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Salmonella in Bovine lymph nodes survey

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Executive Summary

Salmonella contamination of ground beef has traditionally been viewed as originating from the surface of carcasses. A new hypothesis has identified lymph nodes as a significant source of Salmonella contamination since these tissues play an active role in containment of pathogens in the live animal and because some lymph nodes are unavoidably present in manufacturing beef trimmings or primal cuts that may be incorporated into ground beef. A survey was conducted of the microbiological status of lymph nodes from Australian cattle at the time of slaughter to determine the prevalence of microbiological contamination. Sets of lymph nodes (n=197), consisting of the superficial cervical (pre-scapular), pre-pectoral, axillary, pre-sternal, popliteal, ischiatic, subiliac (pre-crural), coxalis and iliofemoralis (deep Inguinal) were collected, from five geographically separated Australian abattoirs over a period of 14 months. Samples were tested for the presence of Salmonella spp. and Shiga toxin-producing Escherichia coli (STEC). Aerobic plate count, E. coli and coliforms were enumerated with a lower limit of detection of 80 cfu/node. The observed prevalence of Salmonella within peripheral lymph nodes was 0.48% (7/1464). Two of the seven lymph nodes in which Salmonella was detected came from the same animal. Grass-fed, grain-fed and cull dairy cattle were all found to have detectable Salmonella in lymph nodes. All Salmonella detections occurred during cooler months of the year. No STEC were detected. Aerobic microorganisms were detected above the limit of quantification in 3.2% of nodes (median count 2.24 log10/node) and E. coli was detected in 0.8% of nodes (median count 3.05 log10/node). The low prevalence and low potential concentration of Salmonella in the lymph nodes of Australian cattle at the time of slaughter, suggests that the risk of lymph nodes contributing significantly to the presence of Salmonella in ground beef is low.

Salmonella, and to a lesser extent, some strains of Shiga toxin-producing Escherichia coli (STEC) are significant causes of foodborne disease throughout the world (24), and a continuing problem in the United States of America (31) with beef and beef products being recognized vehicles for illness in humans. In the USA, ground beef has been estimated to be responsible for 28% of salmonellosis arising from consumption of eggs, pork, poultry and beef (19) and 30% of cases of foodborne E. coli O157 illness (21) .

In beef processing, fecal contamination of hides is recognized as the major source of contamination by enteric pathogens such as E. coli O157:H7 (15) and Salmonella (8). In the USA, considerable effort has been made to treat the carcass with antimicrobial interventions to inactivate enteric pathogens that may be present. Processing establishments seek to minimize transfer of Salmonella from hides to carcass, and then treat the carcass to inactivate any Salmonella that may be present (11). However, Salmonella continues to be detected in ground beef. In 2014, the US Food Safety and Inspection Service detected Salmonella in 1.6% of ground beef samples (5). In Australia, the application of Good Agricultural Practice, Good Hygienic Practice and HACCP principles, have resulted in a very low prevalence of Salmonella and indicator microorganisms (29) in both manufacturing beef (beef intended for grinding) and primals.

Lymph nodes have been identified as a source of Salmonella in ground beef, first by inference from the low prevalence of Salmonella on carcasses but relatively high prevalence of Salmonella in ground beef, and then through microbiological analysis of lymph nodes from cattle at the time of slaughter (6). Studies of lymph nodes of cattle collected at the time of slaughter have found significant differences in the prevalence of Salmonella in different lymph nodes (18), between feedlots (20),

seasons (12), and between grain-fed and cull cattle (6, 17). The reason for the differences is not clear, nor is it clear how these production factors may interact to result in the observed prevalence. While attention has been focused on Salmonella in lymph nodes due to the desire to explain the presence of Salmonella in ground beef, a better understanding of the occurrence in lymph nodes of other enteric pathogens (such as the seven serogroups of STEC of regulatory interest in the USA), is also needed to assess the overall safety risks posed by inclusion of lymph nodes in beef products. The present study was designed to produce an estimate of the prevalence of Salmonella and STEC in the lymph nodes of Australian cattle at the point of slaughter, taking into account possible geographic and seasonal variation and the distribution of microorganisms in lymph nodes at differing anatomical locations.

MATERIALS AND METHODS

Survey design. Samples were collected from five processing facilities, one in each of the states of Queensland, New South Wales, Victoria, Tasmania and South Australia. Collectively, these states produce over 80% of the cattle raised in Australia through the period of the survey (25), so the processing facilities were well situated to collect specimens from cattle representing a wide geographical range across major cattle-producing areas. Samples were collected over a 14 month period from October 2015 – December 2016 recording the state from which cattle were consigned to the slaughter facility. One sample set was collected each week (Monday to Thursday) at each establishment. In the middle of August 2016, the sampling rate was increased to 2 sample sets per establishment per week.

Lymph node collection. Chilled carcasses that had been inspected and passed for human consumption were selected randomly. Lymph nodes and surrounding fat were dissected from their location and collected by trained quality assurance staff at each processing facility using equipment sterilised in water at 82°C and placed in sterile bags. From each carcass side a set of nine lymph nodes was collected consisting of the superficial cervical (pre-scapular), pre-pectoral, axillary, pre-sternal, popliteal, ischiatic, subiliac (precrural), coxalis and iliofemoralis (deep Inguinal) lymph nodes were collected.

Storage and transportation. Samples were stored at 4°C and shipped to the laboratory with an icepack to maintain a temperature of < 10^{o}C, with most samples arriving at the laboratory within 48 hours of sample collection.

Sample preparation. Lymph nodes were removed from surrounding tissue and submerged in a boiling water bath for 3-5 seconds to disinfect the outside of the node (10). Nodes were cut into approximately 1 cm x 1 cm pieces with a sterile knife. The lymph node pieces were placed into a sterile stomacher bag (Daniels Health, Australia), weighed and pulverised with a rubber mallet.

Enumeration. 80ml Modified Tryptic Soy Broth (Oxoid, Basingstoke, UK) was added to each sample and mixed for 60 seconds with a BagMixer 400 laboratory blender (Interscience, Saint Nom, France). A 1mL aliquot was removed to perform serial dilutions to 10-3 in 9mL Peptone Saline Solution (Oxoid, Basingstoke, UK), 1mL of each dilution and 1mL of the initial suspension was used to inoculate single Petrifilm™ Aerobic Count Plates (3M, Minnesota, USA) (3) and single Petrifilm™ E. coli/Coliform Count Plates (3M, Minnesota, USA) (4) with plates incubated at 35°C ± 1 °C for 46 h to 50 h, and 35 °C ± 1 °C for 44 h to 52 h respectively. This method resulted in a lower-limit of quantification of 80 cfu per lymph node.

Salmonella and STEC Detection. After removing the 3ml aliquot for enumeration, the remaining sample was incubated at 42°C ± 1 °C for 15 to 24 h. Detection of Salmonella spp. was performed using the Salmonella BAX® PCR Assay (DuPont Qualicon, USA). STEC detection was by PCR screening for stx and eae genes using the BAX® System Real-time PCR Assay STEC Screening (DuPont Qualicon, USA). STEC screening positive results were then further analysed using the BAX® System Real-time PCR Assay STEC Panel 1, STEC Panel 2, and assay for E. coli O157:H7 (DuPont Qualicon, USA) (1, 2).

Data analysis. Detection rates were calculated based on the number of samples analysed, and the number of samples with results above the minimum limit of quantification. Other quantitative statistics were calculated only for those samples that had results above the minimum limit of quantification. To estimate the relationship between lymph node weight and contamination with Salmonella spp, Stata version 14.2 (Stata Corporation, TX, USA) was used to fit a generalised linear model with binomial error structure and logit link function to the observed data defined by mean lymph node weight (grouped by class intervals) and number of positive samples.

RESULTS

The 197 sets of lymph nodes collected contained only 1,464 individual nodes rather than the theoretical maximum of 1,773 (82.6% of quota). Only 41 sets set included all of the targeted nodes owing to the difficulty of locating nodes and excising from carcasses while they were being processed. For example, the coxalis lymph node was frequently omitted because due to its difficult-to-excise location at the proximal part of the knuckle tip. Other nodes were discarded because they were inadvertently incised during collection (and thus were potentially contaminated). The weights of the lymph nodes varied substantially between anatomical sites (Table 1), and also within each site. The weight data were skewed to the right with lymph nodes several times heavier than the median weight not being uncommon. Weight data were therefore summarised using box plots (Figure 1). Since the lymph node is the unit of analysis, all microbiological results are expressed per lymph node.

Table 1 Prevalence and numbers of indicators and enteric pathogens per lymph node

Lymph node	n	Media n weight (g)	Aerobic Plate Count			Coliforms			*E. coli*			STEC	*Salmonella*
			Number (%) detected	Media n log_{10}	Maximum log_{10}	Number (%) detected	Media n log_{10}	Maximum log_{10}	Number (%) detected	Media n log_{10}	Maximum log_{10}	Number (%) detected	Number (%) detected
Superficial cervical	182	24.8	13 (7.18)	2.24	5.29	9 (4.97)	2.21	3.91	6 (3.31)	1.92	3.91	0	3 (1.65)
Pre pectoral	179	1.7	1 (0.56)	1.90	1.90	1 (0.56)	1.90	1.90	0			0	1 (0.56)
Axillary	167	2.1	2 (1.20)	4.4	5.34	1 (0.60)	3.38	3.38	0			0	0
Pre sternal	162	2.2	4 (2.48)	3.04	5.56	1 (0.62)	3.20	3.20	0			0	2 (1.23)
Popliteal	185	10.0	15 (8.15)	3.2	5.68	9 (4.89)	3.51	5.12	6 (3.26)	3.96	5.12	0	0
Ischiatic	172	3.6	5 (2.91)	1.92	3.20	0			0			0	0
Subiliac	174	24.2	3 (1.72)	1.86	1.89	1 (0.57)	1.91	1.91	0			0	1(0.57)
Coxalis	69	1.9	1(1.45)	6.15	6.15	1 (1.45)	1.89	1.89	0			0	0
Iliofemoralis	175	11.3	3 (1.72)	2.16	5.32	1 (0.57)	2.50	2.50	0			0	0
TOTAL	1465		47 (3.21)	2.24		24 (1.64)	2.4		12 (0.82)	3.05		0	7 (0.48)

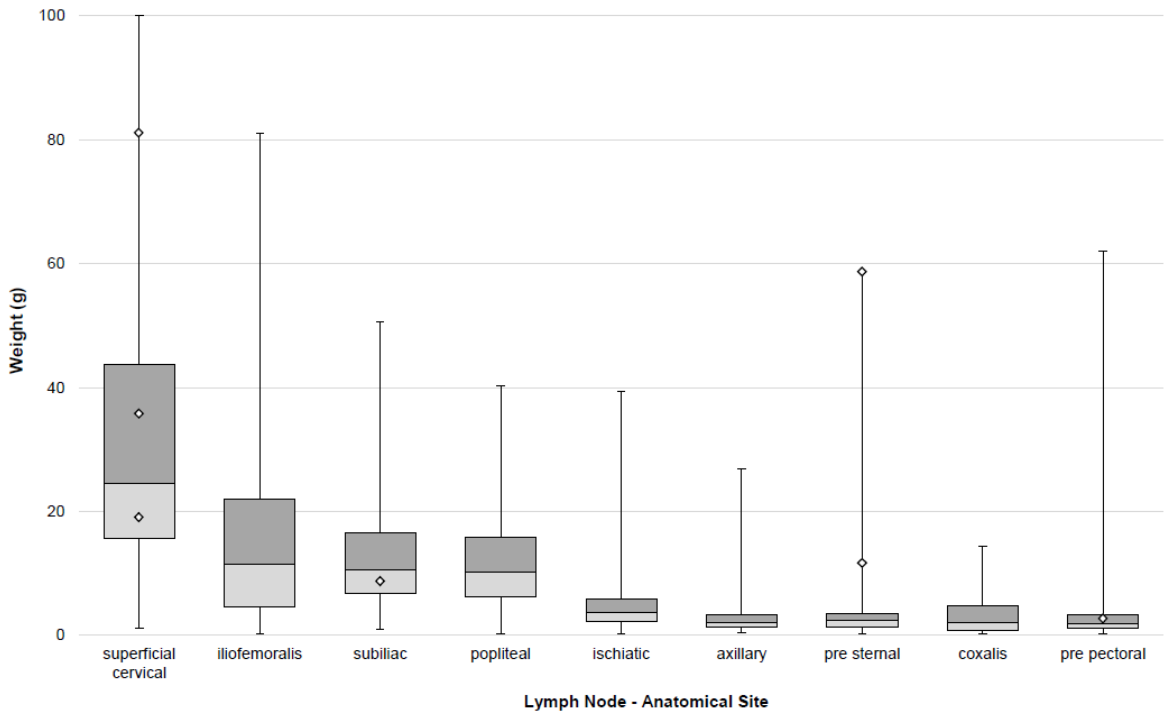


Figure 1 Distribution of lymph node weight at each anatomical site. The box defines the 25th and 75th percentile, with the median (50th percentile) being marked by a horizontal line. The vertical lines indicate the extent of data expected by the distribution. Red dots indicate the weight of the nodes in which Salmonella spp. were detected.

Table 2: Characteristics of bovine source for samples in which *Salmonella* was detected

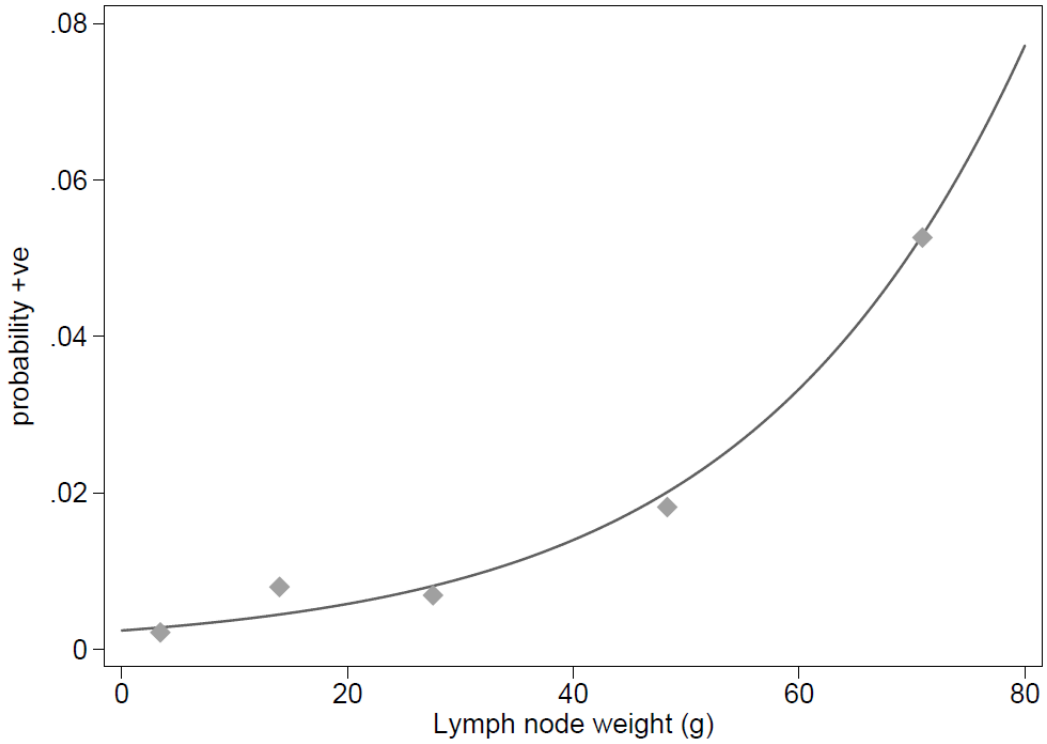
Raising system	State	Month	Node	Serotype	Weight	Number of organisms per lymph node		
						APC	Coliforms	*E. coli*
Dairy	Tas	April	pre-sternal	Kentucky	11.6	<80	<80	<80
Grain-fed	Qld	May	subiliac	Chailey	8	<80	<80	<80
Dairy	Tas	August	pre-sternal	Dublin	58.6	14,064	1582	<80
Grass-fed*	NSW	August	superficial cervical	Virchow	19.2	<80	<80	<80
Grass-fed*	NSW	August	superficial cervical	Virchow	36.2	<80	<80	<80
Grain fed	Qld	September	superficial cervical	Typhimurium	81.1	81	162	<80
			pre-pectoral	Typhimurium	2.4	<80	80	<80

* same lot of animals

Bacteria usually considered as indicator organisms in food microbiology were quantified, using standard food microbiology methods, with a limit of detection of 80 cfu/lymph node. The prevalence of contamination was expressed as the proportion of nodes having bacterial contamination above the lower limit of quantification (Table 1). Bacteria were detected in lymph nodes from all anatomical sites, coliforms and E. coli in a few nodes, and Salmonella in a few nodes that did not necessarily have high prevalence or concentration of contamination by other bacteria (Table 1). The overall prevalence of lymph nodes detected as APC positive was 3.2% and no lymph node site had more than 9% of nodes APC positive.; only the popliteal and superficial cervical nodes had more than 5% of samples positive for APC. Amongst APC positive nodes the median APC count was 2.24 log10/node. Similarly, coliforms were detected in 1.6% of nodes overall and no anatomical site had more than 5% of lymph nodes positive for coliforms. Only popliteal and superficial cervical nodes were found to be contaminated with coliforms at near this level (> 4.5% of samples). The median coliform count for nodes in which coliforms were detected was 2.4 log10/node. E. coli was detected in 0.8% of lymph nodes. Only popliteal and superficial cervical nodes were found to be contaminated with E. coli at prevalence between 3 and 4%. The median E. coli count for nodes in which E. coli was detected was 3.05 log10/node. No STEC were detected on enrichment. Salmonella spp. were detected in 7 nodes (0.48%) in four different anatomical sites (superficial cervical, pre-sternal, subiliac, pre-pectoral) though two of those sites (subiliac and pre-pectoral) were infrequently contaminated by other microorganisms (Table 1).

The seven Salmonella isolates came from six different animals, and five different lots of animals. The samples in which Salmonella were detected were from animals originating from three geographically disparate locations, all in late autumn through to early spring (winter months). The proportion of positive lymph nodes in the winter months (7/684 = 1.02%) was significantly higher than the proportion positive in the summer months (0/781 = 0%; P= 0.005, Fishers exact test). The serotypes of the two isolates from the same animal were both S. Typhimurium, and S. Virchow was isolated from two animals from the same herd. The maximum level of Salmonella in these nodes could be inferred from the difference between the APC and coliform count (since Salmonella are non-coliform organisms). In 6/7 nodes the maximum possible Salmonella level was below the limit of detection of 80 cfu/node.

The detection of Salmonella was related to the weight of the lymph node that was enriched. Salmonella was more likely to be detected in larger nodes, irrespective of the anatomical position. The logistic model revealed a weak although significant relationship (P=0.005) between weight (g) of lymph node enriched and probability of detection of Salmonella, there being an increase in the odds of detecting Salmonella of 1.04 times for every gram increase in lymph node weight in the enrichment (figure 2). Three out of seven nodes in which Salmonella were detected were heavier than the 75th percentile of nodes from that anatomical site.



Firgure 2 Relationship between lymph node weight and the probability of detecting Salmonella spp. Points represent prevalence of Salmonella spp. for lymph node samples in a weight range expressed as the mean weight of those samples.

DISCUSSION

Prevalence and characterization of Salmonella. The prevalence of Salmonella in bovine lymph nodes has been highly variable from study to study. Probably the most comprehensive and thorough US study of prevalence recorded a median prevalence of 1.3% in the subiliac lymph node (17) compared to 0.57% in this study. The overall prevalence of Salmonella found in this study was 0.48% which is notably lower than the prevalence of Salmonella spp. observed similar studies (6, 10) reporting prevalences of 1.6% and 0.80%.

The quantity of Salmonella cells found in lymph nodes in this study was also comparatively low. One study in Nebraska, USA found mean concentration of 3.57 log10 per node in animals raised in an experimental feedlot (32). A broader survey in the USA with sensitive quantification of Salmonella found a geometric mean concentration of 56 cfu/g; however, over 40% of nodes in which Salmonella was detected had levels above 80 cfu/g (17). In this study only 1/7 nodes had the potential to have a Salmonella concentration above 80 cfu/node.

The Salmonella serotypes found in this survey are mostly consistent with those previously found in the feces of Australian cattle at slaughter (9). Salmonella Typhimurium was the most common serotype found in feces with S. Virchow, S. Chailey, and S. Dublin found infrequently. S. Kentucky was not isolated from the comprehensive fecal survey. S. Typhimurium and S. Virchow are amongst the five most highly reported serotypes isolated from humans in Australia in 2011 (28), though there is little evidence of beef being implicated to a significant degree in foodborne illness in Australia (16).

Concerns have been expressed about the presence of multi-drug resistant Salmonella in ground beef (13) particularly S. Typhimurium, S. Hadar, S. Newport, and S. Heidelberg. Only S. Typhimurium was found in this survey. The antimicrobial resistance (AMR) of Salmonella isolated from lymph nodes has been determined in previous US-based studies (6, 17). Although AMR was not determined in this study, a recent large-scale study on the prevalence of AMR Salmonella in the feces of Australian cattle at slaughter (9) found that the prevalence of resistance to common first line treatment antimicrobials such as ampicillin, trimethoprim-sulfamethoxazole and tetracycline were all less than 10% and resistance to antimicrobials usually held in reserve for treatment of human infections such as third-generation cephalosporins and fluoroquinolones, was not detected at all.

Ecology. In comparison to other studies, the prevalence and concentration of Salmonella in lymph nodes examined in this study appear to be lower, As well, Salmonella were isolated from lymph nodes from late autumn through to early-spring which contrasts with the situation in the USA, where prevalence of Salmonella in lymph nodes has appeared to be higher in summer (8, 17), and also affected by seasonal influences particular to the geographic area. This survey sampled animals over a 2,000km north-south gradient. Different ecological factors are likely to affect Salmonella ecology and therefore infection in those different regions, but it is of interest that no Salmonella were isolated from nodes of cattle harvested in summer months, even in the lower latitudes. One theory proposed to explain the presence of Salmonella in lymph nodes is transdermal or intradermal introduction of Salmonella through abrasions or biting flies which are more prevalent in summer (14, 27). Consequently, differences in the occurrence of biting flies or other factors between geographic locations and times could explain differences in the prevalence of Salmonella occurrence in bovine lymph nodes.

Risk of Salmonella contamination of ground beef. The risk posed by Salmonella originating in lymph nodes is managed in a number of ways. Clinically-affected animals should be excluded from slaughter through ante-mortem inspection, fevered carcasses, and those affected from gross abnormalities are required to be excluded from human consumption if detected at post-mortem inspection. Animals systemically infected with Salmonella were not found during this survey, though one animal had the same serotype detected in two adjacent lymph nodes. It is highly unlikely that clinically affected animals would enter the human food supply. However, it is possible that clinically normal animals with multiple affected lymph nodes could enter, such as occurred in this study. It is possible that herds of animals coming to slaughter may be affected by a common serotype, as seen in this study. The low prevalence and low count of Salmonella cells in lymph nodes provide evidence of the favourable hygienic status of Australian cattle at slaughter, and the low potential for any lymph node to pose a high risk of human exposure.

Some lymph nodes are unlikely to be incorporated into manufacturing beef. Lymph nodes, such as superficial cervical, popliteal, subiliac, prescapular, iliofemoralisand axillary nodes are easily, and therefore, frequently removed when trimming primal cuts to specification (7) and are discarded for rendering. The coxalis lymph node was found difficult to recover in the present work, but was infrequently a source of microorganisms.

Overall, it is unlikely that Salmonella present in lymph nodes contribute substantially to the occurrence of Salmonella in Australian manufacturing beef. Fundamentally, this is due to the low prevalence and concentration of Salmonella in lymph nodes as demonstrated in this study, the purposeful removal of many accessible nodes at processing, and the predominant emphasis on production of lean manufacturing meat involving exclusion of lymph-nodes associated with adipose tissues. The low concentrations of Salmonella measured in both manufacturing beef (26) and in lymph nodes (this study), suggests that if Salmonella do enter a batch of product destined for grinding then the process will inevitably dilute these organisms to a very low concentration which may be difficult to detect.

Confidence in these predictions may be gained through considering Salmonella prevalence in ground beef. In the USA in 2014, Salmonella was isolated from 1.6% of ground beef samples (5). A retail survey of ground beef in Australia conducted in 2005 found Salmonella in 4 (1.1%) of 360 ground beef samples (30). For various reasons including the potential for temperature abuse through the retail supply chain, the sourcing of raw materials from a variety of establishments, and the improvements in industry since this survey was conducted, ground beef produced from lean Australian manufacturing beef in the USA in the current period would likely have much lower Salmonella prevalence.

Although a curvilinear relationship between detection of Salmonella and lymph node weight was found, the risk of detection increases only very slowly over the range of weights observed. For example, when the lymph node mass is 40 grams the slope of the curve given in Figure 1 is 0.0001 indicating that the probability of Salmonella detection is increasing by only 0.01% for each gram of increase in node mass. Similarly, at lymph node weights of 60g the probability of detection is increasing by only 0.15% for each gram of node mass. Thus, while the probability of detection does increase quickly in relative terms the scale is small in absolute terms and represents a trivial change in overall risk involving larger lymph nodes. The reason why Salmonella was more likely to be detected in larger lymph nodes is uncertain: possibly it is not just a matter of larger capacity for processing invading organism but because some large nodes are situated at anatomical location where there is a greater challenge from invading pathogens. Regardless, given the very small increases in risk associated with larger lymph nodes it is difficult to justify their excision from carcases given that many

are difficult to locate and access. The extra cutting and handling during the removal process would likely contribute to the increased in contamination as has been shown in the management of lymph node infections in sheep at slaughter (22). Thorough cooking remains an effective terminal step for the control of enteric pathogens in the preparation of ground beef products (23).

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Appendix 1-

Table of Contents

1 Background

1.1 **Strategic Purpose**

Establish the prevalence of *Salmonella* spp. and other pathogenic bacteria in the peripheral lymph nodes which may be incorporated into manufacturing beef, and thereby, into ground beef products.

1.2 **Industry Significance**

There is a developing hypothesis, in the United States of America (USA), that *Salmonella* found in ground beef does not only come from faecal contamination of the external meat surface, but from lymph nodes that are found within beef primals and manufacturing beef that are used to produce ground beef. It is estimated that approximately 26 nodes are located adjacent to muscle that may be specifically included in ground beef and at least six of these are recommended for removal to exclude their presence in ground beef e.g. superficial cervical, axillary, subiliac, popliteal, coxalis, and iliofemoralis.

Given the physiological function of lymph nodes are to filter fluids and substances collected in the lymphatic system including pathogenic microorganisms, lymph nodes are considered a potential harborage site in the carcass of infected animals.

Gragg *et al.* (2013), suggest that higher rates of carriage are associated with warmer climates, and that there was a correlation between *Salmonella* prevalence on cattle hides, in the cattle environments and in the peripheral lymph nodes. They concluded that the infection is likely to be transdermal via abrasions or biting insects with passage of *Salmonella* to the peripheral nodes via afferent lymphatic vessels.

Meat & Livestock Australia (MLA) commissioned advice to provide background information about location of the peripheral lymph nodes in the bovine carcass, and establish the likelihood of the nodes being incorporated into primal cuts or trim.

Analysis of Carton Meat Assessment records shows 5% of sampled cartons contain relatively large lymph nodes (averaging 25 x 15 mm) rather than smaller ones. The survey also found that of the 26 nodes, 12 nodes (Axillary, Prescapular, Pre-pectoral, Pre-pectoral, Pre-sternal, Deep inguinal, Superficial inguinal, Internal iliac, External iliac, Ischiatic, Popliteal, Coxalis and Subiliac) were readily accessible for removal in the boning room. Of the 12 nodes, 3 (Superficial inguinal, Internal iliac and External iliac) pose little risk of being included into manufacturing beef as they are usually trimmed out by operators.

A 2014 *Salmonella* in Manufacturing Beef Survey (MLA, 2014) determined a national *Salmonella* prevalence of 0.7% in manufacturing beef from 1255 samples. The *Salmonella* concentration of the positive samples was low (<0.3 MPN/g) with no apparent correlation between indicator microorganisms (such as TVC, E.coli & coliforms) and *Salmonella.*

Phillips et al. (2012) during an Australian National Survey of the microbiological quality of frozen bonelss beef and beef primal cuts, observed no detectable *Salmonella* or *E. coli* O157:H7 within 1,144 primal cut samples, and no detectable *Salmonella* from 1,165 boneless beef samples.

The Australian Beef Industry has a global reputation as clean and green. In addition to previous MLA food safety studies, determining the prevalence of *Salmonella* spp. in lymph nodes from Australian cattle presents a significant opportunity to assess the health of our cattle herds.

1.3 **Project Aims**

The aim of the *Salmonella* spp. in bovine lymph nodes survey is to representatively sample Australian beef carcases to determine the prevalence of *Salmonella* in nine pre-determined lymph nodes. Additional microorganisms, Shiga toxin-producing *E. coli* (STEC), TVC, *E. coli* and Coliforms were also determined to further profile the food safety risk of lymph nodes and assist with clarifying the food safety position on the Australian beef industry.

A lymph node set included nine pre-determined lymph nodes namely: Pre-pectoral; Pre-sternal; Ischiatic; Axillary, Prescapular; Deep inguinal; Popliteal; Coxalis; and Subiliac. These lymph nodes have been highlighted as of concern in the USA and/or being present in Australian manufacturing beef.

A target of 200 lymph nodes sets was to be collected from bovine carcases from a limited number of the export establishments to provide 1800 lymph node samples.

Participating establishments were volunteers and only one establishment was chosen from the states of QLD, SA, VIC, TAS and NSW. Each participant was provided with lymph node sample collection training, carried out on site with relevant QA personnel or qualified meat inspectors. Trained staff were instructed to randomly sample carcasses.

For each lymph node sample set, a sample submission form was prepared to capture corresponding carcass information such as:

- Collection date
- State of carcase origin
- Carcase ID
- Age
- Gender and type of stock
- Breed
- Sampling point
- Pathology observations
- Samples collected by
- Samples submitted by
- Date submitted
- Cattle condition before hide removal (insect bites / wounds)

Information obtained from the survey can assist in determining the position of Australian cattle compared to US cattle and the consideration to invest in practices to exclude lymph nodes from US export trim.

2 Project Objectives

Determine the prevalence of *Salmonella* in the 9 lymph nodes (Pre-pectoral, Pre-sternal, Ischiatic, and including the 6 recommended by the USDA Agricultural Research Service (Axillary, Pre scapular, Deep inguinal, Popliteal, Coxalis and Subiliac) that have been highlighted as a concern and with the potential of being included into manufacturing beef.

3 Methodology

Research project aims to collect 9 lymph nodes sets from 200 bovine carcases collected at five establishments, to provide approx. 1800 lymph nodes samples. All nine lymph nodes were collected from the one carcass. Samples were collected by trained QA personnel or a qualified meat inspector capable of accurate identification and removal of bovine lymph nodes.

Collection of lymph nodes was requested to be performed before any carcass trimming. Samples were not strictly collected aseptically from the carcasses as this would have increased collection time; however, the samples were collected as aseptically as possible with the surrounding fat tissue encapsulating it.

Lymph nodes were collected approximately weekly from slaughter floors and boning rooms. Sampling kits for each sampling event were provided to each participant and samples were returned to the laboratory within 24 hours of sampling to ensure temperature upon receipt was <10□C and not frozen.

3.1 **Location of lymph nodes**

Pre-scapular – is located from the fat of the chuck in the front pint of the shoulder, under the superficial musculature of the shoulder, at the anterior border of the chuck tender.

Pre-pectoral – are located near the thoracic inlet, on or between the lower anterior borders of the first two ribs, usually embedded in fat.

Axillary – are sometimes paired and located in the axilla under the scapula in the loose tissue between the muscles of the chest wall and those of the shoulder tender.

Pre-sternal – are found in the spaces between the costal cartilages of the first 8 to 9 ribs, under a covering of a sheet of muscle on the floor of the thorax.

Popliteal – is located deep in the intermuscular fat in the popliteal space of the hind leg, on the level of the stifle joint on the hell muscle between the bottom round and eye of the round.

Ischiatic – Loctaed outside the pelvic cavity, on the outer aspect of the sacro-iliac ligament at the posterioir end of the cavity.

Pre-crural/subiliac – is embedded in the fat in the fold of the flank below the stifle joint. Situated in-between the surface flank muscle and bottom edge of the knuckle muscle, where it is in close contact with thi tri-tip.

Coxalis – located in the proximal part of the knuckle tip under cover of the upper end of the tri-tip.

Deep Inguinal – located at the brim of the pelvis, close to the inguinal canal in the pevic channel.

3.2 Collection and sample delivery to laboratory

Following collection, each lymph node was individually bagged, and stored at 4°C until a complete set of lymph nodes from the carcass had been collected. Each set of lymph nodes was then placed in transport esky with hydratable ice sheets to ensure a transport temperature of 0°C to 10°C and dispatched to the testing laboratory. Samples were received at the laboratory within 24hrs of sampling. Once received by the laboratory, the time temperature of receival was documented.

3.3 Sample data collected

The following data was collected via sample submission form, for each lymph node set collected:

- Collection date
- State of carcase origin
- Carcase ID
- Age
- Gender and type of stock
- Breed
- Samples collected by
- Samples submitted by
- Date submitted

3.4 Preparation of lymph nodes for enrichment

Lymph nodes were aspectically removed form the sample bags, and placed on sterile stainless steel trays. Using sterile tweezers and scalpel, any surrounding adipose and/or connective tissue was removed. The lymph node was then surface sterilised in a boiling water bath for 3 to 5 seconds, following surface sterilisation the lymph node was then cut into 1cm x 1cm cubes, placed into a sterile stomacher bag and weighed. The stomacher bag was then heat sealed, and the lymph node pulverised via a rubber mallet for 30 seconds to produce a homogenised paste like consistancey. The stomacher bag was then aseptically opened and 80ml of Modified Tryptic Soy Broth was added, this primary enrichment was then homogenised by stomaching for 60 secs. A total of 2ml was removed from the primary enrichment for Total Viable Count (1ml), *E. coli* and Coliform (1ml) analysis, resulting in a limit of quantification of <80 cfu/Lymph node. The primary enrichment sample was then incubated at 42°C for 24hrs.

3.5 Microbiological methods for testing lymph nodes collected

The methods utilised during this study are detailed below:

- *Salmonella spp.* detection - MLG 4C
- *Salmonella spp.* confirmation - AS5103.10 (2009)
- *E. coli* STEC detection initial screening MLG 5B, E. coli O157:H7 MLG 5A
- Confirmation *E. coli* STEC MLG 5B, *E. coli* O157:H7 ISO 16654 (2001)

- Serotyping *Salmonella* isolates – subcontracted to Queensland Health Scientific Services, Microbiology – Public Health Laboratories 39 Kessels Road, Coopers Plains QLD 4108
- Serotyping *E. coli* STEC isolates – subcontracted to Melbourne Diagnostic Unit (MDU), Public Health Laboratory, Department of Microbiology and Immunology, University of Melbourne, VIC 3010
- Total Viable Count AOAC 990.12
- *E. coli* enumeration AOAC 991.14
- Coliforms enumeration AOAC 991.14

3.6 **Project Management**

The project requirements for the laboratory were as follows:

- Receive samples, record arrival temperatures, register samples and store samples prior to testing.
- Test samples on same day as received.
- Test samples according to approved methods.
- Report results in standard certificate of analysis format.
- Report any factor such as sample temperature and sample transit delays that may unduly influence test results.
- Determining the prevalence and concentration in lymph nodes over a 12-18 month period for:
    - *Salmonella*,
    - TVC,
    - Coliforms, *E.coli*
    - Shiga toxin-producing *E. coli* (STEC)

Performance of Project Manager Responsibilities were as follows:

- Obtained agreement of five establishments in QLD, SA, NSW, TAS, VIC to conduct sampling
- Provided instructions for submission of samples to laboratories.
- Provided detailed instructions and specifications for collection of intact lymph nodes to each sampler.
- Trained each establishment on sample collection method
- Arranged and managed sample collection, transport to the laboratory and tests to be performed on each sample set
- Worked closely with samplers and operational laboratory teams to ensure that collection, sample preparation and testing is performed according to requirements.
- Prepared monthly spreadsheets containing laboratory data for further analysis in conjunction with MLA.
- Performed data accuracy checking and preliminary data analysis.
- Where appropriate, referred any matter to MLA that may compromise the conduct of the sampling and analysis, the quality of the analytical data or compliance with the survey design.

DTS and project partners collected a total of 197 lymph node sets with a total of 1464 lymph nodes tested. The most common lymph node that was missing from sample sets was the coxalis, which is located in the proximal part of the knuckle tip, under the cover of the upper end of the tri-tip. All participants experienced difficulty in locating and incising this node successfully on a routine basis.

A total of 41 complete lymph node sample set were collected and tested.

4 Results

4.1 Summary - Lymph nodes received and tested (197 sample sets)

Lymph Node Type	No. Tested	Not Received[A]	Missing[B]	Cut[C]	Not Tested (%)
Pre scapular	182	2	1	12	7.61%
Axillary	167	8	7	15	15.23%
Deep inguinal	175	6	4	12	11.17%
Ischiatic	172	1	5	19	12.69%
Popliteal	185	1	1	10	6.09%
Pre-crural/subiliac	174	7	3	13	11.68%
Pre pectoral	178	5	7	7	9.64%
Pre sternal	162	7	9	19	17.77%
Coxalis	69	114	4	10	64.97%
Total	1464	151	41	117	17.43%

[A] Participant did not take this sample, [B] Lymph node not present in meat sample received, [C] Lymph node significantly cut during collection and was unable to be tested

4.2 Summary - Lymph nodes tested per State[A].

Lymph Node Type	QLD	SA	TAS	VIC	NSW	Total
Pre scapular	57	12	27	43	43	182
Axillary	55	11	29	38	34	167
Deep inguinal	53	11	29	41	41	175
Ischiatic	52	12	27	44	37	172
Popliteal	56	12	28	43	46	185
Pre-crural/subiliac	52	12	29	40	41	174
Pre pectoral	57	11	29	43	38	178
Pre sternal	57	9	28	34	34	162
Coxalis	3	1	28	0	37	69
Total	442	91	254	326	351	1464

[A] "State" refers to the origin of the beast, not the processing facility location

4.3 Summary – Lymph Nodes per Lymph Node Set (Single Animal)

No. Lymph Nodes (per set)	State					Total % of All Sets
	QLD	VIC	TAS	SA	NSW	
Nine	2	0	23	1	15	21%
Eight	29	28	5	5	12	40%
Seven	10	9	1	6	5	16%
Six	11	5	0	0	11	14%
Five	7	1	0	0	3	6%
Four	4	1	0	0	1	3%
Three	1	0	0	0	0	1%
Two	1	0	0	0	0	1%
One	0	0	0	0	0	0%
Total	65	44	29	12	47	-

4.4 Summary – Lymph Nodes per Set (Single Animal) per Month/Season

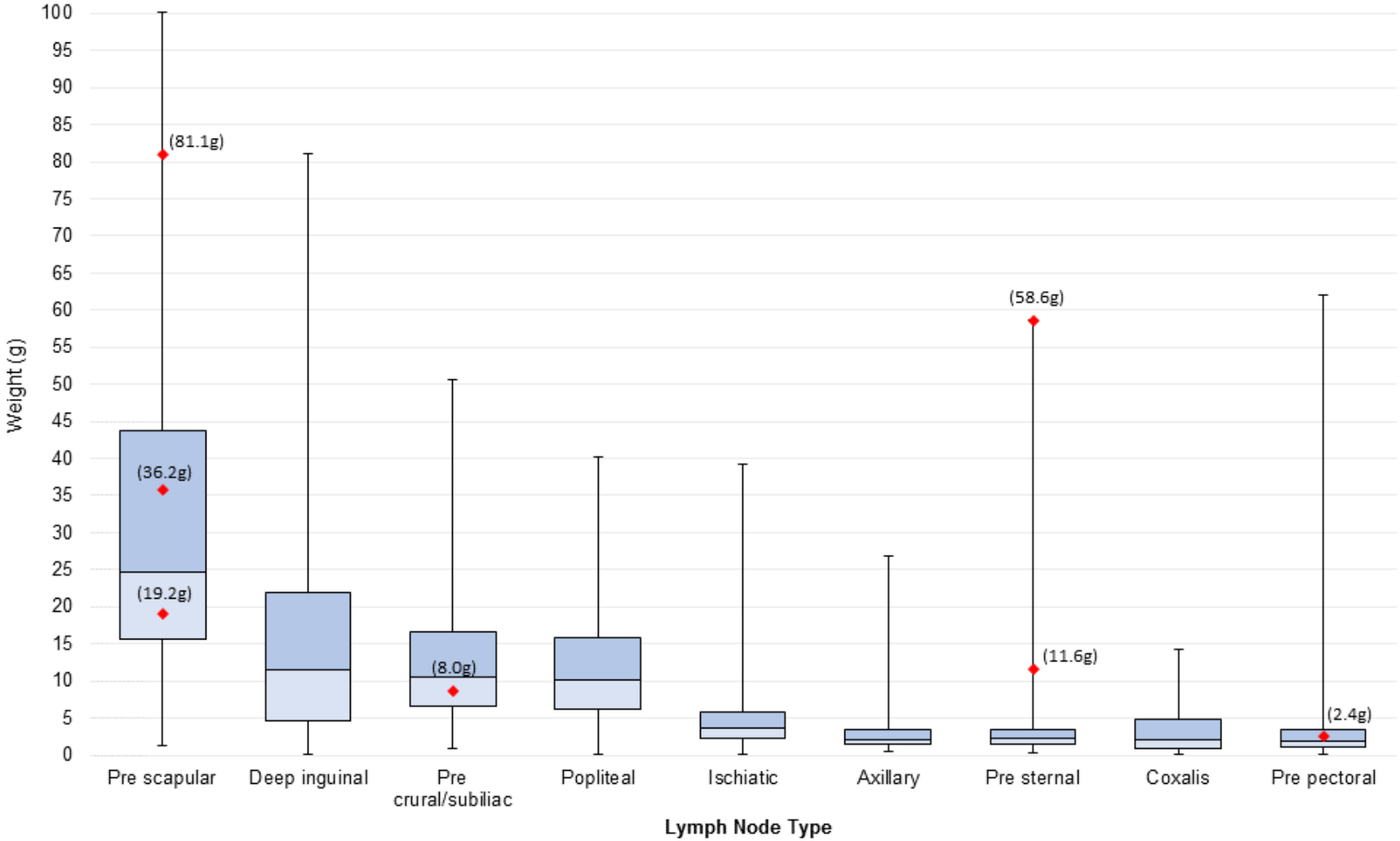
No. Lymph Nodes (per set)	Summer			Autumn			Winter			Spring			Total % of all Sets
	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	
Nine	2	5	4	3	4	3	3	2	3	3	6	3	20.8%
Eight	9	4	7	9	5	6	9	1	8	3	8	10	40.1%
Seven	2	0	2	1	3	1	1	1	6	5	3	6	15.7%
Six	0	0	4	1	1	2	0	2	4	6	4	3	13.7%
Five	0	1	1	0	0	3	1	0	1	2	2	0	5.6%
Four	0	0	0	0	1	0	0	1	1	2	1	0	3.0%
Three	0	0	0	0	0	0	0	0	0	0	0	1	0.5%
Two	0	0	0	0	0	0	0	1	0	0	0	0	0.5%
One	0	0	0	0	0	0	0	0	0	0	0	0	0.0%
Total	13	10	18	14	14	15	14	8	23	21	24	23	-

4.5 Summary - Lymph node types and weights submitted for testing

Lymph Node Type	Weight (g)				
	Min	Max	Median	Mean	SD[A]
Pre scapular	1.1	100	24.5	30.5	19.4
Axillary	0.3	26.7	2	2.9	3.0
Deep inguinal	0.1	80.9	11.4	14.9	13.7
Ischiatic	0.1	39.2	3.6	4.7	4.2
Popliteal	0.1	40.1	10.1	12.0	8.2
Pre-crural/subiliac	0.8	50.6	10.4	12.7	9.0
Pre pectoral	0.1	62.0	1.7	3.0	5.4
Pre sternal	0.2	58.6	2.3	3.4	5.7
Coxalis	0.1	14.2	1.9	3.2	3.4

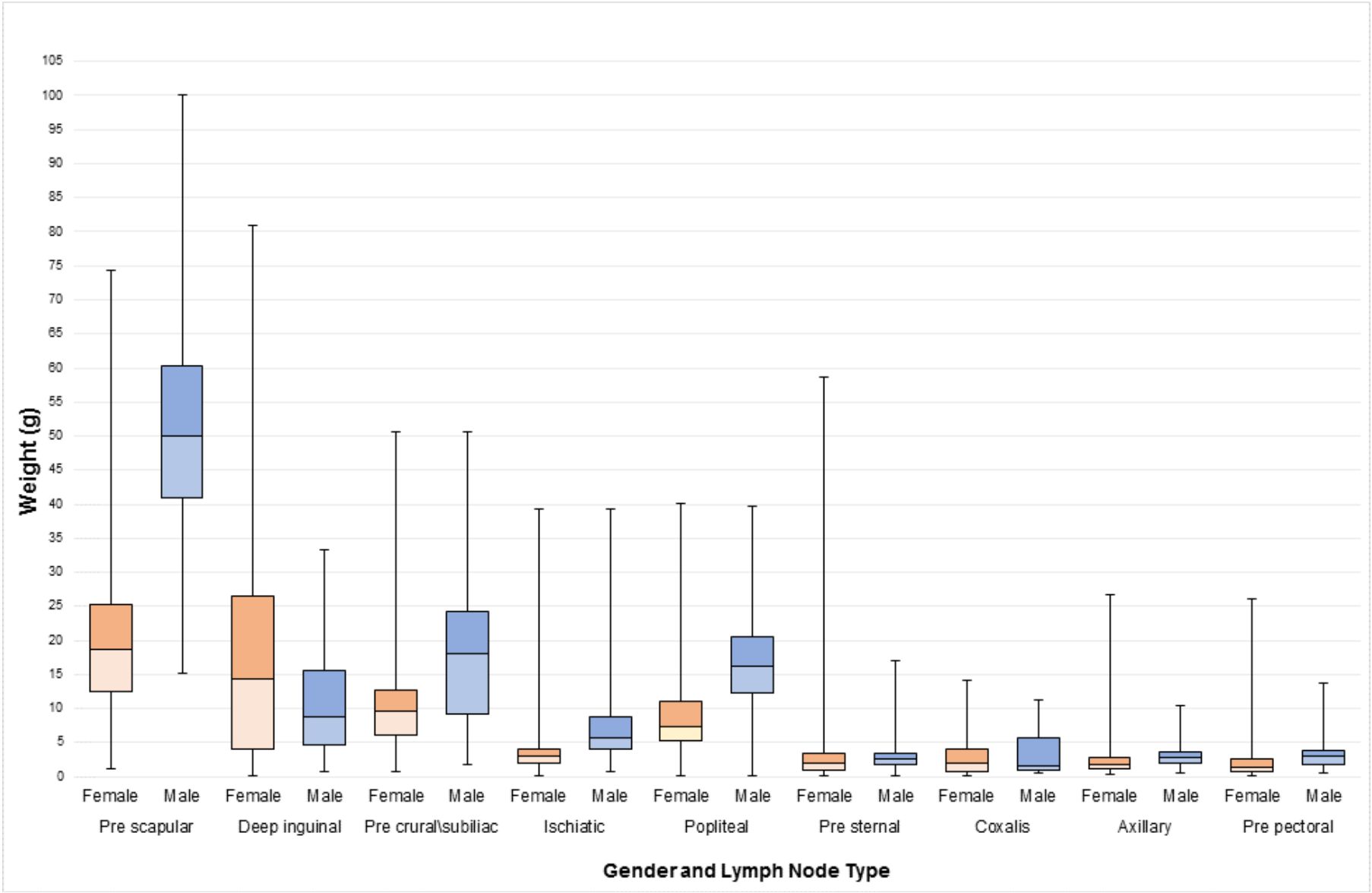
[A] Standard Deviation.

4.6 **Box-Whisker - Weight Distribution of Lymph Nodes – Including *Salmonella* spp. positive sample weights**



Key: ◆ denotes a Salmonella positive (with weight).

4.7 Box and Whisker - Weight Distribution of Lymph Nodes per Gender



4.8 Overall summary of testing data - all samples

Test	Tested	Negative[A]	Positive	Prevelance	Mean[B]	SD[B,C]
Total Viable Count	1458	1409	49	3.36%	1.97	1.63
Coliforms	1459	1434	25	1.71%	1.78	1.22
E.coli	1459	1446	13	0.89%	2.02	1.35
STEC E.coli	1464	1464	0	0.00%	-	-
Salmonella	1464	1457	7	0.48%	-	-

[A] Negative for enumeration methods is a result below the detection/quantification limit of the test method in question, [B] Mean counts of positive samples and SD are in Log10 cfu/g, [C] Standard Deviation

4.9 *Salmonella spp*. and STEC *E. coli* results summary per lymph node type

Lymph Node Type	Tested	Salmonella		STEC E.coli	
		Positive	Prev[A]	Positive	Prev[A]
Pre scapular	182	3	1.65%	0	-
Axillary	167	0	-	0	-
Deep inguinal	175	0	-	0	-
Ischiatic	172	0	-	0	-
Popliteal	185	0	-	0	-
Pre-crural/subiliac	174	1	0.57%	0	-
Pre pectoral	178	1	0.56%	0	-
Pre sternal	162	2	1.23%	0	-
Coxalis	69	0	-	0	-
Totals	1464	7	0.48%	0	-

[A] Prevalence (%)

4.10 Total Viable Count, Coliform & *E. coli* results summary per lymph node type

Lymph Node Type	Total Viable Count[A]					Coliforms[A]					E. coli				
	No Tested	Positive[B]	Prev[D]	Mean[E]	SD[C]	No Tested	Positive[E]	Prev[D]	Mean[E]	SD[C]	No Tested	Positive[E]	Prev[D]	Mean[E]	SD[C]
Popliteal	183	16	8.74%	2.32	1.45	184	10	5.43%	2.72	1.26	184	7	3.80%	3.01	0.85
Pre scapular	181	13	7.18%	1.19	1.15	181	9	4.97%	0.83	0.61	181	6	3.31%	0.86	0.73
Pre sternal	161	4	2.48%	2.53	2.47	161	1	0.62%	1.43	-	161	0	-	-	-
Ischiatic	172	5	2.91%	1.58	0.63	172	0	0.00%	-	-	172	0	-	-	-
Deep inguinal	174	3	1.72%	2.35	2.77	174	1	0.57%	1.15	-	174	0	-	-	-
Axillary	167	2	1.20%	3.91	1.87	167	1	0.60%	1.90	0.66	167	0	-	-	-
Coxalis	69	1	1.45%	5.60	-	69	1	1.45%	1.74	-	69	0	-	-	-
Pre crural/subiliac	174	4	2.30%	0.68	0.25	174	1	0.57%	1.08	-	174	0	-	-	-
Pre pectoral	177	1	0.56%	1.70	-	177	1	0.56%	1.90	-	177	0	-	-	-
Totals	1458	49	3.36%	1.97	1.63	1459	25	1.71%	1.78	1.22	1459	14	0.96%	2.02	1.35

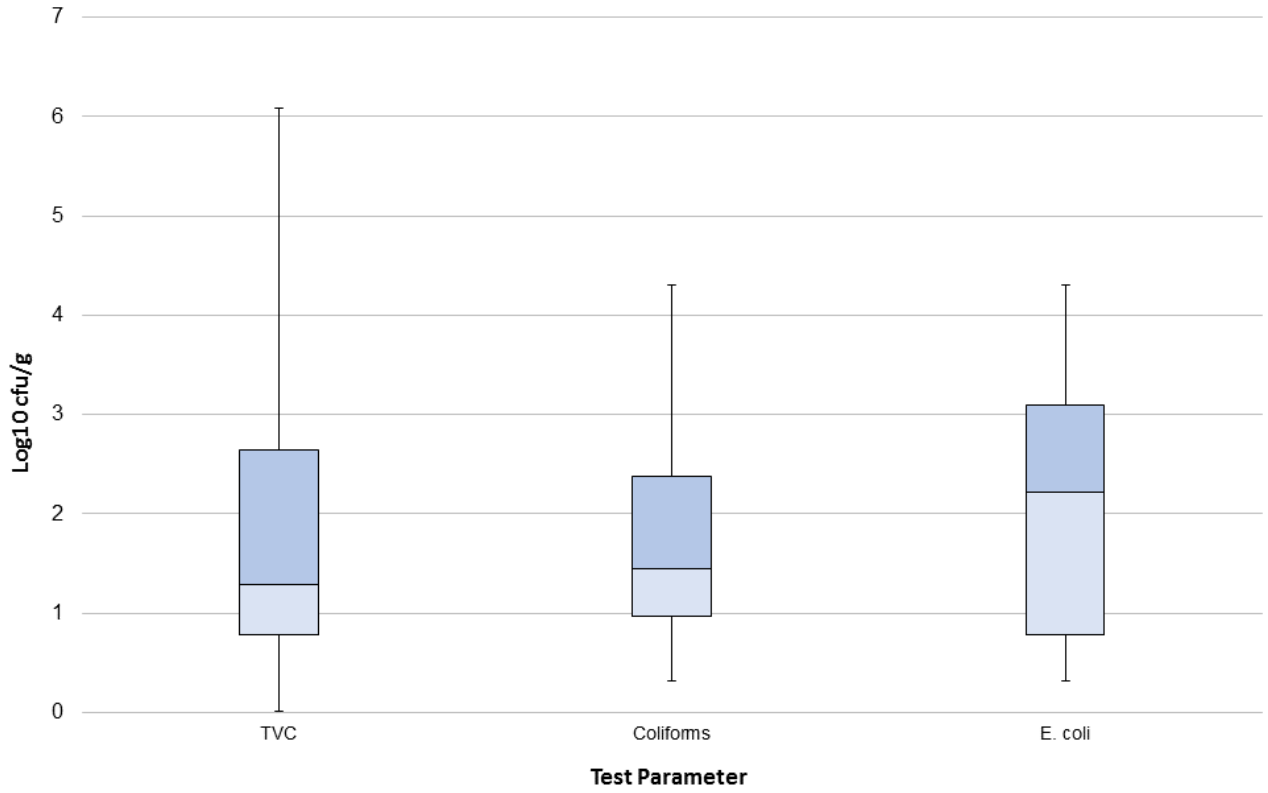
[A] Enumeration units are in Log_{10} cfu/g, [B] Positive enumeration result is a result above lower limit of quantification for the test methodology, [C] Standard Deviation, [D] Prevalence (%), [E] Individual result when only one positive test recorded. [F] 3 Total Viable Counts not reported due to error.

4.11 **Total Viable Count, Coliform and *E. coli* results summary per State[F]**

Lymph Node Type	Total Viable Count[A]					Coliforms[A]					E. coli				
	No Tested	Positive[B]	Prev[D]	Mean[E]	SD[C]	No Tested	Positive[B]	Prev[D]	Mean[E]	SD[C]	No Tested	Positive[B]	Prev[D]	Mean[E]	SD[C]
QLD	436	25	5.73%	1.22	0.99	437	11	2.52%	1.30	0.97	437	5	1.14%	1.27	1.29
VIC	326	4	1.23%	0.86	0.24	326	3	0.92%	1.22	0.31	326	1	0.31%	1.57	-
TAS	254	3	1.18%	1.59	0.99	254	2	0.79%	1.67	0.33	254	0	0.00%	-	-
NSW	351	17	4.84%	3.31	1.76	351	9	2.56%	2.59	1.43	351	7	1.99%	2.61	1.27
SA	91	0	0.00%	-	-	91	0	0.00%	-	-	91	0	0.00%	-	-
Totals	1458	49	3.36%	1.97	1.63	1459	25	1.71%	1.78	1.22	1459	13	0.89%	2.02	1.35

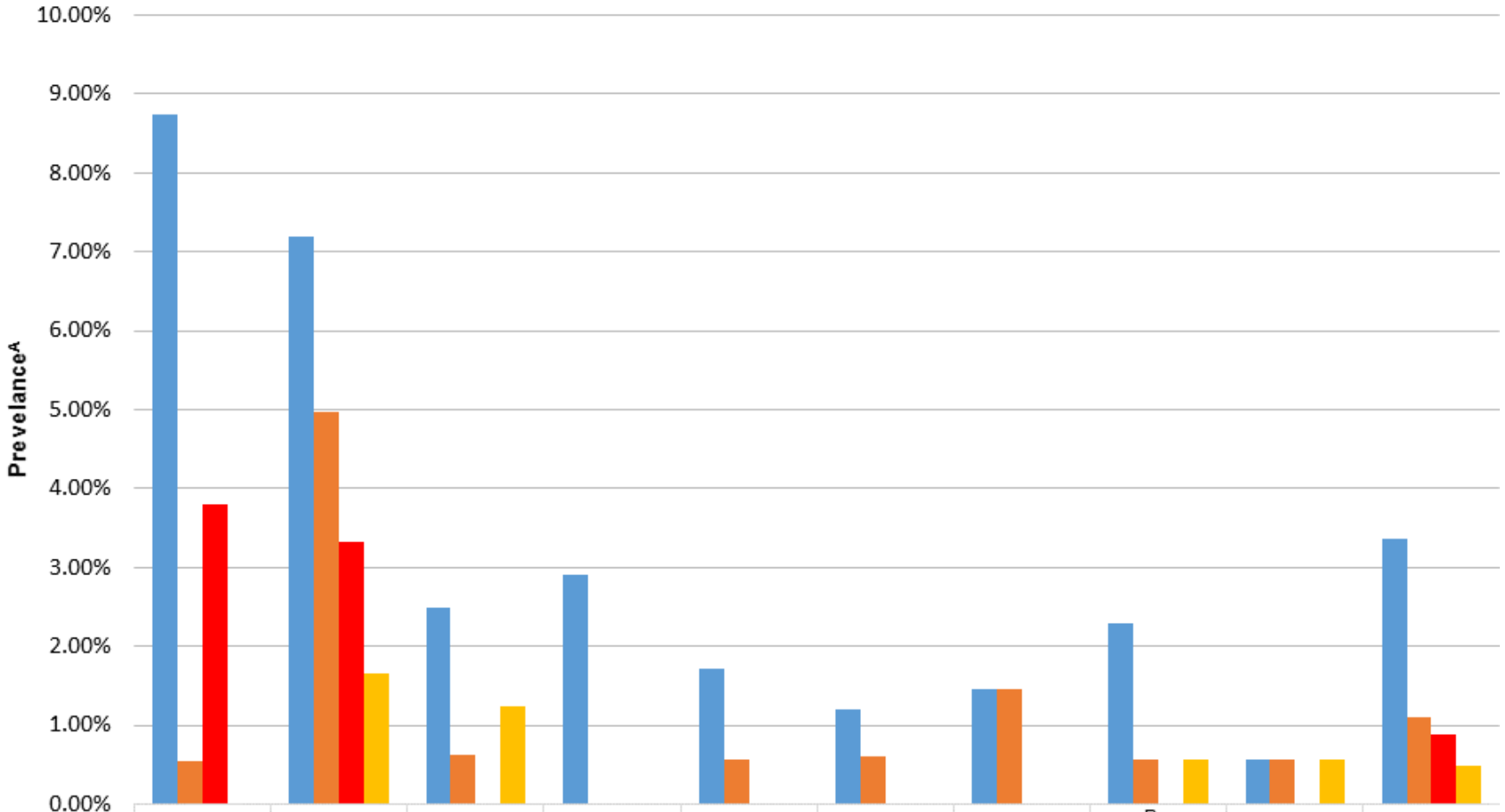
[A] Enumeration units are in Log10 cfu/g, [B] Positive enumeration result is a result above lower limit of quantification for the test methodology, [C] Standard Deviation, [D] Individual result when only one positive test recorded, [E] Prevalence (%).[F] "State" refers to the origin of the carcass, not the processing facility location

4.12 **Box-Whisker – Distribution of Counts for Positive[A] Enumeration Samples**



[A]Positive enumeration result is a result above lower limit of quantification for the test methodology

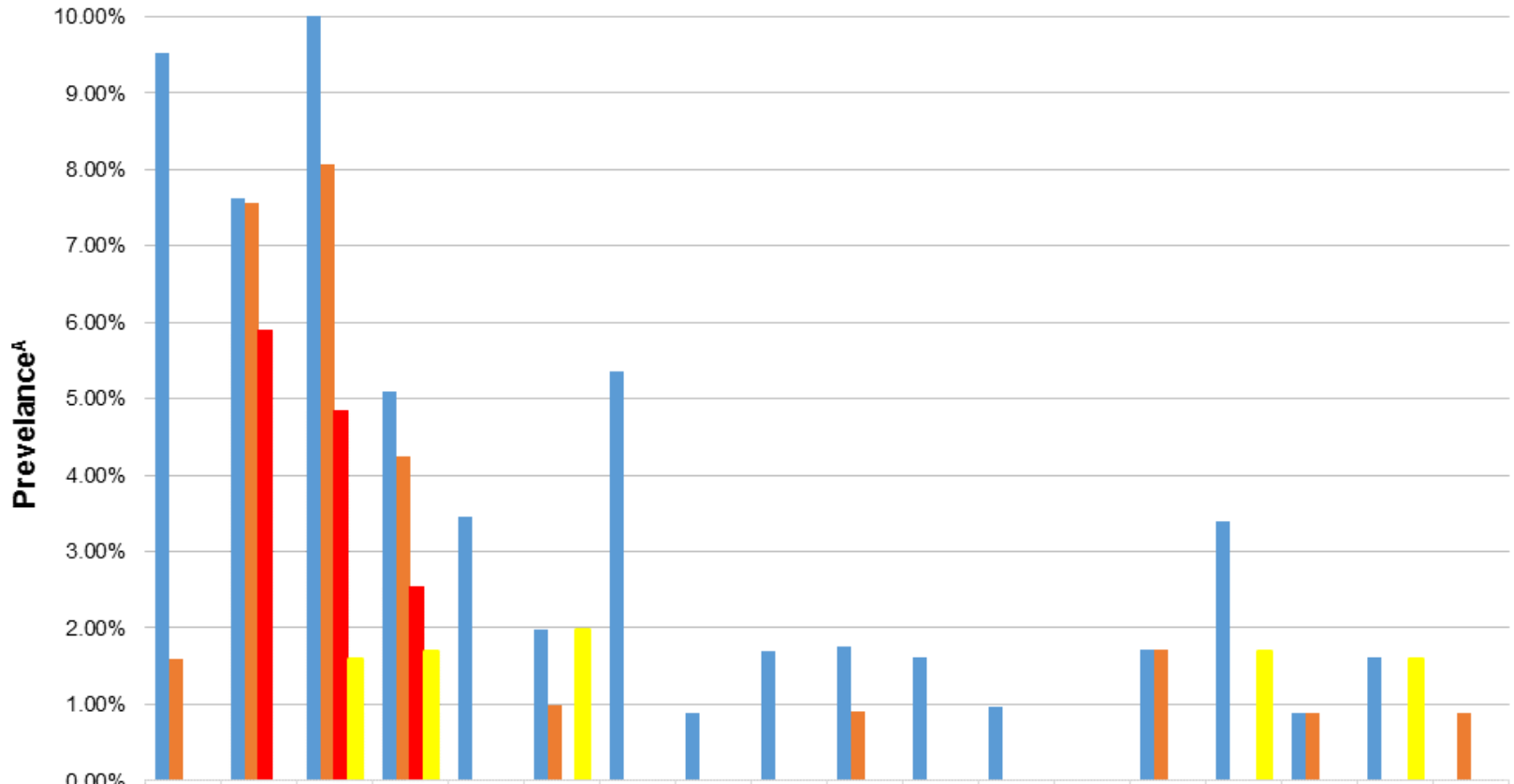
4.13 **Prevalence of TVC, Coliforms, *E.coli* and *Salmonella* per lymph node type**



	Popliteal	Pre scapular	Pre sternal	Ischiatic	Deep inguinal	Axillary	Coxalis	Pre crural/subiliac	Pre pectoral	All
TVC	8.74%	7.18%	2.48%	2.91%	1.72%	1.20%	1.45%	2.30%	0.56%	3.36%
Coliforms	0.54%	4.97%	0.62%	0.00%	0.57%	0.60%	1.45%	0.57%	0.56%	1.10%
E.coli	3.80%	3.31%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.89%
Salmonella	0.00%	1.65%	1.23%	0.00%	0.00%	0.00%	0.00%	0.57%	0.56%	0.48%

[A] Prevalence for quantitative parameters is defined as: a positive enumeration result which is a result above lower limit of quantification for the test methodology.

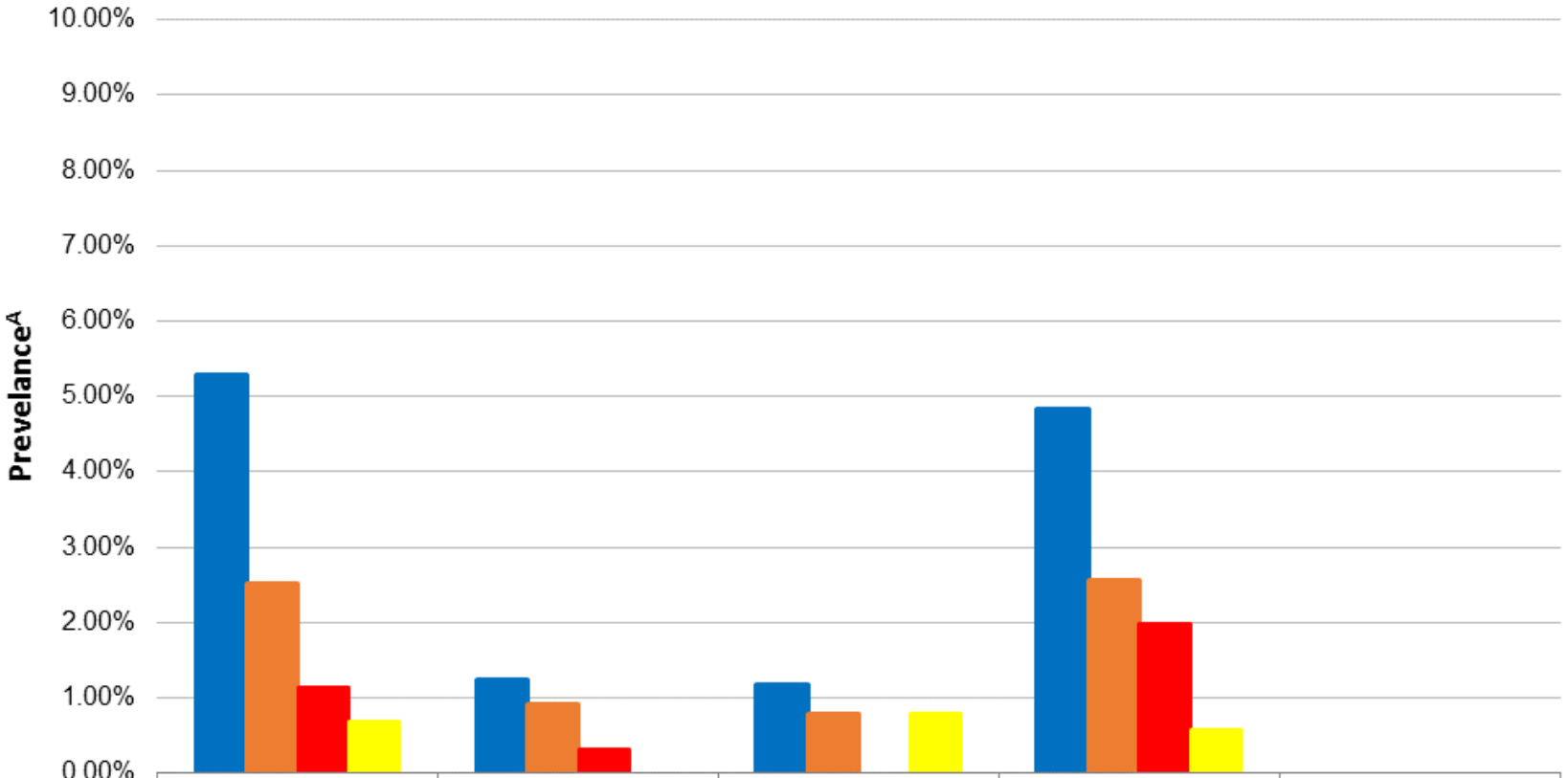
4.14 **Prevalence of TVC, Coliforms, *E.coli* and *Salmonella* per lymph node type and gender**



	Popliteal		Pre scapular		Pre sternal		Ischiatic		Deep inguinal		Axillary		Coxalis		Pre crural/subiliac		Pre pectoral	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
TVC	9.52%	7.63%	11.29%	5.08%	3.45%	1.98%	5.36%	0.88%	1.69%	1.75%	1.61%	0.96%	0.00%	1.72%	3.39%	0.88%	1.61%	0.00%
Coliforms	1.59%	7.56%	8.06%	4.24%	0.00%	0.99%	0.00%	0.00%	0.00%	0.91%	0.00%	0.00%	0.00%	1.72%	0.00%	0.88%	0.00%	0.88%
E. coli	0.00%	5.88%	4.84%	2.54%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Salmoenlla	0.00%	0.00%	1.59%	1.69%	0.00%	1.98%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	1.69%	0.00%	1.59%	0.00%

[A] Prevalence for quantitative parameters is defined as: a positive enumeration result which is a result above lower limit of quantification for the test methodology.

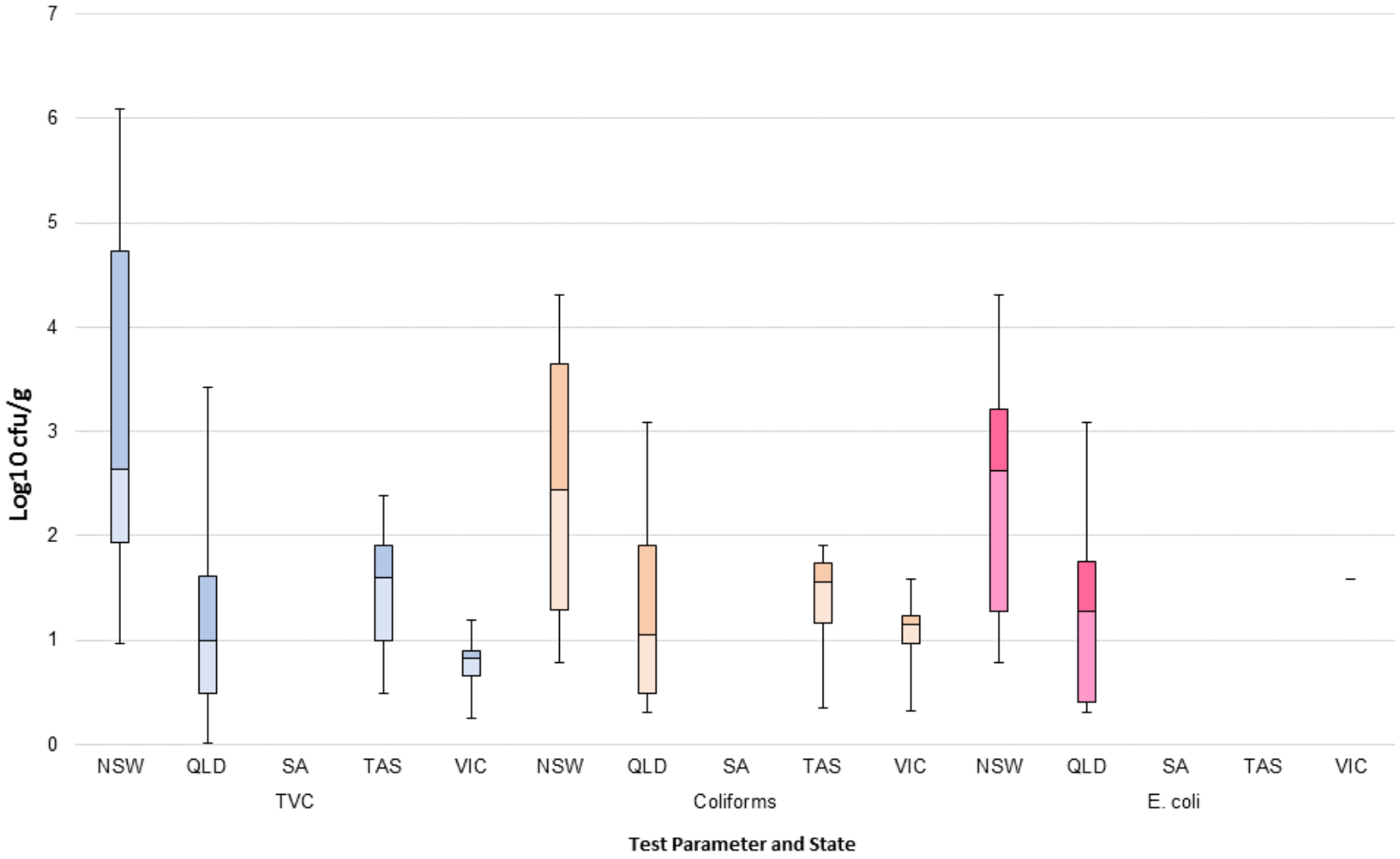
4.15 **Prevalence of TVC, Coliforms, *E.coli* and *Salmonella* per State[B]**



	QLD	VIC	TAS	NSW	SA
TVC	5.28%	1.23%	1.18%	4.84%	0.00%
Coliforms	2.52%	0.92%	0.79%	2.56%	0.00%
E. coli	1.14%	0.31%	0.00%	1.99%	0.00%
Salmonella	0.68%	0.00%	0.79%	0.57%	0.00%

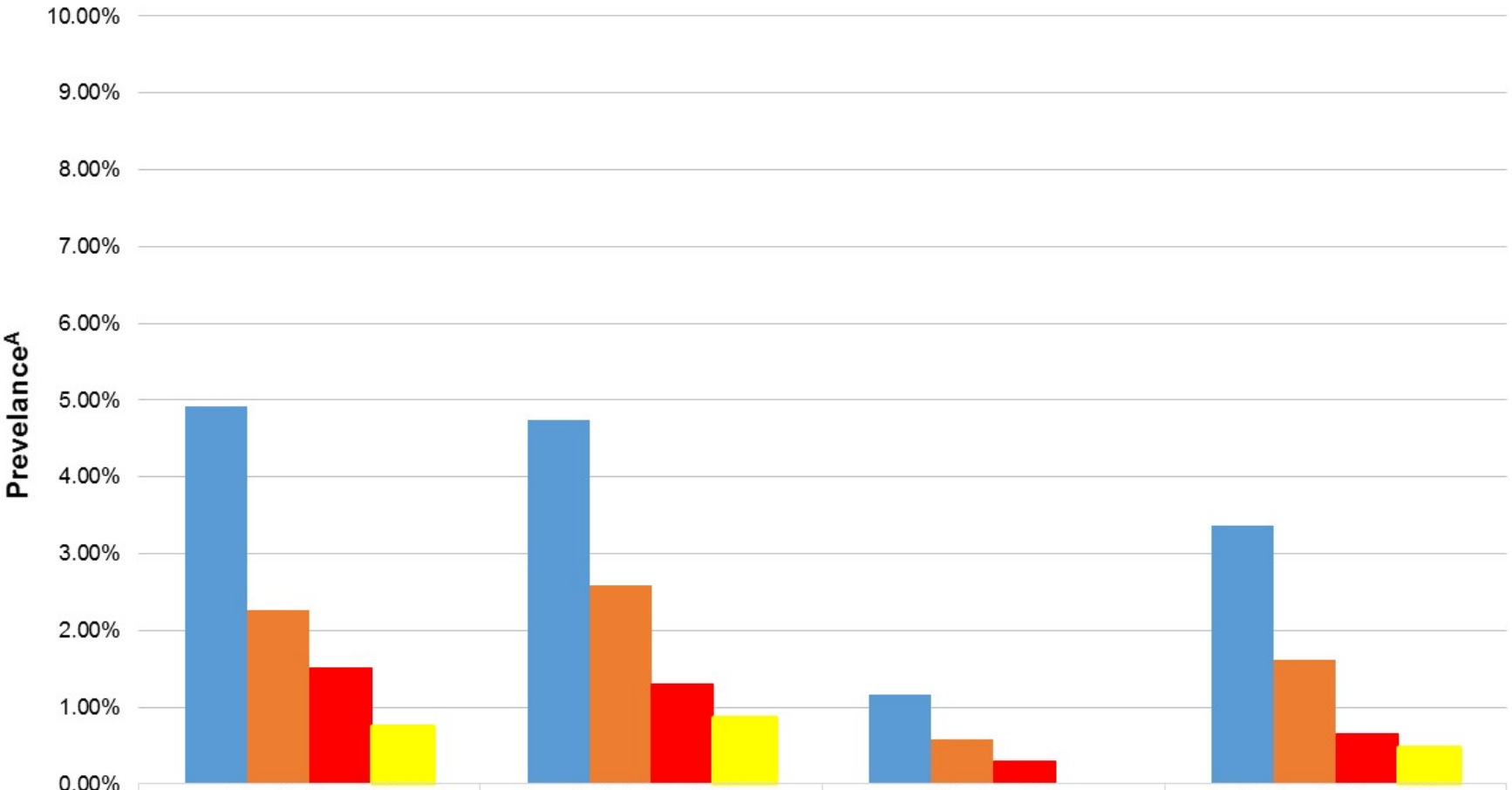
[A] Prevalence for quantitative parameters is defined as: a positive enumeration result which is a result above lower limit of quantification for the test methodology. [B] "State" refers to the origin of the carcass, not the processing facility location

4.16 **Box-Whisker – Distribution of indicator counts per State[A]**



[A] "State" refers to the origin of the carcass, not the processing facility location

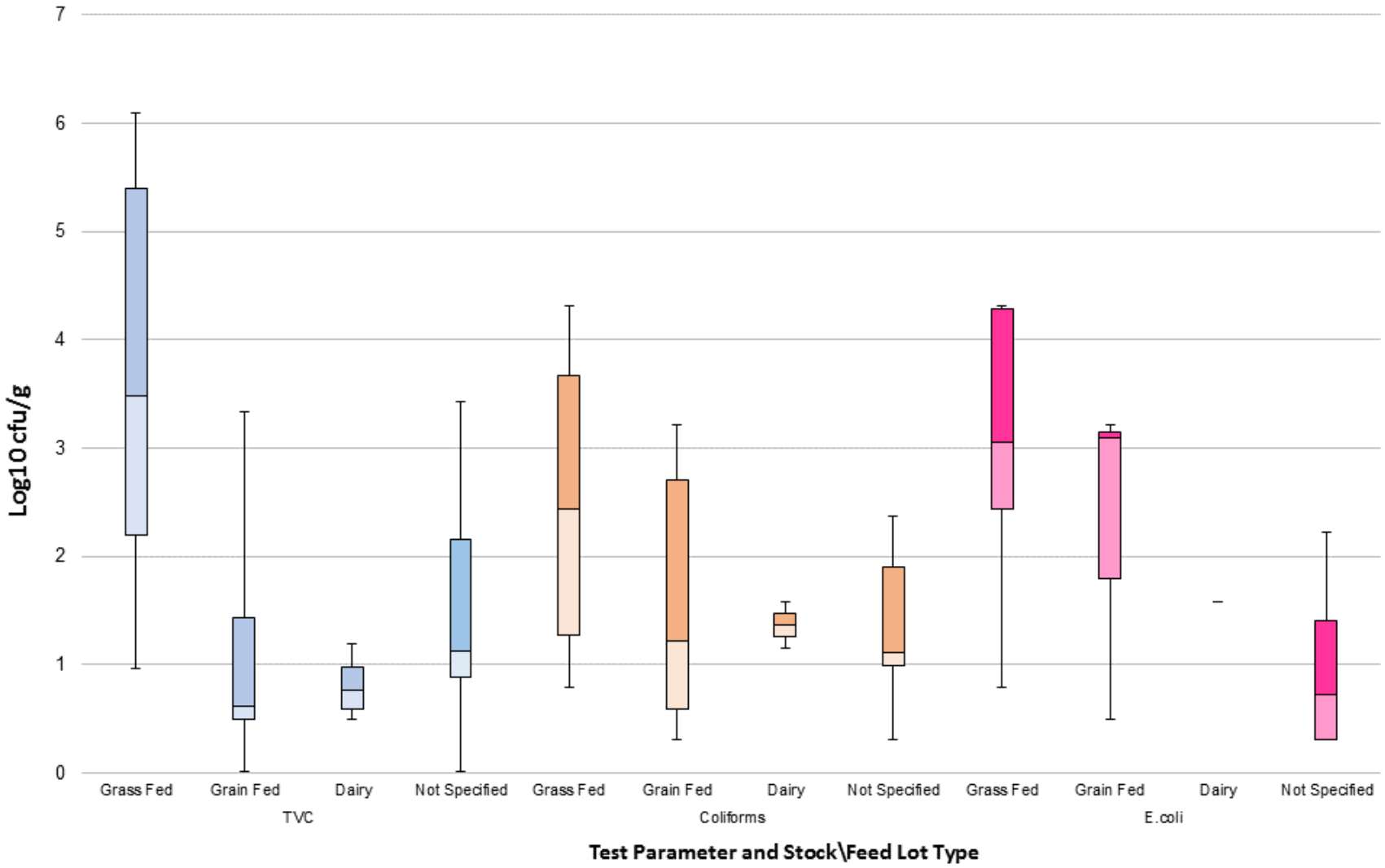
4.17 **Prevalence of TVC, Coliforms, *E.coli* and *Salmonella* per stock\feed lot type**



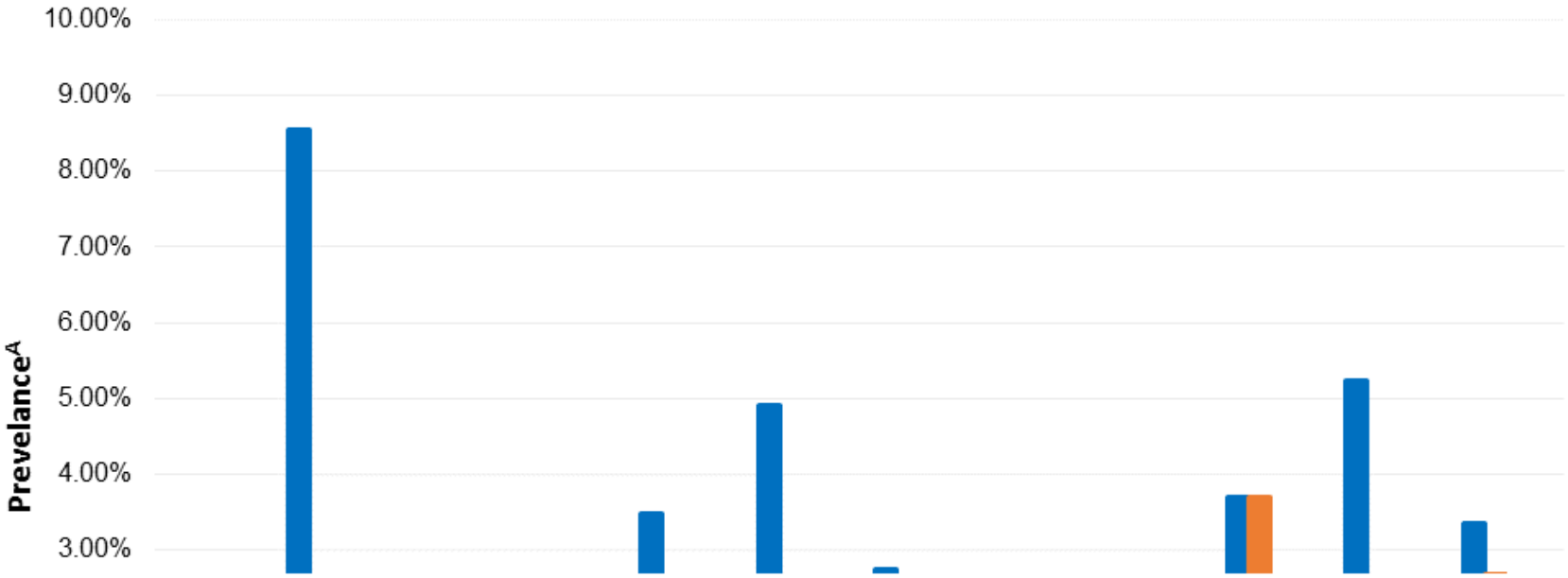
	Grass Fed (n = 265)	Grain Fed (n = 232)	Dairy (n = 344)	Not Specified (n = 623)
TVC	4.92%	4.74%	1.16%	3.37%
Coliforms	2.26%	2.59%	0.58%	1.62%
E. coli	1.51%	1.29%	0.29%	0.65%
Salmonella	0.75%	0.86%	0.00%	0.48%

[A] Prevalence for quantitative parameters is defined as: a positive enumeration result which is a result above lower limit of quantification for the test methodology, n = number of individual lymph nodes tested

4.18 **Box-Whisker – Distribution of indicator counts per stock\feed lot type**

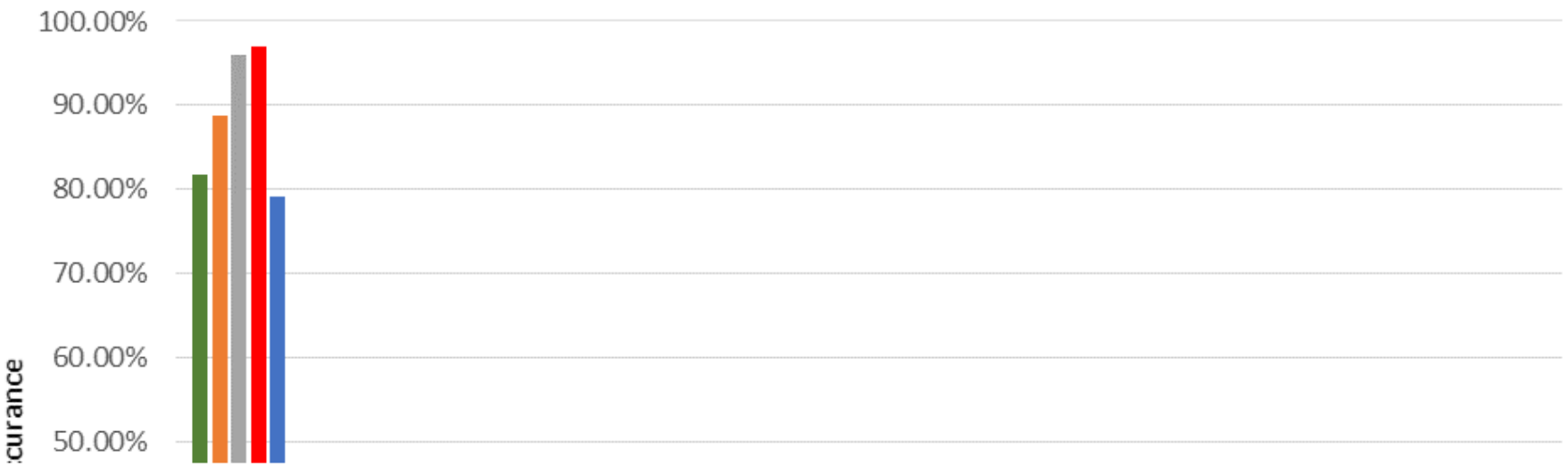


4.19 **Prevalence of TVC, Coliform, *E. coli* and *Salmonella* by month and season**

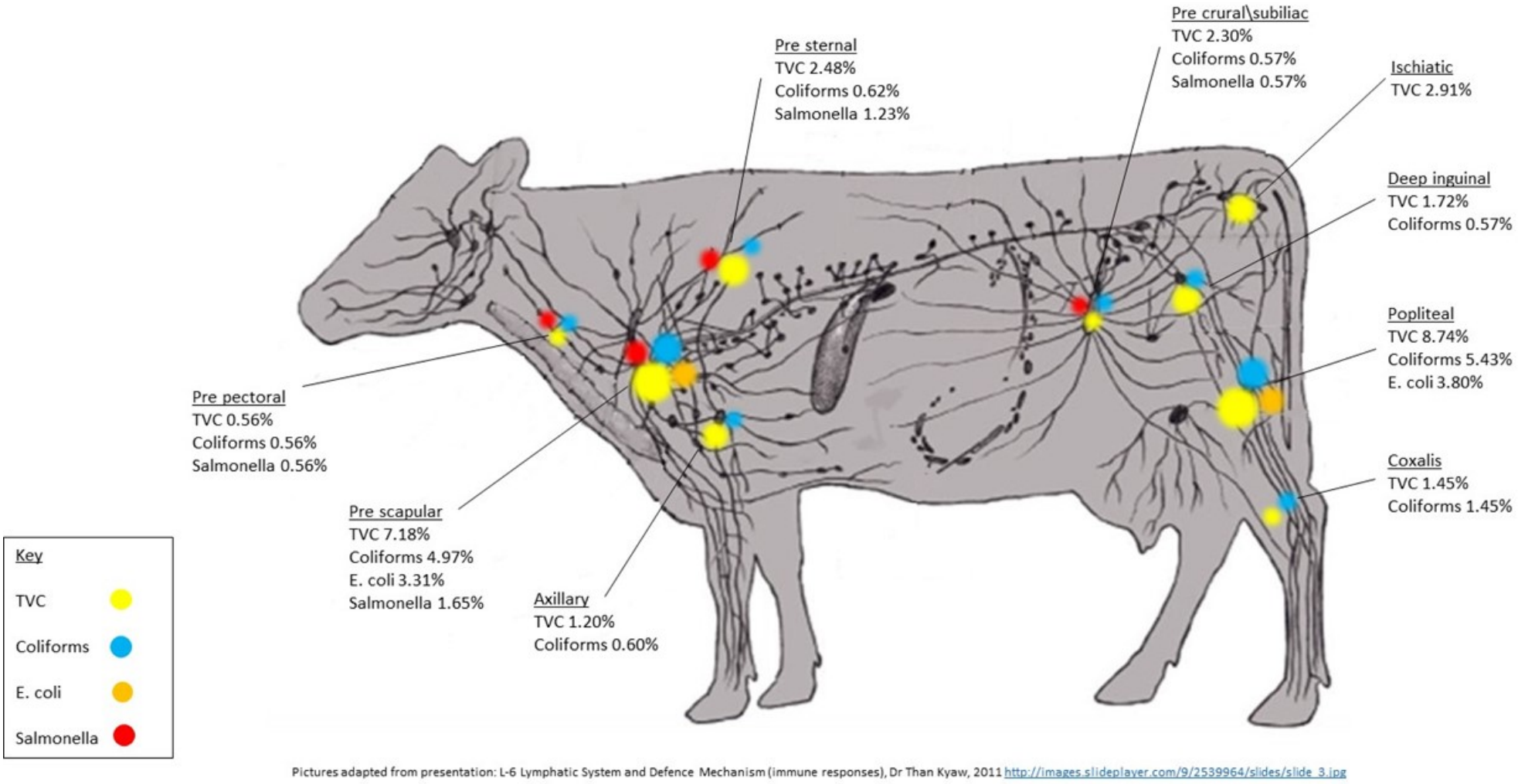


[A] Prevalence for quantitative parameters is defined as: a positive enumeration result which is a result above lower limit of quantification for the test methodology.

4.20 **Positive result per parameter – Occurrence across lymph node sets, complete and incomplete sets (n = 197)**

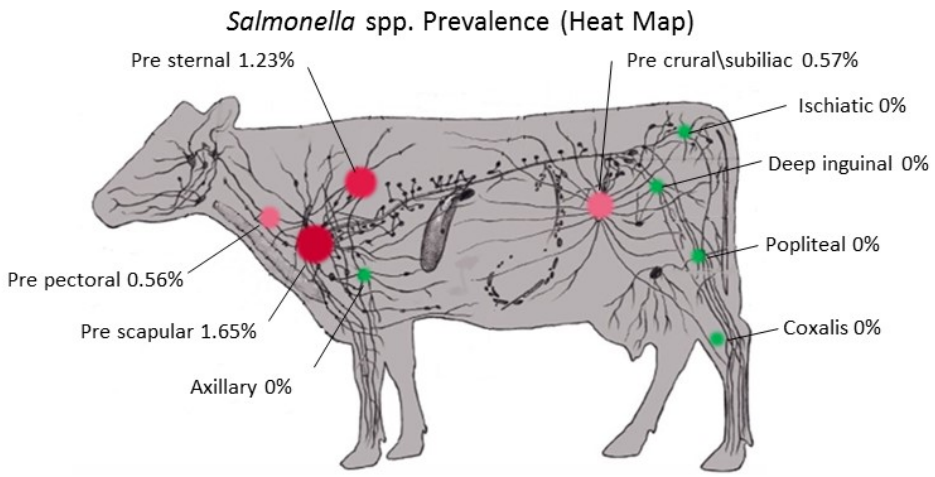


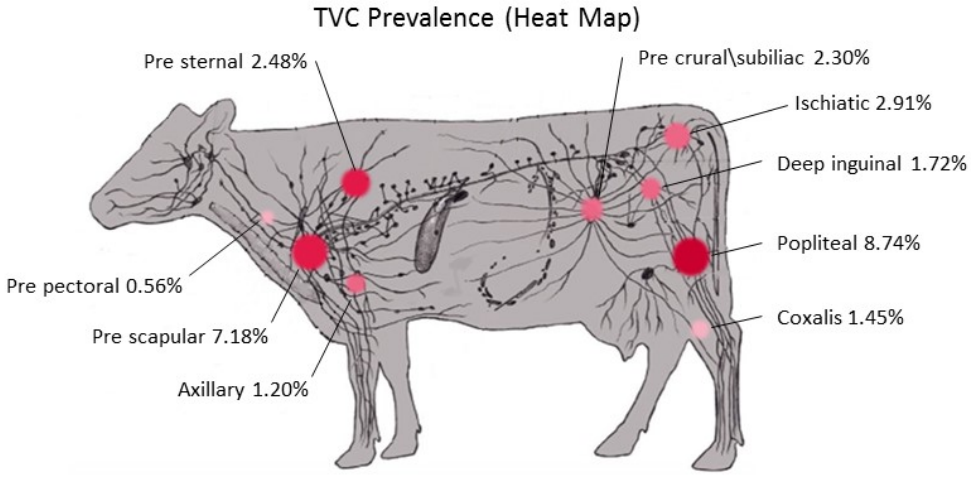
4.21 **Heat Map – Prevalence of positive samples, all lymph node sets complete and incomplete (n = 197)**

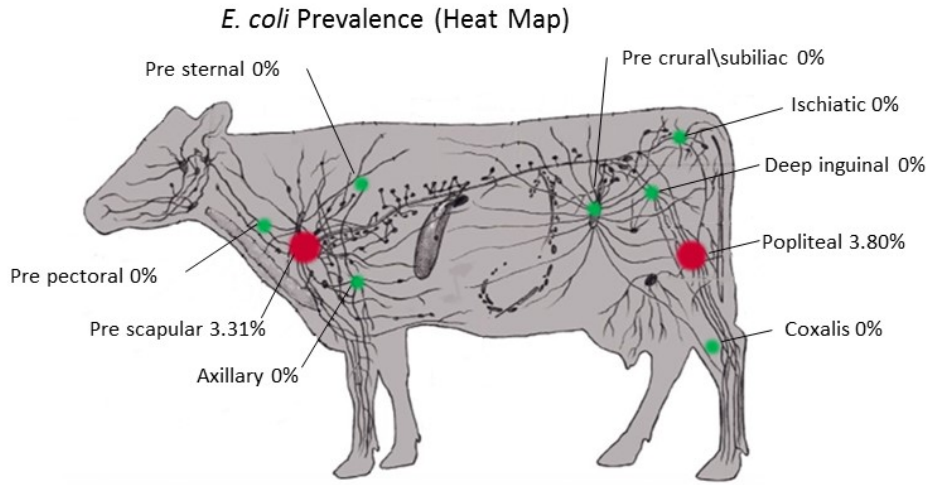


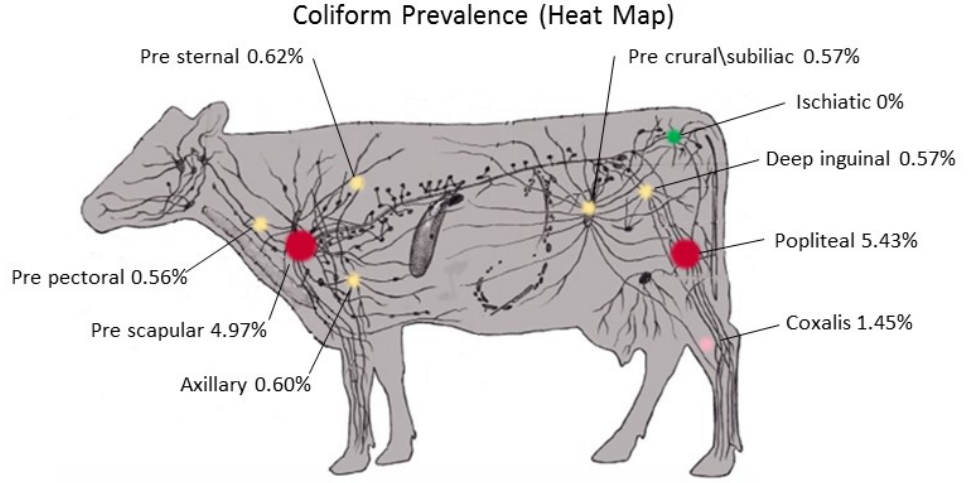
Pictures adapted from presentation: L-6 Lymphatic System and Defence Mechanism (immune responses), Dr Than Kyaw, 2011 http://images.slideplayer.com/9/2539964/slides/slide_3.jpg

4.22 **Heat Maps - *Salmonella*, Total Viable Count, Coliforms and *E. coli* prevalence per lymph node type**









Pictures adapted from presentation: L-6 Lymphatic System and Defence Mechanism (immune responses), Dr Than Kyaw, 2011 http://images.slideplayer.com/9/2539964/slides/slide_3.jpg

4.23 *Salmonella* serotypes isolated from *Salmonella* positive samples[A]

Date Received	Sample Number	Age	Gender	Breed	Stock Type	Lymph Node	Salmonella Serotype
14-Apr-16	8533775	8	Female	Fresian	Cow	Pre sternal	Salmonella kentucky
25-May-16	8675716	1	Male	Bos indicus	Short Fed	Pre crural/subiliac	Salmonella chailey
31-Aug-16	9006350	2.5	Female	Fresian	Cow	Pre sternal	Salmonella dublin
1-Sep-16	9009534	3.5	Female	Brahman	Grass Fed	Pre scapular	Salmonella virchow
1-Sep-16	9009562	3.5	Female	Brahman	Grass Fed	Pre scapular	Salmonella virchow
21-Sep-16	9079193	1.5	Male	Bos indicus	Grain Fed	Pre scapular	Salmonella typhimurium
21-Sep-16	9079194	1.5	Male	Bos indicus	Grain Fed	Pre pectoral	Salmonella typhimurium

[A] Results highlighted in colour, are from the same lymph node set (carcass).

NOTE: Sample numbers 9009534 & 9009562 are from different lymph node sets despite having the same carcass ID numbers reference. Participant has confirmed and recorded evidence (different submission forms, and sampling times, weights etc) demonstrates that these are from different lymph node sets.

5 Discussion

5.1 Sampling Summary

Throughout 2016, beef supply in Australia has decreased with cattle herd numbers at a record low. Given the tight beef supply, beef prices are also at record high levels.

Twenty four lymph node sample sets were received outside of time (within 24 hours) or temperature specification (>10°C) due to a delay in overnight delivery or incorrect packing procedure. On each occasion the out of specification event has been recorded and can be traced to results and where sample packing is identified as the cause, participants were notified. Following the review of the out of specification sample results, two lymph node sets were removed from data analysis. These sets had a high temperature excursion >22°C during transport, and contained a signficnatly high proportion of positive indicator results. Indicating that the temperature abuse had affected the validity of the results.

Project feedback from a number of participants has identified the following details regarding missing lymph nodes: Pre-crural lymph node can detach from carcass upon hide removal from skinny carcasses; Splitter saw has on occasion cut / sliced lymph nodes; Least valuable carcass is randomly selected and placed on retain rail; Lymph nodes in grain feed cattle are typically more difficult to locate due to the higher level of fat than grass feed; One participant collects popliteal, coxalis and ischiatic LNs from the boning room (cold bone) and the remaining 6 LN's are sampled (hot boned) from the slaughter floor.

5.2 Survey Results

5.2.1 *Salmonella spp.*

A total of 1464 lymph nodes were tested for the presence of *Salmonella spp*. with 7 positive results observed; detailed in Table 4.9; The overall prevalence of *Salmonella spp.* in lymph nodes collected and tested was 0.48% (n= 1482, 95% CI; 0.23% - 0.99%). Historically the reported prevalence of *Salmonella spp.* in bovine lymph nodes and trimmings has been highly variable (Brown et. al 2015). However the low prevalence of *Salmonella spp.* observed is in keeping with similar studies undertaken by Arthur *et al*. (2008) and Brichta-Harhay et al. (2012), which reported a prevalence of 1.6% (n = 1140 95% CI: 0.85% – 2.30%) and 0.80% (n = 906) respectively. Higher prevalence has been reported in literature, however Brichta-Harhay et al. (2012) showed during inoculation studies that on average 23% to 43% of lymph nodes are cross-contaminated by adipose tissue during the trimming process prior to testing. The findings by Brichta-Harhay et al. (2012) reinforce the benefits of surface sterilisation before the analysis of lymph nodes for *Salmonella spp*. and the reduction in cross contamination by adipose tissue. Both studies by Arthur *et al*. (2008) and Brichta-Harhay et al. (2012) used similar surface sterilisation techniques to this study.

The low prevalence of *Salmonella spp*. in lymph nodes is also in line with the findings of MLA (2014), which reported a *Salmonella spp.* prevalence rate of 0.70 % (n = 1255) in manufacturing beef products from Australia.

Salmonella was only isolated from 4 of the lymph nodes types, the Pre-scapular had the highest prevalence rate of *Salmonella* (1.65%), followed closely by the Pre-sternal Lymph

node (1.23%). Prevalence of *Salmonella* from Pre-crural\subiliac and Pre-pectoral were similar, 0.57% and 0.56% respectively. No *Salmonella* was isolated from Axillary, Deep inguinal, Ischiatic, Popliteal or Coxalis lymph nodes.

Of the 197 Lymph node sets collected, only 1 Lymph node set contained multiple *Salmonella* detections across the 9 lymph nodes. For this lymph node set the Pre-scapular and Pre-pectoral Lymph nodes were positive, and contained the same serotype; *Salmonella* typhimurium.

Salmonella serotypes; *Salmonella* kentucky (pre sternal), *Salmonella* chailey (pre crural\subiliac), *Salmonella* dublin (pre sternal), *Salmonella* virchow (pre scapular) and *Salmonella* typhimurium (pre scapular and pre pectoral) were isolated.

The objective of this project was to determine the prevalence of *Salmonella* in 9 lymph nodes (Pre-pectoral, Pre-sternal, Ischiatic, and including 6 recommended by the USDA research department Axillary, Pre scapular, Deep inguinal, Popliteal, Coxalis and Subiliac) these lymph nodes have been highlighted as high risk of containing *Salmonella* spp. and with the potential of being included into beef trim. Overall the observed prevalence of *Salmonella* spp. in the 9 selected lymph nodes was 0.48% (n = 1464), the following prevalence within individual lymph nodes was observed; Pre-pectoral 0.56%, Pre-sternal 1.23%, Ischiatic 0.00%, Axillary 0.00%, Pre scapular 1.65%, Deep inguinal 0.00%, Popliteal 0.00%, Coxalis 0.00% and Subiliac 0.57%

5.2.2 **Indicators**

As part of this study, the enumeration of indicator microorganisms; Total Viable Count, Coliforms and *E. coli* was conducted. Overall the prevalence of these indicators in all lymph nodes was low; Total Viable Count 3.36% ($\bar{x}$ = 1.97 Log_{10} cfu/g), Coliforms 1.71% ($\bar{x}$ = 1.78 Log_{10} cfu/g) and E. coli 0.89% ($\bar{x}$ = 2.02 Log_{10} cfu/g). These results demonstrate that the overall microbiological quality of the lymph nodes was good.

The correlation between positive indicator result and positive *Salmonella* result from a single lymph node and/or lymph node sets was poor. Of the 7 *Salmonella* positive lymph nodes observed within this study, 2 had corresponding Total Viable Counts, and 3 had corresponding Coliform counts. Generally, positive enumeration results from Indicators were not reliable in predicting the presence of *Salmonella*. Total Viable Counts had a positive predictive value for *Salmonella* of 4.08% and Coliforms had a positive predictive value of 12.00%. The results of KAPPA analysis showed poor correlation between quality indicators and presence of *Salmonella* spp. (TVC; KAPPA = 0.064, Coliforms; KAPPA = 0.181). No *Salmonella* spp. positive lymph node was accompanied by a positive *E. coli* enumeration result.

6 Conclusion

In conclusion, the prevalence of *Salmonella* within peripheral lymph nodes was extremely low 0.48% (n=1464), with only 3.05% of the 197 lymph nodes sets tested being positive for *Salmonella*. No shiga toxin producing *E. coli* (STEC) were detected from any of the lymph nodes samples.

No correlation between indicators (TVC, Coliforms and E.coli) and *Salmonella* positive lymph nodes was observed.

The Pre-scapular and Pre-sternal lymph nodes produced 5 out of the 7 *Salmonella* positive results, these lymph nodes along with the Popliteal lymph node accounted for 71.2% of all positive results across all parameters tested. As such, any further study and risk profiling should concentrate on the Pre-scapular, Pre-sternal and Popliteal lymph nodes.

Due to the difficulty in locating and sampling the Coxalis lymph node and its relative low risk, further investigation of this node may provide little value; consistent removal of this node may provide a challenge.

7 Bibliography

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MLA (2014a) G.MFS.0305, Location of peripheral bovine lymph nodes, Jonathan Webber 21.08.2014

8 Appendix

8.1 **Acknowledgements**

DTS acknowledges the significant support with conducting this MLA project from the following export participants:

Oakey Beef Exports Pty Ltd Oakey QLD 4401

Midfield Meat International Pty Ltd Warrnambool VIC 3280

JBS Australia Pty Ltd Longford TAS 7301

Teys Australia Pty Ltd Naracoorte SA 5271

Bindaree Beef 7307 Inverell NSW 2360

The following establishments were also willing to participate in the project:

Wingham Beef Exports Pty Ltd (NH Foods) Wingham NSW 2429

Greenmountain Food Processing Pty Ltd Coominya QLD 4311

John Dee Pty Ltd Warwick QLD 4370

Kilcoy Pastoral Company Kilcoy QLD 4515

Nolan Meats Pty Ltd Gympie QLD 5470

Thomas Borthwick & Sons Pty Ltd (NH Foods) Mackay QLD 4740

Greenham Tasmania Pty Ltd Smithton TAS 7330

HW Greenham & Sons Pty Ltd Tongala VIC 3621

GBP Australia Pty Ltd Poowong VIC 3988

Ralphs Meat Company Pty Ltd Seymour VIC 3660

Wodonga Abattoir Pty Ltd Wodonga VIC 3690

8.2 **Supporting project documentation**

The following documentation has been provided as separate attached to this final report:

8.2.1 MLA Lymph node training module FINAL
8.2.2 MLA Submission Form_Lymph Node Salmonella v1 FINAL
8.2.3 SAMP 10 MLA Project: Salmonella in Bovine lymph node survey FINAL
8.2.4 SAMP 11 MLA SOP Summary: Excision and collection of lymph nodes FINAL
8.2.5 SAMP 12 MLA SOP Summary: Transport of collected lymph nodes FINAL
8.2.6 MQ GL 89 Preparation and testing of Bovine lymph nodes MLA

8.3 Summary of Positive Results

Positive sample summary OCT 2015 – MAR 2016[A]

Date Received	Sample Number	Age	Gender	Breed	Stock Type	Lymph Node	Test	Result	Unit
23-October-2015	7914410	8	Female	Angus	Grassfed	Pre Pectoral	Total Viable Count	620	cfu/g
29-October-2015	7936152	1.5	Male	Angus	Grassfed	Popliteal	Total Viable Count	125	cfu/g
12-November-2015	7986129	2	Female	Cross Brarhan/Heref	Grassfed	Popliteal	Total Viable Count	270	cfu/g
12-November-2015	7986129	2	Female	Cross Brarhan/Heref	Grassfed	Popliteal	Coliforms	270	cfu/g
12-November-2015	7986129	2	Female	Cross Brarhan/Heref	Grassfed	Popliteal	E.coli	270	cfu/g
18-November-2015	8008807	1.5	Female	Angus	Grassfed	Pre Crural/subiliac	Coliforms	12	cfu/g
24-November-2015	8029905	2	Male	Bos indicus	Grainfed	Popliteal	Coliforms	8	cfu/g
04-December-2015	8072322	4	Male	Angus Cross	-	Ischiatic	Total Viable Count	340	cfu/g
16-December-2015	8116434	3.5	Female	Fresian	Grainfed	Deep Inguinal	Total Viable Count	3	cfu/g
16-December-2015	8116814	1.5	Female	Grain Feed - MSA	Grainfed	Pre Scapular	Coliforms	3	cfu/g
16-December-2015	8116814	1.5	Female	Grain Feed - MSA	Grainfed	Pre Scapular	E.coli	3	cfu/g
16-December-2015	8116825	1.5	Female	Grain Feed - MSA	Grainfed	Popliteal	Total Viable Count	1200	cfu/g
16-December-2015	8116825	1.5	Female	Grain Feed - MSA	Grainfed	Popliteal	Coliforms	1200	cfu/g
16-December-2015	8116825	1.5	Female	Grain Feed - MSA	Grainfed	Popliteal	E.coli	1200	cfu/g
06-January-2016	8179971	3.5	Female	Santa	Grassfed	Pre Scapular	Total Viable Count	4300	cfu/g
06-January-2016	8179975	3.5	Female	Santa	Grassfed	Axillary	Total Viable Count	170000	cfu/g
06-January-2016	8179976	3.5	Female	Santa	Grassfed	Pre Sternal	Total Viable Count	1200000	cfu/g
06-January-2016	8179985	3.5	Female	Santa	Grassfed	Coxalis	Total Viable Count	400000	cfu/g
06-January-2016	8179987	3.5	Female	Santa	Grassfed	Deep Inguinal	Total Viable Count	350000	cfu/g
20-January-2016	8230198	1	Male	Bos indicus	Grainfed	Pre Scapular	Total Viable Count	3	cfu/g
20-January-2016	8230217	1	Male	Bos indicus	Grainfed	Pre Crural/subiliac	Total Viable Count	3	cfu/g
11-February-2016	8310880	3.5	Female	Fresian	Dairy	Pre Scapular	Total Viable Count	4	cfu/g
25-February-2016	8363741	4	Female	Angus	-	Pre Scapular	Total Viable Count	13	cfu/g
01-March-2016	8380220	1	Male	Bos indicus	Leaf	Pre Scapular	Total Viable Count	2600	cfu/g
01-March-2016	8380220	1	Male	Bos indicus	Leaf	Pre Scapular	Coliforms	11	cfu/g
01-March-2016	8380220	1	Male	Bos indicus	Leaf	Pre Scapular	E. coli	2	cfu/g
01-March-2016	8380223	1	Male	Bos indicus	Leaf	Axillary	Total Viable Count	110	cfu/g
01-March-2016	8380223	1	Male	Bos indicus	Leaf	Axillary	Coliforms	68	cfu/g

[A] Results highlighted in colour, are from the same lymph node sample.

Positive sample summary MAR 2016 (con) – AUG 2016[A]

Date Received	Sample Number	Age	Gender	Breed	Stock Type	Lymph Node	Test	Result	Unit
01-March-2016	8380224	1	Male	Bos indicus	Leaf	Pre Sternal	Total Viable Count	17	cfu/g
06-April-2016	8504335	1	Male	Bos indicus	AGRH	Pre Scapular	Coliforms	2	cfu/g
06-April-2016	8504340	1	Male	Bos indicus	AGRH	Pre Sternal	Total Viable Count	6	cfu/g
06-April-2016	8504341	1	Male	Bos indicus	AGRH	Popliteal	Total Viable Count	8	cfu/g
06-April-2016	8504343	1	Male	Bos indicus	AGRH	Ischiatic	Total Viable Count	8	cfu/g
14-April-2016	8533775	8	Female	Fresian	Cow	Pre Sternal	Salmonella	Detected	per LN
28-April-2016	8577433	4.5	Female	Angus	Grassfed	Pre Scapular	Total Viable Count	26	cfu/g
28-April-2016	8577433	4.5	Female	Angus	Grassfed	Pre Scapular	Coliforms	6	cfu/g
28-April-2016	8577433	4.5	Female	Angus	Grassfed	Pre Scapular	E. coli	6	cfu/g
10-May-2016	8623512	2	Male	Bos indicus	Grainfed	Pre Pectoral	Total Viable Count	50	cfu/g
10-May-2016	8623524	2	Male	Bos indicus	Grainfed	Popliteal	Total Viable Count	3	cfu/g
17-May-2016	8647680	1	Male	Bos indicus	Leaf	Pre Scapular	Coliforms	2	cfu/g
17-May-2016	8647680	1	Male	Bos indicus	Leaf	Pre Scapular	E. coli	2	cfu/g
17-May-2016	8647700	1	Male	Bos indicus	Leaf	Ischiatic	Total Viable Count	19	cfu/g
25-May-2016	8675716	1	Male	Bos indicus	Shortfed	Pre Crural\subiliac	Salmonella	Detected	per LN
26-May-2016	8680798	1	Male	Angus Cross	Grassfed	Popliteal	Total Viable Count	190	cfu/g
26-May-2016	8680853	3.5	Female	Fresian	Dairy	Popliteal	Total Viable Count	15	cfu/g
26-May-2016	8680853	3.5	Female	Fresian	Dairy	Popliteal	Coliforms	37	cfu/g
26-May-2016	8680853	3.5	Female	Fresian	Dairy	Popliteal	E. coli	37	cfu/g
01-June-2016	8700693	2	N/A	Bos indicus	EUHQB	Ischiatic	Total Viable Count	11	cfu/g
07-June-2016	8720869	2	Male	Bos indicus	Grainfed	Pre Scapular	Total Viable Count	3	cfu/g
10-June-2016	8734043	4	Female	Brahman	Grassfed	Popliteal	Coliforms	27	cfu/g
10-June-2016	8734076	3.5	Female	Fresian	Dairy	Popliteal	Total Viable Count	8	cfu/g
05-June-2016	8821543	3.5	Female	Fresian	Dairy	Deep Inguinal	Coliforms	14	cfu/g
22-July-2016	8873360	2	Male	Bos indicus	Grainfed	Deep Inguinal	Total Viable Count	11	cfu/g
25-August-2016	8986285	2.5	Female	Angus	Beef	Coxalis	Coliforms	110	cfu/g

[A] Results highlighted in colour, are from the same lymph node sample.

Positive sample summary AUG 2016 (con) – OCT 2016[A]

Date Received	Sample Number	Age	Gender	Breed	Stock Type	Lymph Node	Test	Result	Unit
31-August-2016	9006350	2.5	Female	Fresian	Cow	Pre Sternal	Salmonella	Detected	per LN
31-August-2016	9006350	2.5	Female	Fresian	Cow	Pre Sternal	Total Viable Count	240	cfu/g
31-August-2016	9006350	2.5	Female	Fresian	Cow	Pre Sternal	Coliforms	27	cfu/g
01-September-2016	9009328	1	Male	Bos indicus	Shortfed	Popliteal	Total Viable Count	7	cfu/g
01-September-2016	9009534	3.5	Female	Brahman	Grassfed	Pre Scapular	Salmonella	Detected	per LN
01-September-2016	9009544	3.5	Female	Brahman	Grassfed	Popliteal	Total Viable Count	4300	cfu/g
01-September-2016	9009544	3.5	Female	Brahman	Grassfed	Popliteal	Coliforms	2700	cfu/g
01-September-2016	9009544	3.5	Female	Brahman	Grassfed	Popliteal	E. coli	2700	cfu/g
01-September-2016	9009562	3.5	Female	Brahman	Grassfed	Pre Scapular	Salmonella	Detected	per LN
01-September-2016	9009569	3.5	Female	Brahman	Grassfed	Popliteal	Total Viable Count	3000	cfu/g
01-September-2016	9009569	3.5	Female	Brahman	Grassfed	Popliteal	Coliforms	1100	cfu/g
01-September-2016	9009569	3.5	Female	Brahman	Grassfed	Popliteal	E. coli	1100	cfu/g
21-September-2016	9077302	3.5	Female	Brahman	N/A	Pre-scapular	Total Viable Count	6	cfu/g
21-September-2016	9077302	3.5	Female	Brahman	N/A	Pre-scapular	Coliforms	9	cfu/g
21-September-2016	9079193	1.5	Male	Bos indicus	OMUGI	Pre-scapular	Salmonella	Detected	per LN
21-September-2016	9079193	1.5	Male	Bos indicus	OMUGI	Pre-scapular	Total Viable Count	1	cfu/g
21-September-2016	9079193	1.5	Male	Bos indicus	OMUGI	Pre-scapular	Coliforms	2	cfu/g
21-September-2016	9079194	1.5	Male	Bos indicus	OMUGI	Pre-pectoral	Salmonella	Detected	per LN
21-September-2016	9079194	1.5	Male	Bos indicus	OMUGI	Pre-pectoral	Coliforms	33	cfu/g
21-September-2016	9079398	4.8	Female	Fresian	Cow	Popliteal	Total Viable Count	12	cfu/g
21-September-2016	9079398	4.8	Female	Fresian	Cow	Popliteal	Coliforms	12	cfu/g
06-October-2016	9132960	3.5	Female	Hereford	Grass Fed	Pre-crural/subiliac	Total Viable Count	9	cfu/g
06-October-2016	9133135	3	Male	Bos indicus	Short Fed	Pre-scapular	Total Viable Count	1300	cfu/g
06-October-2016	9133135	3	Male	Bos indicus	Short Fed	Pre-scapular	Coliforms	160	cfu/g
06-October-2016	9133135	3	Male	Bos indicus	Short Fed	Pre-scapular	E.coli	160	cfu/g
06-October-2016	9133141	3	Male	Bos indicus	Short Fed	Popliteal	Total Viable Count	40	cfu/g

[A] Results highlighted in colour, are from the same lymph node sample.

Positive sample summary OCT 2016 (con) – NOV 2016[A]

Date Received	Sample Number	Age	Gender	Breed	Stock Type	Lymph Node	Test	Result	Unit
13-October-2016	9154292	3	Female	Brahman Cross	MSA	Pre-scapular	Total Viable Count	15	cfu/g
13-October-2016	9154292	3	Female	Brahman Cross	MSA	Pre-scapular	Coliforms	13	cfu/g
13-October-2016	9154292	3	Female	Brahman Cross	MSA	Pre-scapular	E.coli	13	cfu/g
13-October-2016	9154448	2.5	Female	Brahman Cross	Grain Fed	Popliteal	Total Viable Count	2100	cfu/g
13-October-2016	9154448	2.5	Female	Brahman Cross	Grain Fed	Popliteal	Coliforms	1600	cfu/g
13-October-2016	9154448	2.5	Female	Brahman Cross	Grain Fed	Popliteal	E.coli	1600	cfu/g
13-October-2016	9158463	3.5	Male	Bos indicus	Grain Fed	Ischiatic	Total Viable Count	14	cfu/g
18-October-2016	9174558	3	Male	Bos indicus	OMUGI	Pre-crural/subiliac	Total Viable Count	4	cfu/g
20-October-2016	9184175	1	Male	Bos indicus	Short Fed	Pre-scapular	Total Viable Count	2	cfu/g
03-November-2016	9232384	1	Male	Bos Indicus	Short Fed	Pre-scapular	Total Viable Count	1	cfu/g
04-November-2016	9236849	3.5	Female	Hereford	Grass Fed	Popliteal	Total Viable Count	80000	cfu/g
04-November-2016	9236849	3.5	Female	Hereford	Grass Fed	Popliteal	Coliforms	>20000	cfu/g
04-November-2016	9236849	3.5	Female	Hereford	Grass Fed	Popliteal	E.coli	>20000	cfu/g

[A] Results highlighted in colour, are from the same lymph node sample.