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Process Control Data and Analysis for Market Access

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Abstract

The Process Control Data and Analysis for Market Access project supports the red meat industry in continually improving slaughter processes and reducing the risk of microbial contamination of carcases and hence, end product, which in turn helps safeguard overseas market access. This project consists of five sub-projects: (1) evaluation of alternate indicator organisms and sampling sites on sheep carcases at four export slaughter establishments, (2) nine investigation training workshops held around Australia, (3) provision of support to processors to undertake process investigations – "Ask SARDI", (4) an investigation of how establishments investigate a STEC detection and (5) continuation of the ESAM Analysis Reporting Service. Case studies for the 3rd edition of The Processor's Guide to Improving Microbiological Quality were also provided before its public release at the 2017 MINTRAC Meat Inspection & Quality Assurance conference.

Executive summary

The Australian meat production and processing industry is an important part of the Australian economy, in particular, the export of meat and meat products to overseas markets. Access to markets is based on meat processing establishments being able to meet the safety and suitability requirements set by overseas countries. It is the Department of Agriculture and Water Resources' (DAWR) responsibility to certify safety and suitability of product, although ultimately, responsibility resides with each processing establishment to demonstrate ongoing compliance with the relevant market access or commercial trade requirements. Outbreaks of foodborne illness caused by meat products can have wide-spread and significant effects on sales / profits and reduce consumer confidence. End-product testing, such as robust N-60 testing for Australian manufacturing beef exported to the US, has been shown to be ineffective at ensuring product safety, especially when contamination levels are low and infrequent (Kiermeier et. al, 2015). For this reason, increased attention is being placed internationally on verifying process control.

There is a move, both domestically and internationally, to have outcomes-based regulation that allows industry flexibility in how product safety (and suitability, in the case of meat) is achieved. These trends provide the opportunity to merge industry's desire for product that meets country and customer demands (managing its market access and commercial risks) with the competent authority's desire for validated and verified processes that underpin official certification. Both industry and competent authorities require objective data to verify the safety and suitability of product.

Evaluation of alternate sampling sites on sheep carcases

Since 1998, Australian regulations have required regular testing of carcases for generic *E. coli* and *Salmonella* Monitoring system. There have been suggestions that the sites sampled under the ESAM system may not reflect the true level of contamination on the carcases and this present project comprises an investigation to 'map' contamination on ovine carcases.

Sampling was carried out between May 2016 and January 2017 at four abattoirs. A total of 512 samples were collected, thirty-two samples per sample site (rump, belly, shank and neck and 100cm^2 each) per abattoir. The swab samples were transported to the SARDI Food Safety and Innovation laboratories for microbiological testing the following day and tested for Total Viable Counts, coliforms, *E. coli* and Enterobacteriaceae; enriched samples were also tested for *E. coli*.

A general comparison of the concentration of TVC and prevalence of *E. coli* for the ovine industry during the sampling period concludes that it would appear that ESAM swabs give lower TVC and *E. coli* levels than some of the sites sampled in this present study, for example, the rump, however the different sampling methodologies make it difficult to compare directly.

This study estimated prevalence of microbial indicators on sheep carcases and over all sites, the prevalence in order was *Enterobacteriaceae* > Coliforms > *E. coli*. *Enterobacteriaceae* and coliforms may be suitable for process control monitoring, depending on whether their levels correspond with *E. coli* levels temporally, but regardless, *E. coli* remain the indicator of choice for faecal contamination.

Standardised STEC investigations and questionnaire

Most establishments investigate "MHA records, process and product monitoring/ verification, pre-shipment reviews, ESAM test results, other test results, pro-operational and operational monitoring, HACCP records, cleanliness of, and type and source of, animals slaughtered, and national establishment verification system records as appropriate" as outlined in Meat Notice 2012/01 (DAWR, 2012). However, this has now been superseded by the Microbiological Manual (DAWR, 2017). This study was mooted to gather information on how establishments respond to DAWR requirements in the event of a STEC detection, and to develop a standardised response document.

A novel approach for this investigation was developed, stemming from foodborne illness investigations, where epidemiologists typically use standardised questionnaires to elicit information about potential foods and consumption settings and use the answers to generate hypotheses about the likely sources of the outbreak. They then test these hypotheses using a case-control methodology, where foodborne illness cases and control subjects (sourced from the same consumption setting – restaurant, hospital, etc) are compared in relation to their food consumption.

An analogous approach was planned for the present sub-project, namely creating a standardised questionnaire for investigating STEC detections in beef and identifying potential risk factors contributing to end-product contamination. In parallel, the intention was to use the questionnaire to elicit information on similar lots where no STECs were detected. Together, the parallel investigations would comprise case-control pairs similar to those used in informing food poisoning outbreaks.

The intended methodology planned for undertaking this investigation was to:

1. Collect approximately ten (10) investigation reports from different establishments and identify common and unique elements for potential inclusion in a standardised report / form.
2. Use the reports to develop a data capture spreadsheet to be populated from the information provided in establishment STEC investigation reports, known as *cases*.
3. Collect the same information about suitably similar lots in which no STEC contamination was detected, from the same abattoir (matched *controls*).
4. Aim to collect data for 30 cases and 60 controls (1:2 ratio of cases to controls for each investigation). Because cases occur relatively infrequently, a greater number of controls was sought to increase the statistical power of the analysis.

5. Perform a case-control analysis to identify potential risk factors for STEC contamination.

Establishments were contacted with feedback regarding additional information required to populate the data capture spreadsheet. This primarily involved locating raw data and observations from the relevant production dates and forwarding the information on to SARDI. Some establishments provided the raw data and observations relating to cleared production periods, but it was difficult to gather enough information to perform a case-control analysis.

A major finding from this investigation is that the majority of establishments are unable to identify a specific root cause for the STEC contamination. However, based on the 24 investigation reports received, a standardised reporting format is proposed which should be used as an investigation summary to which detailed information for implicated days/shifts (livestock sheets, processing summaries, microbiological test results etc) are appended. It is recommended that this standardised reporting format is adopted by industry and that each area is addressed by stating:

1. What the criterion for assessment is, i.e. what is the plant normal practice (e.g. standard operating procedure);
2. The documents / files reviewed;
3. The evidence / data, including how the affected dates compare with usual practice, e.g. similar dates around the detection.
4. The finding / conclusion.

Investigation training workshops around Australia

Eight workshops were delivered by Andreas Kiermeier and John Sumner and covered microbiological and statistical aspects of undertaking an investigation; slaughter floor and boning room interventions; assessing shelf-life of vacuum packed meat; the effects of slaughter hygiene and cool-chain on shelf-life; and an opportunity for one-on-one support for attendees, including on-going support through the 'Ask SARDI' service. A total of 86 people attended the workshops and the evaluation responses were very positive and highlighted the value of such training and information for establishment staff. Given the interest in these training workshops and subsequent attendance, they are a valuable training resource and activity for QA establishment staff and other industry participants / stakeholders.

ESAM Analysis Reporting Service

Within the Department of Agriculture and Water Resources (DAWR), data on *E. coli* and *Salmonella* from each export slaughter establishment in Australia, as well as *E. coli* O157 and *Shiga* toxin-producing *E. coli* (STECs), have been collected in the National Microbiological Database and now the Product Hygiene Index database. Since 2007, SARDI Food Safety and Innovation has provided monthly reports on ESAM (*E. coli* and *Salmonella* monitoring) results to red meat export slaughter establishments, in addition to national reports sent to MLA and DAWR each month.

Feedback and communication with recipients of the ESAM reports has been received on an ongoing basis and a feedback survey on the ESAM Analysis Reporting Service was conducted in early 2017. Based on the results of the feedback survey which indicated that value is still being received by industry from the monthly ESAM reports, a three-year contract between MLA and SARDI has been signed for the continued provision of the ESAM Analysis Reporting Service, with an annual review (Go/No-Go milestones) and the inclusion of the development of a training webinar and other improvements to the ESAM reports.

"Ask SARDI" – continuation of processor support to undertake process investigations

SARDI has provided assistance to establishments to encourage them to develop and undertake process improvement related projects under the "Ask SARDI" banner, providing ongoing statistical support to processors and providing statistical and data analysis.

Elements of the "Ask SARDI" service include:

- Delivery of case studies for the 3rd edition of the Processor's Guide to Improving Microbiological Quality
- An analysis of the MLA Carcase Baseline Survey with STEC data
- Presentation of case studies from the "Ask SARDI" support service at the MINTRAC Meat Inspection and Quality Assurance Managers Network meetings and conference
- Request for the Coles standard with microbiological specifications for fresh meat, mince and sausages
- Promotion of the advisory service for predicting shelf life of VP meat, provided by MLA, AMPC and University of Tasmania
- In consultation with MLA, direction to the AusMeat Handbook of Australian Meat, MSA criteria, the MLA "Guidelines for developing a method for estimating shelf life of chilled raw vacuumed meat products" and the Shelf life of Australian red meat book.

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1 Background

The Australian meat production and processing industry is an important part of the Australian economy and in particular, the export of meat and meat products to overseas markets. Access to markets is based on meat processing establishments being able to meet the safety and suitability requirements set by overseas countries. It is the Department of Agriculture and Water Resources' (DAWR) responsibility to certify safety and suitability of product, although ultimately, responsibility resides with each processing establishment to demonstrate ongoing compliance with the relevant market access or commercial trade requirements. Outbreaks of foodborne illness caused by meat products can have wide-spread and significant effects on sales / profits and reduce consumer confidence. End-product testing, such as robust N-60 testing for Australian manufacturing beef exported to the US, has been shown to be ineffective at ensuring product safety, especially when contamination levels are low and infrequent (Kiermeier et. al, 2015). For this reason, increased attention is being placed internationally on verifying process control.

As part of completed work by the South Australian Research and Development Institute (SARDI) (G.MFS.0294), several attempts were made to analyse existing national microbiological data sources to identify trends and times of increased risk with Shiga toxin-producing *E. coli* (STEC), with little success. This was mainly related to the low levels of hygiene indicators, e.g. *E. coli*, zero tolerance detects, etc. and the lack of "matching" between microbial hygiene indicators on carcases (e.g. *E. coli*), visual hygiene indicators on carcases (e.g. Meat Hygiene Assessments – MHA), and hazard detections in carton product (e.g. STEC).

SARDI has been commissioned by Meat and Livestock Australia (MLA) to undertake a range of studies, under five sub-project headings:

- Sub-project 1: Evaluation of Alternate Sampling Sites on Sheep Carcases
- Sub-project 2: Standardised STEC investigations and questionnaire
- Sub-project 3: Investigation training workshops around Australia
- Sub-project 4: Continuation of ESAM Analysis Reporting Service
- Sub-project 5: Continuation of processors support to undertake process investigations – "Ask SARDI"

2 Project objectives

1. Evaluate alternative sampling sites on sheep carcases to generate and analyse novel data to:
    - Identify carcase areas or sites with higher microbiological levels than standard ESAM sites
    - Identify potential hygiene indicators more suitable than current indicators
2. Deliver investigation training workshops to processors.
3. Provide ongoing statistical support (experimental design and data analysis) to QA managers and processors to encourage the undertaking of investigations / PIPs and continual process improvement of the industry.

4. Develop a standardised *E. coli* O157 / STEC investigation questionnaire.
5. Collect and analyse standardised *E. coli* O157 / STEC investigation questionnaire data.

3 Subproject 1: Evaluation of Alternate Sampling Sites on Sheep Carcases

3.1 Background

Since 1998, Australian regulations have required regular testing of carcases for generic *E. coli* and *Salmonella* at specific sites on the carcase under the *E. coli* and *Salmonella* Monitoring (ESAM) system. There have been suggestions that the sites sampled under the ESAM system may not reflect the true level of contamination on the carcases. MLA Project V.MFS.0401 reported an investigation of contamination levels on beef carcases at sites other than those specified by the ESAM system, and the present project comprises an investigation to 'map' contamination on ovine carcases.

3.2 Aim

Evaluate alternative sampling sites on sheep carcases over two seasons, to generate and analyse novel data to:

- Identify carcase sites with higher microbiological levels than ESAM sites,
- Identify whether there are hygiene indicators more suitable than those currently used (Total Viable Counts and generic *E. coli*).

3.3 Methodology

3.3.1 Sample collection

Sampling was carried out between May 2016 and January 2017 at abattoirs in South Australia, Victoria and New South Wales. At the South Australian abattoir, carcases were sampled on one day a week over four weeks, and at the other abattoirs, from Monday to Thursday in a single week using the following approach:

- Eight sheep carcases/day were sampled over the processing day – alternating between left and right sides of the carcase for sequential carcases.
- Four sites (100cm^2 each) were aseptically swabbed on the carcase – rump, belly, shank and neck (see Figure 1) using a Whirlpak sponge and according to standard ESAM sampling procedure (Microbiological Manual (DAWR, 2017)).
- A total of 512 samples were collected, thirty-two samples per sample site per abattoir.
- The swab samples were transported to the SARDI Food Safety and Innovation laboratories for microbiological testing the following day. Samples collected at interstate establishments were flown overnight under refrigeration to SARDI for testing – the temperature of the samples upon arrival at SARDI did not exceed 4°C.

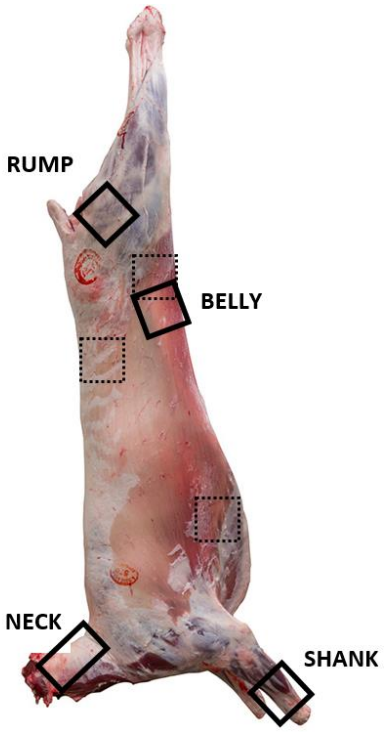

Figure 1: Swab sites on sheep carcase – the project sites are named, bold and black; ESAM sites are represented by dashed boxes.

3.3.2 Microbiological testing

All samples were tested for Total Viable Counts (TVCs), coliforms, *E. coli* and *Enterobacteriaceae*; enriched samples were also tested for *E. coli*. The contents of each Whirlpak bag were 'squished' for 30 seconds, the sponge stripped of excess fluid and an aliquot (1mL) removed to produce serial dilutions in Buffered peptone (0.1%) which were plated as follows:

- Total Viable Counts were performed using Petrifilm (AOAC® Official Method 990.12) incubated for 48 h ± 3 h at 35°C ± 1°C; the limit of detection was 2.5 cfu/cm^2.
- Coliform and *E. coli* counts were determined using Petrifilm (AOAC Offical Method 991.14) incubated for 24 h ± 2 h at 35°C ± 1°C (coliforms) and 48 h ± 2 h at 35°C ± 1°C (*E. coli*); the limit of detection was 0.25 cfu/cm^2.
- Enterobacteriaceae counts were determined using Petrifilm™ incubated for 24 h ± 2 h at 35°C ± 1°C (3M Petrifilm™ Enterobacteriaceae Count Plate Interpretation Guide); the limit of detection was 0.25 cfu/cm^2.
- *E. coli* presence/absence (via enrichment) were performed by incubating the contents of the Whirlpak bag for 6h at 35°C and plating on Petrifilm as described above.

3.3.3 Statistical analysis

All microbiological concentration results were $\log_{10}$ transformed and the log-transformed results were used as response variables for summary statistics, significance tests and regression models.

Statistical analyses were performed with the statistical software R (R Core Team, version 3.1.3, 2015) and a significance level of 5% was used to assess significance tests.

3.4 Results

3.4.1 Total Viable Counts

A summary of TVC counts by establishment and sampling site is provided in Table 1 and Figure 2. In interpreting results such as these, the statement by Gill *et al.* (1998) should be considered "*It is generally recognised that bacterial counts which differ by less than one log are similar (Jarvis, 1989)*". As a result, it may be said that while there are statistically significant differences between sites both within and between establishments, these rarely point to meaningful differences. What may be observed, however, is that:

- Total bacterial loadings on carcases were higher at establishments 2 and 3.
- The belly site had generally lower counts than the other sites.

Table 1: Summary average (standard deviation) of log_{10} TVC/cm^2 (n=32) by establishment and sample site.

Sample Site	Estab 1	Estab 2	Estab 3	Estab 4	Overall
Rump	2.01 (0.61)	2.66 (0.69)	2.40 (0.76)	2.20 (0.62)	2.32 (0.71)
Belly	1.31 (0.68)	2.09 (0.81)	2.54 (0.69)	1.88 (0.49)	1.96 (0.80)
Shank	2.51 (0.53)	2.96 (0.62)	2.97 (0.71)	1.70 (0.52)	2.54 (0.79)
Neck	2.07 (0.64)	2.81 (0.54)	3.07 (0.63)	1.99 (0.72)	2.48 (0.78)

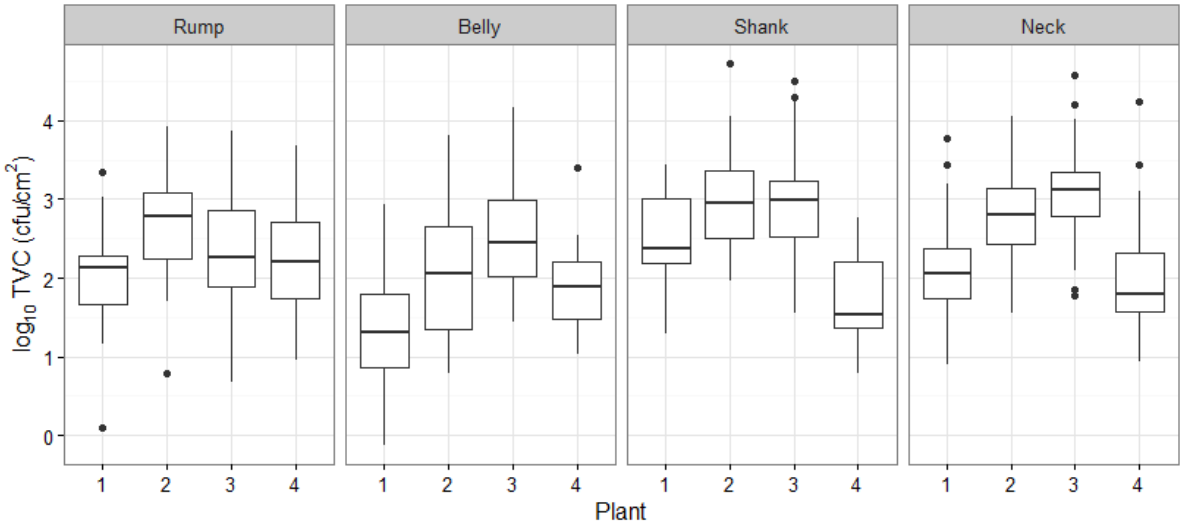

Figure 2: Box plots of log_{10} TVC/cm^2 by establishment and sample site.

3.4.2 Coliforms

For each establishment and sampling site, a summary of the coliform prevalence is given in Table 2, and of mean concentration in Table 3 and Figure 3. In general:

- The rump had a higher prevalence and concentration of coliforms than other sites.
- Establishments 2 and 3 had generally higher prevalence and concentration of coliforms than establishments 1 and 4.

Table 2: Prevalence (%) of coliforms by establishment and sample site.

Sample Site	Establishment 1	Establishment 2	Establishment 3	Establishment 4	Overall
Rump	84	100	94	84	91
Belly	28	81	94	62	66
Shank	53	91	69	44	64
Neck	66	87	78	59	73

Table 3: Summary average (standard deviation) of log_{10} coliforms/cm^2 by establishment and sample site.

Sample Site	Establishment 1	Establishment 2	Establishment 3	Establishment 4	Overall
Rump	0.79 (0.62)	1.64 (0.82)	0.48 (0.75)	0.26 (0.52)	0.82 (0.87)
Belly	-0.19 (0.27)	0.88 (0.95)	-0.01 (0.56)	-0.02 (0.63)	0.24 (0.81)
Shank	0.05 (0.48)	0.48 (0.64)	0.19 (0.72)	-0.31 (0.35)	0.18 (0.64)
Neck	0.19 (0.64)	0.63 (0.90)	0.08 (0.69)	0.19 (0.46)	0.31 (0.75)

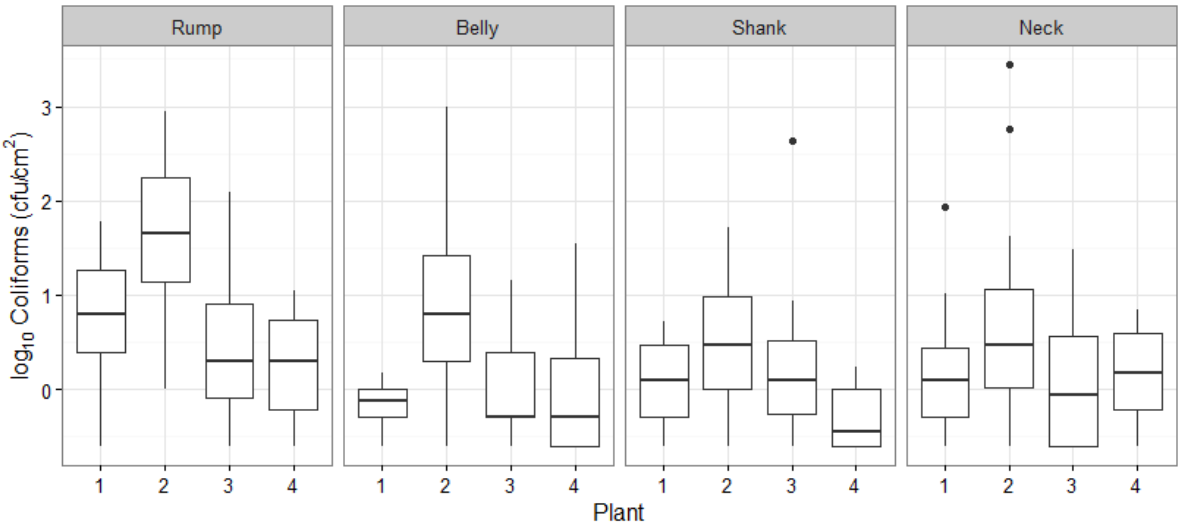

Figure 3: Box plots of log_{10} coliforms/cm^2 by establishment and sample site.

3.4.3 E. coli

For each establishment and sampling site, a summary of *E. coli* prevalence is presented in Table 4, and of mean concentration in

Table 5 and Figure 4. In general:

- The rump had a higher prevalence and concentration of *E. coli* than other sites.

- Establishments 2 and 3 had generally higher prevalence and concentration of *E. coli* than establishments 1 and 4.

Table 4: Prevalence (%) of E. coli *by establishment and sample site.*

Sample Site	Establishment 1	Establishment 2	Establishment 3	Establishment 4	Overall
Rump	84	100	94	81	90
Belly	25	75	87	53	60
Shank	44	91	66	41	60
Neck	56	81	78	56	68

Table 5: Summary average (standard deviation) of log_{10} E. coli*/cm^2 by establishment and sample site.*

Sample Site	Establishment 1	Establishment 2	Establishment 3	Establishment 4	Overall
Rump	0.75 (0.61)	1.48 (0.83)	0.32 (0.76)	0.13 (0.51)	0.82 (0.87)
Belly	-0.33 (0.24)	0.78 (0.88)	-0.09 (0.55)	-0.09 (0.54)	0.24 (0.81)
Shank	0.06 (0.52)	0.35 (0.68)	0.09 (0.73)	-0.41 (0.30)	0.18 (0.64)
Neck	0.20 (0.55)	0.52 (0.88)	-0.03 (0.68)	-0.01 (0.49)	0.31 (0.75)

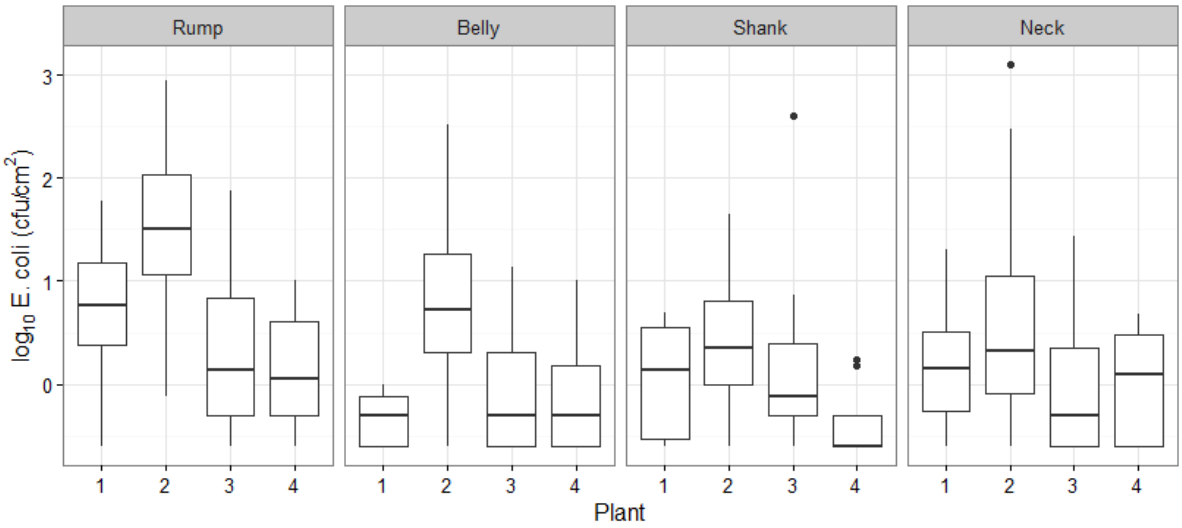

Figure 4: Box plots of log_{10} E. coli*/cm^2 by establishment and sample site.*

3.4.4 Enterobacteriaceae

For each establishment and sampling site, a summary of *Enterobacteriaceae* prevalence is presented in Table 6, and of mean concentration in Table 7 and Figure 5. In general:

- The rump had a higher prevalence and concentration of *Enterobacteriaceae* than other sites.
- Establishments 2 and 3 had generally higher prevalence and concentration of *Enterobacteriaceae* than establishments 1 and 4.

Sample Site	Establishment 1	Establishment 2	Establishment 3	Establishment 4	Overall
Rump	91	100	94	81	91
Belly	50	94	84	75	75

Shank	59	91	91	56	74
Neck	81	94	72	84	83

Table 6: Prevalence (%) of Enterobacteriaceae *by establishment and sample site.*

Table 7: Summary average (standard deviation) of log_{10} Enterobacteriaceae*/cm^2 by establishment and sample site.*

Sample Site	Establishment 1	Establishment 2	Establishment 3	Establishment 4	Overall
Rump	0.91 (0.73)	1.71 (0.90)	0.65 (0.77)	0.39 (0.61)	0.95 (0.91)
Belly	-0.07 (0.63)	0.86 (0.96)	0.21 (0.61)	0.07 (0.62)	0.33 (0.82)
Shank	0.02 (0.51)	0.51 (0.67)	0.15 (0.68)	-0.24 (0.28)	0.16 (0.64)
Neck	0.09 (0.65)	0.70 (0.96)	0.59 (1.03)	0.22 (0.69)	0.40 (0.87)

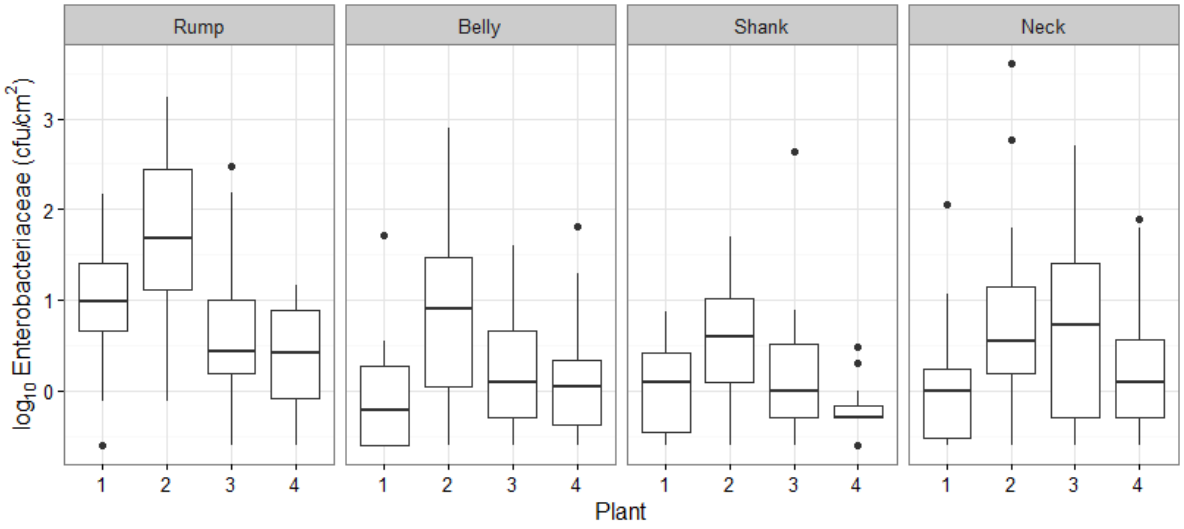

Figure 5: Box plots of log_{10} Enterobacteriaceae*/cm^2 by establishment and sample site.*

3.4.5 *E. coli* Enrichment

Table 8 presents the prevalence of *E. coli* following enrichment of the sponge fluids at each site. The total area sponged at each site was 3,200 cm^2 (100 cm^2 on each of 32 carcases). Almost all sites at each establishment had at least one viable *E. coli* cell.

Table 8: Prevalence (detects/samples) of log_{10} E. coli enrichment/cm^2 by establishment and sample site.

Sample Site	Establishment 1	Establishment 2	Establishment 3	Establishment 4	Overall
Rump	100% (32/32)	100% (32/32)	100% (32/32)	100% (32/32)	100% (128/128)
Belly	94% (30/32)	97% (31/32)	97% (31/32)	100% (32/32)	97% (124/128)
Shank	100% (32/32)	100% (32/32)	94%(30/32)	100% (32/32)	98% (126/128)
Neck	100% (32/32)	100% (32/32)	84% (27/32)	100% (32/32)	96% (123/128)

3.5 Discussion / Recommendations

3.5.1 Are there sites with higher microbiological levels than ESAM sites?

It is important to note that:

- ESAM data are based on a pooled sample from three samples as indicated in Figure 1, while individual sites were tested in the present study.
- Each ESAM site is sponged over an area of 5cm x 5cm, compared with 10cm x 10cm in the present study, resulting in different limits of detection (0.33 cfu/cm^2 and 0.25 cfu/cm^2, respectively).
- ESAM samples are taken from chilled carcases while the present study took samples from carcases before they entered the chillers.

Given the foregoing, a general comparison is made between data for concentration of TVC and prevalence of *E. coli* for the ovine industry during the period of the sampling program of the present study. In Figure 6, ESAM data for TVC and *E. coli* concentration from the days of sampling are presented with site data for each establishment. It would appear that ESAM swabs give lower TVC and *E. coli* levels than some of the sites sampled in this present study, for example, the rump, however, the different sampling methodologies make it difficult to compare directly.

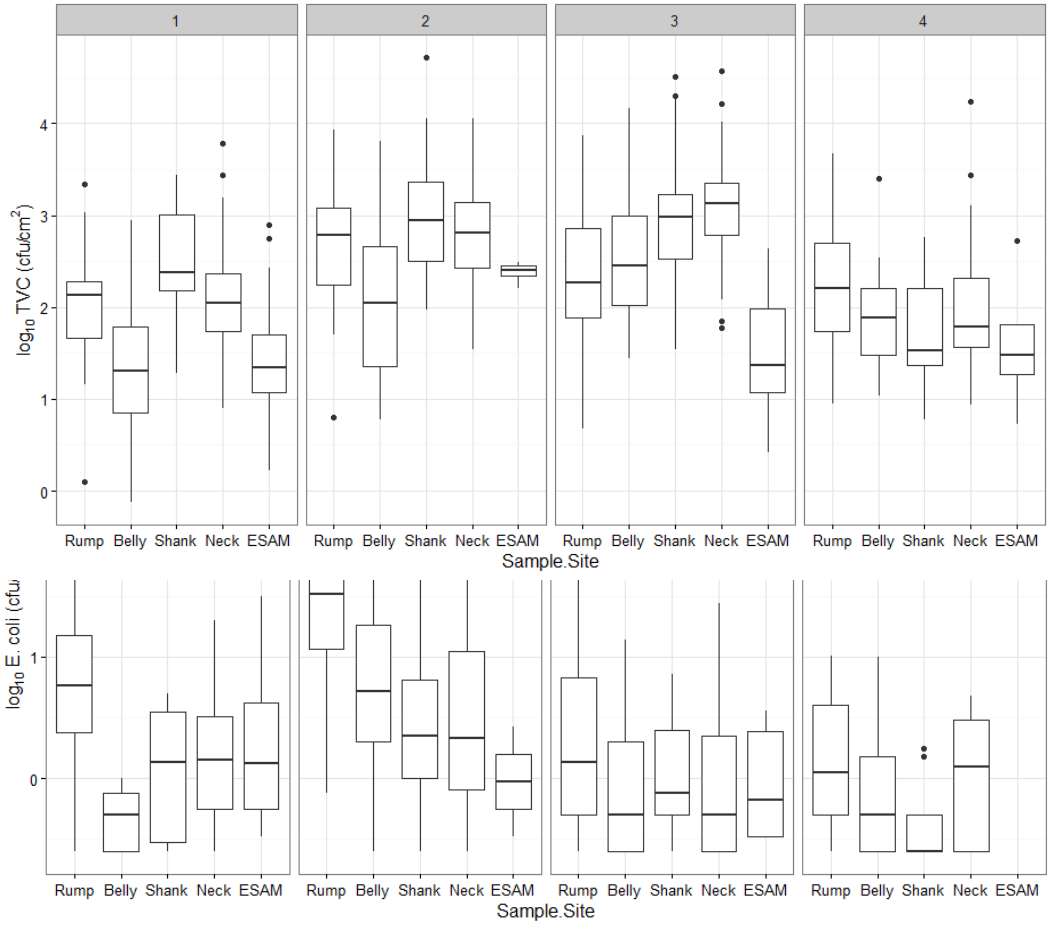

Figure 6: Boxplots of TVC and E. coli for sample sites versus ESAM sites.

3.5.2 Are there hygiene indicators more suitable than those currently used?

Monitoring hygienic processing of red meat products is hampered because *E. coli* is detected only rarely and usually at low concentrations, which has led to suggestions that alternate indicators such as coliforms and *Enterobacteriaceae* might have more utility.

This study estimated prevalence of microbial indicators on sheep carcases, for which a summary across all establishments is presented in Table 9. From this, it can be seen that over all sites, the prevalence in order was *Enterobacteriaceae* > Coliforms > *E. coli*.

Table 9: Summary of the prevalence (%) of indicator organisms by sample site across all establishments.

Sample Site	n	*E. coli*	Coliforms	*Enterobacteriaceae*
Rump	128	90	91	100
Belly	128	60	66	97
Shank	128	60	64	98
Neck	128	68	73	96

In the review of faecal indicator organisms in food by Craven *et al*. (2003), the criteria for suitability as an faecal indicator organism (Buttiaux & Mossel, 1961) is stated to be that "an organism:

1. Should occur naturally only in intestinal environments
2. Should be in high numbers in faeces
3. Have a high resistance to the external environment
4. Should be detectable at low concentration by reliable methods."

Craven *et al*. (2003), after evaluating the suitability of *Enterobacteriaceae*, Coliforms, *E. coli* and enterococci as indicators, concluded that: "No species or group, including *E. coli*, fulfils these (above) conditions perfectly, but none is superior to *E. coli* in most circumstances."

In the context of the Australian meat industry, the work of Jordan *et al*. (2007) is relevant. The authors undertook an analysis of a national survey of beef and sheep carcases which included several indicator organisms and concluded that:

1. Under conditions of 'good processing' when rates of *E. coli* contamination are low, Coliform or *Enterobacteriaceae* counts can perform as useful surrogates of indicators of faecal origin, simply because of their increased prevalence.
2. However, using Coliforms or *Enterobacteriaceae* as a surrogate for *E. coli* would result in substantial false-positive misclassifications.

Based on the foregoing, *Enterobacteriaceae* and coliforms may be suitable for process control monitoring, but regardless, *E. coli* remain the indicator of choice for faecal contamination.

4 Sub-project 2: Standardised STEC investigations and questionnaire

All establishments exporting beef trim to the US for grinding are required to test each individual lot (up to 700 cartons) for Shiga Toxin-producing *E. coli* O157, and many also test for an additional six Shiga toxin-producing *E. coli* serotypes (STECs). An STEC detection is seen as a failure of the establishment's HACCP plan, requiring an investigation to identify reason(s) for the detection and a review of the establishment's HACCP methodology to identify appropriate corrective or preventive actions; actions implemented must be verified as effective (DAWR, 2017).

Unfortunately, little guidance is provided in relationship to what specific aspects (if any) need to be reviewed – the current Microbiological Manual (DAWR, 2017, p46) only notes that establishments upon being informed of a positive result in a department verification test must: "investigate their process and test records from the relevant production periods including livestock, slaughter, refrigeration and boning processes".

Most establishments investigate "MHA records, process and product monitoring/verification, pre-shipment reviews, ESAM test results, other test results, pro-operational and operational monitoring, HACCP records, cleanliness of, and type and source of, animals slaughtered, and national establishment verification system records as appropriate" as outlined in Meat Notice 2012/01 (DAWR, 2012). However, this has now been superseded by the Microbiological Manual (DAWR, 2017).

Based on the foregoing, a study was mooted to gather information on how establishments respond to DAWR requirements in the event of a STEC detection, and to develop a standardised response document.

A novel approach for this investigation was developed, stemming from foodborne illness investigations, where epidemiologists typically use standardised questionnaires to elicit information about potential foods and consumption settings and use the answers to generate hypotheses about the likely sources of the outbreak. They then test these hypotheses using a case-control methodology, where foodborne illness cases and control subjects (sourced from the same consumption setting – restaurant, hospital, etc) are compared in relation to their food consumption.

An analogous approach was planned for the present sub-project, namely creating a standardised questionnaire for investigating STEC detections in beef and identifying potential risk factors contributing to end-product contamination. In parallel, the intention was to use the questionnaire to elicit information on similar lots where no STECs were detected. Together, the parallel investigations would comprise case-control pairs similar to those used in informing food poisoning outbreaks.

In particular, this sub-project was aimed at addressing Objectives 4 & 5 of this project:

Objective 4. Develop a standardised *E. coli* O157 / STEC investigation questionnaire.
Objective 5. Collect and analyse standardised *E. coli* O157 / STEC investigation questionnaire data.

4.1 Methodology

The methodology planned for undertaking this investigation was described in the research proposal, and included the intention to:

1. Collect approximately ten (10) investigation reports from different establishments and identify common and unique elements for potential inclusion in a standardised report / form.
2. Use the reports to develop a standardised reporting form for investigating STEC detections.
3. Get companies to undertake STEC investigations using the standardised form and report them to SARDI, which will provide the information on *cases*.
4. Collect the same information about suitably similar lots in which no STEC contamination was detected, from the same abattoir (matched controls).
5. Aim to collect data for 30 cases and 60 controls (1:2 ratio of cases to controls for each investigation). Because cases occur relatively infrequently, a greater number of controls was sought to increase the statistical power of the analysis.
6. Perform a case-control analysis to identify potential risk factors for STEC contamination.

4.2 Constraints to the proposed study methodology

As the study progressed, a number of constraints were encountered which necessitated changes to the study methodology as outlined in elements 1-6 (above) including:

1. As reported in V.MFS.0400 Milestone 3.1 Report (December 2016), the standardised reporting form was abandoned because establishments were collecting very similar information and they had all developed reporting formats that suited their internal needs.
2. When establishments were contacted with a request for information and records from control cases (with no *E. coli* O157 /STEC detection) that were processed around the same time as the lots in which *E. coli* O157 /STEC had been detected, most stated that it would be too time consuming to extract and compile all investigative information for cleared lots.
3. While SARDI Food Safety and Innovation gained the in-principle participation of 15 establishments to provide information on lots where STECs were and were not detected, for the case-control analysis, despite repeated follow up attempts, information on only 24 cases (from ten establishments) and 5 controls (from two establishments) could be obtained.
4. In addition, not all information could be obtained from establishments, despite repeated follow up. As a result, no case-control analysis could be performed – instead only descriptive summaries are provided below. The various aspects covered by the investigations, and recorded in the spreadsheet, were reported in the Milestone 3.1 report (December 2016).

5. While follow-up with various establishments revealed that STEC investigations were often more thorough and detailed than the information in the investigation reports suggested, some reports generally provided only broad summaries of the information and factors considered and the level of detail provided in these reports was insufficient for us to conduct effective data capture.
6. In many instances, the raw data and observations relevant to each investigation were archived by each establishment, requiring substantial and repeated follow-up to obtain this information (if at all).
7. Furthermore, no establishment actively used information from cleared lots as a point of comparison when undertaking investigations into lots with an *E. coli* O157 /STEC detection. This lack of 'baselining' contributed to the difficulty in obtaining a sufficient number of controls for this sub-project.

4.3 Amendments to the proposed study methodology

- As a consequence of constraint 1 (above), a data capture spreadsheet was developed which could be populated from the information provided in establishment STEC investigation reports.
- Establishments were contacted with feedback regarding additional information required to populate the data capture spreadsheet. This primarily involved locating raw data and observations from the relevant production dates and forwarding the information on to SARDI.
- Some establishments provided the raw data and observations relating to cleared production periods, but it was difficult to gather enough information to perform a case-control analysis.

4.4 Results

4.4.1 Traceability information

A summary of traceability information is provided in Table 10, with the following observations made about lots where STEC were detected:

- Product types are primarily trimmings (TRMG: 18 of the 24 lots) and primarily involved Bull ("B-") or Cow ("C-"), and only 3 lots were classified only as beef ("A-"). However, these are likely due to the fact that these product types are those destined for grinding in the US and hence are required to be tested for STEC.
- A wide range of chemical lean was represented in affected lots; the higher proportion of high CL likely reflects the volume of high CL trim exported to the USA market.
- Only two affected lots were produced from a single slaughter date; the rest involved several slaughter dates over an average period of 11.6 days.

Table 10: Summary of lot traceability information

Information	STEC detected
Number of establishments	10
Number of lots (n)	24
Range of number of cartons per lot	15,* 217, 292, 322, 325, 333, 345, 350 (x5), 660, 690, 700 (×7) Not provided (×4)
Range of product types*	A-F (×1) A-FH (×1) A-TRMG (×1) B-FH (×3) B-TRMG (×3) C-F (x1) C-TRMG (×10) TRMG (×4)
Range of Chemical Lean (CL)	65 (×2) 80 (×2) 85 (×5) 90 (×4) 95 (×11)
Number of slaughter dates in lot	1-71**
Time period covered by slaughter dates (days)	1-71

* Based on AUS-MEAT PART 6 – APPENDIX 6.1 - Your Guide To The Australian Common Code
** For 10 lots, only the total period was provided over which the lot was produced, rather than the actual number of implicated dates.

4.4.2 Livestock information (for each lot processed)

A summary of information about the livestock processed is provided in Table 11, from which the following observations are made about lots where STEC were detected:

- The majority of livestock was clean prior to washing, but received a routine wash, presumably to remove dust and moisten the hide for processing.
- Some animals were considered dirty and received extra washing to remove dags and faecal matter. It is unknown how clean livestock were on entry to slaughter, although all must have been deemed acceptable.
- Few establishments had information, or commented on, the time off-feed / transport distance /duration. In particular, time off-feed may affect the concentration of *E. coli* O157/ STEC in faeces (Pointon *et al*., 2012).

Table 11: Summary of livestock information

Information	STEC detected
Time off feed	Unknown (×23) Fed hay & silage during holding until day prior to slaughter (×1)
Cleanliness of livestock prior washing	Unknown (×2) Clean (×14) Dirty (×8)
Were livestock washed – routine or extra	Unknown (×4) Routine wash (×20) Extra (×6)
Water issues	Unknown (×4) No (×17) Low pressure (×2) Mud pools in laneway (×1)
Unusual observation(s) reported about stock processed, e.g. distance travelled, very large animals, etc.	No (×13) Very dirty, needing additional wash (x6) Cattle processed earlier were rated dirty (×2) Large bulls processed (×3)

4.4.3 Slaughter information

A summary of information about the slaughter performance is provided in Table 12 from which the following observations are made about lots where STEC were detected:

- For the majority of lots, there were no slaughter problems, with acceptable MHA scores (where reported) and no zero tolerance (ZT) defects.
- One establishment noted an MHA score of 2.0, which is considered marginal.
- Some establishments identified problems, but to what extent they did/may have contributed to the STEC detection is unknown.

Table 12: Summary of slaughter information

Information	STEC detected
Pre-op hygiene issues	Not noted (×6) No (×18)
Slaughterfloor MHA	Unknown (×15) – one noted 45 Minors across 5 slaughter days, but not number inspected 0.13-0.26 (×2, though only single values were provided to cover multiple slaughter dates) Three establishments (six reports) specified scores for each slaughter date – one include a score of 2.0; all others were <1.0
Number of ZT's detected over slaughter days	0 (×18) 1 (×3; inc. one where DAWR identified multiple ZTs) 2 (×1) 3 (×1)

Information	STEC detected
	Unknown (×1)
Problems identified during slaughterfloor operation	No (×17) 1.5 hr chain breakdown (x2) Legs/neck dragging on floor (x1) Process score provided (×4): 85-100%
Problems identified during chilling operation	No / RI compliant (x24)

4.4.4 Production (boning / packing) information

A summary of information about the boning room performance is provided in Table 13, from which the following observations are made about lots where STEC were detected:

- Given the sometimes long period over which cartons were collected for a lot, it is not surprising that extended chilling (i.e. weekend) was commonly observed. For example, as indicated in Table 10, the number of slaughter days ranged between 1 and 71, through for 10 of these, only the first and last slaughter dates were provided, which does not imply that every slaughter date in that range was represented in the implicated lot. From the 10 lots for which individual dates were provided, the average number of slaughter days in the lots was seven. In addition, since only a single pooled sample is tested for *E. coli* O157/STEC, it is not possible to ascertain whether the detection originated from a carton that was produced after extended chilling or not.
- Most affected lots had no zero tolerance defects associated with them, though one had a large number (7), which should have, and may have, raised concerns about the lot of animals to be boned for the US market. It is unknown what/if any action was taken with respect to this detection.
- Carton Meat Assessment (CMA) indicates that overall large numbers of cartons are assessed, and that various defects are found, though establishments generally did not specify the nature of these defects i.e. contamination or manufacturing.

Table 13: Summary of boning room information

Information	STEC detected
Hot or cold boned	Hot (×1) Mixed (×1) Cold (×22)
Extended chilling (weekend/pub. hol.)	Yes (×14) No (×7) Unknown (×3)
Boning room MHA (cold boned only)	Unknown (×14) 0.025-0.3 (×4, though only single values were provided to cover multiple processing dates) Two establishments (five reports) specified scores for each of the processing dates implicated - scores ranged from 0.025-0.5.
Number of ZTs detected	0 (×17)

Information	STEC detected
	1 (×3) 2 (×1) 7 (×1) Unknown (×1)
Carton Meat Assessment	n=95, 10 minors, 1 major* n=920, 2 majors (hair & bone fragment) n=20, no defects n=152, minor defects* n=461, 1 critical, 4 major, 120 minor* n=477, 1 critical, 4 major, 171 minor* n=1346, 2 critical, 8 major, 367 minor* n=1129, 6 major, 325 minor* n=?**, 12 minors detected* n=8, no defects detected n=?**, 7 minors detected* n=?**, minors detected (mostly hair/bone chips)

* No information was provided about the type of defects.

** Number of cartons sampled not provided.

4.4.5 Microbiological testing results

A summary of information about the microbiological performance (slaughter and boning) is provided in Table 14, from which the following observations are made about lots where STEC were detected:

- Overall slaughter performance is in line with industry performance i.e. Cow/Bull (5.3%) and Steer/Heifer (3.3%) over the last 3 years.
- Some slaughter dates resulted in generic *E. coli* detection on carcases, though these were at very low levels.
- Only one establishment failed an *E. coli* window related to any of the slaughter dates.
- While establishments are not required to test for *E. coli* in carton meat, it appears that many do and, in all cases, *E. coli* was not detected, and coliforms were detected infrequently, and at low concentrations.

Table 14: Summary of microbiological information

Information	STEC detected
Number of ESAM tests	2-66
Number of *E. coli* detections	1/28 (3.6%); 2/66 (3.0%); 1/51 (2.0%); 1/11 (9.1%); 1/16 (6.3%); 1/31 (3.2%)
Average *E. coli* concentration (cfu/cm^2)	<0.02
Failed *E. coli* window	Yes (×1) No (×18) Unknown (×5)
Number of carton *E.* coli tests*	1-41 Not tested (×10)

Information	STEC detected
Number of *E. coli* detections	All non-detects
Average *E. coli* concentration (cfu/cm^2)	NA
Number of carton coliform tests*	1-41 Not reported (×13)
Number of coliform detections	1/2 (50%); 2/27 (7.4%); 1/20 (5.0%)
Average coliform concentration (cfu/cm^2)	1.1; 10; 1.5

* Establishments are not required to test for *E. coli* in carton meat, only for coliforms.

4.5 A standardised reporting format

A major finding from this investigation is that the majority of establishments are unable to identify a specific root cause for the STEC contamination. However, based on the 24 investigation reports received, a standardised reporting format is proposed (below) which could be used as an investigation summary to which detailed information for implicated days/shifts (livestock sheets, processing summaries, microbiological test results etc) are appended along with how those days compare to "normal" slaughter and dressing days (i.e. baseline information).

1. **Traceability Information**
    a. Number of cartons affected
    b. List of slaughter and production dates related to the cartons produced
    c. Product type(s) / detail
2. **Livestock information**
    a. Time-off feed / distance travelled
    b. Cleanliness of stock prior to washing
    c. Routine washing applied and any extra washing needed
    d. Cleanliness of stock entering the slaughter floor
    e. Any water supply problems
    f. Any unusual observations about the incoming livestock (e.g. long hauls, delay in slaughter).
3. **Slaughter floor information** – for each affected slaughter run/shift/date (these could effectively be summarised in a table, with one row per affected run/shift/date)
    a. Detailed visual (MHA) results, including number of carcases/sides inspected, day/shift MHA score, number of ZTs (for company and DAWR verification)
    b. Process monitoring results and any problems during slaughter operations
4. **Boning room information** – for each affected boning run/shift/date (these could effectively be summarised in a table, with one row per affected run/shift/date)
    a. Detailed visual (CMA) results, especially those defects related to contamination, including number of cartons inspected, defect type and severity, number of ZTs (for company and DAWR verification)
    b. Process monitoring results and any problems during boning operations

5. **Chilling information**
    a. Temperature / Refrigeration Index information / logs for affected carcases and cartons
    b. Other relevant chilling information, e.g. breakdowns, delays
6. **Microbiological test results**
    a. Carcase ESAM test results, especially number of tests collected from affected slaughter/ boning dates, number of generic *E. coli* detections, *E. coli* concentrations (for detections) and how these results compare with normal operations (e.g. from monthly SARDI ESAM reports).
    b. Carton microbiological results, especially for *E. coli* (if tested) or coliforms, including number of tests collected from affected boning dates, number of detections, concentrations (for detections) and how these results compare with normal operations (e.g. from monthly SARDI ESAM reports).
    c. Any additional microbiological test results, including number of tests during affected periods, number of detections and concentrations (for detections), e.g. carcase excision sampling or harvest monitoring results.
7. **Any addition information**, such as pre-shipment reviews, HACCP records, etc.
8. **Detailed information**, sheets for each lot of production are appended.

4.6 Conformance with the standardised test reporting format

A total of 24 reports from 10 establishments were received and an assessment was made of how each report conformed with the standardised approach; the results are summarised in **Error! Reference source not found.**. Note that there is an element of interpretation (value judgement) by the project team on how well the establishment conformed with each criterion.

From **Error! Reference source not found.**, it can be seen that:

1. Some reports did not state the number of cartons affected.
2. Reports from the same establishment do not necessarily provide the same information or to the same detail.
3. With respect to slaughter floor and boning room MHA results, some plants considered only Zero Tolerance (ZT) defects, and not the overall process and/or product results.
4. Several reports only included carcase microbiological results, but no carton microbiological data.
5. Provision of evidence was lacking in several reports. Some reports noted that the results are held on file, others included the detailed information in the report and others provided no evidence for their findings.

In addition, it was apparent that reports are commonly prepared by modifying the most recent investigation and changing the information. However, this practice has the potential for contradictions to occur if some of the information is not updated. For example, in one report it was stated that for carcase inspections in the boning room “no zt deviations detected during MHA

inspection", yet shortly after it was noted that "7 zts were identified by pre trimmers". Similarly, in another report, it was noted that "all lots on the slaughter days involved were rated as good", while it was noted in the following paragraph that "[dirt yards which can turn quickly to mud in wetter weather] could have contributed to excess contamination on the hide of animals."

It was also apparent that almost none of the investigations considered how the affected slaughter/production dates compared with normal practice, around the time of the detection. For example, an MHA score of 0.8 complies with the MHA guidelines (AQIS, 2002), but may still be an indication of something unusual if the plant normally operates with an MHA score of 0.2, say. Only a single report provided information to this effect, and then only about MHA results. The same applies for all other information that is assessed – whether the finding is unusual or not can only be assessed in the context of 'normal' operation.

Table 15: E. *coli O157 / STEC investigation report conformance summary with proposed standardised reporting format (reports from the same establishment are shading with the same colour; if a report number is not shaded then only a single report was provided by an establishment); 'N' denotes that no information was not provided about the report aspect having been investigated, 'Y' denotes that information was provided about the report aspect having been investigated, 'P' denotes that partial information was provided about the report aspect having been investigated.*

	Report																							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Traceability Information																								
Number of cartons affected	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N	N	N
Slaughter and production dates related to the cartons produced[1]	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	P	P	P	Y	Y	Y	Y	Y	Y
Product type(s) / detail	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Livestock information																								
Time-off feed / distance travelled	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Stock cleanliness prior to washing	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N	N	N	N	N	N	N	Y	Y	Y	Y
Routine / extra washing	N	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	N	N	Y	Y	Y	Y	Y	N	Y	Y	Y	Y
Stock cleanliness entering slaughter floor	Y	Y	N	N	Y	Y	Y	Y	Y	Y	Y	N	N	Y	Y	N	N	N	Y	Y	Y	Y	Y	Y
Water supply problems	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y	N	N	N	N	N
Any other/unusual observations about livestock	N	Y	Y	Y	N	N	N	N	N	N	N	N	Y	N	N	N	N	N	N	Y	N	N	N	Y
Slaughter floor information – for each affected slaughter run/shift/date																								
MHA results, including number of carcases/sides inspected, day/shift MHA score, number of ZTs[2]	P	P	P	P	P	P	Y	P	P	P	P	P	N	Y	Y	Y	Y	Y	Y	P	Y	Y	Y	Y
Slaughter process monitoring, including any additional problems during slaughter operations	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	Y
Boning room information – for each affected boning run/shift/date																								
CMA results, especially those defects related to contamination, including number of cartons inspected, defect type and severity, number of ZTs[3]	Y	P	P	P	P	P	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	P	Y	Y	Y	Y
Boning process monitoring, including any problems during boning ops.	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	P	Y	Y	Y	N
Chilling information																								

	Report																							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Temperature/RI information/logs for affected carcases and cartons	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	Y
Other relevant chilling information (e.g. breakdowns, delays)	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N	Y	Y	Y	Y	Y	N	N	N	N	Y
Microbiological test results																								
Carcase ESAM test results	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Carton microbiological result	N	N	N	N	N	N	Y	Y	N	N	N	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Any additional micro. test results[4]	Y’	Y’	Y’	Y’	Y’	Y’	Y	Y’	Y’	Y’	Y’	Y	N	N	Y	N	N	N	Y	Y	Y	Y	Y	Y
Any addition information, e.g pre-shipment review, HACCP records, etc.[5]	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	P	Y	P	P	P	Y	P	Y	Y	Y	Y
Detailed evidence provided for each aspect investigated, either appended or included in report[6]	P	OF	OF	OF	OF	OF	OF	OF	OF	OF	OF	OF	P	P	P	P	P	P	Y	Y	Y	Y	Y	Y

[1] P denotes that only production dates were identified

[2 & 3] P denotes that only ZT detections were reported

[4] Y’ denotes that (only) STEC testing/detection history was provided

[5] P denotes that only HACCP plan/records were reassessed, but no pre-shipment review information was noted.

[6] P denotes that some detailed evidence was provided; OF denotes that records /documents investigated were noted to be “on file” (amount of detailed evidence in the report varied).

4.7 Conclusions / Recommendations

A detection of *E. coli* O157 / STECs in beef meat destined for grinding in the US is seen as a failure of an establishment's HACCP plan, requiring an investigation to identify reason(s) for the detection and a review of the establishment's HACCP methodology to identify appropriate corrective or preventive actions; actions implemented must be verified as effective (DAWR, 2017).

After reviewing 24 investigation reports from 10 establishments, all of which had been signed off by the establishment and the department's On-Plant Vet, it was apparent that the reports varied considerably in detail, and that even the best reports had scope for improvement. From these reports, a standardised reporting format was produced. It is recommended that this standardised reporting format (i.e. items to be addressed as per Section 4.5) is adopted by industry and that each area is addressed by stating:

1. What the criterion for assessment is, i.e. what is the plant normal practice (e.g. standard operating procedure);
2. The documents / files reviewed;
3. The evidence / data, including how the affected dates compare with usual practice, e.g. similar dates around the detection.
4. The finding / conclusion.

This approach allows plants to prepare a reporting "template" where items 1 and 2 do not require changing, and items 3 and 4 are completed for each investigation. It is also suggested that a "true template" is prepared (with blank space for items 3 and 4), rather than copying and modifying the most recent report. This should help avoid the failure to modify information for the most recent investigation.

With respect to item 3, it is suggested that the data / information is included either directly in the report or included as an appendix. This approach is preferred over simply referring to the source documentation that is held on file, because it ensures that all the relevant information is included in the report and thus makes the report more transparent.

5 Sub-project 3: Investigation training workshops around Australia

Training on how to run and analyse small, focussed investigations in establishments was delivered in April/May 2013 in the form of workshops by Andreas Kiermeier and John Sumner. With changes in QA staff, other staff and companies who may be interested in attending similar training, as well as some states not visited in 2013, expressions of interest were solicited from processors around the country in January 2016 and sufficient interest for a repeat of the workshops was received. In consultation with Ian Jenson, MLA, the format of the workshop was amended slightly from the original plan to be one-day workshops, consisting of four sessions.

Workshops in eight locations were delivered by Andreas Kiermeier and John Sumner and covered microbiological and statistical aspects of undertaking an investigation; slaughter floor and boning room interventions; assessing shelf-life of vacuum packed meat; the effects of slaughter hygiene and cool-chain on shelf-life; and an opportunity for one-on-one support for attendees, including on-going support through the 'Ask SARDI' service. The workshops were delivered as shown below.

- Rockhampton, Qld – Tuesday, 31st of May 2016
- Brisbane, Qld – Wednesday, 1st of June 2016
- Tamworth, NSW – Thursday, 2nd of June 2016
- Wagga Wagga, NSW - Friday, 3rd of June 2016
- Launceston, Tas – Monday, 6th of June 2016
- Melbourne, Vic – Tuesday, 7th of June 2016
- Adelaide, SA – Wednesday, 8th of June 2016
- Bunbury, WA – Thursday, 9th of June 2016

A total of 86 attended the workshops and participants included QA managers and staff, laboratory managers and technicians (on-plant and commercial), training facilitators, establishment managers, slaughter and boning supervisors, R&D project managers, HACCP coordinators and MINTRAC staff.

All attendees received USBs containing:

- The Shelf life of Australian Red Meat book
- Guidelines for developing a method for estimating shelf life of chilled raw vacuumed meat products
- 2nd Edition of the Processor's Guide to Improving Microbiological Quality
- Validation of Antimicrobial Interventions for Small and Very Small Processors: A How-to Guide to Develop and Conduct Validations
- Reporting template – template for reporting the conduct and results of an investigation
- Testing template v7 – Excel templates for evaluating microbiological data
- StatPak – Excel statistical add-in
- Copy of the presentation slides

The evaluation responses from the workshops attendees were positive and highlighted the value of such training and information for establishment staff. The average scores for each of the four sessions are given below, where a score of 5 was 'OK' and a score of 10 was 'extremely useful'.

	Stats & Micro Review	Investigations	Shelf-life	Ask SARDI
Average score	8.3	8.5	9.1	8.4

In response to a request from an establishment, a tailored workshop was run by Andreas Kiermeier and John Sumner on the 29th of July, assisting them with their processes (slaughter and boning), interventions and product shelf-life.

5.1 Conclusions / Recommendations

From these workshops, a number of case studies were developed in conjunction with the respective establishments, for publication in the 3rd edition of the Processor's Guide to Improving Microbiological Quality.

Given the interest in these training workshops and subsequent attendance, they are a valuable training resource and activity for QA establishment staff and other industry participants / stakeholders. MLA may wish to revisit these training workshops again in a few years' time or on a regular basis.

6 Sub-project 4: Continuation of ESAM Analysis Reporting Service

Within the Department of Agriculture and Water Resources (DAWR), data on *E. coli* and *Salmonella* from each export slaughter establishment in Australia, as well as *E. coli* O157 and *Shiga* toxin-producing *E. coli* (STECs), have been collected in the National Microbiological Database and now the Product Hygiene Index database. Since 2007, SARDI Food Safety and Innovation has provided monthly reports on ESAM (*E. coli* and *Salmonella* monitoring) results to red meat export slaughter establishments, in addition to national reports sent to MLA and DAWR each month.

Monthly ESAM and *E. coli* O157:H7/STEC establishment reports were sent to all fifty-nine participating establishments from January 2016 to June 2017. National reports were also sent monthly to Ian Jenson and Long Huynh at MLA and Christine Coulson, Mark Salter, Arefin Chowdhury, Dugald MacLachlan and Maged Tawadros at DAWR. Group ESAM reports have been distributed to the relevant QA managers, as well as the tailored ESAM report comparing between shifts for one individual establishment.

Feedback and communication with recipients of the ESAM reports has been received to update the email distribution details of four establishments and the Explanatory Guide for the ESAM Reports has been distributed to a number of QA and establishment staff. Changes have also been made to introduce a day versus night shift comparison report for hot boned cow/bulls. Quality assurance staff from one establishment contacted SARDI with a request for consolidated ESAM testing data for the last year – copies of the latest ESAM reports were sent.

A number of processing establishments contacted Jessica Jolley, SARDI Food Safety and Innovation, with respect to analysis and interpretation of the ESAM data. These enquires included:

- Inclusion of three “new” establishments on the ESAM mailing list, one of which is a hot boning establishment interested in seeing how their results compare with other establishments that hot bone.
- Updating and regenerating an establishment’s reports at the request of a QA manager.
- Follow up an establishment’s missing data in July and August with the Department of Agriculture and Water Resources (DAWR).
- Receiving ESAM data that had missed the DAWR submission deadline, directly from the establishment and generating up-to-date ESAM reports.
- Exchanging emails and phone calls with a QA manager over dilutions and limit of detections for Total Viable Counts, as their reported TVC prevalence was approximately 35%.
- Correcting data entry errors in *Salmonella* detections for a sheep establishment and regenerating the corresponding ESAM reports.
- Receiving confirmation from a beef establishment that a *Salmonella* positive result was in fact, negative – amended the SARDI ESAM database and conveyed the change to DAWR.
- Receiving a query from a sheep establishment as to whether there are certain months of the year in which there are more failures of the *E. coli* window criteria, compared to other months – analysed the ESAM database and reported back.

Jessica Jolley also supplied information in response to the following requests:

- Ian Jenson, MLA – a list of hot boning establishments and contacts in order to investigate chilling rates and product quality.

- Jay Kocharunchitt, University of Tasmania – ESAM reports for an establishment, to assist in a MLA/UTas project.
- Mark Salter, DAWR – national prevalence values for *Salmonella* per species from the 2015 ESAM data.
- Ian Jenson and Paul Vanderlinde, MLA – ESAM data for comparison of hot boned meat and conventionally chilled meat.
- Mark Salter, DAWR – graphs of national TVC and *E. coli* per species, carcase/carton over the last 12 months on a quarterly basis for EMIAC meeting papers.
- Mark Salter, DAWR and Fiona Culley, Charles Sturt University – national graphs from 2012-2016.
- Ian Jenson, MLA – graphs of carcase TVC for an individual establishment, as an anonymous illustration.

A copy of all email correspondence is provided in Appendix A.

6.1 Feedback survey on ESAM Analysis Reporting Service

In early 2017, 75 QA managers and staff from the 58 establishments currently receiving the monthly ESAM reports were invited to provide feedback on the reports and the service provided via an online survey hosted by SurveyMonkey. Of these, a total of 32 responses were returned.

The questions from the previous survey carried out in December 2014 / January 2015 were used as the basis for the questions in the current feedback survey. The survey asked how recipients use the reports, what value they find from them and any suggested improvements. The questions of the feedback survey, the survey results and the proposed action plan are reported in the Milestone 4.1-4.5 Report.

Some key findings are:

- 50% of respondents read the ESAM reports fully every time.
- 81% of respondents read the ESAM reports fully if time permits or every time.
- 80% of respondents have a “pretty good understanding” or “a very good understanding” of the ESAM reports.
- 72% of respondents “get a fair bit of value” or “get a lot of value (i.e. have made changes based on reports; use to benchmark performance)” from the ESAM reports.

6.2 Conclusions / Recommendations

Over the past 20 years, a history of detailed, long-term results from the ESAM database and the ESAM reports provided by SARDI Food Safety and Innovation has provided valuable information and resources to establishments and the red meat industry. Scientific background monitoring information on the industry is supported by over a million data points accumulated by ESAM / PHI.

Tailored reports are available to QA staff, MLA and DAWR and continue to be developed based on industry needs. Feedback from QA managers is still regularly received and are integral to maximising full value from the ESAM Analysis Reporting Service.

The ESAM Analysis Reporting Service under this project concluded with the June 2017 ESAM reports. Based on the results of the feedback survey which indicated that value is still being received by industry from the monthly ESAM reports, a three-year contract between MLA and SARDI has been signed for the

continued provision of the ESAM Analysis Reporting Service, with an annual review (Go/No-Go milestones) and the inclusion of the development of a training webinar and other improvements to the ESAM reports.

7 Sub-project 5: Continuation of processor support to undertake process investigations – "Ask SARDI"

As part of MLA project G.MFS.0294, SARDI provided assistance to establishments to encourage them to develop and undertake process improvement related projects. This support program has continued in the present project under the "Ask SARDI" banner, providing ongoing statistical support to processors and providing statistical and data analysis.

Elements of the "Ask SARDI" service include:

- Delivery of case studies for the 3rd edition of the Processor's Guide to Improving Microbiological Quality
- An analysis of the MLA Carcase Baseline Survey (V.MFS.0332) with STEC data
- Presentation of case studies from the "Ask SARDI" support service at the MINTRAC Meat Inspection and Quality Assurance Managers Network meetings and conference
- Request for the Coles standard with microbiological specifications for fresh meat, mince and sausages
- Promotion of the advisory service for predicting shelf life of VP meat, provided by MLA, AMPC and University of Tasmania
- Responded to a query on shelf-life of chilled carcases in consultation with MLA, by recommending the AusMeat Handbook of Australian Meat, the MSA criteria, the MLA "Guidelines for developing a method for estimating shelf life of chilled raw vacuumed meat products" and the Shelf life of Australian red meat book.

7.1.1 Case studies for the 3rd edition of the Processor's Guide to Improving Microbiological Quality

SARDI received a number of datasets from in-house trials for analysis and publication as case studies in the 3rd edition of the Processor's Guide to Improving Microbiological Quality. The topics covered in these case studies were:

- Hot water washing
- Effect of hot water decontamination carcase wash on ESAM results
- Chlorine dioxide and peracetic acid as *E. coli* decontaminants of beef carcases
- Lactic acid spray trial on beef carcases
- Chemical decontamination of calf carcases
- Mapping of *E. coli* on beef carcases
- Mapping of *E. coli* on sheep carcases
- Sheep brains – batch collection versus individually processing
- Massage technique to estimate bacterial loading of primal cuts (2 separate case studies)
- Carcase baseline study
    - Trimming
    - Trimming plus hot water

- Trimming plus lactic acid
- Trimming plus steam vacuum

In addition to these case studies, the 3rd edition of the Processor's Guide contains additional information and guidance on shelf-life for processors (supplied by John Sumner, University of Tasmania and MLA). The 3rd edition of the Processor's Guide was publically released at the 2017 MINTRAC MI&QA conference (http://publications.mla.com.au/go/E5SKUmsmcjsxsZ00) where Alexander Howard from SARDI gave a presentation on new case studies and focussed on the lessons learnt from the results and how to plan future plant investigations. In the survey after the MINTRAC MI&QA presentation, two people said "I have data I need SARDI to help me analyse", one person said "I want assistance with running or setting up a case study", five people said "I am interested in comparing case studies from the guide" and six people said "I am interested in doing some in-house case studies". This is an indication of industry's continued interest in case studies.

7.1.2 Comparison between carcase baseline survey and STEC data

From June 2015 to October 2016, MLA conducted a survey of beef and veal carcases from Australian export meat processing establishments to demonstrate the level of process control in slaughter operations and the resulting hygienic quality of beef/veal carcases (V.MFS.0332). A total of 5,452 beef and veal carcase sponge samples were collected from different meat processing establishments throughout Australia. Samples were collected immediately after the hide was removed and again at the end of processing prior to entering the chiller. The samples were tested for *Salmonella* and indicator microorganisms including *E. coli*, coliforms and Total Viable Counts.

A question posed by MLA was to investigate whether an establishment with high microbiological baseline results for TVC and *E. coli* also has a high incidence of STECs, as reported in the ESAM database. Eighteen beef establishments gave permission for their carcase baseline results to be accessed and analysed by SARDI Food Safety and Innovation for this purpose.

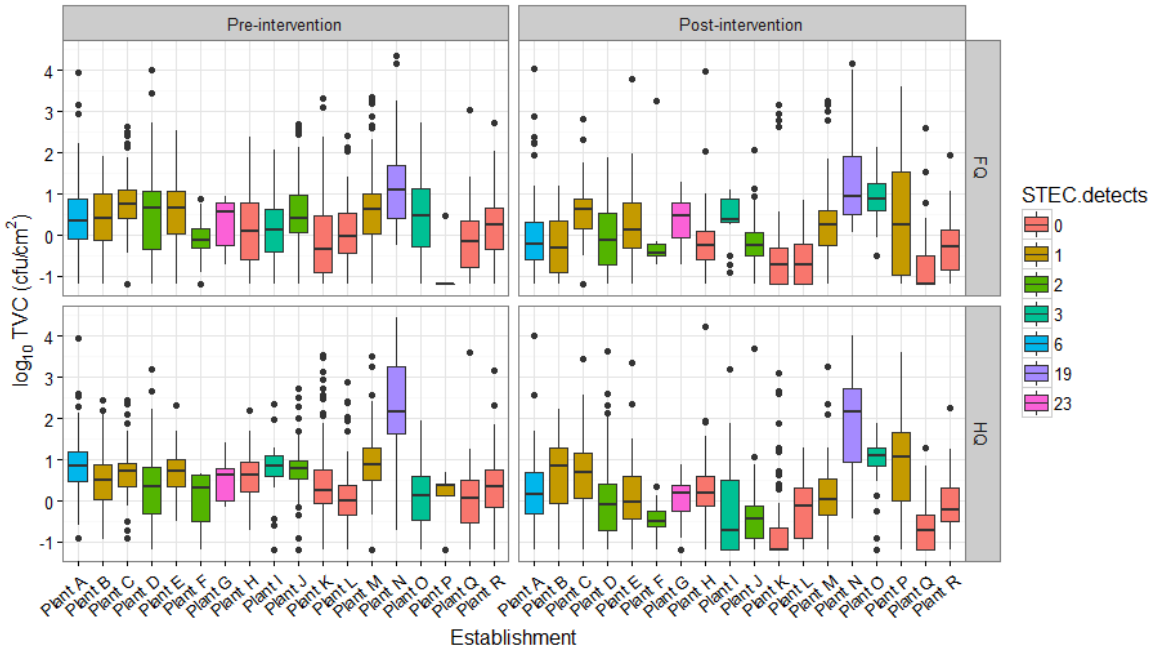

Figure 7: Comparison of log_{10} TVC (cfu/cm^2) and total number of STEC detections across eighteen beef establishments pre and post intervention from forequarter and hindquarter sites (June 2015-October 2016).

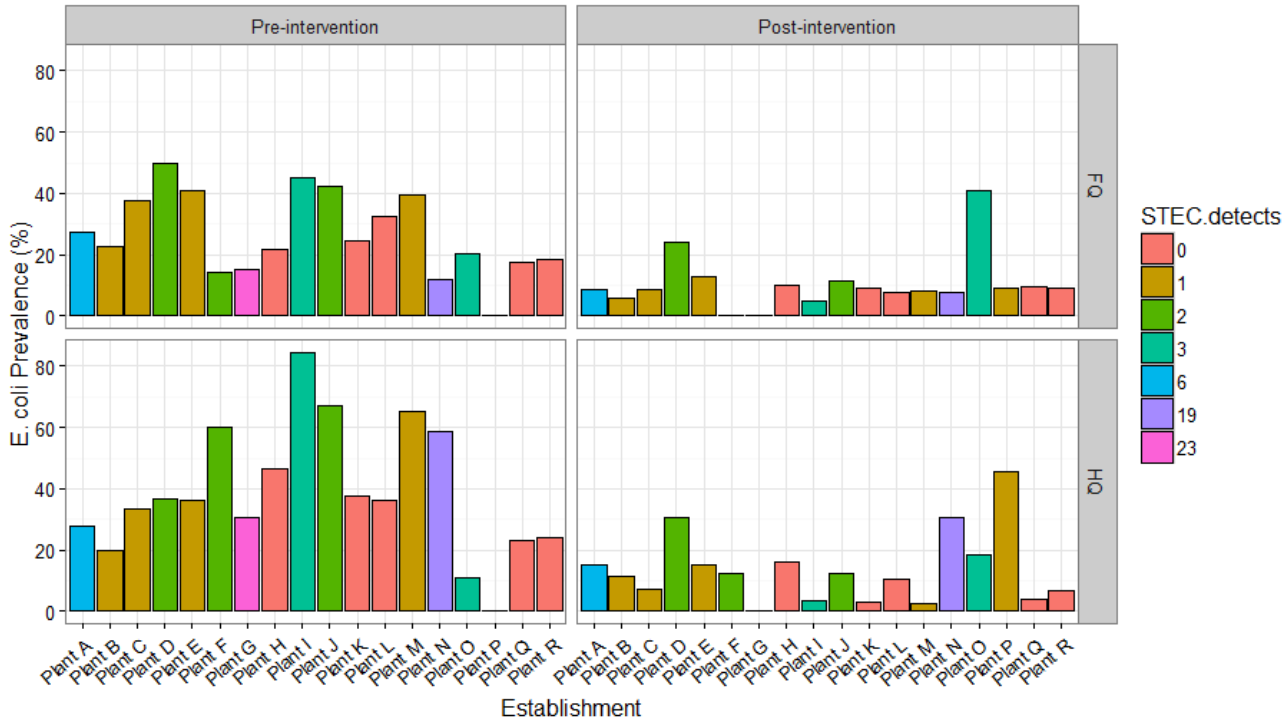

Figure 8: Comparison of generic E. coli *prevalence and total number of STEC detections across eighteen beef establishments (June 2015 – October 2016).*

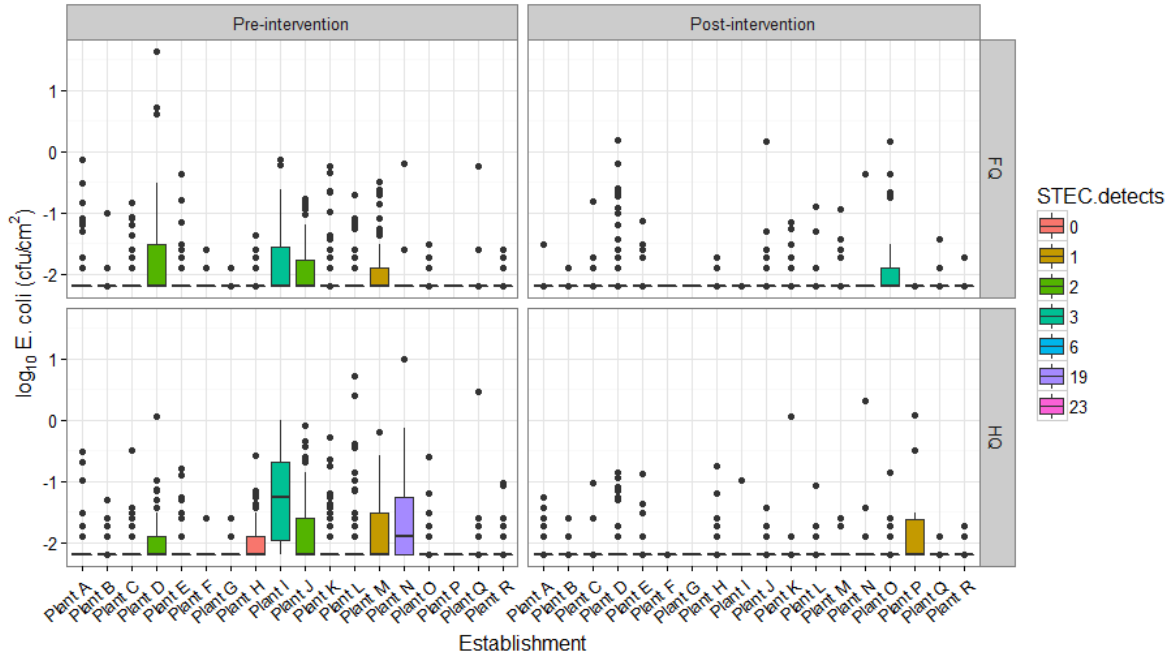

Figure 9: Comparison of log_{10} E. coli *(cfu/cm^2) and total number of STEC detections across eighteen beef establishments pre and post intervention from forequarter and hindquarter sites (June 2015-October 2016).*

Figure 7, 8 and 9 indicate no correlation between the prevalence and concentration of generic *E. coli* or log_{10} TVC and the occurrence of STEC detections within an establishment. The number of STEC detections was used as the measure, rather than detection rate, because it is not mandatory for establishments to report negative results for STEC tests in the PHI system. It is also important to note

that some establishments undertake intensive STEC testing (compared to other establishments) and so have more detections. Figures 10 and 11 are scatter plots of the number of STEC detections against either mean $\log_{10}$ TVC or *E. coli* prevalence for each individual establishment and again, there is no clear relationship such that high TVC averages or prevalence of *E. coli* correspond with more STEC detections at the establishment level.

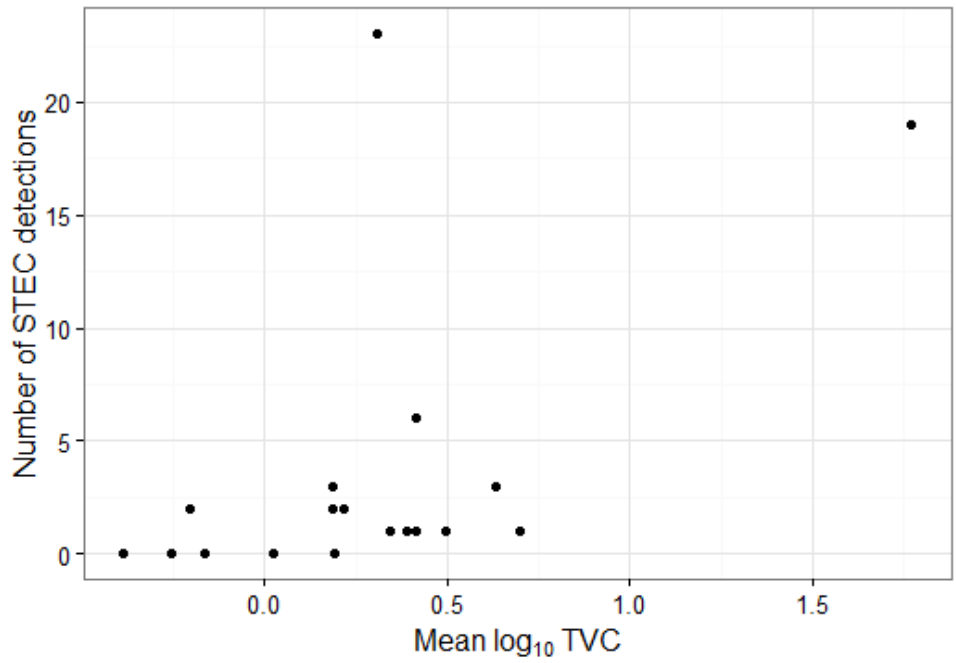

Figure 10: Scatter plot of total number of STEC detections against mean $\log_{10}$ TVC cfu/cm^2 for the eighteen beef establishments (June 2015-October 2016).

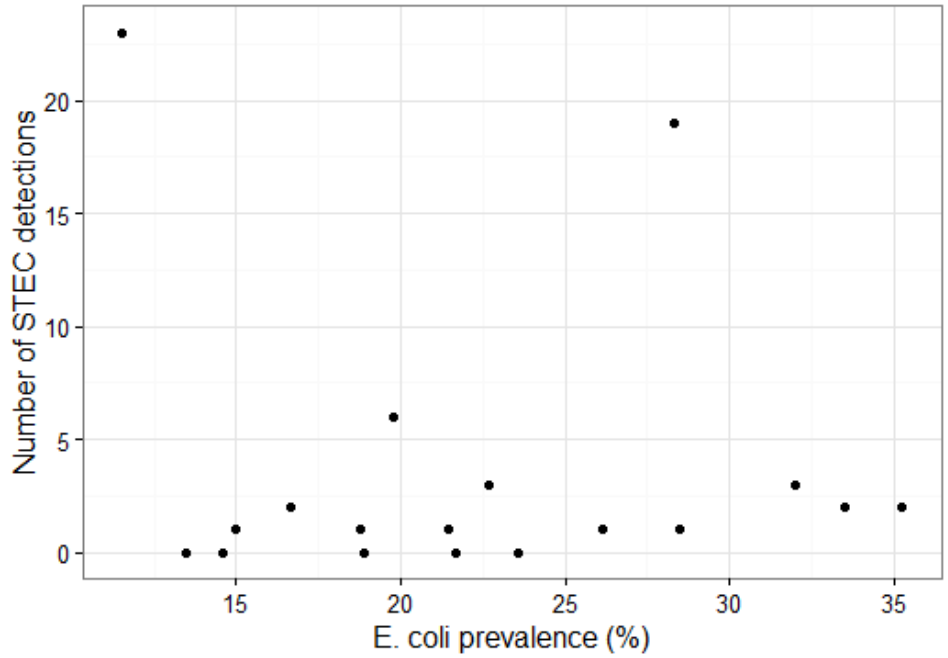

Figure 11: Scatter plot of total number of STEC detections against *E. coli* prevalence for the eighteen beef establishments (June 2015-October 2016).

Discussion between Jessica Jolley, Ian Jenson and Andreas Kiermeier included the following points:

- In a faecal sample, the ratio of STECs to total *E. coli* can vary widely, probably due to large variation in both the number of STECs and total *E. coli* (Fegan *et al*., 2004). Similarly, transfer of *E. coli* and STECs from hide or faeces onto the carcase must vary widely too. Could it be that both of these factors are so variable, that a positive correlation is not feasible/observable?
- Large area sampling is good at detecting low levels of *E. coli* on carcases at some stage of dressing and even possibly after chilling.
- STEC testing (and hence, detections) occurs after meat has been boned, placed in cartons and then sampled according to a sampling program – it could be seen as a "game of chance" that the particular sample is positive for STECs.
- Quantifying "STEC issues" is difficult – the ESAM database may not have all the required information to accurately estimate % STEC positive, as negative test results do not have to be reported. This could be ascertained by contacting the establishments directly and validating the sampling numbers.
- Rather than using number of detections or % positive as an accurate measure of STEC prevalence, it is more important to know the number and results of "independent" STEC events – currently, some establishments divide a contained load into more than one port mark and test each one; several detections from the same container may actually indicate the same STEC event. To ascertain a correct picture from the current data, each reported detection and the corresponding boning/production dates would need to be investigated, in order to know whether they are related or independent.
- An establishment with high prevalence (but low concentration) of generic *E.* coli has a different problem than one with low prevalence (but high contamination). High *E. coli* prevalence should result in more contaminated meat pieces and thus "potentially" increase the chance of having a STEC detection. However, there are numerous boning operations between an *E. coli* detection on the carcase and a STEC detection from a carton. Very little or no correlation has been observed between carcase and carton microbiology – maybe it would be reasonable to investigate the relationship between the prevalence of *E. coli* and STECs, both from cartons.
- Given how samples are collected (different carcases and cartons, different points in the slaughter and boning process, etc.), we are dealing with very large sources of variability, yet we have few observations (primarily establishments, and STEC events per establishment), so ultimately, we are dealing with low power.
- Highly variable data result in prediction or uncertainty bounds which are huge and so are not of practical use or relevance.
- One suggestion was comparable testing, so results of *E. coli* and STEC from the same sample, in order to compare like-with-like, but the above comments would still hold.

7.2 Conclusions / Recommendations

Funding for the "Ask SARDI" program has concluded with the conclusion of this project, so future funding would need to be provided in order for SARDI to continue assisting establishments.

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9 Appendix A: ESAM Analysis Reporting Service Correspondence

Date: 29th February 2016

Query: Please add XXX to this e-mail. Thanks

SARDI Response: Added XXX to the ESAM mailing list and sent a copy of the Explanatory Guide for the ESAM Reports.

Date: 2nd March 2016

Query: Hi Jessica, could you please send these reports to XXX as well. She has taken over the role of QA Manager.

SARDI Response: Added XXX to the ESAM mailing list and sent a copy of the Explanatory Guide for the ESAM Reports.

Date: 6th April 2016

Query: Hi Jessica, Please replace me with XXX as she is now the QA Manager at Place X.

SARDI Response: Added XXX to the ESAM mailing list and sent a copy of the Explanatory Guide for the ESAM Reports.

Date: 25th May 2017

Query: Hi Jess. Our cow/bull vs. night shift comparison needs to be calculated for our hot cow/bulls too, not only the cold cow/bulls. Also with the lambs we only need the comparison between carton day shift vs. carton night shift (for TVCs and coliforms only, we don't do E. coli). We don't have day vs night kill, only boning.

Email Response: Thanks for your email. I'll get working on a day vs night shift comparison report for your hot cow/bulls in the next month or so – busy with the end of the financial year approaching, but it is now on my list of things to do. With the lamb day vs night shift report, I noticed that you have almost no night carton data in the last year or so – do you expect that to continue?

Email Response: Hi Jess. Yes, that is likely to continue…so probably not worth doing. Thanks for that, Jess.

SARDI Response: Developed a day vs night shift comparison report for Hot Swabbed Cow/Bull and am sending it on a monthly basis.

Date: 27th August 2017

Query: Hi XYZ, I asked Jenny to put me in touch with you, because Ian Jenso seaid that he met you in Darwin last week and you want to be able to compare your results with other hot boning plants. I can help! As part of the monthly ESAM reporting that I do, I have developed a Hot Swabbed ESAM report which I send out to all the plants which do hot boning. I don't believe Company XYZ are currently on my mailing list for ESAM, while I presume you are doing ESAM testing…would you like to receive monthly ESAM reports?

Actually, what is probably easiest is for us to have a quick chat about the ESAM reports I generate and what you are after in terms of hot swabbing results – feel free to give me a call, my phone number is below.

Email Response: Hi Jessica, Thank you for contacting me. I would love to be added to your monthly ESAM reports. We are currently collecting ESAM samples we are a hot boning plant that swabs the carcases hot with no intervention step prior to swabbing. I was just interested to see how our results compare with plants that have a similar swabbing process.

SARDI Response: Added Plant XYZ to the ESAM mailing list, emailed a copy of the Explanatory Guide for the ESAM Reports and am sending ESAM reports on a monthly basis.

Date: 24th August 2016

Query: Hi Jessica, I'm trying to sort out some problems that the Malaysian Department of Veterinary Services has with our chilling regimes – including hot boning.