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MEAT & LIVESTOCK AUSTRALIA

Processor's Guide to Improving Microbiological Quality and Shelf Life of Meat

3rd Edition

Government of South Australia
Primary Industries and Regions SA

SARDI
SOUTH AUSTRALIAN RESEARCH AND DEVELOPMENT INSTITUTE

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Contents

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What's new in this edition?

Our second edition was published in 2015 and distributed at the MINTRAC Meat Inspection and Quality Assurance Conference. Since then, a number of establishments have provided the South Australian Research and Development Institute (SARDI) with in-house data for analysis and writing up as case studies for this third edition. Some establishments have also agreed to share their data from the large carcase survey for further analysis.

Asking SARDI to process and analyse their data has given QA Managers in these establishments real insights into their process control, as well as showing them how their processes rate, not just on a month-to-month basis but over long time periods.

In addition to the new studies, shelf life is becoming increasingly important in the meat trade, as new export markets become available. This edition contains a new section on shelf life which details case studies on trials, on measuring storage temperature accurately and on determining shelf life. This is extremely important as the industry seeks to assure shelf life in export markets with unknown or unreliable cold chain infrastructure.

Origin and Contents of the guide

In mid-2013, a national training program on how to do investigations in meat establishments was run.

The training included:

1. Identifying a particular unit operation which required investigation. For example, does the use of a steam vacuum make a difference to microbial counts?

2. Designing an investigation – including consideration of the logistics and factory floor difficulties – that would provide the required information and be scientifically credible.

3. Performing the investigation and obtaining relevant data.

4. Handling the data generated - this included an introduction to statistics.

5. Setting up spreadsheets so that data could be manipulated – a number of simple tools were provided into which data could be loaded directly. The tools provide key statistical information which tells you whether your unit operations are effective.

6. Writing up a report that documented important aspects of the investigation.

These reports are published here largely as they were written by the workshop participants, though for consistency reasons, we changed the formatting and, where needed, clarified the writing.

While all workshop participants used the same reporting template, it is evident from the reports that the amount of detail provided differs between them. We recommend that you provide as much detail as possible so that other staff at your plant can fully understand what you have done and repeat it if necessary. Although raw data have been omitted from these reports, you should include a table with the raw data in an appendix of your report(s). The following sections provide some detail as to the steps undertaken in preparing these reports.

Planning your investigation

You may have a hygiene problem that needs investigating, or may want to trial a new method, piece of equipment or intervention.

One of the key points in designing an investigation is to keep the aim simple enough so that you focus on a single processing operation or factor. Most investigations fall into one of three broad categories:

1. Before and after, for example before and after trimming
2. With and without, for example, with Twin Oxide application and without Twin Oxide
3. Comparing two groups, for example, using two different processing techniques.

We encourage the KISS principle (Keep It Simple Stupid).

How many samples do I need to take?

You'll see that most of the investigations published in this booklet used a total of 40-50 samples – 20-25 for the current procedure and 20-25 for the proposed procedure. This sample size has been shown to be sufficient to give you an answer to your investigation.

Setting up the work

Taking samples on the factory floor is not easy – operators are doing their unit operations and carcases are moving. Your challenge is to fit in around them, and keep your samples from being contaminated. Sometimes disruptions are unavoidable just make sure that everything and everyone is ready to go, so that the time of any disruptions is as short as possible.

The logistics of sampling need some thought, and it's best to go onto the factory floor beforehand and sort out where it can be done. Think about:

- Is there room?
- How much time do I have?
- What am I testing for?
- Is there room to store my kit safely (sponges etc.)?
- Can I keep my hands sterile and equipment?
- How will I collect the samples?
- Can I do it on my own or do I need a mate to help and take notes

Once you're satisfied you can do the work OK, you need to tee up everyone who needs to know about the project – supervisor, operators, department staff etc. So that everyone is aware of what is going on and agrees to the proposed procedures.

Writing up your investigation

Once the lab sends the results, you can write your report. You may be hoping to convince management to change a procedure, or to assure your regulator that a change in operations has no adverse impact on product hygiene. You'll need to describe why you did the work, the methods you used, and to analyse the data and present them in a businesslike format. This document contains many examples of how to write a report and analyse data.

All of the reports follow a standard template: **Title, Introduction, Methods, Results** and **Conclusions**. To make the reports anonymous, we've taken out details of the establishment, the investigators and the date the work was done – you should include these in your own report.

Data analysis

You'll see that all the micro data are described as logarithmic ($\log_{10}$) counts and there are good reasons for this:

- Micro counts don't have the same accuracy as chemistry or physics data
- We often have high counts with lots of zeros
- Micro counts can be very variable
- Log counts smooth out all these factors by removing the influence of high counts on the mean.

Arithmetic count	$\log_{10}$ count
10	1
100	2
1000	3
10000	4

The first thing you need to do is convert your counts from the lab to log counts. You can do this in Excel by using the formula = log (count) or = log (cell reference), where cell reference is the location of the count data in Excel to be converted (i.e. A1, C23 etc).

To help you analyse the data, SARDI has made some software tools specially designed for handling the results of your investigations.

Using the tools, you can produce tables which tell you the mean (average) count, the standard deviation (variability in your counts) and whether there is a significant difference between your current procedure and your proposed method.

You can also make boxplots, which are a visual representation of all your data.

You can get the tools from SARDI, who will also talk you through how to use them (contact details are supplied below).

Contacts

For further information or advice in planning, running or analysing an investigation like those detailed in this booklet or to obtain the spreadsheet tools for analysis of the investigations, please contact SARDI or MLA.

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Case Studies on beef processing

Opening cuts

1. Effect of Twin Oxide spray on hide of cattle prior to opening the hide

Introduction

The hide of cattle is a known source of Total Viable Count (TVC) and *E. coli*, including STEC, on the carcase. These organisms are detected more often than is desirable from cross contamination during processing. It is thought that these organisms cross contaminate the carcase during the opening of the hide during processing.

Objective

Determine if the application of Twin Oxide spray at above 100ppm to the opening lines of the hide will result in lower contamination with *E. coli*.

Methods

Swabbing of the hide (brisket area) prior to the application of Twin Oxide and again after the spraying of the same area in the cradle after stunning.

Sampling: Fifty samples were gathered by sponging the hide brisket area (~400 cm^2) using the same technique as for ESAM sampling: 25 samples were taken prior to spraying in the cradle and 25 from the same carcasses after the spraying of Twin Oxide.

Testing and analysis: Sponge samples were plated on *E. coli* Petrifilm and incubated at 35°C. After 48 hours, colonies were counted and data entered on a spreadsheet tool.

Results

The results are presented in the tables below from which it can be seen that *E. coli* and TVC were isolated at lower concentrations after Twin Oxide treatment.

Table 1: Summary of difference in log_{10} *E. coli* cfu/cm^2 between before and after Twin Oxide treatment.

Summary	*E. coli* Difference
Mean	0.50
St. Dev.	0.52
n	25
Conf level	95%
CI Lower	0.28
CI Upper	0.71
Significance	Highly significant

Table 2: Summary of difference in log_{10} TVC cfu/cm^2 between before and after Twin Oxide treatment.

Summary	TVC Difference
Mean	0.57
St. Dev.	0.49
n	25
Conf level	95%
CI Lower	0.36
CI Upper	0.77
Significance	Highly significant

Boxplots of the log_{10} TVC and *E. coli* concentrations are presented below.

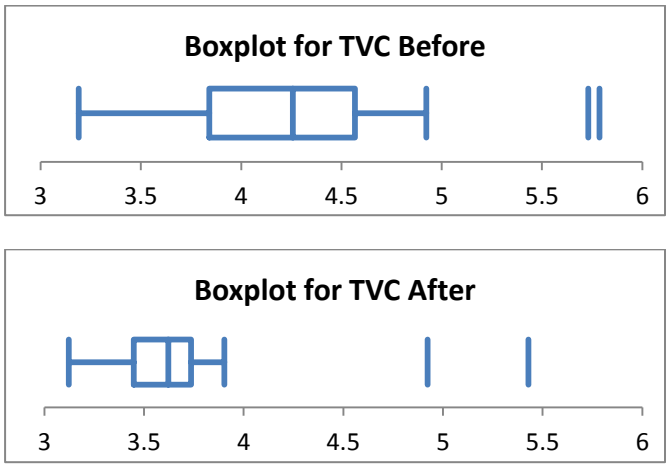


Figure 1: Boxplots showing log_{10} TVC cfu/cm^2 before and after Twin Oxide application.

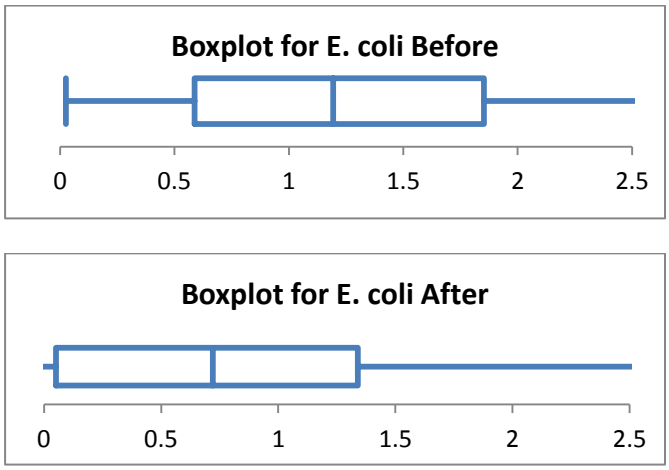


Figure 2: Boxplots showing log_{10} *E. coli* cfu/cm^2 before and after Twin Oxide application.

Conclusion

It was concluded that the application of Twin Oxide to the opening cutting lines of the hide was effective in reducing *E. coli* and TVC concentrations by an average of 0.5 and 0.57 log_{10} cfu/cm^2, respectively.

2. Effect of Twin Oxide treatment on microbial load of cattle hides

Introduction

Having clean cattle would improve the overall hygiene of the kill floor. This in turn would lead to better end products. Recent studies have indicated that most of the *STEC* contamination on carcases can be attributed to poor cattle hygiene and faecal contamination during the process of carcass dressing.

Objective

Determine if Twin Oxide will result in lower contamination of the carcase with *E. coli* and reduce the microbial load.

Methods

Processing: The current procedure is to clean the cattle with chlorinated water of high concentration. With introduction of Twin Oxide treatment, the cattle will be treated with 200ppm of Twin Oxide once the animal is stunned. For the treatment to be effective, the treated animal is held for 10-12 minutes before the next process.

To establish the effect of Twin Oxide, four sampling sites for each animal were swabbed (100cm^2) from sites as illustrated below in Figure 1, before and after treatment. Samples were then analysed for *E. coli*, coliform and TVC to ascertain the effect of Twin Oxide treatment.

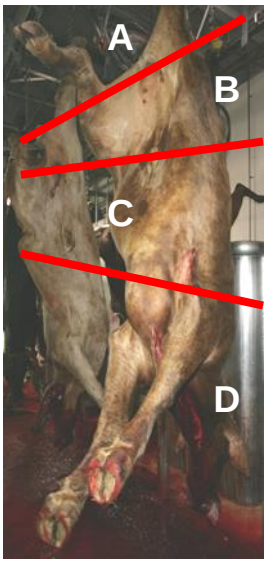


Figure 1: Sampling sites

Sampling: Thirty-eight sets of samples were gathered by sponging each of the four areas illustrated above using the same technique as for ESAM sampling. Sampling was done after stunning before spraying Twin Oxide (pre-treatment). Post treatment sample sets were taken at the legging stand.

Testing and analysis: Sponge samples were plated on *E. coli* Petrifilm and Aerobic count Petrifilm and incubated at 35°C. After 48 hours, colonies were counted and data entered on a spreadsheet tool.

Results

The results are presented in the table below from which it can be seen that total microbial counts were significantly reduced (P-value < 0.001) after treating with Twin Oxide. Boxplots of the $\log_{10}$ *E. coli*, TVC and Coliform concentrations are shown below.

Table 1: Summary of difference in $\log_{10}$ TVC cfu/cm^2 between before and after Twin Oxide treatment.

Summary	TVC Difference
Mean	1.49
St. Dev.	0.96
n	38
Conf level	95%
CI Lower	1.17
CI Upper	1.80
Significance	Highly significant

Table 2: Summary of difference in $\log_{10}$ *E. coli* cfu/cm^2 between before and after Twin Oxide treatment.

Summary	*E. coli* Difference
Mean	1.75
St. Dev.	1.08
n	38
Conf level	95%
CI Lower	1.39
CI Upper	2.10
Significance	Highly significant

Table 3: Summary of difference in $\log_{10}$ Coliforms cfu/cm^2 between before and after Twin Oxide treatment.

Summary	Coliforms Difference
Mean	1.45
St. Dev.	1.08
n	38
Conf level	95%
CI Lower	1.09
CI Upper	1.80
Significance	Highly significant

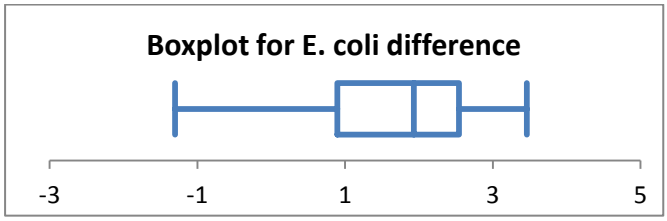


Figure 2: Boxplot showing difference in log_{10} *E. coli* cfu/cm^2 before and after Twin Oxide application.

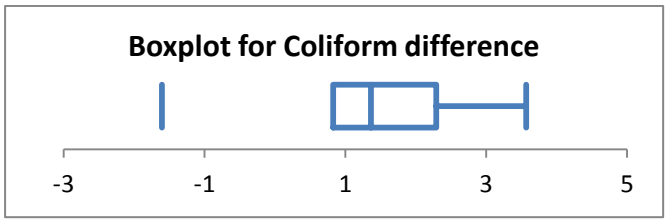


Figure 3: Boxplot showing difference in log_{10} Coliforms cfu/cm^2 before and after Twin Oxide application.

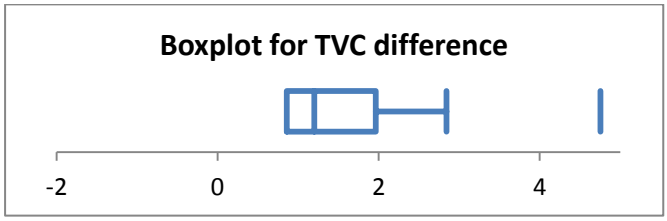


Figure 4: Boxplot showing difference in log_{10} TVC cfu/cm^2 before and after Twin Oxide application.

Conclusion

It was concluded that spraying with Twin Oxide (200ppm) was effective in reducing total TVC by approximately 1.5 log, *E. coli* by 1.75 log and coliforms by 1.45 log.

3. Effect of Twin Oxide on cattle opening lines

Introduction

Cattle hide typically has a high *E. coli* and total viable count. Even though work instructions are being followed, contamination can still occur through airborne particles and through the knife cutting through the hide and contaminating the carcase. By using Twin Oxide, we want to reduce microbial contamination.

Objective

Determine if applying Twin Oxide solution will result in lower contamination with *E. coli* and TVC.

Methods

Processing: Apply Twin Oxide solution to hide cutting lines after shackling and before opening hide.

Sampling: Twenty-five samples were gathered by sponging the hindquarter opening line cut area (100cm^2) using the same technique as for ESAM sampling. 25 samples were taken before applying Twin Oxide on the cradle, and 25 from the same carcasses (opposite leg) before the first leg operation.

Testing and analysis: Sponge samples were plated on *E. coli* Petrifilm and incubated at 35°C. After 48 hours, colonies were counted and data entered on a spreadsheet tool.

Results

The results are presented in Table 1 from which it can be seen that there was a highly significantly reduction of *E. coli* (P-value = 0.0001). It can also be seen that TVC reductions were highly significant (P-value = 0.002).

Table 1: Summary of difference in $\log_{10}$ *E. coli* cfu/cm^2 between before and after Twin Oxide treatment.

Summary	*E. coli* Difference
Mean	0.64
St. Dev.	0.70
n	25
Conf level	95%
CI Lower	0.35
CI Upper	0.93
Significance	Highly significant

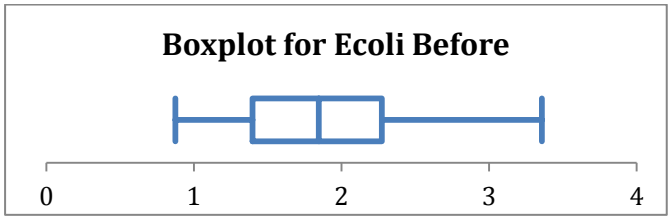


Figure 1: Boxplot showing $\log_{10}$ *E. coli* cfu/cm^2 concentration before Twin Oxide application.

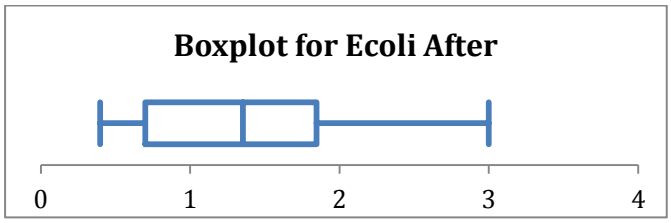


Figure 2: Boxplot showing $\log_{10}$ *E. coli* cfu/cm^2 concentration after Twin Oxide application.

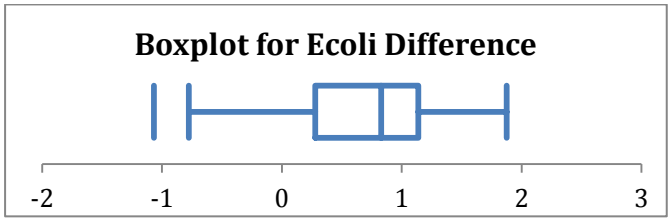


Figure 3: Boxplot showing the difference in $\log_{10}$ *E. coli* cfu/cm^2 concentration before and after Twin Oxide application.

Table 2: Summary of difference in $\log_{10}$ TVC cfu/cm^2 between before and after Twin Oxide treatment.

Summary	TVC Difference
Mean	0.52
St. Dev.	0.75
n	25
Conf level	95%
CI Lower	0.21
CI Upper	0.83
Significance	Highly significant

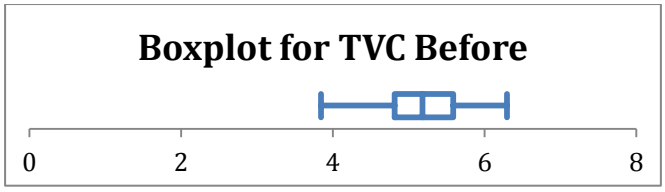


Figure 4: Boxplot showing log_{10} TVC cfu/cm^2 concentration before Twin Oxide application.

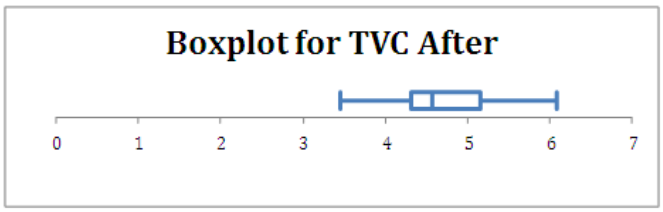


Figure 5: Boxplot showing log_{10} TVC cfu/cm^2 concentration after Twin Oxide application.

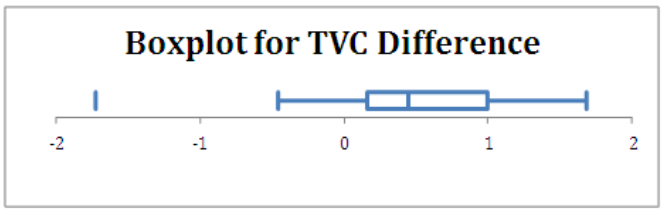


Figure 6: Boxplot showing difference in log_{10} TVC cfu/cm^2 concentration before and after Twin Oxide application.

Conclusion

It was concluded that current procedures applying Twin Oxide to the hide cutting lines are effective in reducing the concentration of *E. coli* and TVC on the carcase by approximately 0.5 log each.

4. Brisket contamination and the effect of spraying hides with Twin Oxide

Introduction

Bulls are considered to be difficult to process, especially at the forequarters, where contamination of the brisket has led to isolation of STECs. It was decided to investigate the level of contamination of brisket hide, and of exposed brisket. In addition, it has been suggested that spraying the hide with Twin Oxide provides a significant reduction in *E. coli* levels, and that this may, in turn, lead to lower levels of the faecal indicator on the carcase. Accordingly, Twin Oxide (200 mg/kg) was sprayed on the briskets of cattle; unfortunately processing of bulls had been completed before the team could prepare for the sampling so the investigation was carried out on cattle.

Objective

Determine if application of Twin Oxide will result in lower contamination of the carcase with *E. coli.*

Methods

Sampling: A total of 100 samples were gathered by sponging areas of approximately 200 cm^2; hide samples (n=25) from sprayed briskets were taken just after carcases had been shackled and hung on the moving rail. A further 25 hide samples of unsprayed briskets were taken at the NLIS stand. Sampling of exposed briskets of carcases which had been sprayed (n=25) and not sprayed (n=25) were taken just prior to hide removal.

Testing and analysis: Sponge samples were plated on *E. coli* Petrifilm and incubated at 35°C. After 48 hours, colonies were counted and data entered on a spreadsheet tool.

Results

The results are presented in Table 1 and Figure 1, from which it can be seen that *E. coli* was detected on all hides, irrespective of whether they were treated with Twin Oxide or not. However, the $\log_{10}$ *E. coli* cfu/cm^2 counts on the hide were significantly lower (difference of 1.73 $\log_{10}$ cfu/cm^2) after Twin Oxide application (P-value < 0.001). No significant differences in *E. coli* prevalence or concentration were detected on exposed briskets.

Table 1: Summary for investigation of brisket contamination and efficacy of Twin Oxide.

	E. coli	**Without TO**	**With TO**
Hide	Detections/n (%)	19/19 (100%)	25/25 (100%)
	Mean ($\log_{10}$ cfu/cm^2)*	2.32	0.59
	SD ($\log_{10}$ cfu/cm^2)*	0.43	0.67
Exposed brisket	Detections/n (%)	9/19 (47%)	11/25 (44%)
	Mean ($\log_{10}$ cfu/cm^2)*	-0.33	-0.66
	SD ($\log_{10}$ cfu/cm^2)*	0.32	0.60

* includes only samples with detectable levels of *E. coli*

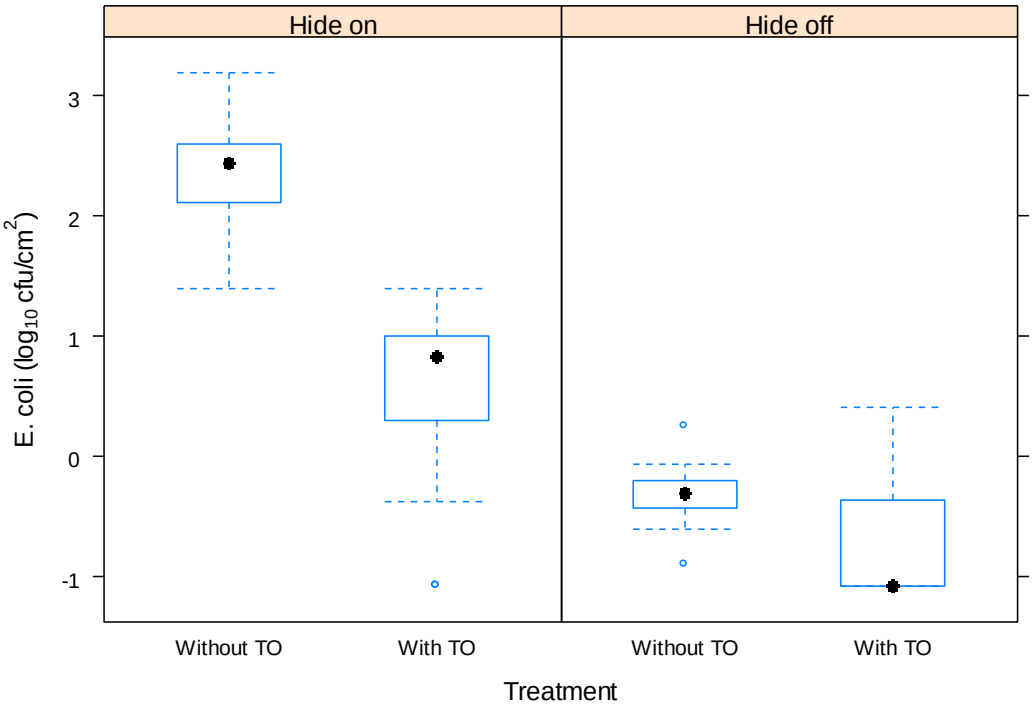


Figure 1: Boxplots of log_{10} *E. coli* cfu/cm^2 collected from brisket hide (left panel) and freshly exposed brisket carcase area (right panel) with and without the application of Twin Oxide on the hide.

Conclusion

It was concluded that Twin Oxide leads to > 1.5 log_{10} cfu/cm^2 reduction of *E. coli* on hides. However, the current procedure for exposing the brisket leads to low levels of contamination and hence it is not possible to determine whether hide application of Twin Oxide has an effect on contamination of the briskets of cattle.

Hide Removal

5. Effect of using sanitizer on hands during hide removal

Introduction

The hide is a major source of contamination and its handling during hide removal operations may have an effect on the hygiene of the carcase.

Objective

Determine if sanitizing hands will result in lower contamination on hands.

Methods

Processing: Our current work instruction does not require the sanitising of hands after washing. However, for this trial the sanitiser to be used is Smart San Instant Mist Hand Sanitiser.

Sampling: Fifty (50) samples were gathered by sponging the hands of a first leg operator, 25 were collected by swabbing hands after washing with soap and a further 25 after sanitizing the hands. The surface area of the hand that was swabbed had an area of 424cm^2. The area was swabbed by passing the swab over the front and back of the hands and between the fingers.

Testing and analysis: Sponge samples were plated on *E. coli* and Aerobic Plate Count (APC) Petrifilm. The plates were incubated at 35°C. After 48 hours, colonies were counted and data entered on a spreadsheet tool.

Results

The results are presented in the table below from which it can be seen that the reduction in TVC was highly significant. Using a sanitizer on hands after hand washing reduced the TVC by 0.48 log.

Table 1: Summary for difference in $\log_{10}$ TVC cfu/cm^2 between hands washed with soap and sanitised hands.

Summary	Difference
Mean	0.48
St. Dev.	0.17
n	24
Conf level	95%
CI Lower	0.41
CI Upper	0.55
Significance	Highly significant

Boxplots of the log_{10} TVC concentrations are shown below.

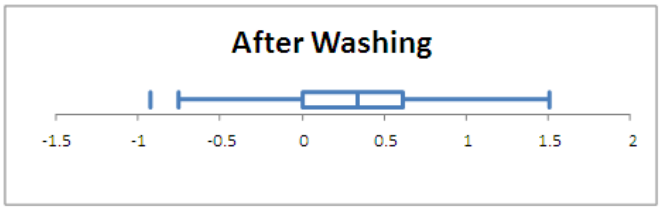


Figure 1: Boxplot of log_{10} TVC cfu/cm^2 after washing hands.

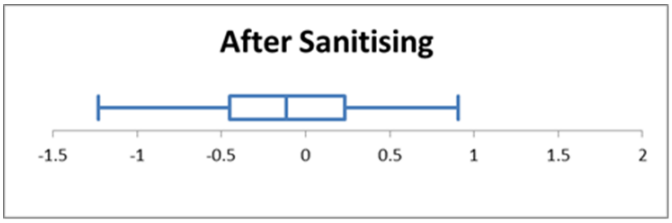


Figure 2: Boxplot of log_{10} TVC cfu/cm^2 after using hand sanitiser.

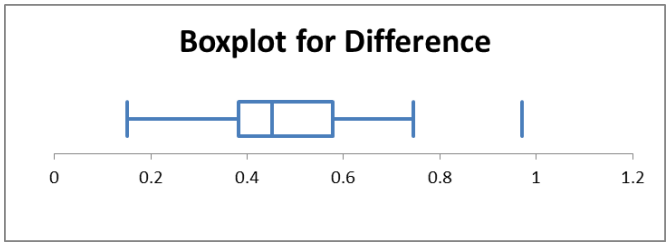


Figure 3: Boxplot of difference in log_{10} TVC cfu/cm^2 after washing hands compared to after using hand sanitiser.

Conclusion

It was concluded that sanitising hands after washing with soap at the first leg position on the slaughter floor is effective in reducing the TVC on the hands.

6. Effect of tail cleanliness on contamination of the loin area

Introduction

Within the last two years, a procedure was put in place to remove all bushy parts of the tail. This was done to control tail contamination transferring to the loin of the carcase.

Objective

Assessing microbiological impact of different amounts of faecal contamination on the bushy part of the tail transferring to the loin area.

Methods

Bush assessment

1- Clean
2- Slightly dirty
3- Dags on bush
4- Dags on bush and tail
5- Dags everywhere

Processing: Our current procedure requires the removal of the bushy part of the tail to eliminate the chance of cross-contamination and *E. coli* spreading to the loin area of the carcase.

Sampling: Fifty samples were gathered by sponging loin areas of the carcases (~400cm^2) immediately after the hide puller and before splitting using the same technique as for ESAM sampling. Ten samples were taken over 5 days on varying tail contamination levels.

Testing and analysis: Sponge samples were plated on Aerobic Plate Count (APC) and *E. coli* Petrifilm and incubated at 35°C (reference to method). After 48 hours, colonies were counted and data entered on a spreadsheet tool.

Results

For the purpose of the analysis, bush assessment scores of 1 and 2 were combined, as were scores 3-5. The results are presented in the tables below from which it can be seen that *E. coli* was isolated significantly more frequently from tails rating 3-5 compared to tails rating 1-2 and the mean TVC was around 1 log higher.

Table 1: Summary of *E. coli* prevalence for tail ratings 1-2 and 3-5.

Summary	Rating 1-2	Rating 3-5
Detect	2	14
n	35	15
Prev	5.7%	93.3%
Conf level	95%	
CI Lower	0.7%	67.8%
CI Upper	19.8%	100.0%
Significance	Highly significant	

Table 2: Summary of log_{10} TVC cfu/cm^2 for tail ratings 1-2 and 3-5.

Summary	Rating 1-2	Rating 3-5
Mean	2.59	3.49
St. Dev.	0.58	0.51
n	35	15
Conf level	95%	
CI Lower	2.39	3.21
CI Upper	2.79	3.77
Significance	Highly significant	

Boxplots of the log_{10} TVC concentrations are shown in Figure 1.

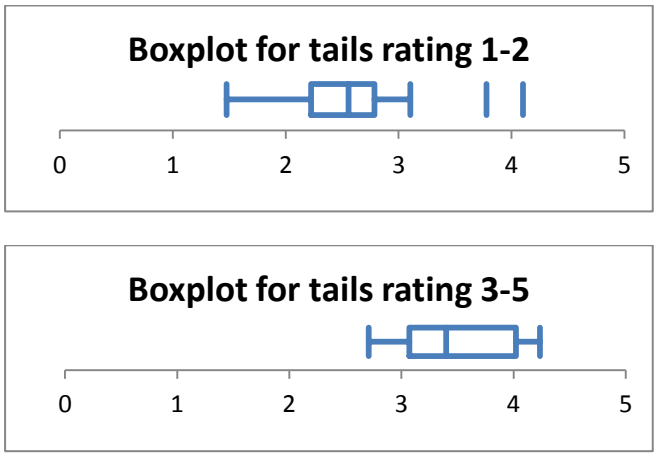


Figure 1: Boxplots of the log_{10} TVC cfu/cm^2 for Tail ratings 1-2 and ratings 3-5.

Conclusion

It was concluded that carcases with tail rating 1-2 have significantly lower TVC levels than carcases with higher tail rating (dirty tails). Current procedures for tail bush removal reduce the frequency of *E. coli* contamination, but do not eliminate *E. coli* from the loin area.

7. Effect of tail flick on carcase hygiene

Introduction

Tails have a high level of bacterial load of both TVC and *E. coli*. Our investigation will look at the effect of the flick of the tail during hide pulling on contamination of the carcase.

Objective

Processing: Our current work instruction 'Rumping' requires the skinning of the underside of the tail and cutting off the brush. An alternative method involving removal of the tail before hide pull was investigated.

Figure 1: Tail On.

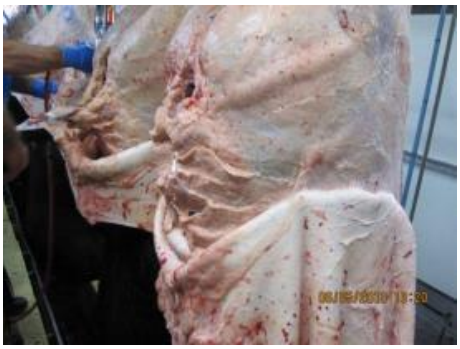


Figure 2: Tail Off.

Methods

Samples (24 each for "Tail On" and "Tail Off") were gathered by sponging the centre back (~200cm^2) from the 6th Lumbar vertebrae down area (as shown in Figure 3) using the same technique as for ESAM sampling. Samples were taken at the evisceration stand and were collected as a set of 6 carcasses for each processing method over four production days.

Sponge samples were plated on TVC & *E. coli* Petri film and incubated at 26°C for TVC & *E. coli* at 35°C. After 48 hours, incubation colonies were counted and data entered on a spreadsheet tool.

Figure 3: Sampling location.

Results

The results are presented in the tables below from which it can be seen that *E. coli* was isolated less frequently by removing the tail prior to the hide puller with zero detection compared to 29% detection with tail on. TVC counts were also significantly reduced by the alternative method (see Table 2 and boxplots).

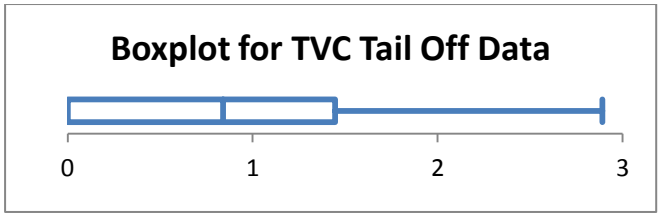
Table 1: Summary for *E. coli* prevalence for Tail on and Tail off.

Summary	Tail on *E. coli*	Tail off *E. coli*
Detect	7	0
n	24	24
Prev	29.2%	0.0%
Conf level	95%	
CI Lower	14.8%	0.0%
CI Upper	49.4%	16.7%
Significance	Highly significant	

Table 2: Summary for log_{10} TVC cfu/cm^2 for Tail on and Tail off.

Summary	Tail on TVC	Tail off TVC
Mean	1.56	0.89
St. Dev.	0.79	0.83
n	24	24
Conf level	95%	
CI Lower	1.23	0.54
CI Upper	1.89	1.24
Significance	Highly significant	

Boxplots of the log_{10} TVC concentrations are shown in Figure 4.



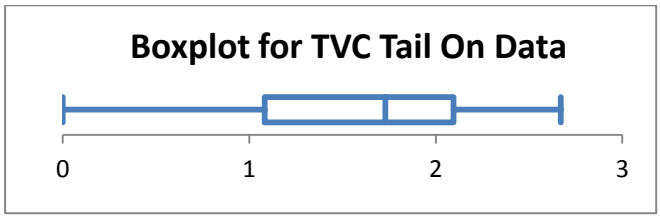


Figure 4: Boxplots of the $\log_{10}$ TVC cfu/cm^2 from Tail Off and Tail On.

Conclusion

It was concluded that removal of the tail prior to hide puller reduced *E. coli* and TVC contamination. Consequently, it is recommended that the alternative procedure of removing the tail becomes the new standard operating procedure.

8. Effects of a controlled tail pull by operator compared with an uncontrolled tail pull

Introduction

Swabbing was carried out to determine whether operator error was causing increased risk of contamination onto carcasses, via the process of the tail brush being controlled whilst being pulled, compared with it being uncontrolled, allowing contaminated water off the brush to flick onto carcass.

Objective

To determine if operator error was causing the spread of contamination off the brush of the tail onto the carcass.

Methods

Processing: Our current work instruction states that the tail must be held and controlled whilst the chain and hydraulic ram are removing the hide off the tail. However because this process relies on human factor, this process is not always carried out; therefore an investigation into the viability of the process was carried out.

Sampling: Twenty-five samples were gathered of each uncontrolled tail pull and controlled tail pull. A sponge was used for the swabbing on the inside of the hind leg (topside area) using the same technique as ESAM sampling, using a 100cm^2 template. Each carcass was only swabbed once. The swabbing was carried out approx. 8 -10 mins after tail pull process was carried out, due to the layout of the slaughter floor.

Testing and analysis: Sponge samples were plated on *E. coli* Petrifilm and incubated at 35°C. After 48 hours, colonies were counted and data entered on a spreadsheet tool.

Results

The results are presented in Boxplot form for the TVC results and a summary table for the *E. coli* results. The boxplot shows very little difference in the controlled and uncontrolled results with the average for controlled and uncontrolled being 2.1 $\log_{10}$ cfu/cm^2 and 2.2 $\log_{10}$ cfu/cm^2, respectively. The *E. coli* prevalence results detected from the summary table below also show very little difference (Controlled: 4 detections from 25 swabs, uncontrolled: 6 detections from 25 swabs).

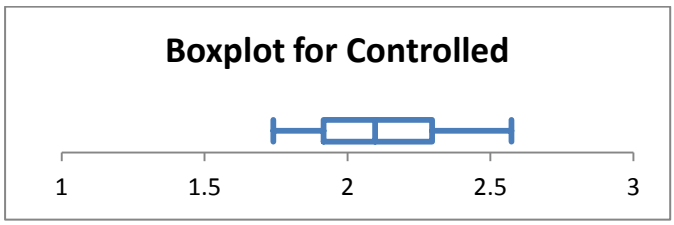


Figure 1: Boxplot for $\log_{10}$ TVC cfu/cm^2 for controlled tail pull.

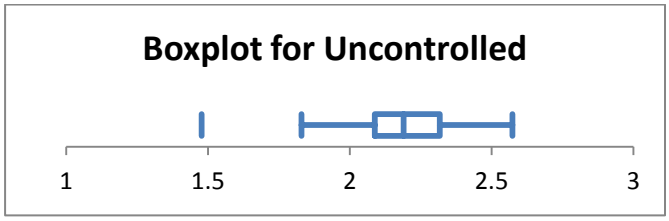


Figure 2: Boxplot of log_{10} TVC cfu/cm^2 for uncontrolled tail pull.

Table 1: Summary of *E. coli* prevalence for controlled and uncontrolled tail pull.

Summary	Controlled	Uncontrolled
Detect	4	6
n	25	25
Prev	16.0%	24.0%
Conf level	95%	
CI Lower	5.9%	11.3%
CI Upper	35.4%	43.9%
Significance	Not significant	

Conclusion

It was concluded that although there is a visible risk of contamination from water flicking off the brush of the tail, our swabbing results show there is little to no effect of the added risk of contamination of *E. coli* or TVC counts on the carcasses. Therefore it will be addressed as to whether this step will be removed from the current work instruction.

9. Hide removal at bunging

Introduction

Incorrect procedure during hide removal at bunging has the potential to contaminate the carcase. This investigation will assess the impact of the incorrect procedure compared to the correct procedure.

Objective

Determine if the correct bunging procedure will result in lower contamination with *E. coli.*

Methods

Sampling: Samples were gathered at the bunging stand, where two operators remove hide and free the bung, and two further operators bag and drop the bung. An area approximately 100cm^2 was sponged from a total of 50 carcases, 25 of which were processed using the current procedure, and 25 using a procedure where the operator was considered likely to contaminate the exposed rim.

Testing and analysis: Sponge samples were plated on *E. coli* Petrifilm and incubated at 35°C. After 48 hours, colonies were counted and data entered on a spreadsheet tool.

Results

The results are presented in Table 1 and

Table 2 from which it can be seen that while *E. coli* was present at a similar prevalence from both bunging techniques, there was a difference of approximately 0.6 $\log_{10}$ cfu/cm^2 between the current and the incorrect technique (Figure 1). This difference resulted in a marginal statistical difference (P-value = 0.05).

Table 1: Summary of *E. coli* prevalence for investigation of correct and incorrect hide removal at bunging.

Summary	Correct	Incorrect
Detect	14	13
n	23	25
Prev	60.9%	52.0%
Conf level	95%	
CI Lower	40.7%	33.5%
CI Upper	77.8%	69.9%
Significance	Not significant	

Table 2: Summary of $\log_{10}$ *E. coli* cfu/cm^2 concentration for correct and incorrect hide removal at bunging.

Summary	Correct	Incorrect
Mean	-0.21	0.38
St. Dev.	0.47	0.85
n	14	13
Conf level	95%	
CI Lower	-0.48	-0.13
CI Upper	0.06	0.89
Significance	Significant	

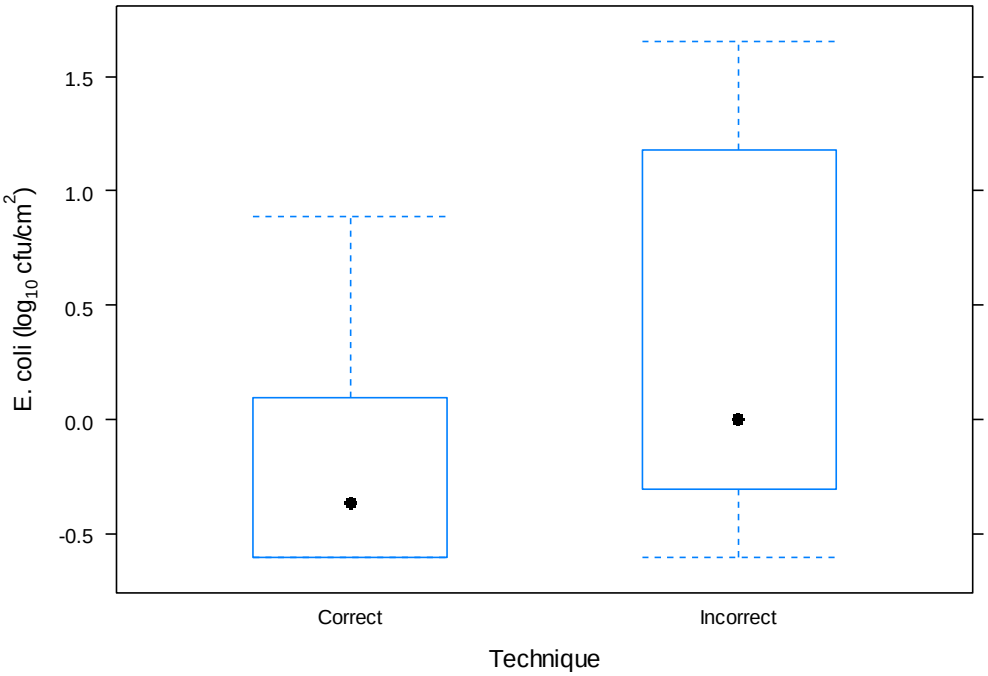


Figure 1: Boxplots of $\log_{10}$ *E. coli* cfu/cm^2 for correct and incorrect bunging technique. Includes only samples with detectable levels of *E. coli*.

Conclusion

It was concluded that the current procedure for hide removal at the bung is effective and that failure to adhere to the operating procedure may lead to greater contamination, which is practically important (difference >0.5 log).

Evisceration and Trimming

10. Trimming as an intervention – how effective is it?

Introduction

Like everyone who exports trim for grinding in the USA, we are concerned about the likelihood of one of our consignments being investigated at Port of Entry and found to be positive for STEC.

We already use Twin Oxide on the cutting lines of hides as an intervention and have considered other interventions such as hot water treatment of the carcase. We have heard that trimming of the cutting lines and adjacent areas might also serve as an intervention to reduce the prevalence and concentration of *E. coli* and therefore of STEC.

Objective

We wanted to know whether trimming carcases immediately before they left the slaughter floor would have a marked effect on their bacterial loading. The way we set up the trial would tell us:

- Where, and how much, contamination we were putting on the carcase
- Whether trimming was going to be effective.

Methods

We set up a trial where our lab staff took samples from various locations on the carcase before and after trimming (Fig 1). We took incision samples (10x10cm^2) at each site and placed the meat in a sterile Stomacher bag with sterile peptone water to give a 1:10 dilution.

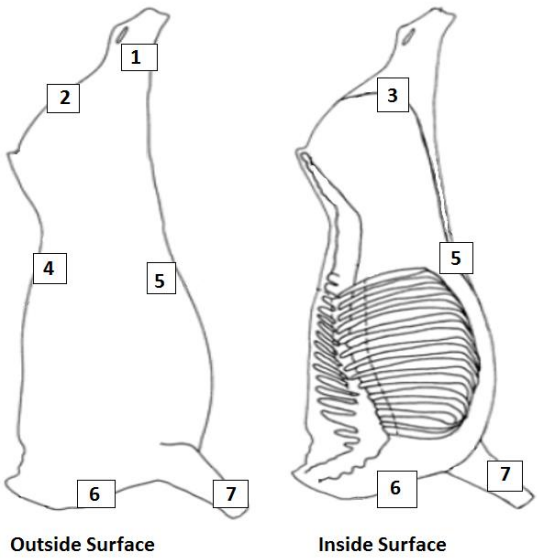


Figure 1: Sampling sites

From this dilution, we made serial dilutions in peptone water blanks and plated out onto APC and E. coli Petrifilm and incubated them at 35°C for 48 hours. We counted colonies and calculated the bacterial count/cm^2 of carcase surface.

We did this on 73 days during a four-month period (December to March) so we had large sample numbers (see data analysis).

Results

Total bacterial loadings at sites 1-7 are shown in Table 1. Sites 1-3, on the rump, rear hock and pelvic rim were more highly contaminated than other sites lower down the carcase. Trimming was effective only at site 3, where an almost 1 log reduction was achieved.

Table 1: Mean TVCs (log_{10} cfu/cm^2; n=784) at sites 1-7 before and after trimming

Carcase position	Pre Trim	Post Trim
1	0.9	0.7
2	1.1	1.0
3	1.9	1.1
4	0.2	0.3
5	0.6	0.7
6	0.8	0.7
7	0.7	0.5

E. coli was recovered from all sites both before and after trimming, with site 3 (pelvic rim) having the highest prevalence. At most sites, trimming was effective in reducing the prevalence of *E. coli*, most notably at site 3.

Table 2: Prevalence of *E. coli* (n=784) at sites 1-7 before and after trimming

Carcase position	Pre Trim	Post Trim
1	4.4	2.7
2	6.1	2.7
3	21.1	7.2
4	0.9	1.8
5	4.4	2.7
6	8.8	3.6
7	0.9	0.9

Conclusions

Trimming reduces the bacterial loading in general and the *E. coli* prevalence in particular at some sites on the carcase, especially at those sites which were the most heavily contaminated by the dressing process.

11. Effect of trimming after the flanking process

Introduction

Flanking procedure has been shown to have a high TVC. It is thought that lack of attention to trimming and poor hygiene procedures by the flanker could lead to contamination of the flank area with STEC due to limited time in allowing flanker to perform trim hygienically at all times.

Objective

Determine if trimming the flank after clearing will result in lower contamination with *E. coli*.

Methods

An extra trimmer was placed after the flanker to allow for trimming of the exposed meat surface. The same carcase was swabbed prior to trimming and then after trimming using a sponge method similar to the ESAM process.

Figure 1: Sampling location near opening cut

Sampling: 48 samples were gathered by sponging the belly hide opening area (~100cm^2) using the same technique as for ESAM sampling. 24 samples were taken at the Flank stand prior to trimming, and 24 from the same carcasses after the area had been trimmed, just prior to the hide puller.

Testing and analysis: TVC sponge samples were plated on Aerobic Plate Count (APC) Petrifilms and incubated at 25°C. After 72 hours, colonies were counted and data entered on a spreadsheet tool.

E. coli samples were plated on Petrifilm plates and incubated at 35°C. After 48 hours, colonies were counted and data entered on a spreadsheet tool.

Results

The results are presented in the tables below from which it can be seen that the TVC were significantly lowered by trimming after flanking.

Table 1: Summary of difference in log_{10} TVC cfu/cm^2 due to trimming after flanking.

Summary	TVC Difference
Mean	0.96
St. Dev.	0.78
n	24
Conf level	95%
CI Lower	0.63
CI Upper	1.29
Significance	Highly significant

The *E. coli* prevalence results are presented below from which it can be seen that *E. coli* was isolated significantly less often from the flank area after trimming (64%).

Table 2: Summary of *E. coli* prevalence due to trimming after flanking.

Summary	*E. coli* Before	*E. coli* After
Detect	25	9
n	25	25
Prev	100.0%	36.0%
Conf level	95%	
CI Lower	83.9%	20.3%
CI Upper	100.0%	55.6%
Significance	Highly significant	

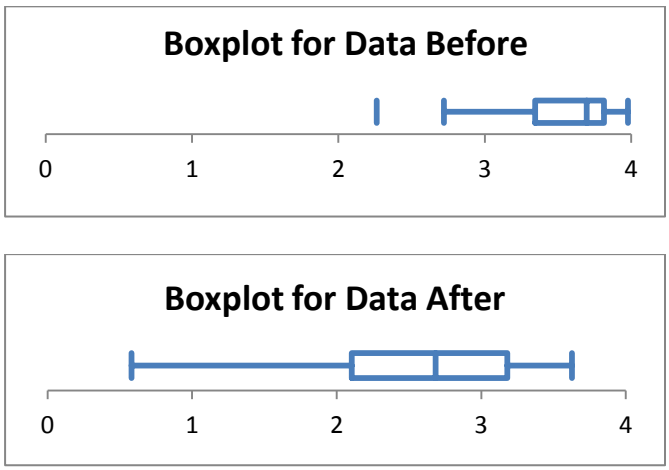


Figure 2: Boxplots of the log_{10} TVC cfu/cm^2 for before and after trimming.

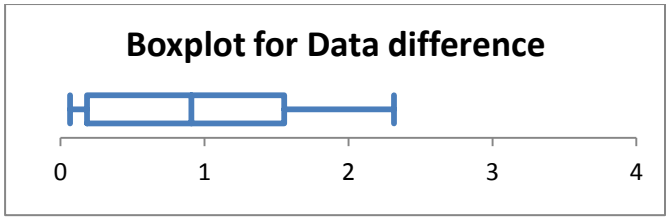
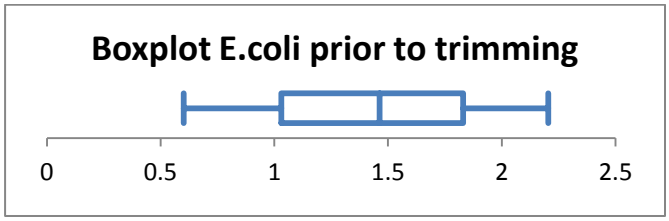


Figure 3: Boxplot of the difference in log_{10} TVC cfu/cm^2 before and after trimming.



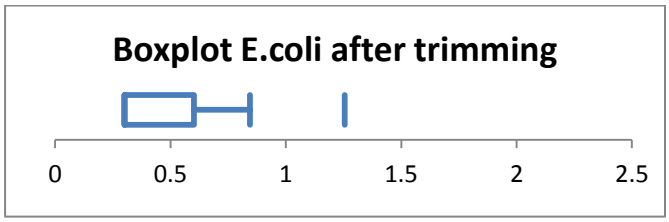


Figure 4: Boxplots of log_{10} *E. coli* cfu/cm^2 for before and after trimming. Includes only samples with detectable levels of *E. coli*.

Conclusion

It was concluded that current procedures for trimming after flanking are effective in reducing the TVC levels and the *E. coli* prevalence at this site.

12. Effect of Halal neck trim

Introduction

Forequarter trim has a high TVC and has STEC detected more often than is desirable. It is thought that lack of attention to trimming after the halal cut could lead to contamination of neck meat with STEC because the knife cuts through the hide and contaminates the wound.

Objective

Determine if trimming the neck wound will result in lower contamination with *E. coli*.

Methods

Sampling: Fifty samples were gathered by sponging the halal cut area (~50cm^2), 25 samples were taken at the low inspection DAFF stand, and 25 from the same carcasses after the wound had been trimmed.

Testing and analysis: Sponge samples were plated on *E. coli* Petrifilm and incubated at 35°C. After 48 hours, colonies were counted and data entered on a spreadsheet tool.

Results

The results are presented in

Table 1 from which it can be seen that *E. coli* was isolated significantly more frequently (P-value = 0.002) from Halal wounds before trimming (40%) compared to after trimming (4%). Boxplots of the $\log_{10}$ *E. coli* concentrations are shown in Figure 1.

Table 1: Summary of *E. coli* prevalence for investigation of efficacy of Halal neck trimming.

Summary	Before Trimming	After Trimming
Detect	10	1
n	25	25
Prev	40.0%	4.0%
Conf level	95%	
CI Lower	23.5%	0.0%
CI Upper	59.3%	21.4%
Significance	Highly significant	

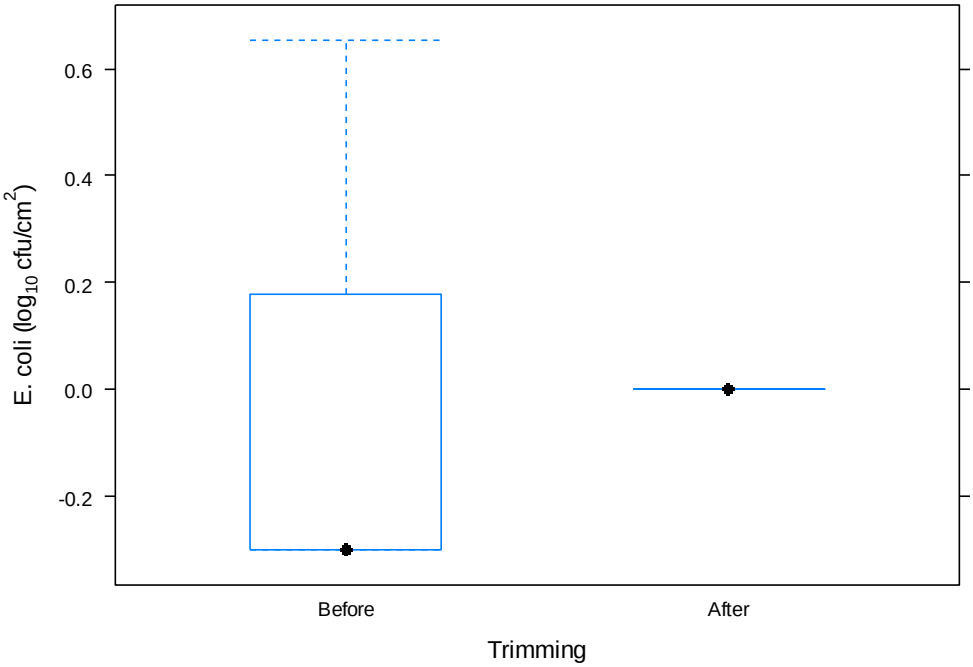


Figure 1: Boxplots of the $\log_{10}$ *E. coli* cfu/cm^2 from before and after Halal neck trimming. Includes only samples with detectable levels of *E. coli* – only one detection was made from the samples collected after trimming.

Conclusion

It was concluded that current procedures for trimming the Halal cut are effective in reducing the prevalence of *E. coli* at this site.

13. Effect of trimming on retain rail

Introduction

The company employs several trimmers on the retain rail and it was questioned whether their role was effective in removing microbial contamination.

Objective

Determine if trimming on the retain rail is effective in removing microbial contamination.

Methods

Sampling: Samples (n=25) were gathered of trimmed areas of meat (approximately 200cm^2) into a stomacher bag, and 25 samples of approximately the same area were taken of the meat surface exposed by trimming.

Testing and analysis: Meat samples were massaged in 25mL Butterfields solution by squeezing the outside of the stomacher bag for 30 seconds. Meat and sponge samples were plated on *E. coli* and APC Petrifilm and incubated at 35°C. After 48 hours, colonies were counted and data entered on a spreadsheet tool.

Results

The results are presented in Table 1. *E. coli* was isolated significantly more frequently (P-value = 0.002) from trimmed meat samples (80%) compared to the freshly-exposed trimmed areas (36%). Boxplots for $\log_{10}$ *E. coli*/cm^2 and $\log_{10}$ APC/cm^2 concentrations are shown in Figure 1 and Figure 2. Meat samples had a significantly higher (P-value =0.002) mean $\log_{10}$ *E. coli* count (1.46 $\log_{10}$ cfu/cm^2) compared to freshly trimmed areas (0.40 $\log_{10}$ cfu/cm^2). The mean $\log_{10}$ APC of meat trimmed from the carcase was 1.14 $\log_{10}$ cfu/cm^2 higher than that of freshly-exposed trim – this difference was significant (P-value < 0.001).

Table 1: Summary for investigation of efficacy of retain rail trimming

	Before trimming	After trimming
***E. coli* Detections/n (%)**	20/25 (80%)	9/25 (36%)
E. coli* Mean ($\log_{10}$ cfu/cm^2)	1.46	0.40
E. coli* SD ($\log_{10}$ cfu/cm^2)	0.93	0.32
APC Mean ($\log_{10}$ cfu/cm^2)	1.42	0.28
APC SD ($\log_{10}$ cfu/cm^2)	0.71	0.77

* includes only samples with detectable levels of *E. coli*

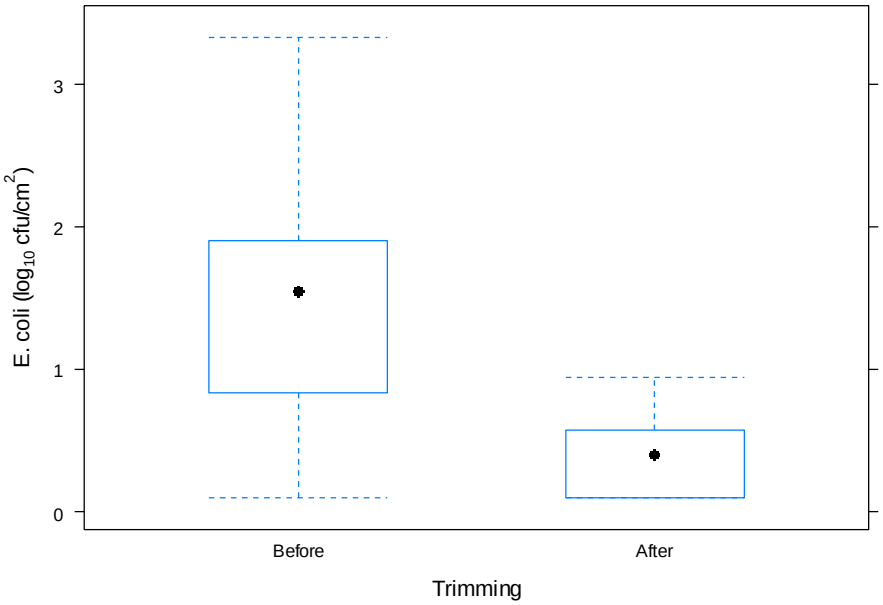


Figure 1: Boxplots of $\log_{10}$ *E. coli* cfu/cm^2 from samples collected before and after retain rail trimming. Includes only samples with detectable levels of *E. coli*.

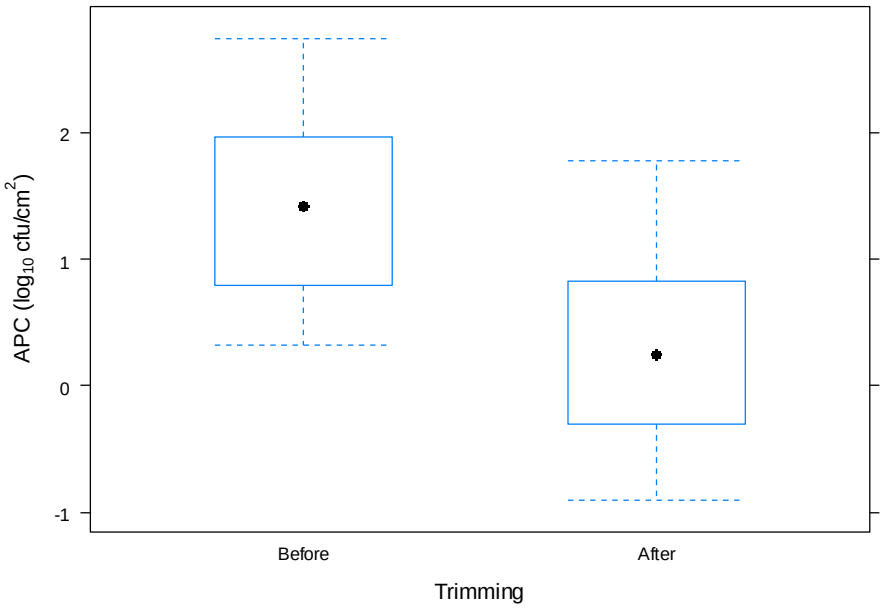


Figure 2: Boxplots of $\log_{10}$ APC cfu/cm^2 from samples collected before and after retain rail trimming.

Conclusion

It was concluded that current procedures for trimming at the retail rail are effective in reducing the prevalence of *E. coli* as well as the concentration of *E. coli* and APC at this site.

14. Microbiological impact of burst paunches after retain trimming

Introduction

Due to a large amount of 'busted paunches', the team felt that this may have a detrimental effect on the carcase hygiene/safety on exit from the retain chain. A comparison was made against our hygiene operations on a moving chain compared to a contaminated carcase, after the intensified hygiene trimming procedure on the stationary retain chain.

Objective

Determine if trimming on the retain rail is effective at removing contaminates (paunch matter) and whether we have to reassess procedures in this area.

Methods

Contaminated carcases are placed on the retain rail for intensified trimming and inspection.

Figure 1: Sampling location

Sampling: Samples were gathered by sponging the PE Brisket area (~225cm^2) using the same technique as for ESAM sampling (see Figure 1 above).

25 samples were taken after the hygiene trimming stand, before the spinal cord removal. 25 samples were taken after trimming on the retain stand and just prior to placement back onto the main chain, before spinal cord removal.

Testing and analysis: Sponge samples were plated on TVC and *E. coli* Petrifilm and incubated at 35°C. After 48 hours, colonies were counted and data entered on a spreadsheet tool.

Results

The results are presented in the table below and in Boxplot form to show the significance of the findings. Figure 2 shows TVC Results on clean carcase while Figure 3 shows TVC results on retain carcases. The average difference was in the order of 1 $\log_{10}$ cfu/cm^2 (10-fold higher on retain carcases) which was highly significant (P-value < 0.01). Table 1 shows prevalence of *E.coli* detections on "clean" carcases and retained carcases – the retain carcases were higher than clean carcases.

Table 1: Summary of $\log_{10}$ TVC cfu/cm^2, after hygiene trimming and after retained carcase trimming

Summary	Clean	Retain
Mean	-0.20	0.91
St. Dev.	0.57	0.80
n	25	25
Conf level	95%	
CI Lower	-0.43	0.58
CI Upper	0.04	1.23
Significance	Highly significant	

Table 2: Summary of *E. coli* prevalence results, after hygiene trimming and after retained carcase trimming

Summary	Clean	Retain
Detect	1	5
n	25	25
Prev	4.0%	20.0%
Conf level	95%	
CI Lower	0.0%	8.6%
CI Upper	21.4%	39.7%
Significance	Marginal significant	

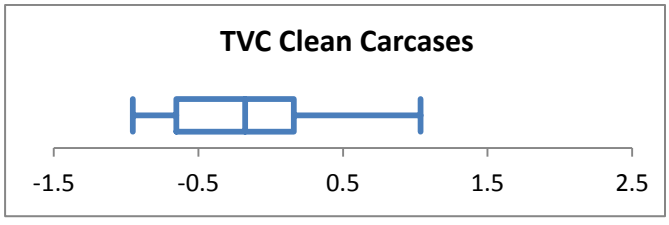


Figure 2: Boxplot of the $\log_{10}$ TVC cfu/cm^2 from clean carcases.

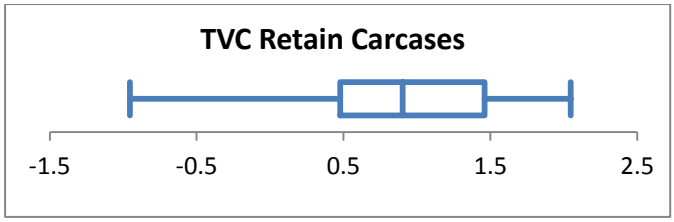


Figure 3: Boxplot of the log_{10} TVC cfu/cm^2 from retain carcases after hygiene and retain carcase trimming.

Conclusion

It was concluded that current procedures for the trimming of retained carcases, after paunch contamination is ineffective in reducing the prevalence of TVC and *E. coli* at this site. An overview of the current procedures and more investigation will give a clearer indication as to where we can improve this system.

Swabbing in the same area, after intensified hygiene on the retain chain but before hosing down of the carcase, will determine whether hosing of the carcase is helping to wash away contaminates or spreading bacteria.

15. Microbiological status of non-ESAM sites

Introduction

We are a bovine slaughtering and boning facility processing stirk animals. The reason for the investigation is to determine the TVC of non-ESAM sites on our carcases.

Objective

Determine the TVC of 4 non-ESAM sites for 0-teeth animals compared to 2-8 teeth animals.

Methods

Sampling: 104 samples were gathered by sponging the area (~300cm^2) using the same technique as for ESAM sampling (reference to method). 104 samples were taken from 24 bodies over several production days. All samples were taken in the chillers after the day of slaughter.

Testing and analysis: Four specific sites on the carcase (see figure below) were sponged to assess the microbial load. Sponge samples were plated on *E. coli* Petrifilm and Aerobic count Petrifilm and incubated at 35°C. After 48 hours, colonies were counted and data entered on a spreadsheet tool.

Figure 1: Sampling locations

Results

The results are presented below in boxplot format.

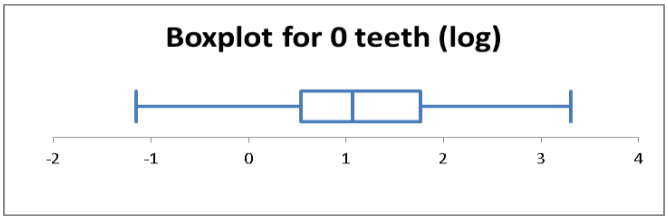


Figure 2: Boxplot of the overall log_{10} TVC cfu/cm^2 for 0 teeth animals.

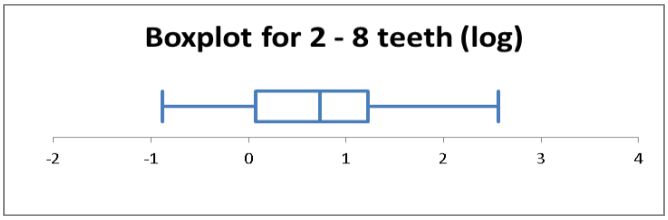
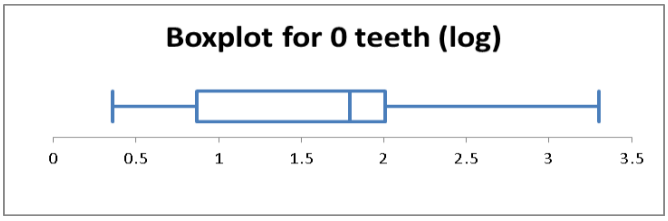


Figure 3: Boxplot of the overall log_{10} TVC cfu/cm^2 for 2-8 teeth animals.

Specific location swabbing results



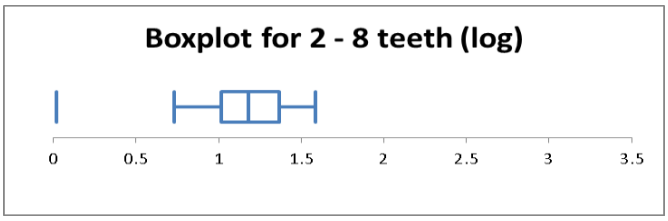
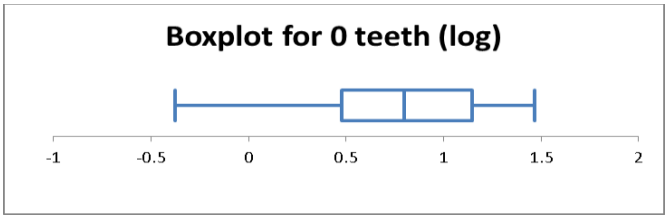


Figure 4: Boxplots for log_{10} TVC cfu/cm^2 for Butt. Marginal difference.



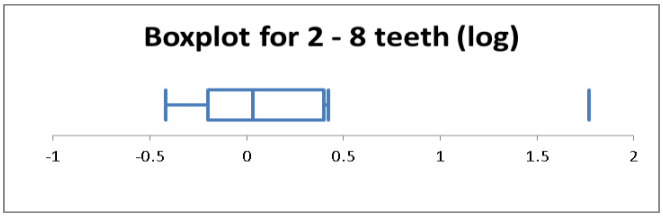
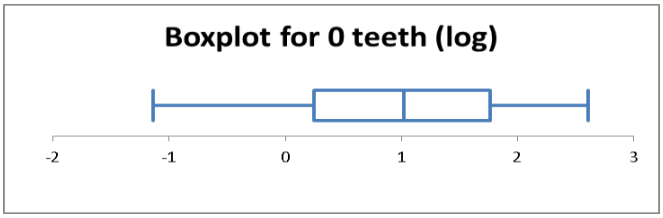


Figure 5: Boxplots of log_{10} TVC cfu/cm^2 for Loin. Marginal difference.



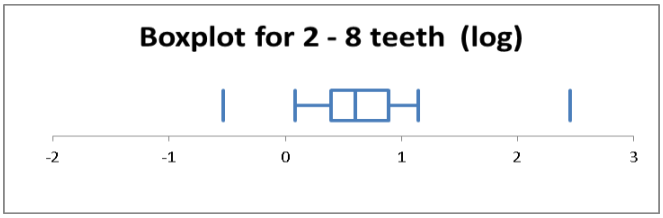
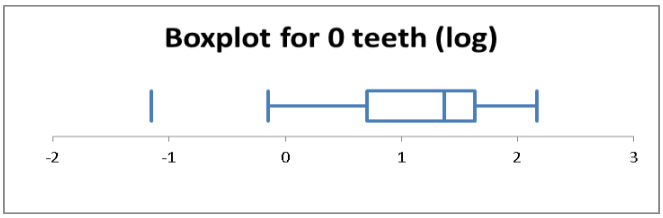


Figure 6: Boxplots of log_{10} TVC cfu/cm^2 for Blade.



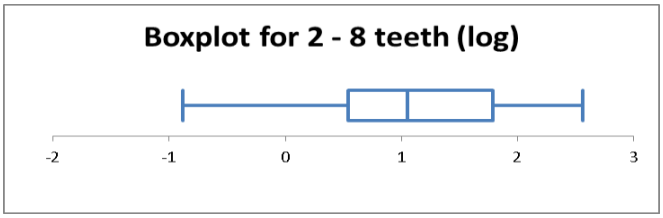


Figure 7: Boxplots of log_{10} TVC cfu/cm^2 for Chuck.

Conclusion

It was concluded that there is a significant difference in results based the age of the animals. There are also marginal differences in the loin and butt location depending on the age of the animals.

16. *E. coli* "hot spots" on beef carcases

Introduction

E. coli and STEC contamination of beef carcases can occur during hide removal, through airborne transmission and contact with working surfaces/operator's equipment but little is known about the deposition and distribution of the bacteria on the carcase itself. We want to be able to map specific carcase "hot spots" for *E. coli* contamination as this information could potentially be used to inform targeted intervention or decontamination steps along our slaughter chain[1].

Objective

1. To determine which beef carcase sites have the highest prevalence of *E. coli.*
2. To compare these results from alternative carcase sites with ESAM data.

Methods

Sampling occurred from October to December 2015, with seven sampling days scheduled, one day each week. Each sampling day, five carcases were randomly selected and sponged individually prior to entry to the chillers. Carcases were sampled at different times throughout the day to capture within day-variation. On each of the carcases, five surface sites (400cm^2) were sponged aseptically; the sites included the rump, loin, belly, neck and shank, both on the left and right sides of the carcase (Figure 1).

Samples were stored below 10°C and transported to SARDI Food Safety and Innovation Microbiology laboratories where they were processed within 24 hours of receipt. Swab samples were plated onto *E. coli* Petrifilm™ and incubated for 48 hours at 35°C. Colonies were then counted and results entered into a spreadsheet. Further enrichment of swabs was performed in 50mL Lauryl Tryptose Broth (37°C for 18-24 hours), subcultured into EC broth (42°C for 48 hours), then examined for Indole production to indicate detection of *E. coli*.

[1] J. Tan, Final Report: 2015 Science and Innovation Awards for Young People in Agriculture, Fisheries and Forestry (Red Meat Processing category) – Wherefore art thou, *E. coli*? An Approach to Mapping the Presence of *E. coli* Contamination on Beef Carcases, April 2016

Results

E. coli was found on all carcase sites with a mean prevalence of 71% (n=350). The highly contaminated sites were the neck and loin (prevalence of 97% and 94% respectively) while the belly was found to have a prevalence of 40%, the lowest of all sites (Table 1, Figure 2).

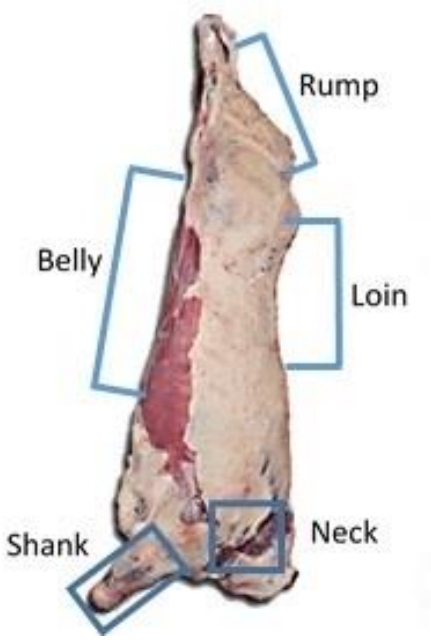


Figure 1: Swab sites on beef carcase, each site was sponged over a 400cm^2 surface area.

Table 1: Number of positive detections of generic *E. coli* on beef carcases at five sites over 7 sampling days.

	Rump	Loin	Belly	Neck	Shank
20/10/2015	8/10	10/10	5/10	10/10	6/10
26/10/2015	8/10	10/10	7/10	10/10	8/10
2/11/2015	10/10	10/10	4/10	10/10	9/10
17/11/2015	6/10	10/10	3/10	10/10	2/10
3/12/2015	6/10	10/10	3/10	10/10	6/10
10/12/2015	5/10	9/10	3/10	8/10	3/10
16/12/2015	5/10	7/10	3/10	10/10	4/10
Total	48/70	66/70	28/70	68/70	38/70
***E. coli* Prevalence**	**69%**	**94%**	**40%**	**97%**	**54%**

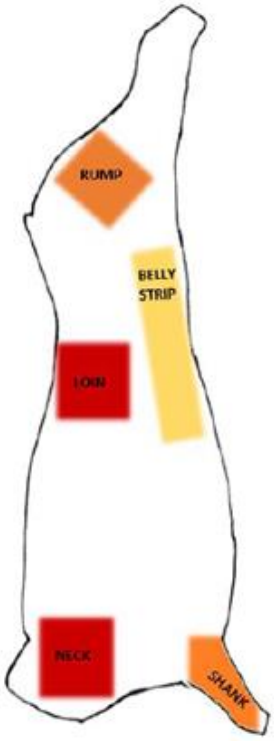


Figure 2: Map of *E. coli* prevalence at beef carcase sites, red indicates *E. coli* prevalence of 94-97%, orange indicates prevalence of 54%-67% and yellow indicates prevalence of 40% and below.

Despite prevalence of *E. coli* across all carcase sites, only 38 out of the total 350 swab samples (10.9%) were found to have *E. coli* at countable concentrations. Mean counts varied from site to site ranging from -1.20 $\log_{10}$ (cfu/cm^2) at the shank to -0.80 $\log_{10}$ (cfu/cm^2) at the loin (Table 2). The highest individual count was -0.03 $\log_{10}$ (cfu/cm^2) which was taken from the loin.

Table 2: Mean *E. coli* (cfu/cm^2) of *E. coli* positive swab samples (n=38).

	$\log_{10}$ *E. coli* (cfu/cm^2)
Rump	-0.85
Loin	-0.80
Neck	-0.95
Shank	-1.20

Conclusion

Results of this trial indicate widespread prevalence of *E. coli*, ranging from 40%-97% on all of the carcase sites tested. The neck and loin were found to have the highest prevalence, followed by the rump and shank, while the belly displayed the lowest level of *E. coli* contamination of the sites tested. Despite high prevalence on our carcases, bacterial counts indicated that *E. coli* was present at very low concentration.

Figures from the regulatory ESAM program claim a national average *E. coli* prevalence of 5.6%, which is much lower than those revealed in this study. This discrepancy is likely due to the differences in swabbing methods under the ESAM program which include swabbing of chilled carcases, swabbing of fewer sites and swabbing of a smaller total surface area.

This study has been successful in mapping the specific carcase “hot spots” for *E. coli* contamination and can potentially be used to inform targeted intervention or decontamination steps along our slaughter chain.

17. How do counts from hot sponging of carcases compare with cold sponging?

Introduction

Our plant exports beef trim to USA for grinding and we are concerned with faecal contamination. As an in-house procedure to inform our operators, we routinely sample hot carcases by sponging at the ESAM sites. We also undertake ESAM sponging of chilled carcases and carton testing of boned-out trim. Over time, we have accumulated a huge amount of in-house data which we've used to inform supervisors at weekly meetings.

Recently at a MINTRAC QA Managers annual conference, we heard from researchers at the University of Tasmania that *E. coli* counts on carcases go down after overnight chilling, but then increase again after 48 hours. At the same meeting, we spoke with SARDI statisticians who said they could help us look at our data in a number of ways.

Objective

Our ESAM counts for TVC and *E. coli* have always been pretty good but we heard that this might be a false sense of security because the counts can increase over the next 48 hours. Because we do in-house testing of carcases before they leave the slaughter floor, we have a good picture of the contamination our operators put on the bodies.

What we needed from SARDI was a comparison of contamination levels on "hot" carcases (this is the real contamination level) compared with the level on chilled carcases.

Methods

Each day, we sample 12 carcases at the MHA stand by sponging at the rump, flank and brisket. All samples are tested in our onsite laboratory, using standard testing procedure and plated on *E. coli* and Aerobic Count Petrifilm and incubated at 35°C. After 48 hours, colonies are counted and data entered into an Excel spreadsheet.

We have a great deal of information (from 2007, a total of more than 15,000 tests) and SARDI analysed the data to give graphs and tables which are presented in the results.

Results

In Figures 1 and 2, we can see how our in-house sponging of carcases as they leave the slaughter floor compares with the same sites after carcases have been chilled (cold sponging for ESAM).

Figure 1 shows the prevalence of *E. coli* with a large reduction following chilling, from around 20% before chilling to around 5% after it.

Similarly for TVC, counts were generally higher on hot-sponged versus cold-sponged carcases, by about log 0.5 cfu/cm^2.

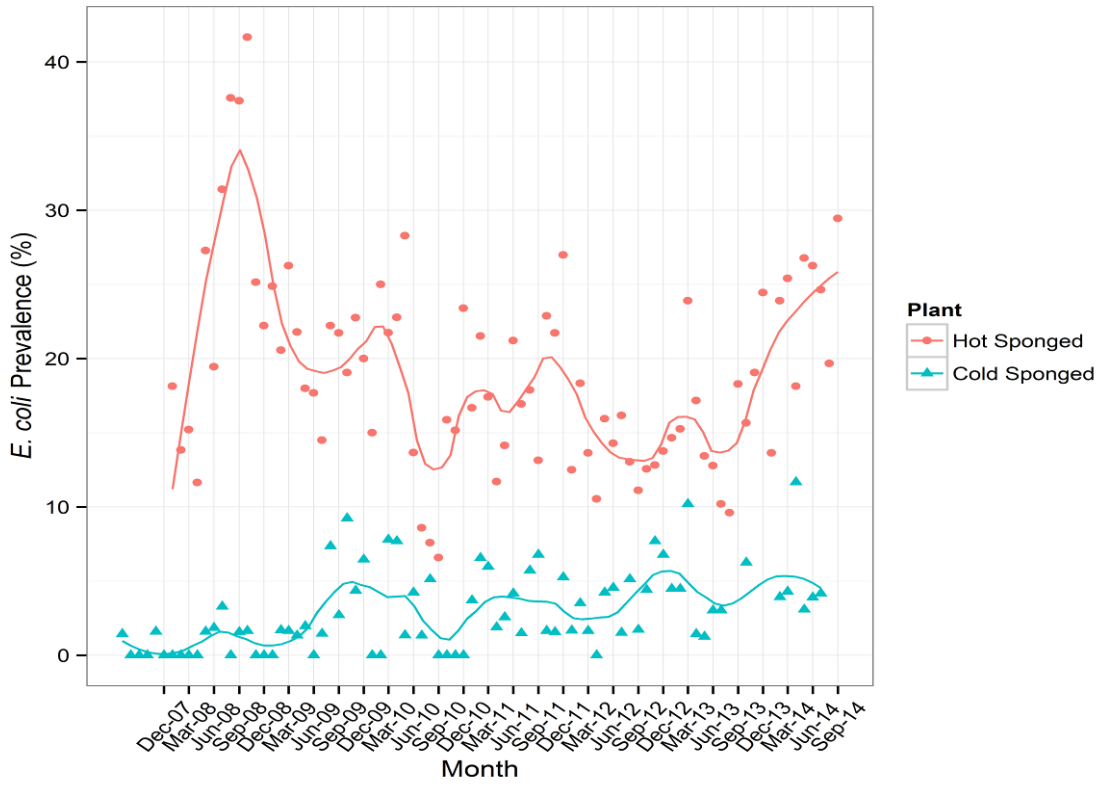


Figure 1: Prevalence of *E. coli* on hot-sponged carcases compared with cold-sponged carcases

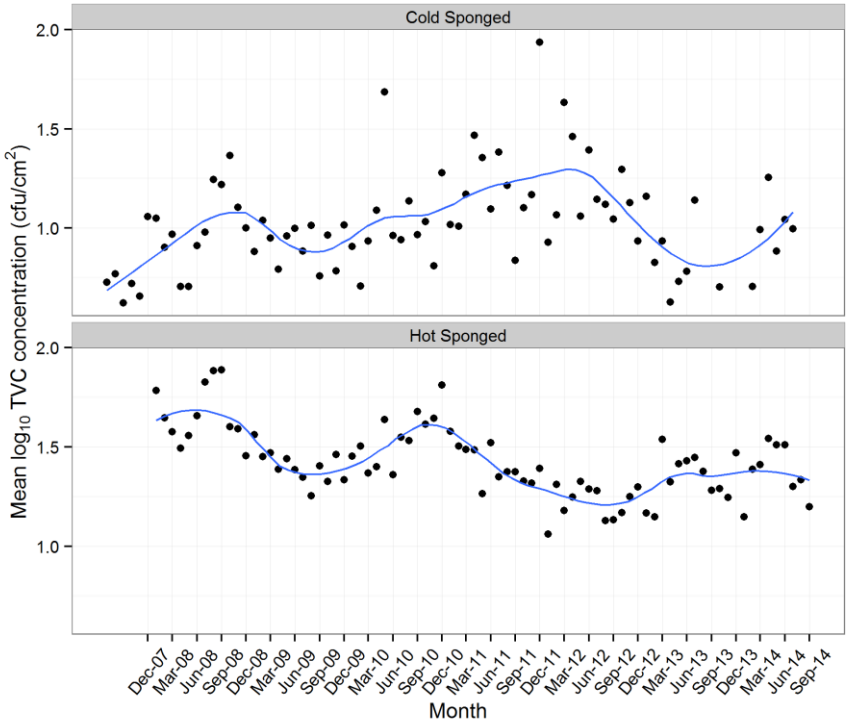


Figure 2: Mean log_{10} TVC cfu/cm^2 for hot-sponged carcases compared with cold (ESAM) carcases

SARDI comments

The E. coli data are interesting. For some parts of your ESAM (cold-sponging) results (see the end of 2010) you apparently don't get even one colony of E. coli for four months. This seems unlikely.

Considering those months when you get very high E. coli prevalence on hot carcases – does that ring alarm bells? Do you ask your supervisors why that may have happened?

You should, because that's making good use of the investment you've made over the years on in-house carcase testing.

18. Where do we put contamination on the beef carcase?

Objective

Our beef slaughter floor is not large and changes direction several times.

We wanted to know:

- Which are our high contamination sites?
- Is there a difference in bacterial loading between the right and left side of the carcase?

To answer these questions, we sampled the same seven sites on both right and left sides of the carcase just before the MHA stand.

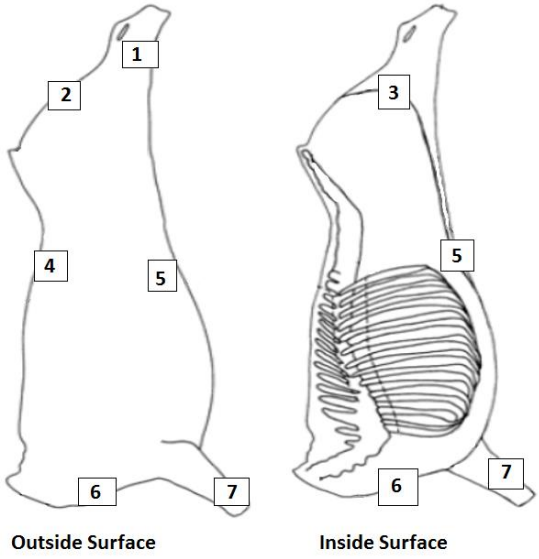


Figure 1: Sampling sites

Methodology

We excised 100cm^2 areas at seven sites on each side of the carcase using a Whirlpak sponge. Samples were transported chilled to the lab for testing where 100mL of buffered peptone was added and the contents of the bag stomached for 30 seconds.

Serial dilutions were prepared in 0.1% buffered peptone water blanks (9 mL) using 1mL aliquots. Aliquots (1 mL) from each dilution were spread on either Aerobic Plate Count Petrifilm to give a Total Viable Count (TVC) or *E. coli* Petrifilm and incubated at 30°C for 2 days.

Colonies were identified and counted as per the manufacturer's instructions. When *E. coli* was absent from Petrifilms, the result was entered as "not detected". Counts were converted to $\log_{10}$ colony forming units (cfu) and the mean of the $\log_{10}$ cfu/cm^2 was calculated.

Results

Total bacterial loading

In total, the right and left sides on each of 58 carcases were tested and, as shown in Figure 2:

- There was little difference in mean TVC for the seven sites on the left versus the right side.
- TVCs varied from log 2.0 cfu/cm^2 at site 3 to log 0.7 cfu/cm^2 at site 4.
- Site 4 (loin) had a lower contamination compared with the other sites.
- Site 3 (topside rim) had the highest level of contamination.

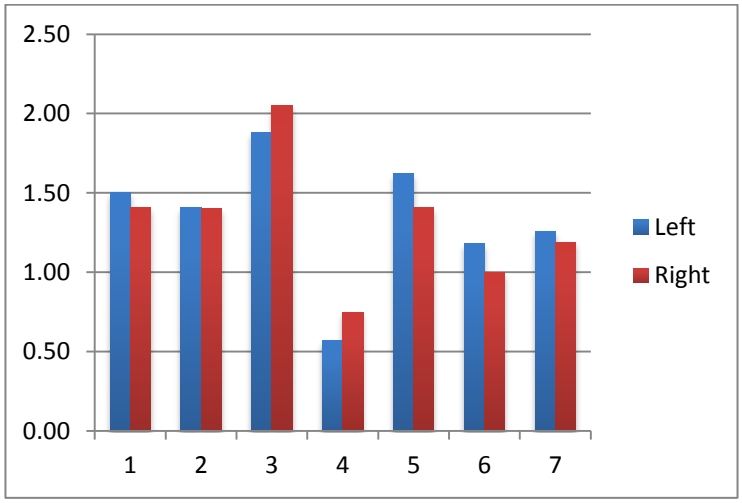


Figure 2: Mean log TVC cfu/cm^2 (vertical axis) at seven sites on the right and left sides of each carcase

E. coli contamination

As seen from Figure 3:

- The right side is more likely to be contaminated with *E. coli*, with 27 detections, compared with 10 detections on the left side of the carcase.
- Site 3 on the right side is more likely to be contaminated with *E. coli.*
- *E. coli* was not detected in any of the 58 tests on the left side at site 2 (outside) and site 5 (flank).

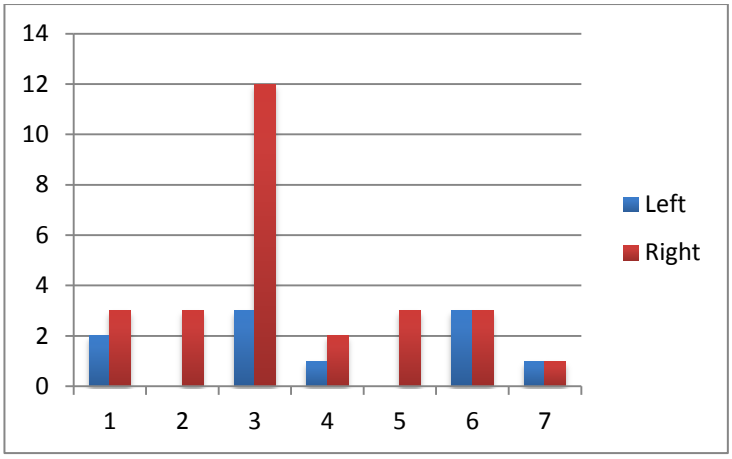


Figure 3: Detections of *E. coli* at seven sites on the right and left sides

What did we learn

We learned our operators on the 2nd leg weren't following standard operating procedures and we rectified this.

We plan to re-assess our procedures in the future.

19. Where do we put contamination onto carcases?

Introduction

Our plant exports beef trim to USA for grinding and we are concerned with faecal contamination. As an in-house procedure to inform our operators, we routinely sample carcases hot by sponging at the ESAM sites. We also undertake ESAM sponging of chilled carcases and carton testing of boned-out trim. Over time, we have accumulated a huge amount of in-house data which we've used to inform supervisors at weekly meetings.

Recently, we spoke with SARDI statisticians who said they could look at our data in a number of ways. So we sent our data to SARDI and they've helped us make better use of it and we have agreed to publish the work in this book.

Objective

Because we keep our sponging separate at the three ESAM sites, we're able to look at how much contamination we put on at each site over time.

Methods

Each day, we sample 12 carcases at the MHA stand by sponging at the rump, flank and brisket. All samples were tested in our onsite laboratory, using standard testing procedure and plated on *E. coli* and Aerobic Plate Count Petrifilm and incubated at 35°C. After 48 hours, colonies were counted and data entered into an Excel spreadsheet.

We have a great deal of information (from 2007, a total of more than 15,000 tests) and SARDI analysed the data to give graphs and tables, which are presented in the results.

Results

In Figures 1 and 2, we have a long-term historical profile of our carcases as they leave the slaughter floor, and the profile is done at three locations: rump, flank and brisket.

Over the seven-year period, we can see that the TVC varies between log 1 and 2 cfu/cm^2 with counts generally being similar at the three sites. If anything, the monthly averages seem to be trending lower over the past three years at the rump and brisket, with the flank site being constant around log 1.6 cfu/cm^2.

We don't see any seasonal effect of TVC or *E. coli* prevalence with the latter generally cycling between 10-20% prevalence, though there are some months where we get 30-40% prevalence, which is of concern as ESAM data we get from SARDI tell us the national *E. coli* prevalence is 4-5%.

SARDI comments

The in-house testing you do on carcases as they leave the slaughter floor gives you a true picture of contamination you put on during hide removal and evisceration, and the contamination you remove during trimming.

During chilling, some bacteria are inactivated and the counts will fall. This may only be a temporary fall if the product remains chilled, but if it's boned and frozen after 24-hour chilling, that process will prevent bacteria being resuscitated.

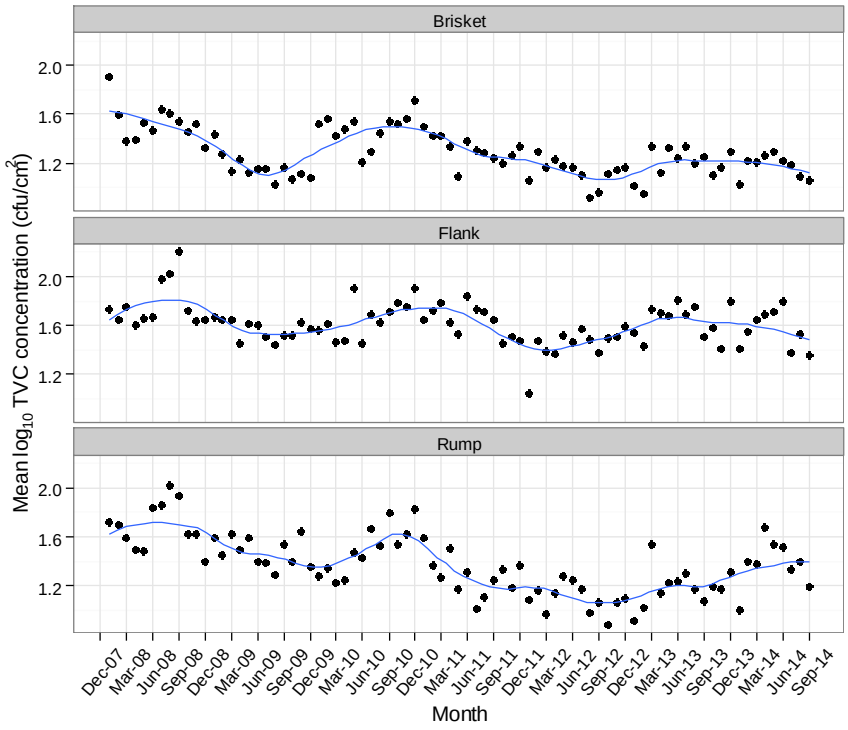


Figure 1: Mean monthly log_{10} TVC cfu/cm^2 for hot carcase sites

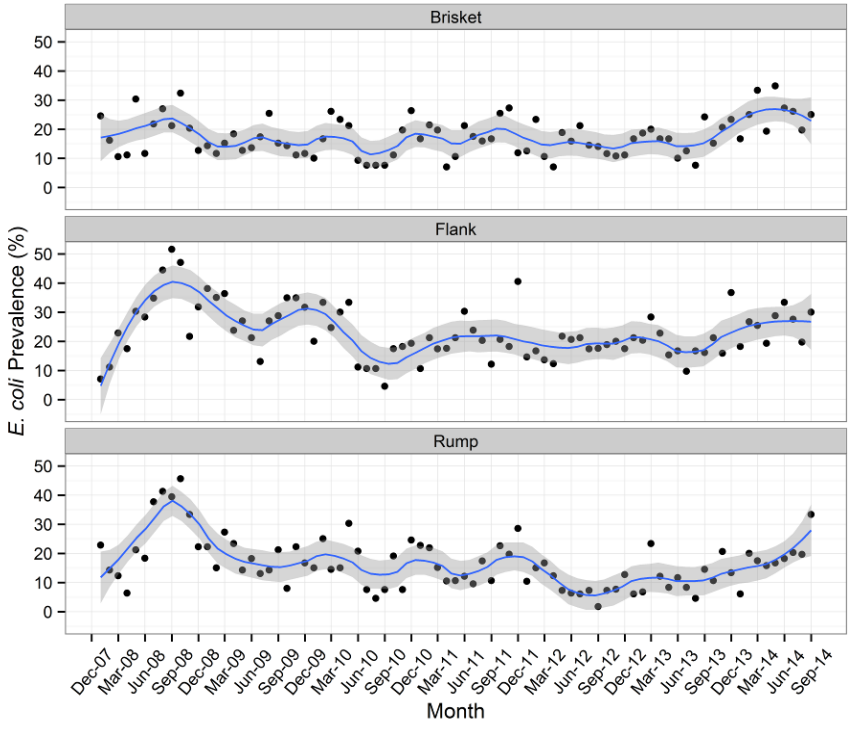


Figure 2: Monthly *E. coli* prevalence for hot carcase sites

20. Microbiological impact of steam vacuum and 82°C wash on beef carcases

Introduction

It is thought that the introduction of a steam vacuum and 82°C wash after trimming could lead to reduction of contamination to the brisket area.

Objective

Determine if vacuuming and 82°C wash will result in reduced TVC counts.

Methods

Processing: Our current work instruction does not include vacuuming and 82°C wash.

Sampling: Two hundred samples were gathered by sponging the brisket area pre-vacuum (100) and post wash (100) using the same technique as for ESAM sampling.

Testing and analysis: Sponge samples were plated on APC Petrifilm and incubated at 35°C. After 48 hours, colonies were counted and data entered on a spreadsheet tool.

Results

The data in the table below indicate that, although statistically significant, no meaningful reduction is obtained by steam vacuum and hot water washing.

Table 1: Summary of difference in log_{10} TVC cfu/cm^2 before and after steam vacuum & 82°C wash.

Summary	Difference (log)
Mean	0.18
St. Dev.	0.51
n	99
Conf level	95%
CI Lower	0.08
CI Upper	0.29
Significance	Highly significant

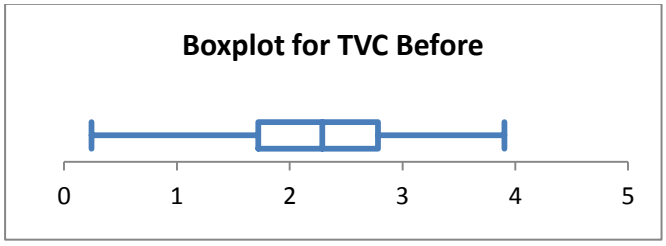


Figure 1: Boxplot of log_{10} TVC cfu/cm^2 for before steam vacuum and 82°C wash.

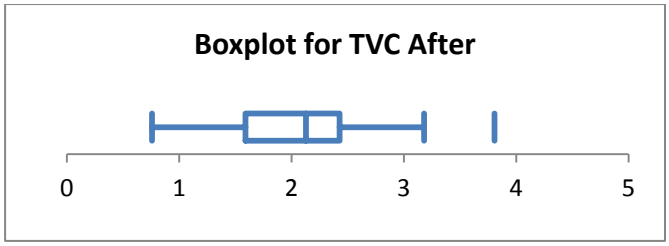


Figure 2: Boxplot of log_{10} TVC cfu/cm^2 for after steam vacuum and 82°C wash.

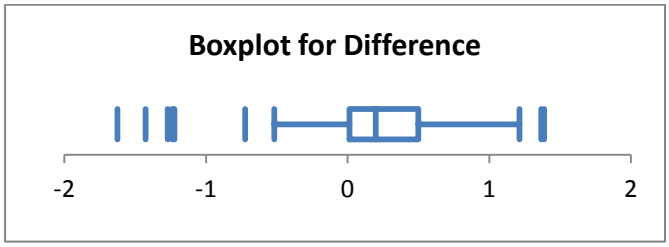


Figure 3: Boxplot of difference in log_{10} TVC cfu/cm^2 for before and after steam vacuum and 82°C wash.

Conclusion

It was concluded that the current procedures for vacuum and 82°C wash are not effective in reducing the TVC of the brisket area.

21. Hot water treatment of carcases – how effective is it at our plant?

Introduction

We have a hot water pasteurising unit, which the USA manufacturers tell us will kill 90-99% of STECs. We have never validated the equipment on our slaughter floor and so we undertook a Plant Initiated Project (PIP), where samples were taken from carcase sides at three stages in the process:

1- Before the hot water cabinet

2- After hot water treatment

3- After active chilling

Objectives

We wanted to establish:

1- The level of contamination our operators put on the carcase

2- The amount of contamination removed by the pasteuriser

3- The contamination level after chilling

Methods

The sampling procedure was as follows:

- Four sites (neck, brisket, loin, butt) from 5 different carcase sides were sampled into separate bags
- Excise surface tissue to generate approximately 25g of tissue
- Sample before the hot water cabinet, after the cabinet and after chilling, sampling from the same body number where possible
- Take samples a minimum of 8 hours after chilling (on one occasion due to a public holiday, samples were taken after 72 hours chilling)
- Sample once a day for one week/month for three months
- Courier the samples to an off-site laboratory for estimation of Total Viable Count and *E. coli* on Petrifilm (incubated 35°C/48 hours and 37°C/48 hours, respectively)
- Count plates according to the manufacturer's instructions and then express them as colony forming units (cfu/g)
- The limit of detection was 10 cfu/g

Data were analysed by SARDI using the open-source statistical software R (R Core Team (2014). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org).

Differences in proportions (i.e. for percentage of TVC detections and *E. coli* prevalence) were tested for significance using a chi-squared test for differences in proportions. Mean TVC concentrations were estimated using Maximum Likelihood Estimation (MLE).

All figures and tables were produced using R and concentrations are reported as $\log_{10}$ cfu/g.

Results

As can be seen from Table 1, before they entered the pasteuriser, most (64-84%) of carcases had a bacterial loading >10 cfu/g. Pasteurising reduced this percentage significantly, especially at the rump and loin sites, however, after transfer to the chiller and overnight chilling, counts were obtained on 29-80% of carcases, depending on their location on the carcase.

Table 1: Percentage of TVC results (n=75) (at each location on the carcase) above the limit of detection (10 cfu/g)

	Site	% Results >10 cfu/g
Before pasteuriser	Bung	84%
	Loin	67%
	Brisket	64%
	Neck	68%
After pasteuriser	Bung	20%
	Loin	29%
	Brisket	44%
	Neck	36%
After Chilling	Bung	29%
	Loin	52%
	Brisket	68%
	Neck	80%

In terms of the total bacterial loading on the carcase at each stage of the process, mean counts are presented in Table 2 and Figure 1 from which it can be seen that:

- Before pasteurising the rump was the most heavily contaminated location
- After pasteurising counts were reduced at all four locations
- After chilling higher counts were found lower down on the carcase

Table 2: Average concentration (log_{10} cfu/g) at each location on the carcase (n=75)

	Site	Mean	SD	Mean raw counts
Before pasteuriser	Bung	2.14	0.73	138
	Loin	1.82	0.71	66
	Brisket	1.72	0.56	52
	Neck	1.6	0.57	40
After pasteuriser	Bung	1.43	0.56	27
	Loin	1.45	0.67	28
	Brisket	1.66	0.65	46
	Neck	1.55	0.45	35
After Chilling	Bung	1.91	0.92	81
	Loin	2.07	1.06	117
	Brisket	2.31	1.22	204
	Neck	2.25	1.11	178

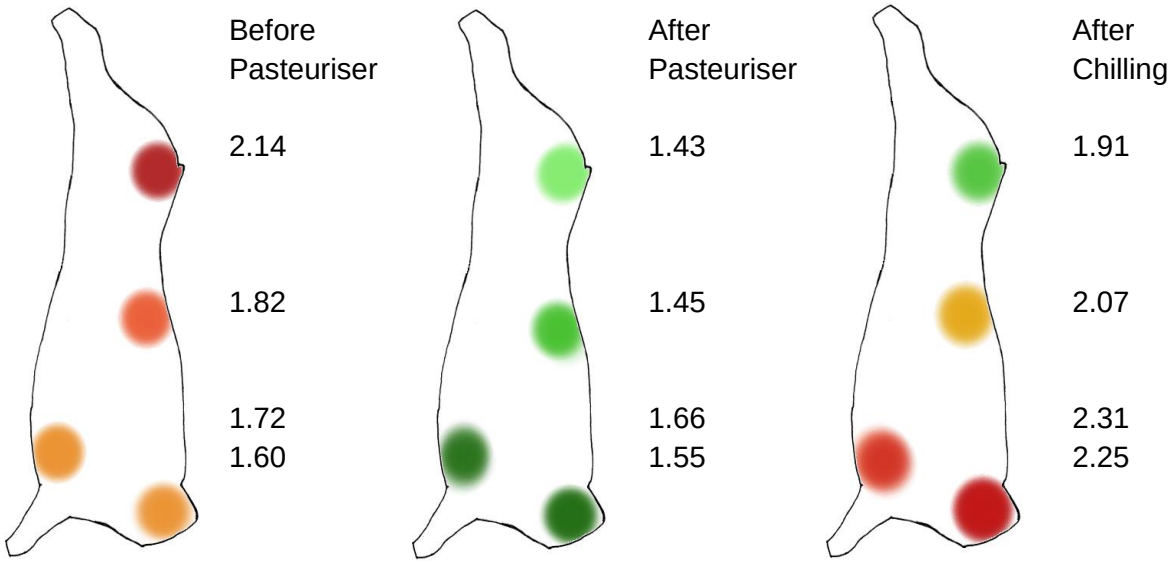


Figure 1: Mean TVCs (log_{10} cfu/g) on carcases before and after pasteurising and after overnight chilling

Prevalence of *E. coli* at each location on the carcase at the three stages in the process is presented in Table 3 and Figure 2. Before pasteurising, *E. coli* was detected at all four locations on the carcase, particularly the rump, and a small number of samples had *E. coli* at this location after pasteurising. After chilling, *E. coli* were not isolated on any of the four carcase locations.

Table 3: Prevalence (%) of *E. coli* results at each location on the carcase (n=75)

	Site	Number of results >10 cfu/g
Before pasteuriser	Bung	21
	Loin	1
	Brisket	1
	Neck	4
After pasteuriser	Bung	2
	Loin	0
	Brisket	0
	Neck	0
After Chilling	Bung	0
	Loin	0
	Brisket	0
	Neck	0

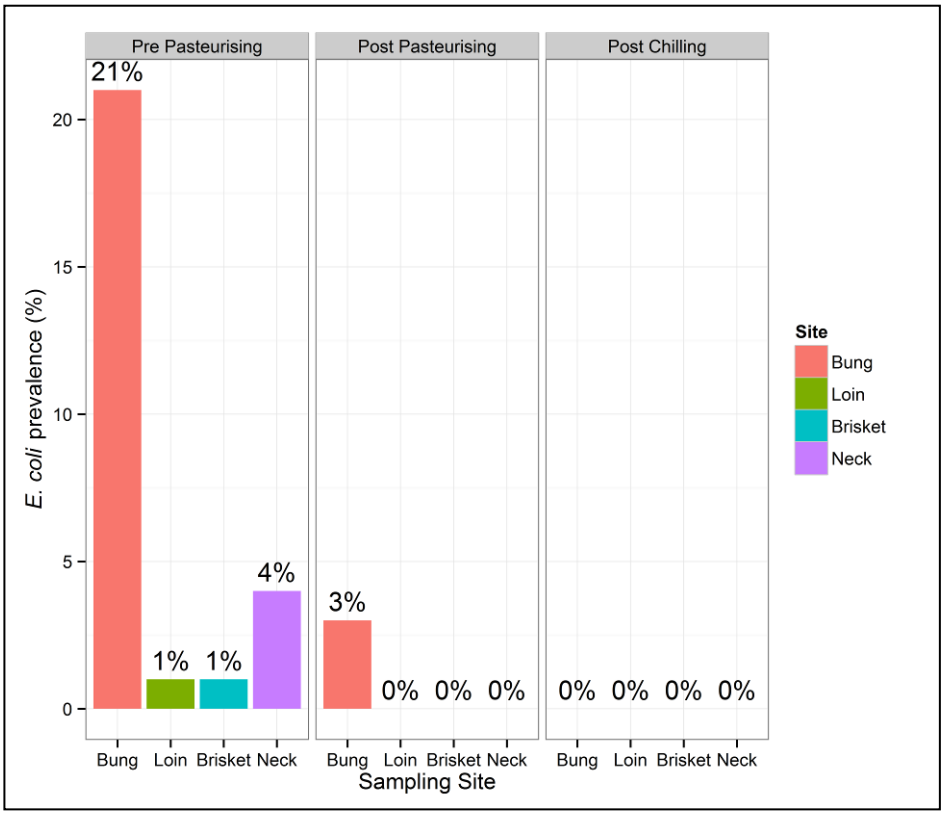


Figure 2: *E. coli* prevalence (%) at the three carcase locations before and after pasteurising and after chilling

Discussion

We now know:

1- The total contamination levels our operators put on carcases – both on the average and on “bad days”

2- Where the contamination is located on the body

3- How effective the pasteuriser is in removing this contamination

4- How much contamination we put back on the carcase during transfer to the chiller and during active chilling

5- How often *E. coli* remains after pasteurising and chilling

6- We should stop saying *E. coli* is “absent” when it is actually “<10 cfu/g”.

7- Also we know from the UTas work that *E. coli* are inactivated immediately after chilling but can apparently resuscitate themselves in the days immediately following e.g. after weekend chilling.

22. Effect of hot water washing on contamination at various sites of beef carcases

Background

Hot water treatments have been extensively researched and shown to remove and/or inactivate bacteria on beef carcases. We have installed a hot water cabinet which supplies water at least 84°C for 14 seconds to each carcase. The cabinet is located after the MHA stand.

Objective

The objective of this study was to determine the effectiveness of a hot water cabinet on the microbial level of beef carcases and our study sampled carcases in 2015 (before installation) and in 2016 (after installation of the cabinet).

We reported the position before installation as Case Study 18 from which we concluded:

- The right side was more likely to be contaminated with *E. coli*, with 27 detections, compared with 10 detections on the left side of the carcase.
- Site 3 (pelvic rim) on the right side was more likely to be contaminated with *E. coli* than any other site.
- *E. coli* was not detected in any of the 58 tests on the left side at site 2 (outside) and site 5 (flank).
- We learned that our operators on the 2nd leg weren't following standard operating procedures and we rectified this.

Because we are monitoring two factors (rectifying legging techniques particularly on the right side, plus the effect of hot water washing) in this study we are making no direct comparisons with Case Study 18.

In addition, in Case Study 18, we were sampling dry carcases at the MHA stand while in the present study, we sampled carcases which were still wet and warm from the hot water wash.

As a result, we will focus on what are the contamination levels of indicator organisms (APC and *E. coli*) currently at our plant.

Methodology

Sampling:

From 16th February 2015 to 8th April 2015, carcases (n=58) were sampled before the cabinet was installed, with sampling being done on carcases at the MHA stand.

From 11th January 2016 to 25th February 2016, a further 58 carcases were sampled after passage through the hot water cabinet, at the Tenderstretch stand.

On each sampling day, incision meat surface samples (100cm^2) were collected at seven sites on both sides of two or three carcases (see Figure 1).

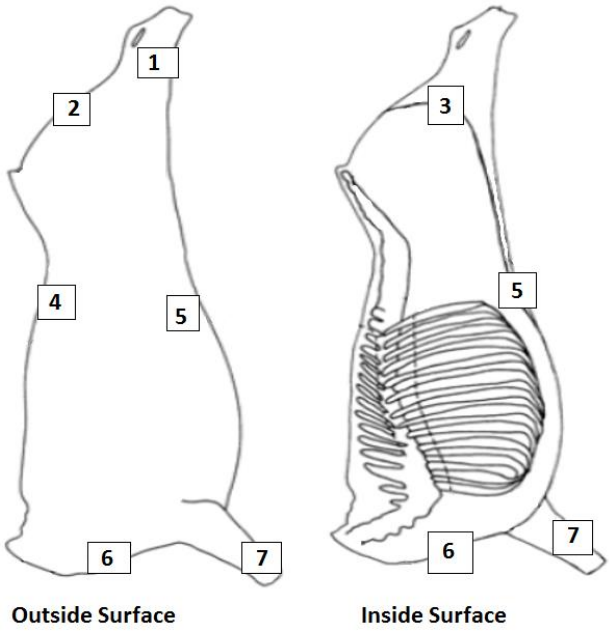


Figure 1: Sites on the carcase that were sampled.

Microbiological testing:

Each incision sample was placed in a sterile bag and diluted in 100ml of Buffered Peptone Water for enumeration and the contents of the bag stomached for 30 seconds.

Serial dilutions were prepared in 0.1% buffered peptone water blanks (9 mL) using 1mL aliquots. Aliquots (1 mL) from each dilution were spread on Aerobic Plate Count Petrifilm, and *E. coli*/ Coliform Petrifilm and incubated at 35°C for 48 hours for APC and 24 hours for *E. coli*/ Coliform.

Colonies were identified and counted as per the manufacturer's instructions. When no growth was observed from Petrifilms, the result was entered as "not detected". Counts were converted to $\log_{10}$ colony forming units (cfu) and the mean of the $\log_{10}$ cfu/cm^2 was calculated.

Statistical Analysis:

The results were analysed by SARDI using the Testing template v7 spreadsheet and the open-source statistical software R (R Core Team (2015). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org).

Results

The results are presented in the tables and figures below from which it can be seen that hot washing had no real effect on the mean APCs before and after

installation of the hot water cabinet (Figure 2). There was also no difference between the mean counts on left and right sides.

Hot water treatment had an effect on prevalence of *E. coli* (Table 1), though the right side sites of the carcase were more likely to have *E. coli* both before and after the hot water wash was installed, particularly on the rump and pelvic rim.

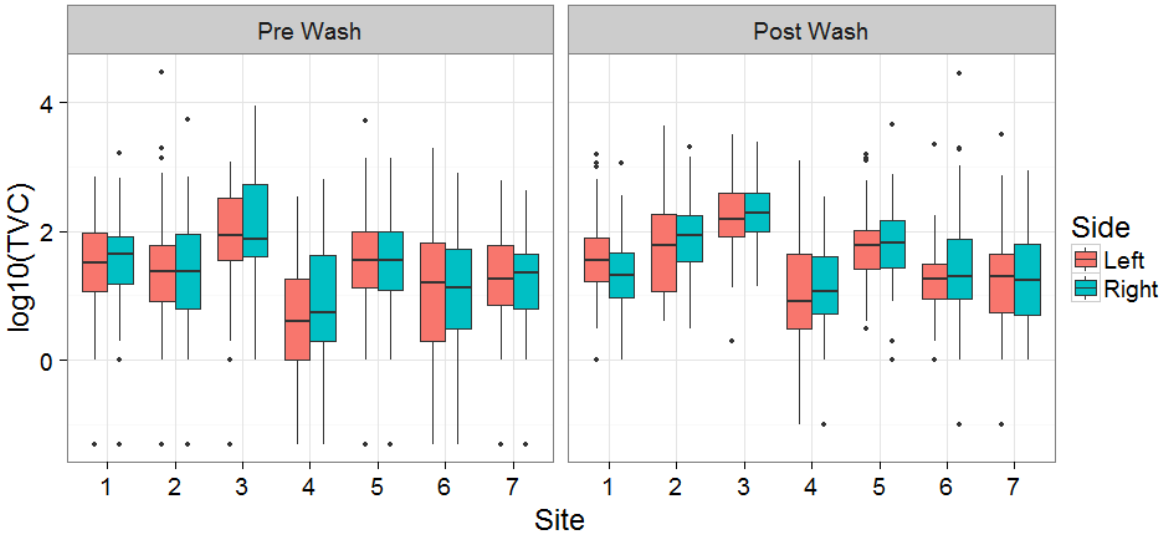


Figure 2: Boxplots showing log_{10} APC cfu/cm^2 of left and right sides on each site before and after hot water wash treatment.

Table 1: Prevalence of *E. coli* on left and right sides of the carcase when sampled before, and after, the hot water wash was installed

	Before wash installed		After wash installed	
Site	Left	Right	Left	Right
1	1/58	3/58	0/58	0/58
2	0/58	3/58	0/58	3/58
3	3/58	12/58	1/58	4/58
4	1/58	2/58	0/58	0/58
5	0/58	3/58	1/58	2/58
6	3/58	2/58	1/58	2/58
7	2/58	1/58	0/58	0/58
Total	10/406 (2.5%)	26/406 (6.4%)	3/406 (0.7%)	11/406 (2.8%)

In addition, monitoring of STEC detections and positives has indicated a downward trend in the number of STEC potentials and confirmed detections. From a year's worth of data before and after the introduction of hot water treatment, STEC potential detections has dropped from 6.8% to 1.7% (Table 2).

Table 2: Number of STEC tests and potentials in the period of 12 months before and after the hot water wash was installed

	Before wash installed	After wash installed
Number of tests	424	536
Number of potentials	29	9
%	**6.8**	**1.7**

Conclusions

Hot water treatment appears effective in reducing *E. coli* and STEC potentials, which then reduces the number of confirmed samples and detections.

23. Effect of hot water decontamination carcase wash on ESAM results

Background

Since 2015, we have routinely collected swab samples from chilled carcases under the *E. coli* and *Salmonella* Monitoring program (ESAM) and have accumulated results over time.

We also introduced a hot water decontamination carcase wash on the beef slaughter chain from March 2016 onwards and are interested in seeing whether there is a difference in our ESAM results.

Objective

The objective was to see whether the hot water decontamination carcase wash has an effect on ESAM micro results.

Methodology

A hot water cabinet with multiple spray nozzles was installed on the post slaughter floor and carcases were sprayed with hot water at 86 °C for 6-8 seconds before entering the chiller passageway.

Each day, we collected and tested ESAM samples as per the AQIS Meat Notice 2003/06 and using standard procedure. All samples were tested for total viable counts (TVC), coliforms and *E. coli* counts by a commercial laboratory and the data entered into an Excel spreadsheet.

SARDI analysed the data to give graphs and tables, which are presented in the results.

Results

In Figure 1, we have the profile of total viable counts from the ESAM sites over time. There appears to be a slight decrease in average TVC in recent months, particularly from May 2016.

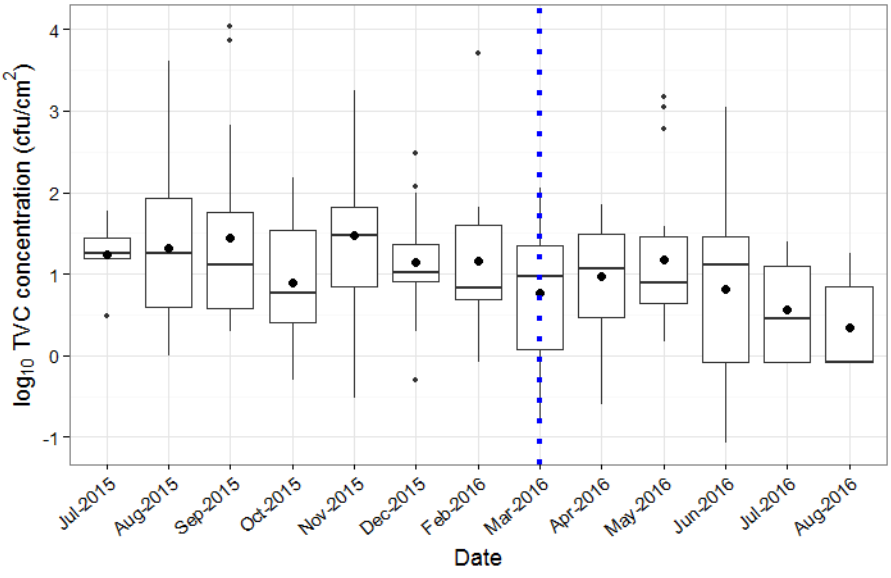


Figure 1: Box plots of log_{10} TVC cfu/cm^2 from ESAM samples over time. The dashed blue line represents the introduction of a hot water decontamination carcase wash in March 2016 and the large black dots represent the mean log_{10} TVC cfu/cm^2 each month.

When comparing the ESAM results before and after the introduction of the hot water wash, there is a decrease of 0.42 log_{10} TVC cfu/cm^2 on average, which is statistically significant, but is only a borderline meaningful/practical reduction (Table 1).

Table 1: Summary of log_{10} TVC cfu/cm^2 before and after the introduction of a hot water decontamination carcase wash.

Summary	Before Wash	After Wash
Mean	1.26	0.84
St. Dev.	0.84	0.85
n	124	112
Conf level	95%	
CI Lower	1.11	0.68
CI Upper	1.41	1.00
Significance	Highly significant	

Similarly, there is a statistically significant reduction in *E. coli* prevalence from 7.1% to 0.9% after the introduction of the hot water wash (Table 2).

Table 2: Summary of *E. coli* prevalence results before and after the introduction of a hot water decontamination carcase wash.

Summary	Before Wash	After Wash
Detect	9	1
n	126	112
Prev	7.1%	0.9%
Conf level	95%	
CI Lower	3.7%	0.0%
CI Upper	13.2%	5.5%
Significance	Significant	

Conclusions

It was concluded that the hot water decontamination carcase wash is having a beneficial effect on the microbiological results of the ESAM sites.

However, the time and temperature of the hot water carcase wash is important in order to maximise effectiveness.

24. Organic lactic acid spray trial

Introduction

We have had a number of issues with positive detections for *E. coli* O157:H7 as well as the other 'Big Six' STECs, primarily on our bull carcases and retains, so we decided to conduct a trial on lactic acid spraying. We wanted to assess whether lactic acid reduces bacterial levels of indicator organisms at different sites on our carcases.

Objective

To test whether there is an effect from lactic acid spraying on TVC and *E. coli* of beef carcases at several sites.

Methods

Industry standard procedures were followed for the application of the lactic acid (2.5%), with two people assigned to spray the fore and hindquarters respectively and 1.6L of lactic acid solution sprayed on each of the carcases to ensure complete coverage.

Sponge sampling was carried out on beef carcases (n=140) at seven surface sites which included the ESAM sites as well as the opening cut of the first leg, rump, neck and forequarter shin. Each carcase was sampled hot prior to the application of the lactic acid spray solution (pre spray), and then sampled at the same sites the following day after a spray chilling cycle (post spray).

Swabs from all of the carcase sites were pooled in the same bag, labelled (pre or post lactic spray) and transported to Symbio Laboratories for testing for TVC, *E. coli* and coliforms.

Results

Three of the post treatment TVC results were removed from the statistical analysis as, these counts were incredibly high, ranging from $3x10^4$ - $9x10^9$ cfu/cm^2 and were likely contaminated in transit.

Results showed an increase in mean log TVC of beef sides from log 0.82 to log 1.23 cfu/cm^2 and an increase in *E. coli* prevalence from 2.86% to 9.29% following the application of the lactic acid spray (Figure 1 & Table 1).

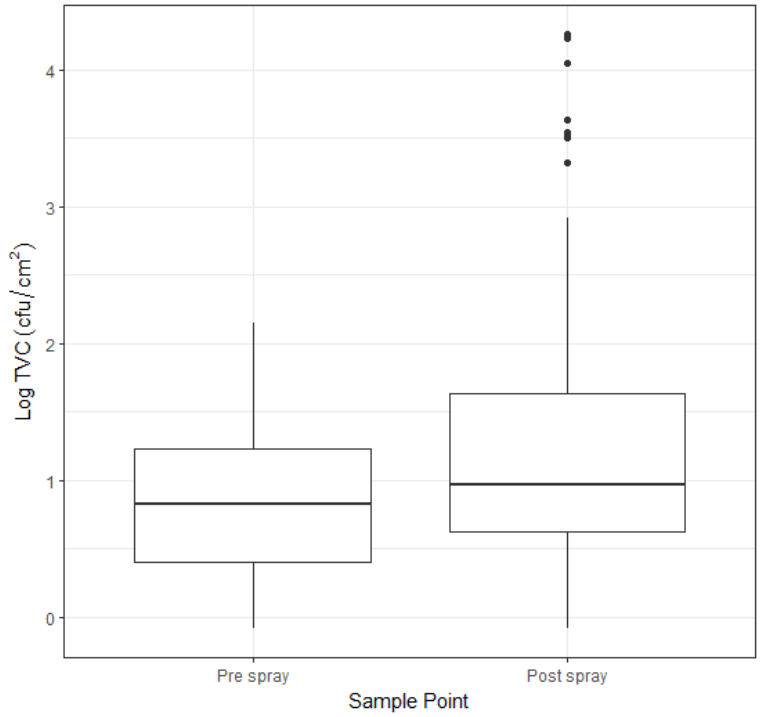


Figure 1: Side by side comparison of mean log_{10} TVC (cfu/cm^2) of beef carcases before and after lactic acid treatment.

Table 1: Summary of the log_{10} TVC (cfu/cm^2) and generic *E. coli* detections in beef carcases before and after lactic acid treatment.

Sample point	TVC		*E. coli* prevalence
	Mean	Standard deviation	
Pre spray	0.82	0.53	4/140 (2.86%)
Post spray	1.23	1.04	13/140 (9.29%)

Conclusion

The objective of this trial was to test whether lactic acid spraying had an effect on the TVC and *E. coli* of our beef carcases. Analysis of the results found an increase in the mean TVC results and *E. coli* prevalence of beef carcases after treatment with the lactic acid spray, indicating that the intervention apparently had the reverse effect.

"ASK SARDI"

We undertook a trial with lactic acid and hope you can help us understand the results please? Often the counts are higher on the side sprayed with lactic acid, compared with the unsprayed side. That shouldn't happen, should it?

SARDI COMMENTS:

You're right, that shouldn't happen and we'll try and work through what might have happened. But first, we'll make some general comments about how you set up this investigation.

1 *It's a big piece of work – you've compared 70 sides before and after lactic acid treatment for a total of 140 samples tested for TVC and E. coli/Coliforms.*

2 *Your aim was straightforward: to identify high contamination areas and you sponged seven individual sites. You also used lactic acid to investigate how effective it is at reducing contamination.*

3 *By sponging all seven sites with one sponge, it is not possible to identify high contamination sites – you can only get an average across the carcase side. If you had kept the sites separate, then you would be able to identify which sites are more contaminated, but at 7 times the cost.*

4 *It seems the laboratory thinks you just did the ESAM sites and they've sent the results back with a limit of detection of 0.083 cfu/cm^2. But you probably sponged seven x 100 cfu/cm^2 sites and the LOD is actually 0.035 cfu/cm^2. As a result, all results need to be adjusted for the larger area swabbed; they need to be multiplied by 3/7.*

5 *Also, another variable in the investigation has been added: the effect chilling has on carcase counts. It's well known that sponging carcases before chilling gives higher counts than after chilling – that's why SARDI distributes special monthly reports for hot-swabbed carcases.*

So those are some general comments, now let's look at your findings in more detail.

First, we took out three post treatment results because something obviously went wrong with them. They were in one batch and the TVC ranged from 30,000 cfu/cm^2 to 1,000,000,000 cfu/cm^2, maybe they were held up with the courier and subjected to some temperature abuse and the counts took off.

Our simple comparison is:

Log TVC/cm^2		***E. coli* prevalence (%)**	
Pre spray	Post spray	Pre spray	Post spray
0.65	1.05	4/70 (5.7%)	13/70 (18.6%)

So we agree that the results don't look right but they would look more "normal" if we reversed the Pre and Post spray data. The data then look in the correct range for TVC and, though the E. coli prevalence is high, don't forget you're sponging large areas (700 cm^2) so you're much more likely to pick up E. coli.

Log TVC/cm^2		***E. coli* prevalence (%)**	
Pre spray	Post spray	Pre spray	Post spray
1.05	0.65	13/70 (18.6%)	4/70 (5.7%)

Could the problem be something simple as your technician having labelled the sponge bags consistently, but in a way that led the outside lab to give you the results the wrong way around – again consistently? It wouldn't be the first time it's happened.

We think this is the best explanation for your results, and there is support from your own STEC data.

In 2015, you sprayed all carcases with lactic acid and compared the prevalence of STECs with 2014 (no lactic acid spray).

Before lactic acid (2014)		**After lactic acid (2015)**	
Tests	Positives	Tests	Positives
4931	17 (0.34%)	5790	4 (0.06%)

So it seems lactic acid works for you, as it does for many other establishments around the world.

25. Chlorine dioxide and peracetic acid sprays as potential *Escherichia coli* decontaminants of beef carcases

Introduction

We export beef trim for grinding in the USA and the possibility of failing a port of entry test for STEC is a constant concern. Hot water treatment of sides is expensive and we are interested in chemical decontamination as a cheaper alternative. We designed this study to examine the effect that two chemical decontaminants – chlorine dioxide (ClO_2) and peracetic acid (PAA) – have on reducing Total Viable Counts (TVC) and *E. coli* counts of our beef sides.

The US, has approved the use of ClO_2 and PAA (21CFR 173.300) as a direct food additive for decontamination of red meat carcases could be applied as a spray or dip at a level not to exceed 3 ppm residual chlorine dioxide, PAA could be used as a spay not to exceed 220 ppm PAA, 162 ppm hydrogen peroxide, and 13 ppm 1-hydroxyethylidene-1, 1-diphosphonic acid (HEDP). However other oversea markets such as Korea and Japan do not accept the use of ClO_2 and PAA as an antimicrobial agent for red meat.

Australia New Zealand Food Standards Code has approved, both sodium chlorite (a precursor used to generate ClO_2) and peracetic acid (or peroxyacetic acid, PAA) as a processing aid under Standard 1.3.3 and Schedule 18.

Objective

This study looks at the effect that ClO_2 and PAA sprays have in reducing Total Viable Counts (TVC) and eliminating *E. coli* contamination of beef carcases at high contamination sites.

The overall question we wanted answered is: *if we invest in antimicrobial treatment, how confident are we that we will eliminate E. coli from high contamination sites*?

Methods

Beef sides (n=72) were used in three treatment groups; 24 untreated (control), 24 sprayed with ClO_2 and 24 sprayed with PAA. Four carcase sites that correspond with cutting lines were sponged aseptically after treatment: the left side leg (300cm^2), right side leg (300cm^2), bung (300cm^2) and midline (1200cm^2).

Rinsates were plated on APC and *E. coli* Petrifilm™ and incubated at 35°C for 4 hours. Colonies were counted and then replated on *E. coli* Petrifilm™ to determine presence of the indicator below the standard Petrifilm™ limit of detection (LOD).

Results were entered into a spreadsheet tool for statistical analysis.

Results

The bung site was found to have the highest mean TVC (cfu/cm^2) and prevalence of *E. coli* both before and after decontamination treatment (Tables 1, 2).

Table 1: Summary of mean log_{10}TVC (cfu/cm^2) at carcase sites.

	Untreated	ClO_2	PAA
1st leg	1.1	0.5	0.5
2nd leg	0.9	0.2	0.2
Bung	1.4	0.8	0.8
Midline	1.1	0.4	0.3
Mean	**1.1**	**0.5**	**0.5**

Table 2: Summary of *E. coli* detections at carcase sites.

	Untreated	ClO_2	PAA
1st leg	6 (6.3%)	0	0
2nd leg	5 (5.2%)	0	0
Bung	12 (12.5%)	4 (4.2%)	1 (1%)
Midline	10 (10.4%)	1 (1%)	1 (1%)
Total	**33 (34.3%)**	**5 (5.2%)**	**2 (2.1%)**

As seen in Table 3, both ClO_2 and PAA decontamination spray treatments were found to significantly reduce TVC at the carcase sites by an average of log0.67 and log 0.70 TVC (cfu/cm^2) respectively.

Table 3: Summary of difference in log_{10} TVC (cfu/cm^2) between untreated, chlorine dioxide and peracetic acid treated beef carcases.

Summary	Untreated	ClO_2	PAA
Mean	1.15	0.48	0.45
St. Dev.	0.67	0.58	0.64
Diff	-	0.67	0.7
P-value	-	4.95E-11	5.87E-11
Significance	**Highly significant**		

As seen in Table 4, carcase *E. coli* concentrations were markedly reduced through the use of the ClO_2 and PAA treatments, with a large increase observed in the number of post-treatment samples with no recoverable amounts of *E. coli.*

Table 4: Summary of *E. coli* (cfu/cm^2) at carcase sites.

E. coli cfu/cm^2	Untreated	ClO_2	PAA
Not detected	12 (12.5%)	64 (66.7%)	61 (63.5)
< LOD	51 (53.1%)	27 (28.1%)	33 (34.4%)
LOD to 1	29 (30.2%)	5 (5.2%)	2 (2.1%)
1 to 10	3 (3.1%)		
> 10	1 (1%)		

Conclusion

Both ClO_2 and PAA were found to be effective in reducing *E. coli* on beef carcases from high contamination sites along cutting lines. The prevalence of *E. coli* detections on beef carcases was reduced from 34% (untreated) to 5.2% (ClO_2) and 2.1% (PAA) with more than 90% of the treated samples found to have *E. coli* at concentrations below the limit of detection.

26. What do we put on carcases and what do we take off? Lessons learned from the carcase baseline study 2016[1]

Background

This study came about because, in the USA, the Food Safety and Inspection Service (FSIS) did a baseline survey of beef and veal carcases. At time of writing the FSIS baseline report has not been published to the public.

They sponged large areas of hind- and forequarters at two points along the processing chain:

1. Immediately after hide pulling
2. At the end of the slaughter floor - after all operations, including interventions, had been done

The Australian industry undertook their own survey to provide comparable data. During 2015 and 2016, sponge samples were collected from 24 beef and 4 veal establishments, and 5296 beef and 156 veal samples tested.

SARDI received a number of follow-up enquiries and it was decided to include case studies from the baseline study in this edition of the Processors' Guide, plus some individual analysis. Some establishments, which contributed a handful of samples, haven't been included because there aren't enough data to analyse.

A word of warning about trying to compare analysis from the Baseline study with ESAM data – they are different in many key ways because the Baseline study:

- Used "hot" carcases while ESAM uses chilled (except for hot boning plants)
- Sampled on the slaughter floor while bodies were moving, compared with chiller sampling as in ESAM
- Sponged large areas (4000cm^2) compared with 100cm^2 sites in ESAM

A number of plants in the survey have a unit operation which they have shown to be lethal for target pathogens such as Shiga Toxic *E. coli* (STEC) or *Salmonella* e.g. washing in hot water or in an organic acid.

Such operations are termed interventions and while they are not Critical Control Points (CCPs) in that they do not prevent, eliminate or reduce target

[1] https://www.mla.com.au/download/finalreports?itemId=3428

pathogens to an acceptable level, they are likely to reduce the likelihood of detecting a target pathogen.

Other operations such as the AusMeat trim and steam vacuuming have been shown to be effective in removing visible contaminants (hair, dirt) but are not able to reliably reduce the bacterial population to an appreciable extent.

In this section, we present data for each plant which describe the Total Viable Count (TVC) concentration and the *E. coli* prevalence at two stages during processing:

- As soon after hide removal as practicable at the particular establishment
- After all slaughter floor operations have been concluded.

Note that sampling points both after hide removal and before the carcase leaves the slaughter floor may vary between establishments because of the practicality of taking the sample safely and without impeding the unit operations of the processing line.

In each case, we compare a specific establishment against data which describe all establishments using the same post-hide removal unit operations e.g. each plant with a hot water system is compared with all other hot water plants combined.

Note that when comparing total counts, most microbiologists do not consider counts to be different unless they are at least 0.5 log different; microbiological counting is not a precise discipline.

We have arranged establishments into four categories according to the degree of intervention following the AUS-MEAT trim:

1. AUS-MEAT trim only
2. AUS-MEAT trim plus hot water wash
3. AUS-MEAT trim plus lactic acid wash
4. AUS-MEAT trim plus steam vacuum

As well as comparing on a plant-by-plant basis, we have made a comparison between the effectiveness of the AUS-MEAT trim alone (category 1), and augmenting this with hot water treatment (category 2). As there was only one plant which was placed in each of the steam vacuum and the lactic acid treatment categories, we haven't made comparisons between these and categories 1 and 2.

Trimming versus Trimming plus Hot Water

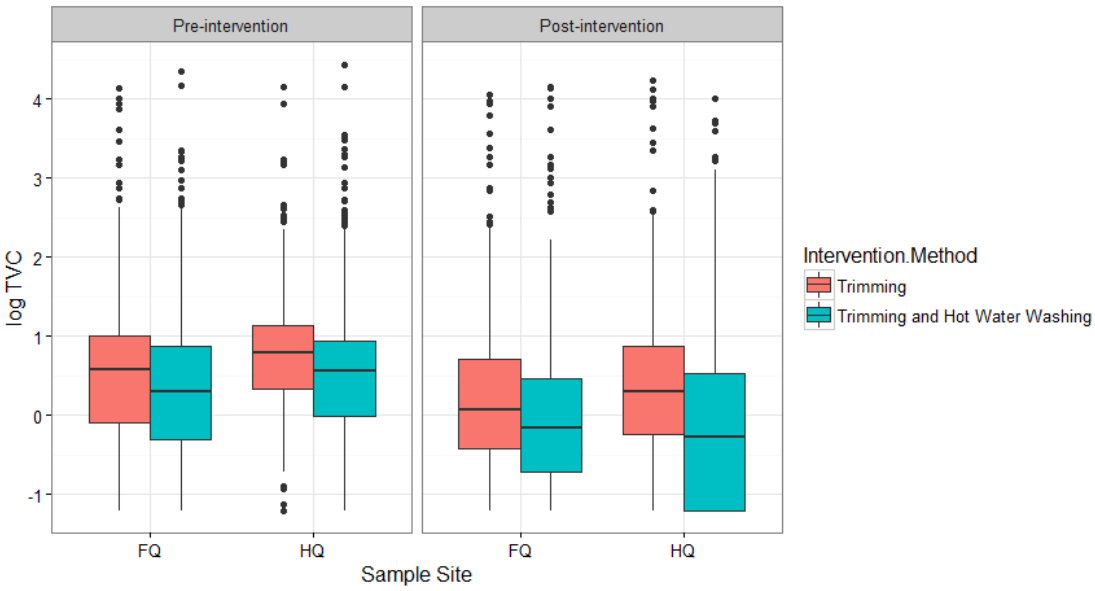
Hot water washes have become more widely used in Australia in recent years, with numerous plants utilising hot water decontamination in addition to standard AusMeat trimming. The Baseline survey provides data which answer the question: *how much improvement in carcase hygiene do we get from washing them in hot water?*

To answer this question, we have summarised the data from the two systems: AusMeat trim alone (Category 1), and augmented by hot water washing (Category 2).

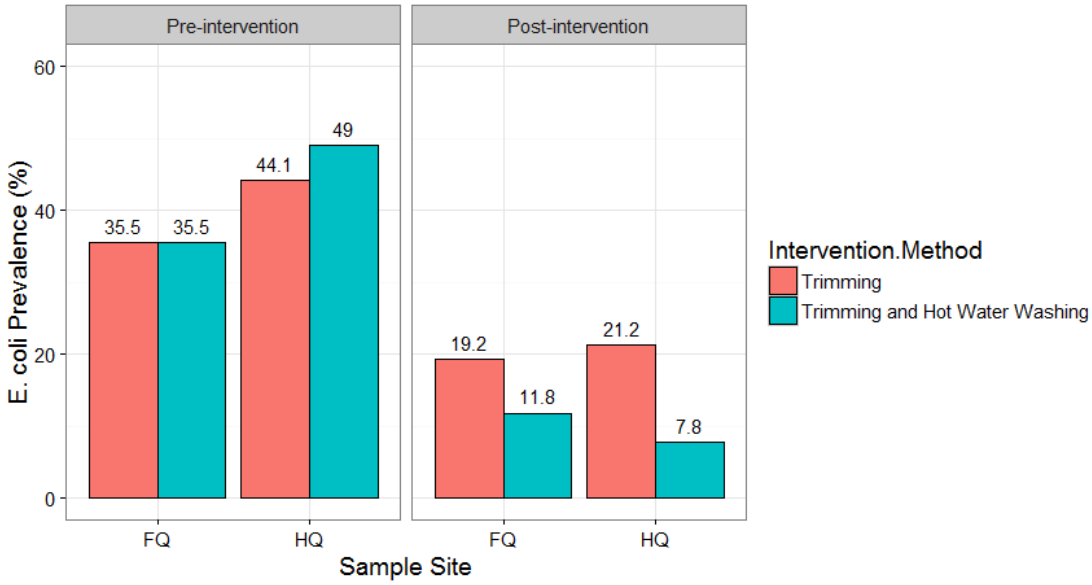
For category 1 plants, a total of 1211 samples were taken immediately after hide removal (606 at the FQ and 605 at the HQ) and 1225 samples after trimming (611 at the FQ and 614 at the HQ).

For category 2 plants, a total of 1219 samples were taken immediately after hide removal (609 at the FQ and 610 at the HQ) and 1225 samples after trimming and hot water treatment (613 at the FQ and 612 at the HQ).

Unsurprisingly, there was little difference between total bacterial loadings of Category 1 and 2 plants immediately after hide removal, though Category 2 plants had slightly lower loadings. There were small reductions in TVCs for the two categories after trimming and after trimming plus hot water treatment with Category 2 plants slightly lower.



The prevalence of *E. coli* was similar at both Category 1 and 2 plants immediately after hide removal and, while trimming alone led to significant reductions in prevalence at both FQ and HQ, augmenting trimming with hot water treatment resulted in much greater reduction in prevalence.



SARDI comments: Clearly, as a group, plants using a hot water wash gain real benefit in reduction of E. coli prevalence, particularly at the HQ. However, a closer analysis of all plants in the survey indicates that some which do not have hot water treatment have lower E. coli prevalence on their carcases than those which do (Table 1).

Table 1: Summary of forequarter E. coli prevalence of beef carcases as they leave the slaughter floor at different slaughter establishments.

Establishment	*E. coli* prevalence	Category
Plant G	0/13 (0%)	Trim
Plant F	0/7 (0%)	Trim
Plant I	1/21 (4.8%)	Trim + hot water
Plant B	2/35 (5.7%)	Trim
Plant L	8/105 (7.6%)	Trim + hot water
Plant N	1/13 (7.7%)	Trim + hot water
Plant M	7/88 (8%)	Trim + hot water
Plant C	6/71 (8.5%)	Trim
Plant A	6/68 (8.8%)	Trim
Plant Q	8/90 (8.9%)	Trim + steam vacuum
Plant K	10/108 (9.3%)	Trim + hot water
Plant P	5/52 (9.6%)	Trim + lactic acid
Plant H	9/88 (10.2%)	Trim
Plant J	12/106 (11.3%)	Trim + hot water
Plant E	12/93 (12.9%)	Trim
Plant D	15/62 (24.2%)	Trim
Plant O	22/54 (40.7%)	Trim + hot water

In considering the relative performance results from Plants F, N and G, these should be treated with caution given they contributed only 33 post treatment

forequarter samples to the study. As well, unusual results were observed at Plant O and it is speculated that the sponge bags may have been mislabelled (see later).

However, Plants A, B and C had *E. coli* prevalence at the FQ comparable with a number of plants with a hot water intervention. It is known that Plant A undertakes deep trimming of all cutting lines but it may be worthwhile investigating the unit operations at Plants B and C, which also led to relatively low *E. coli* prevalence.

As indicated from Table 2, at the hindquarter, Category 2 (hot water) plants appear to perform better, with the addition of the hot water wash to trimming reducing the post treatment *E. coli* prevalence over that of Category 1 (trim only) plants.

Again, the small sample sizes from Plants F, N and G should be taken into account when considering these results.

Table 2: Summary of hindquarter *E. coli* prevalence of beef carcases as they leave the slaughter floor at different slaughter establishments.

Establishment	*E. coli* prevalence	Category
Plant G	0/13 (0%)	Trim
Plant M	2/86 (2.3%)	Trim + hot water
Plant K	3/103 (2.9%)	Trim + hot water
Plant I	1/29 (3.4%)	Trim + hot water
Plant P	2/52 (3.8%)	Trim + lactic acid
Plant Q	6/92 (6.5%)	Trim + steam vacuum
Plant C	5/71 (7%)	Trim
Plant L	11/105 (10.5%)	Trim + hot water
Plant B	4/35 (11.4%)	Trim
Plant J	13/105 (12.4%)	Trim + hot water
Plant F	2/16 (12.5%)	Trim
Plant A	10/66 (15.2%)	Trim
Plant E	14/92 (15.2%)	Trim
Plant H	14/88 (15.9%)	Trim
Plant O	10/54 (18.5%)	Trim + hot water
Plant D	19/62 (30.6%)	Trim
Plant N	4/13 (30.8%)	Trim + hot water

This publication contains an investigation (#20: *Hot water treatment of carcases – how effective is it?*), a Plant Initiated Project (PIP), which is a comprehensive examination of what happens in hot water washing. In summary, hot water treatment was most effective at the hindquarter and loin and, after chilling, the highest TVCs are obtained at the brisket and neck. Importantly, the study showed that hot water treatment was effective in reducing *E. coli* to below the limit of detection except at the bung.

Regarding the analysis presented here, you may be interested in how your establishment was placed in Table 1 and 2 (above), contact SARDI for more information from page 4.

Category 1: Trimming to AUS-MEAT specifications

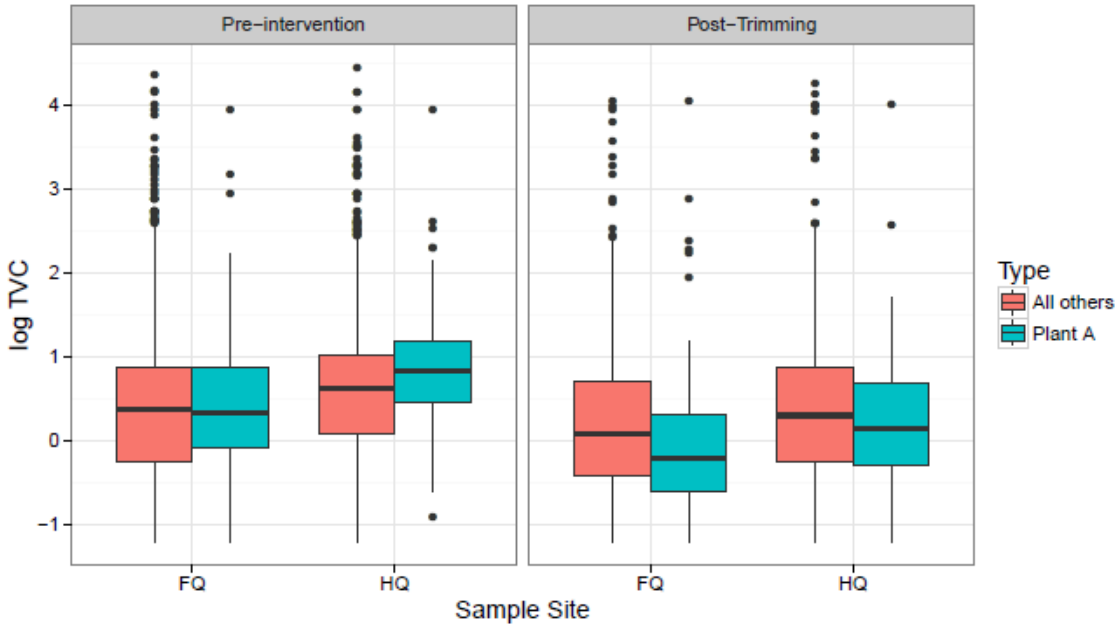
When organs, appendages, excess fat and tissue surrounding the Halal cut are excised as part of standard trim, it is thought that their removal may also reduce the population of surface bacteria on the carcase.

In the Baseline survey, seven plants which trim to AUS-MEAT specifications, plus one plant which deep trims all selvedge, provided sufficient samples to support an analysis.

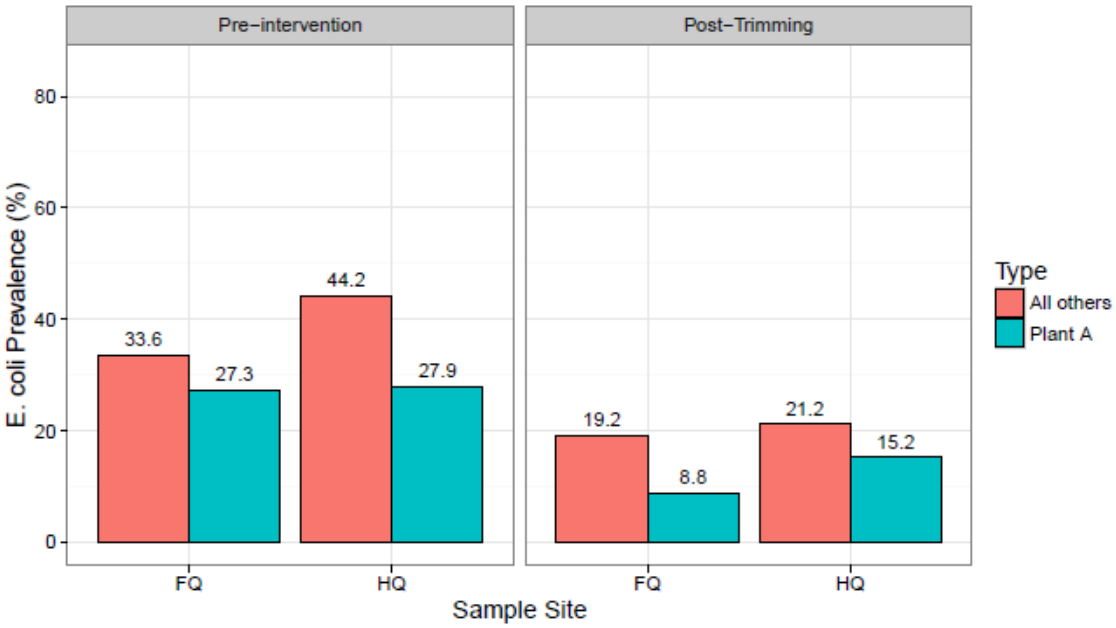
Plant A

At Plant A, a total of 134 samples were taken immediately after hide removal (66 from the forequarter (FQ) and 68 from the hindquarter (HQ)); after trimming, a further 134 samples were taken (68 from the FQ and 66 from the HQ).

The total bacterial loading at Plant A was similar to that of other plants in this group before trimming, and slightly lower after trimming, at both the FQ and HQ.



Immediately after hide removal, *E. coli* prevalence was lower at Plant A compared with the other seven plants in the group, particularly at the HQ. After trimming, *E. coli* prevalence was much lower at both FQ and HQ, compared with its peers.

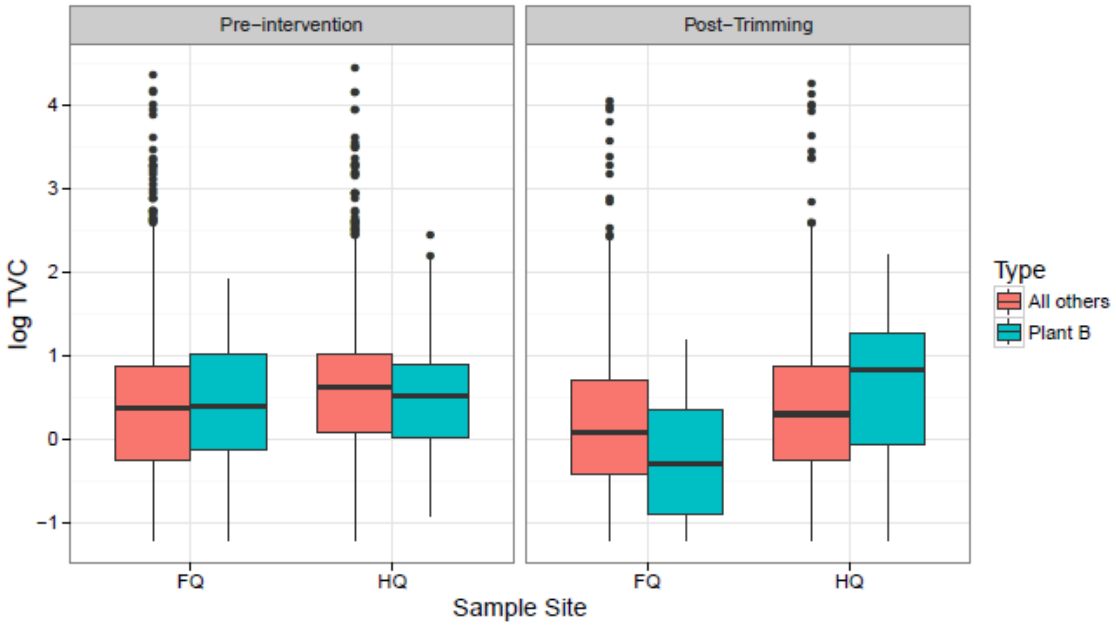


SARDI comments: It should be emphasised that Plant A is unique in this group, in that the selvedge is removed from all cutting lines by deep trimming. This results in E. coli prevalence similar to that from trimming plus hot water treatment at some plants.

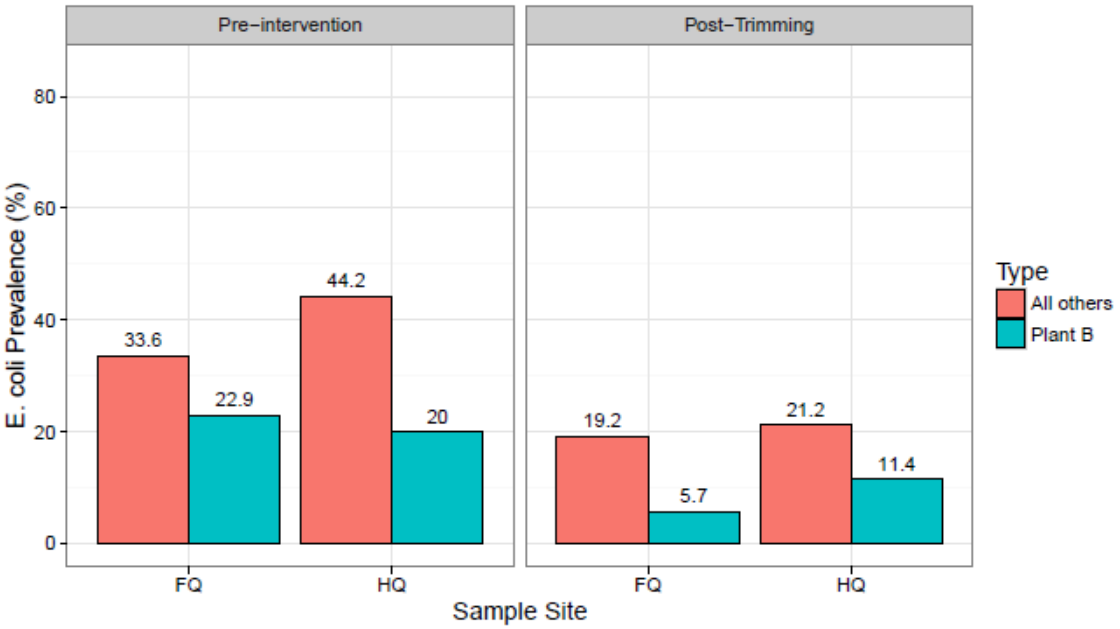
Plant B

At Plant B, a total of 70 samples were taken immediately after hide removal (35 from the FQ and 35 from the HQ); and a further 70 samples after trimming, 35 at each site.

The total bacterial loading at Plant B was similar to that of other plants in this group before trimming, then was reduced at the FQ and increased at the HQ after trimming; the same post-trim trend was similar compared with other plants in the group.



Immediately after hide removal, *E. coli* was much less prevalent at Plant B than at other plants in the group, particularly at the HQ, a difference which persisted after trimming.



SARDI comments: The E. coli prevalence on carcases leaving the slaughter floor at Plant B approximates that of the average for plants which also use a hot water wash.

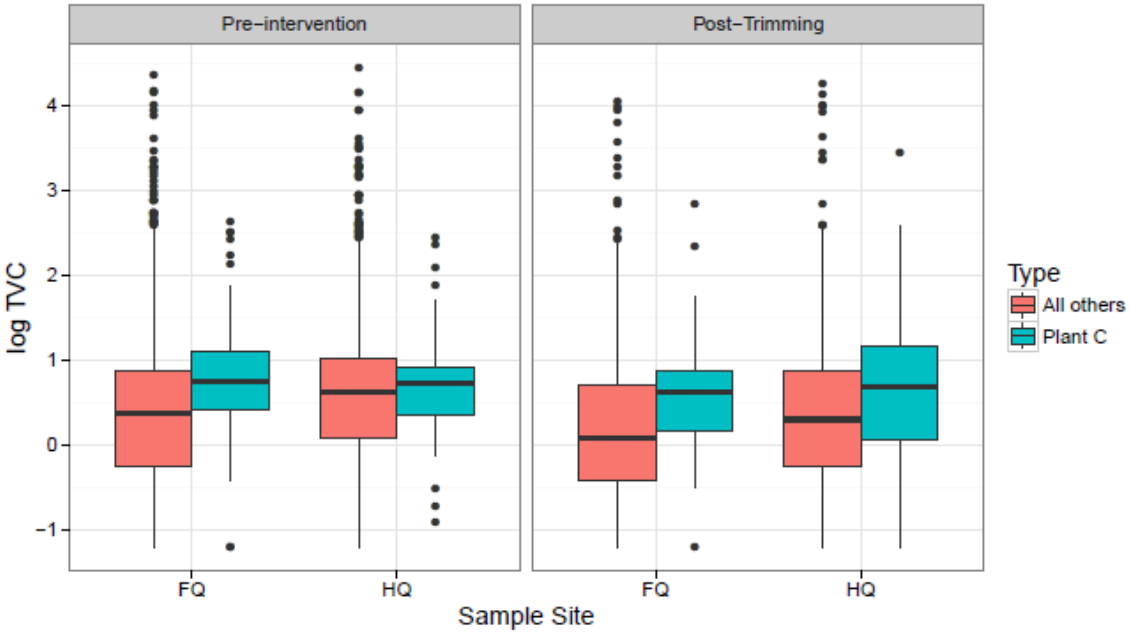
Plant C

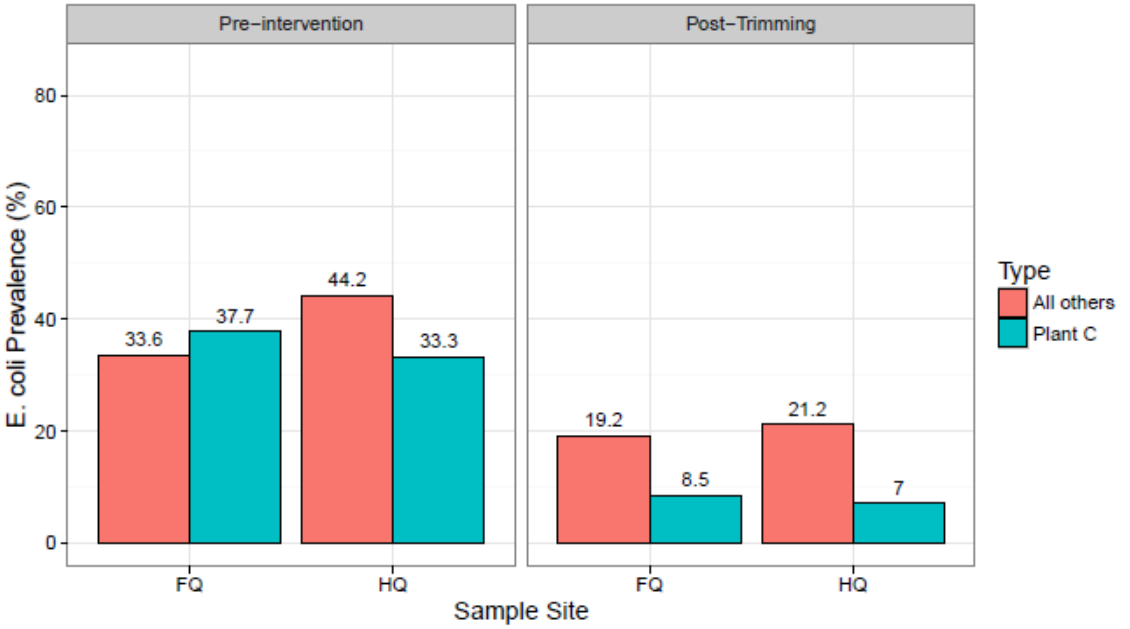
At Plant C, a total of 138 samples were taken immediately after hide removal (69 from the FQ and 69 from the HQ); after trimming, a further 142 samples were taken, 71 from each site.

The total bacterial loading at Plant C was higher than that of other plants in this group, both before and after trimming, with trimming having no effect on the TVC.

Immediately after hide removal, *E. coli* was much less prevalent at Plant C than at other plants in the group, particularly at the HQ, a difference which persisted after trimming.

SARDI comments: The E. coli prevalence on carcases leaving the slaughter floor at Plant C approximates that of the average for plants which also use a hot water wash in addition to trimming.

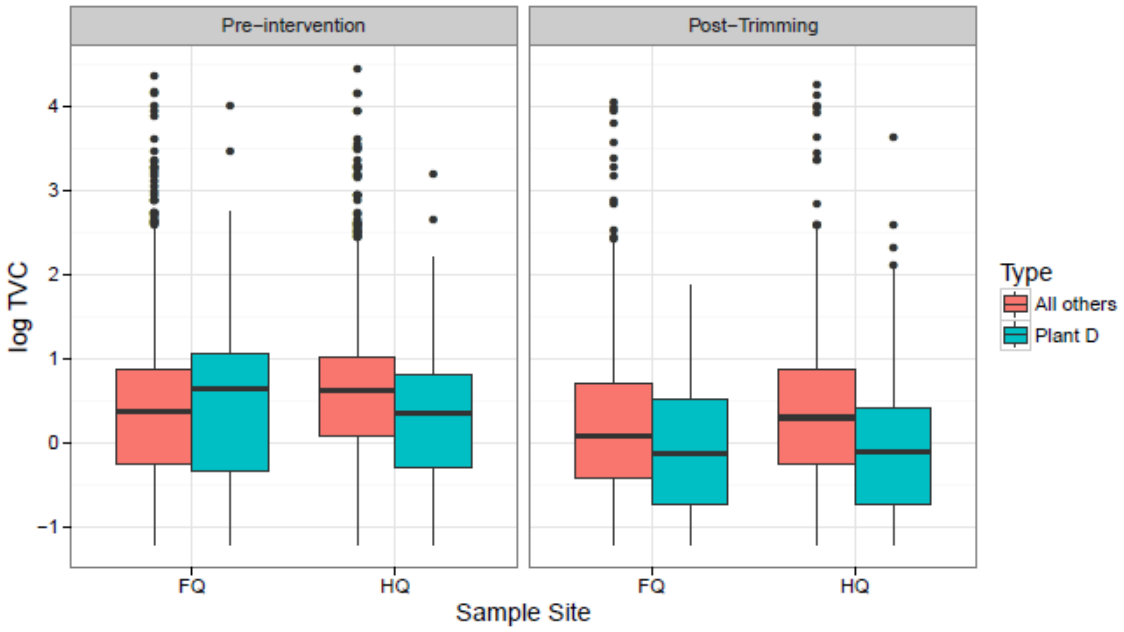




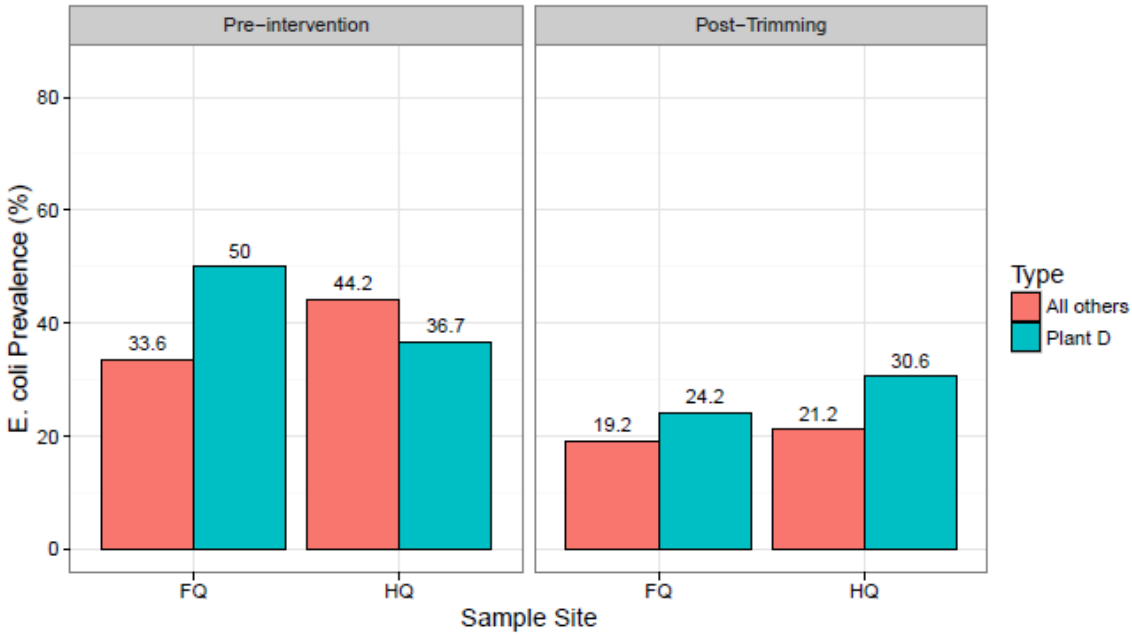
Plant D

At Plant D, a total of 120 samples were taken immediately after hide removal (60 from each of the FQ and the HQ); after trimming, a further 124 samples were taken (62 from each site).

The total bacterial loading at Plant D was similar to that of other plants in this group, both before trimming, but slightly lower after trimming at both FQ and HQ.



Immediately after hide removal, *E. coli* was highly prevalent, particularly at the FQ. After trimming, *E. coli* prevalence at Plant D was much higher at both FQ and HQ, compared with its peers.

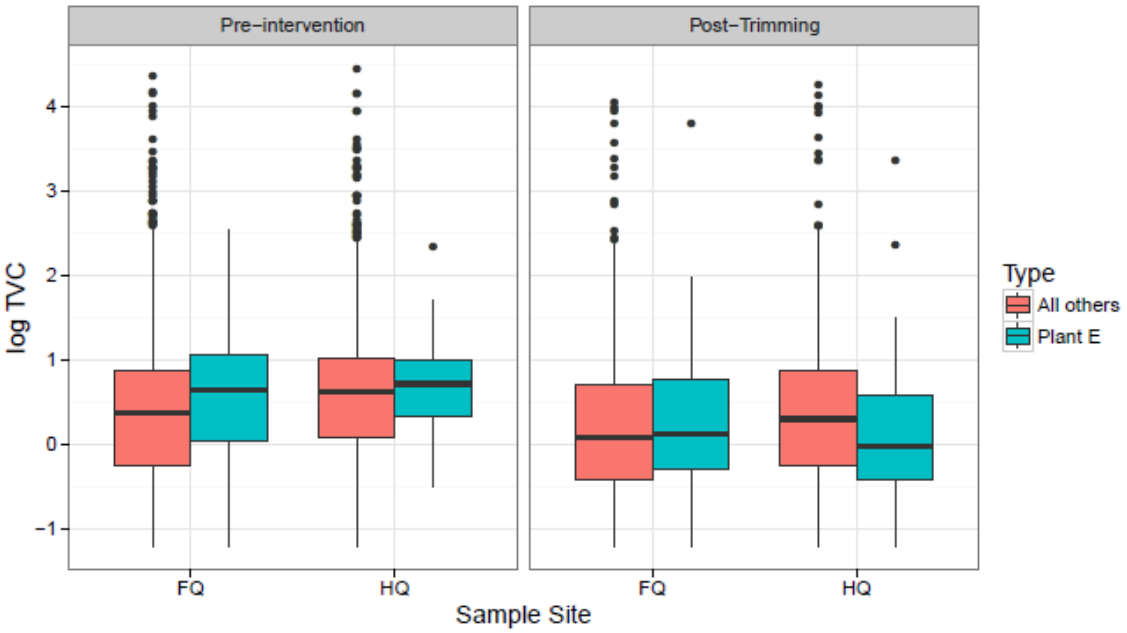


SARDI comments: Within this group of eight plants which either deep trim (Plant A) or trim to AusMeat specifications, Plant D is an outlier with E. coli prevalence much higher than its peers.

Plant E

At Plant E, a total of 88 samples were taken immediately after hide removal (44 from both FQ and HQ sites); after trimming, a further 92 samples were taken (46 from each site).

The total bacterial loading at Plant E was similar to that of other plants in this group, both before and after trimming.

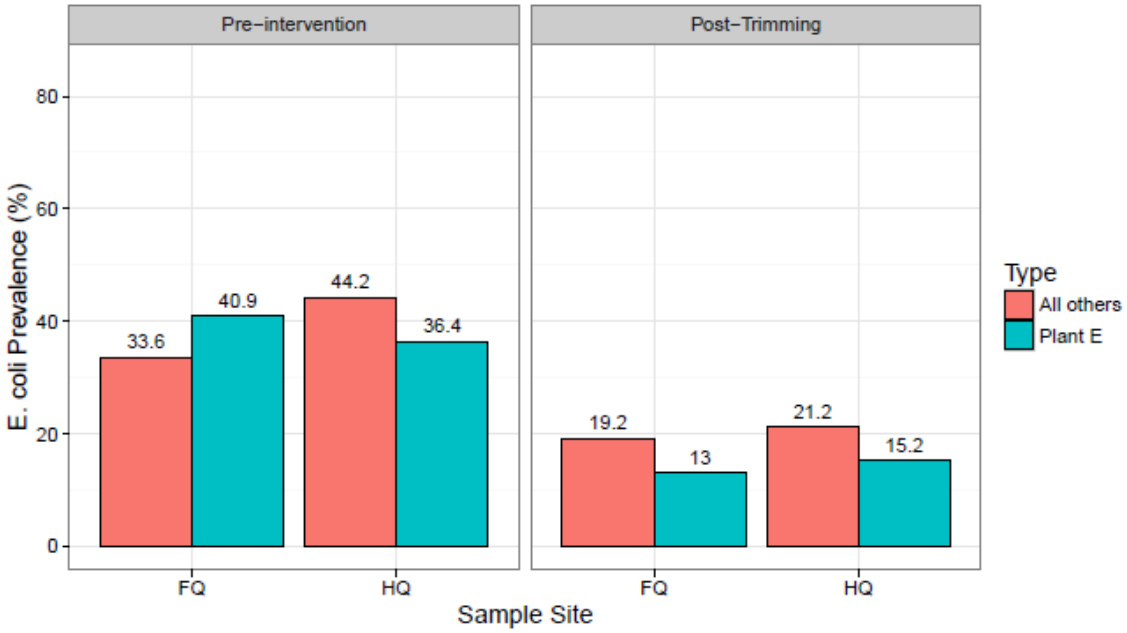


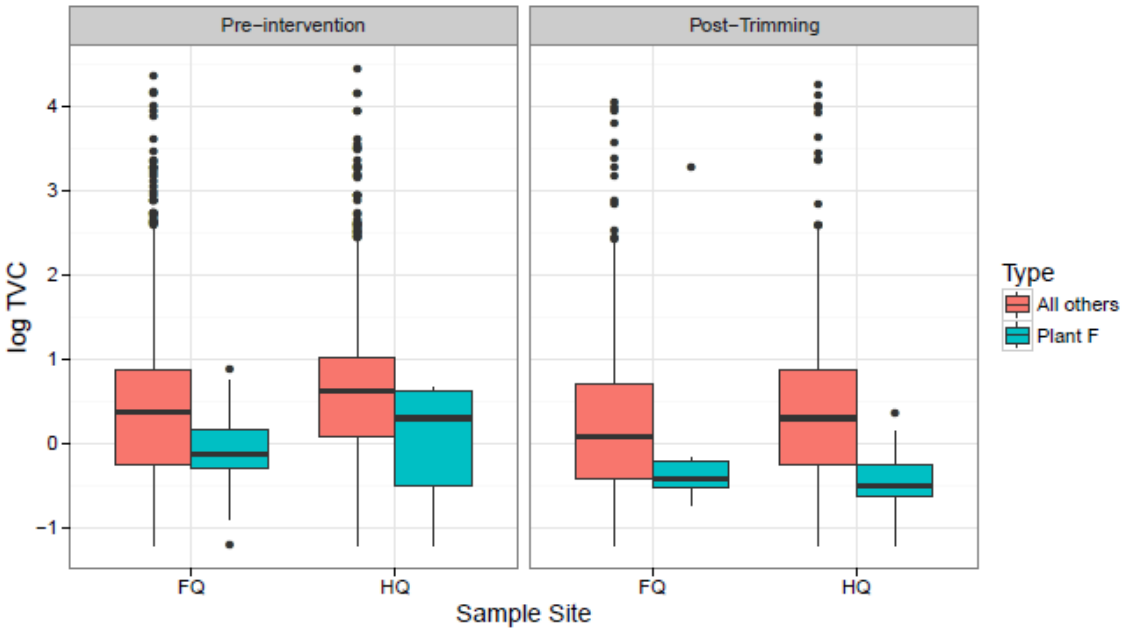
Immediately after hide removal, *E. coli* prevalence at Plant E was similar to other plants in the group and trimming resulted in slightly lower *E. coli* prevalence compared with its peers.

Plant F

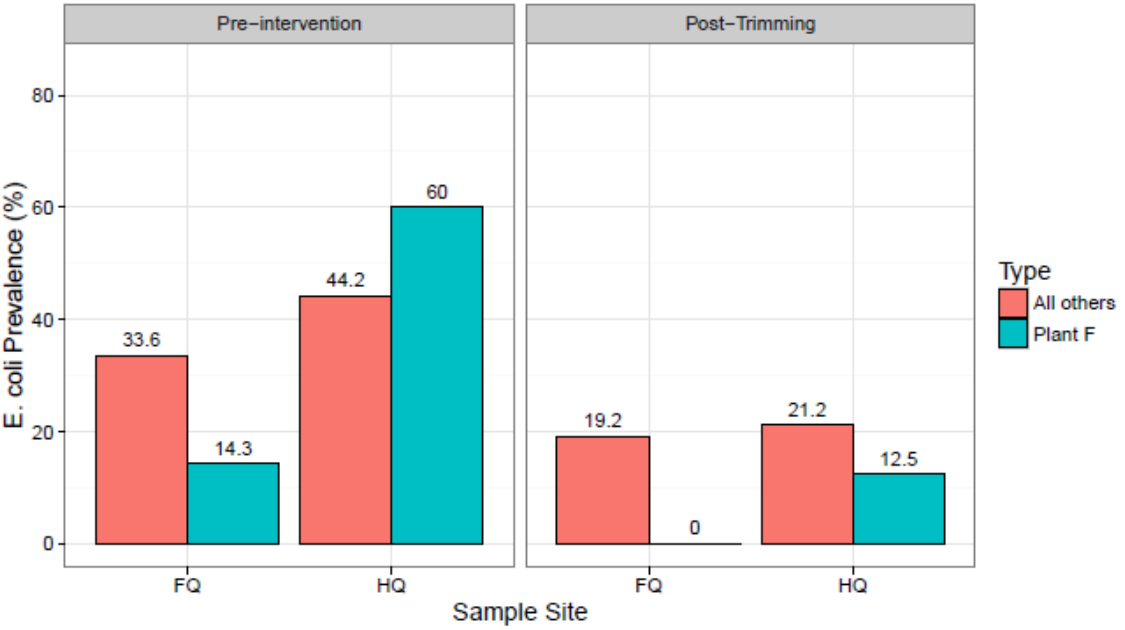
At Plant F, a total of 19 samples were taken immediately after hide removal (14 from the FQ and 5 from the HQ); after trimming, a further 23 samples were taken (7 from the FQ and 16 from the HQ).

The total bacterial loading at Plant F was lower than that of other plants in this group, both before and after trimming at both sites.





Immediately after hide removal, *E. coli* was much more prevalent at the HQ and much less prevalent at the FQ than at other plants in the group. After trimming, *E. coli* was not detected in FQ samples and prevalence was lower at the HQ.

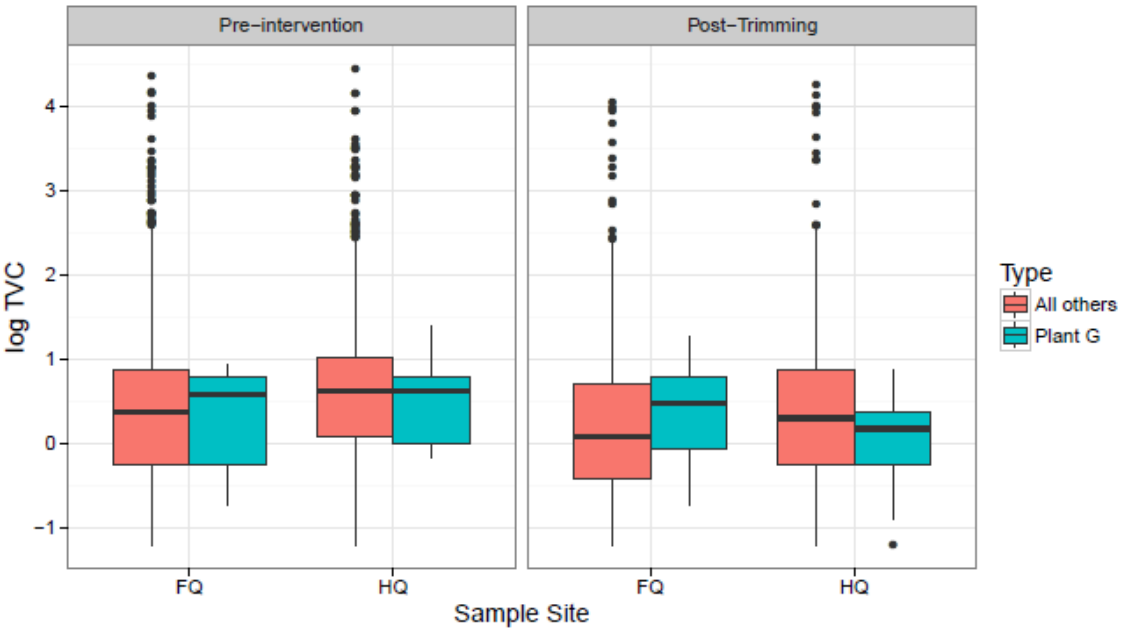


SARDI comments: Plant F contributed very few samples to the survey, which may account for extremes in E. coli prevalence.

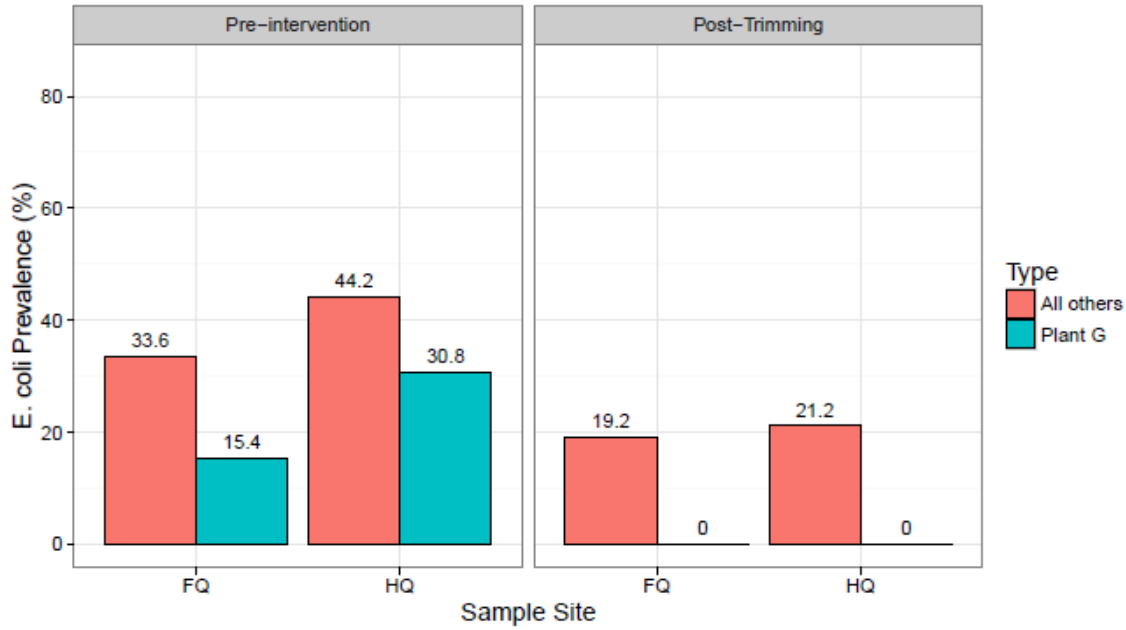
Plant G

At Plant G, a total of 52 samples were taken immediately after hide removal and after trimming (13 from the FQ and the HQ on both occasions).

The total bacterial loading at Plant G was similar to that of other plants in this group, both before and after trimming, with the latter reducing the loading only slightly at the HQ.



Immediately after hide removal, *E. coli* was much less prevalent at Plant G than other plants in the group. After trimming, *E. coli* was not isolated from any of 26 samples.

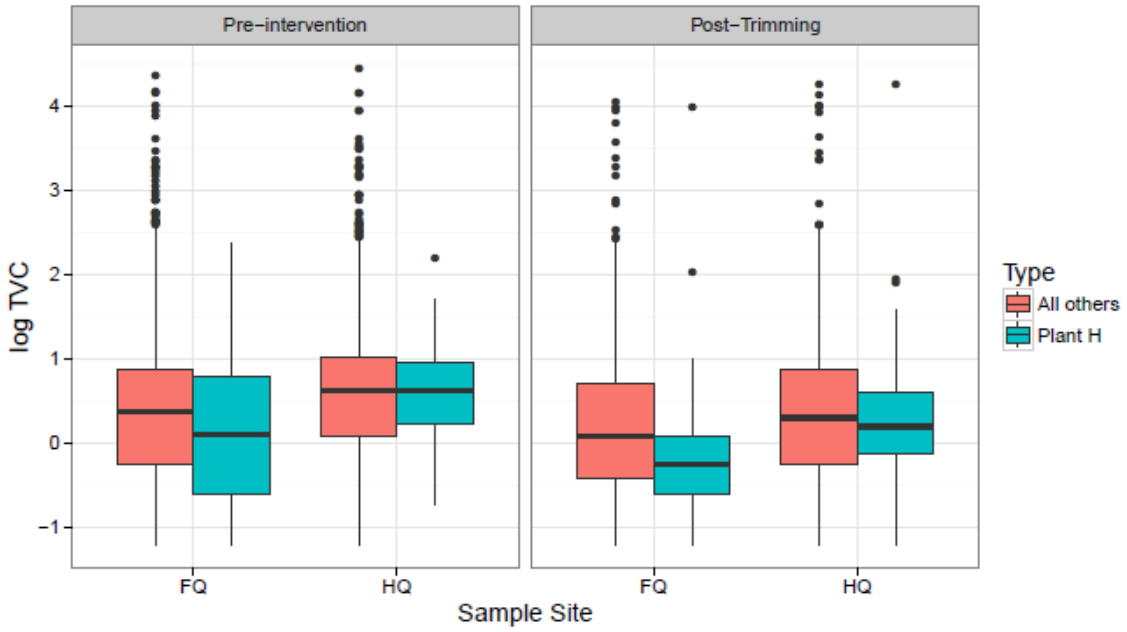


SARDI comments: Plant G contributed very few samples to the survey, but it is surprising that no E. coli was detected in any of the 26 post-trim samples.

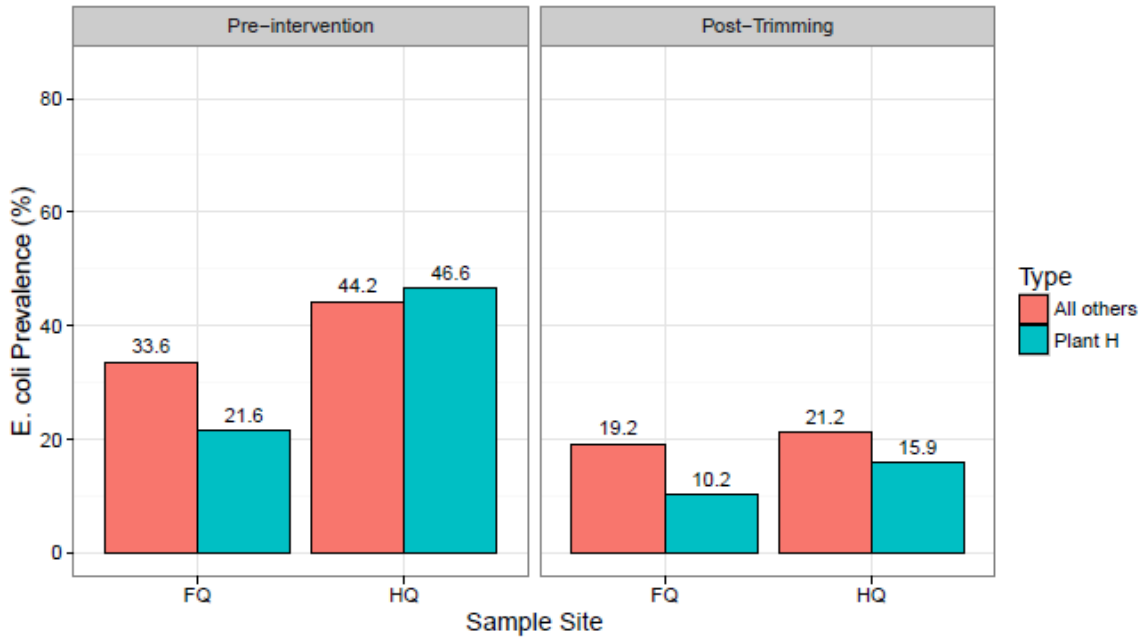
Plant H

At Plant H, a total of 352 samples were taken immediately after hide removal and after trimming (88 from the FQ and the HQ on both occasions).

The total bacterial loading at Plant H was similar to that of other plants in this group before trimming and slightly lower after trimming, both in terms of cfu/cm^2 and compared with its peers.



Immediately after hide removal, *E. coli* was of similar prevalence to other plants in the group and trimming resulted in *E. coli* prevalence lower than its peers, both at the FQ and HQ.



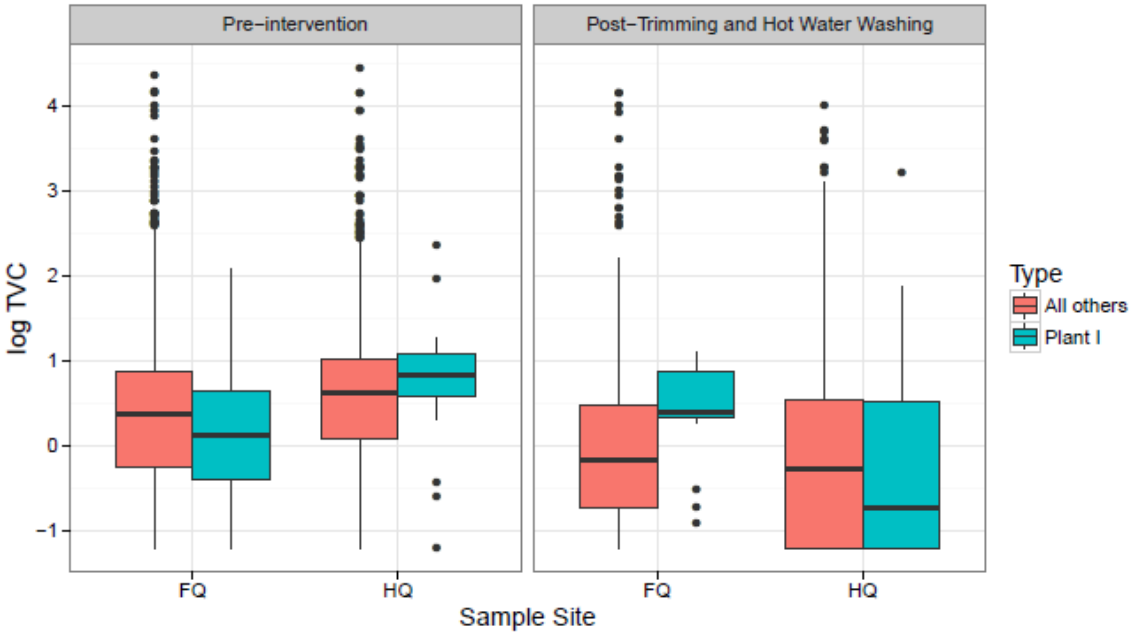
Category 2: AUS-MEAT trim plus hot water wash

Seven establishments which participated in the Baseline survey had hot water cabinets at the end of the processing line and the effect of this intervention is presented on a plant-by-plant basis.

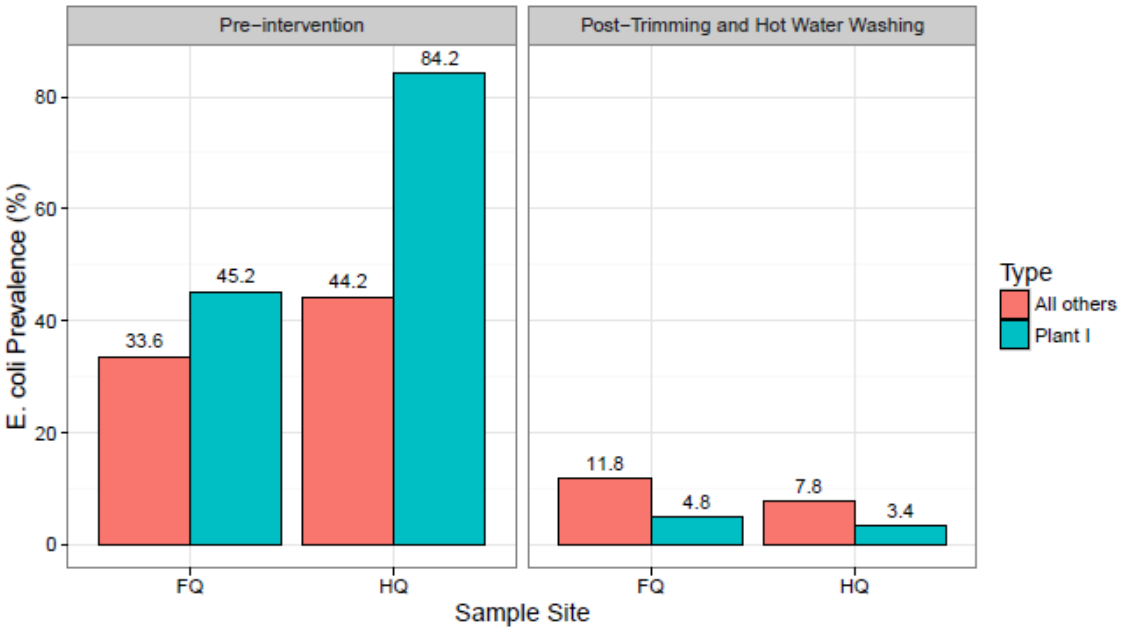
Plant I

At Plant I, a total of 50 samples were taken immediately after hide removal (31 from the FQ and 19 from the HQ); after trimming and hot water treatment, a further 50 samples were taken (21 from the FQ and 29 from the HQ).

The total bacterial loading at Plant I was similar to that of all plants in this group after hide removal, being higher at the HQ. Trimming and hot water treatment reduced the TVC by approximately 1 log (90%) at the HQ, but at the FQ, processing did not reduce the TVC, probably because bacteria were washed to the lower part of the carcase.



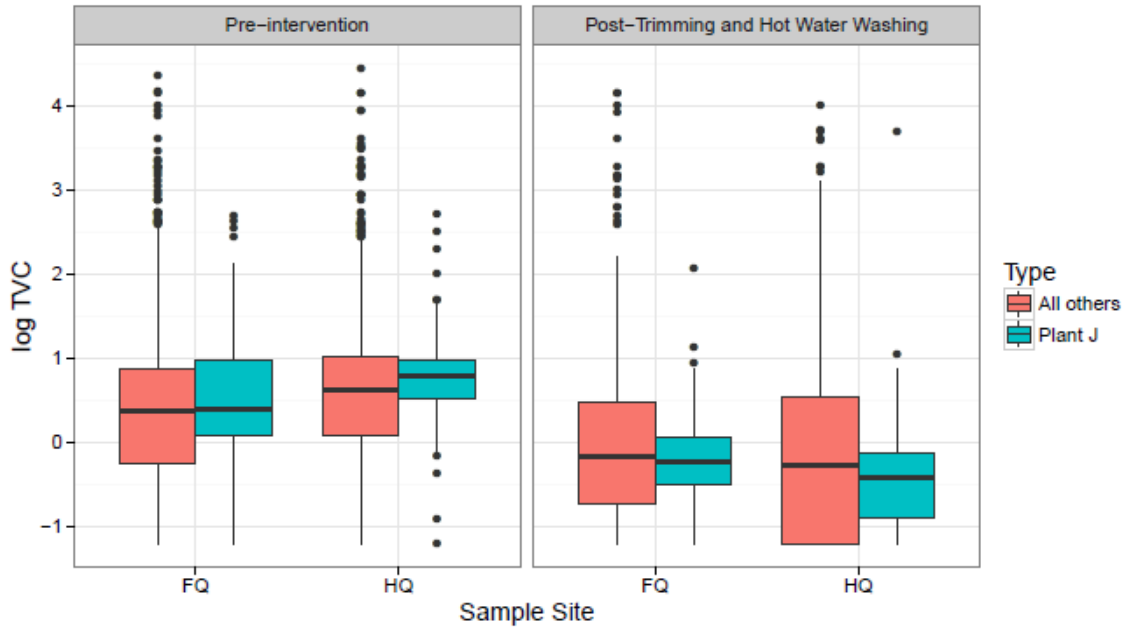
Immediately after hide removal, *E. coli* was more prevalent at Plant I than other plants in the group, particularly at the HQ. After trimming and hot water treatment, however, *E. coli* prevalence at Plant I was lower at both FQ and HQ, compared with its peers.



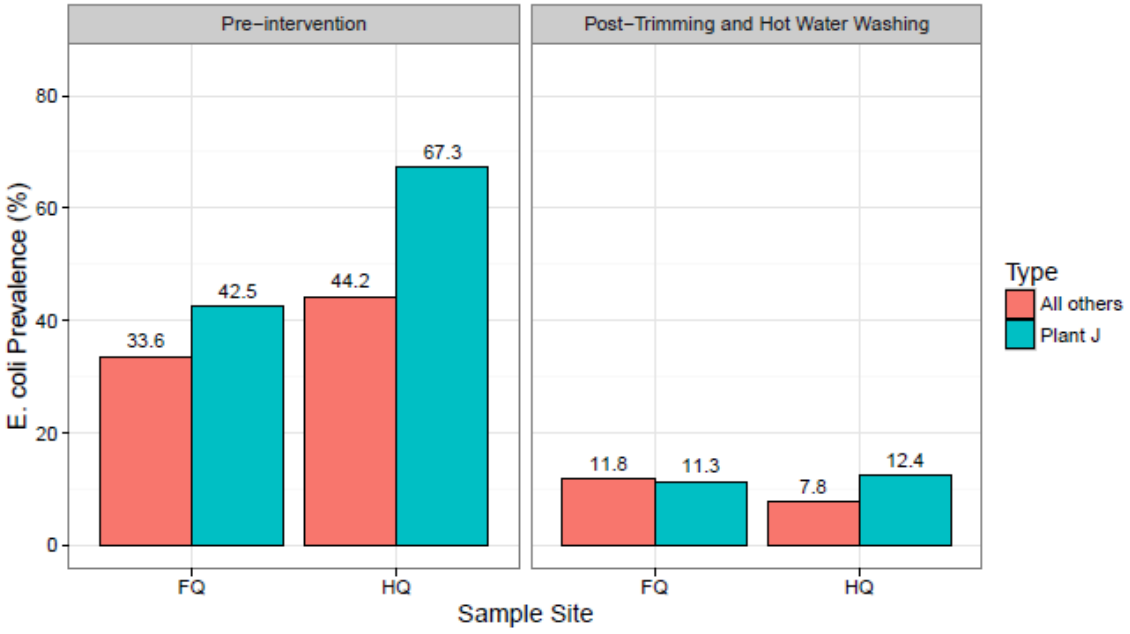
Plant J

At Plant J, a total of 213 samples were taken immediately after hide removal (106 from the FQ and 107 from the HQ); after trimming and hot water treatment, a further 213 samples were taken (106 from the FQ and 107 from the HQ).

The total bacterial loading at Plant J was similar to that of other plants in this group, both before and after trimming plus hot water washing. At the HQ, trimming and hot water treatment reduced the TVC by more than 1 log (>90%) and at the FQ by around 0.5 log.



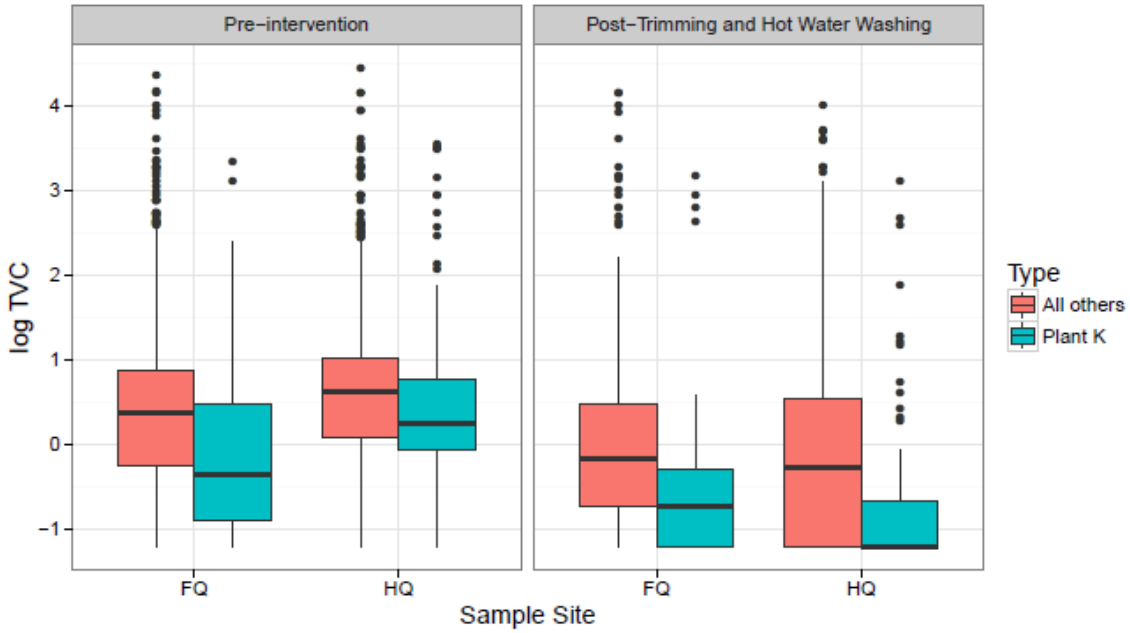
Immediately after hide removal, *E. coli* was more prevalent at Plant J than other plants in the group, particularly at the HQ. Trimming and hot water treatment reduced *E. coli* prevalence to a level similar to its peers.



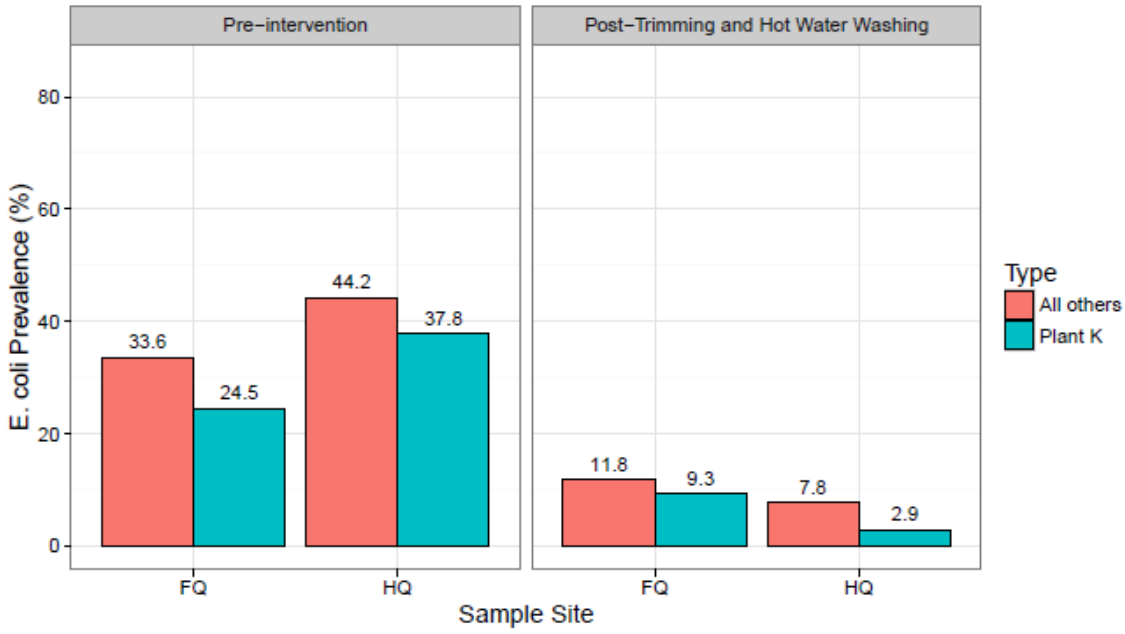
Plant K

At Plant K, a total of 213 samples were taken immediately after hide removal (102 from the FQ and 111 from the HQ); after trimming and hot water treatment, a further 211 samples were taken (108 from the FQ and 103 from the HQ).

The total bacterial loading at Plant K was lower than that of other plants in this group immediately after hide removal. Trimming plus hot water washing further reduced the TVC at the HQ by around >1 log (>90%), though only slightly at the FQ.



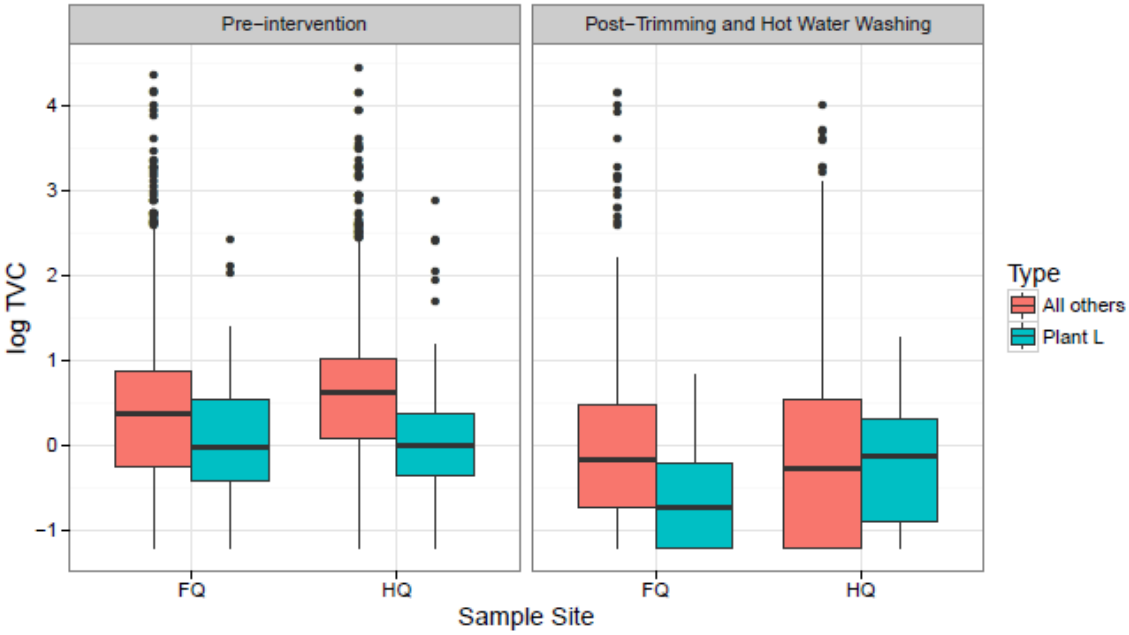
Immediately after hide removal, *E. coli* was less prevalent at Plant K than other plants in the group, with further reductions after trimming and hot water treatment, making *E. coli* prevalence at Plant K lower at both FQ and HQ, compared with its peers.



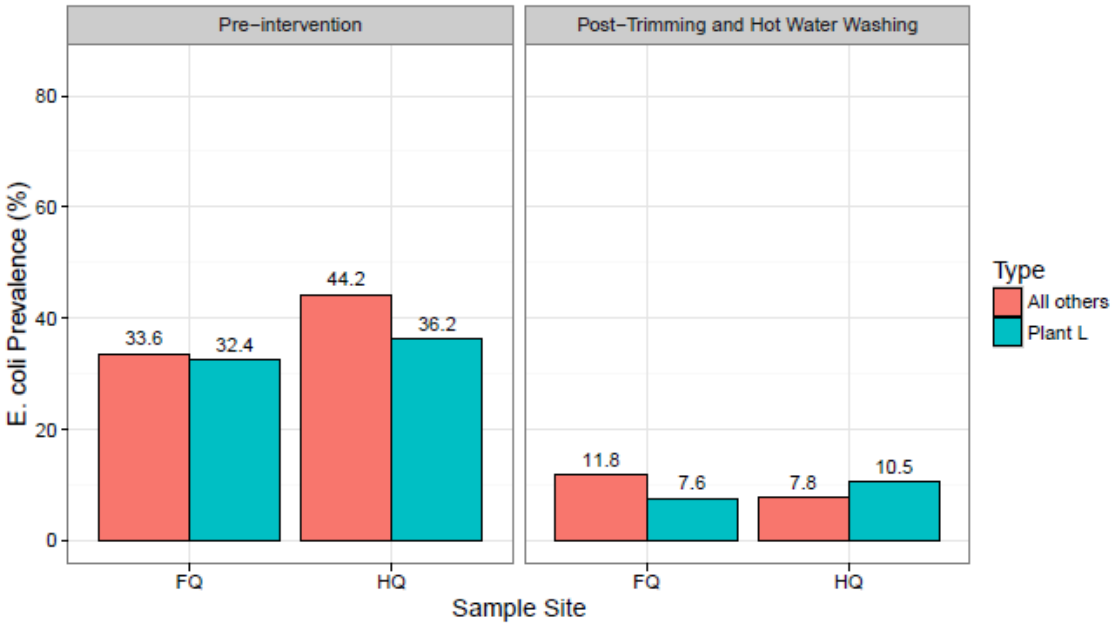
Plant L

At Plant L, a total of 210 samples were taken immediately after hide removal and after trimming and hot water treatment, with 105 samples from both FQ and HQ on both occasions.

The total bacterial loading at Plant L was lower compared with that of other plants in this group immediately after hide removal. After trimming and hot water treatment, the TVC was reduced by around 0.5 log at the FQ but not at the HQ.



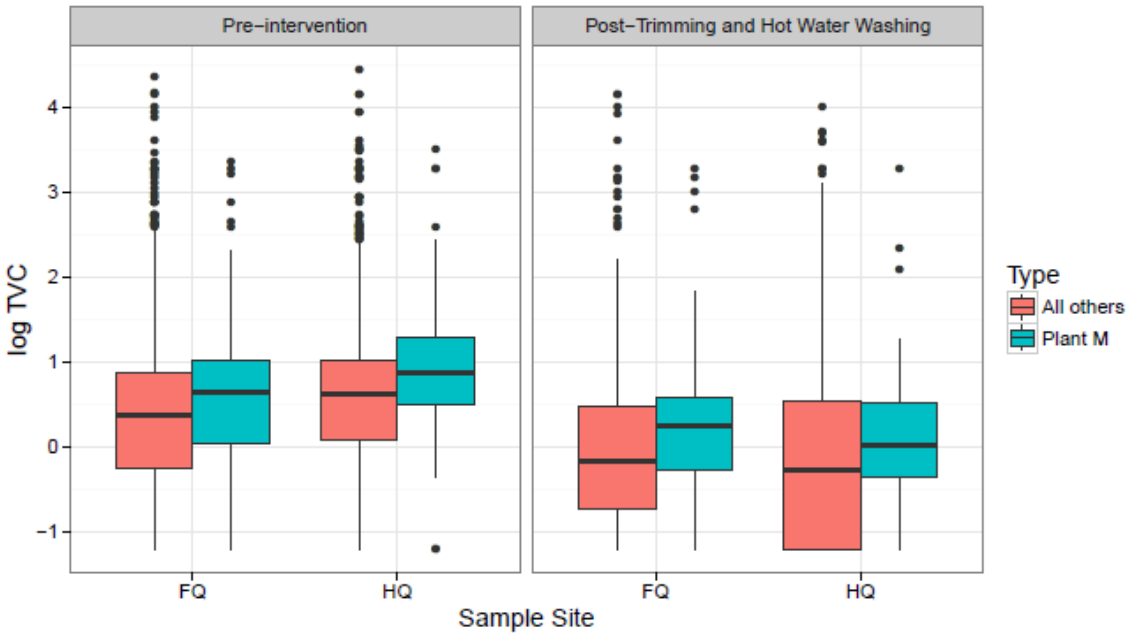
Prevalence of *E. coli* was similar at Plant L to other plants in the group both after hide removal and after trimming and hot water treatment.



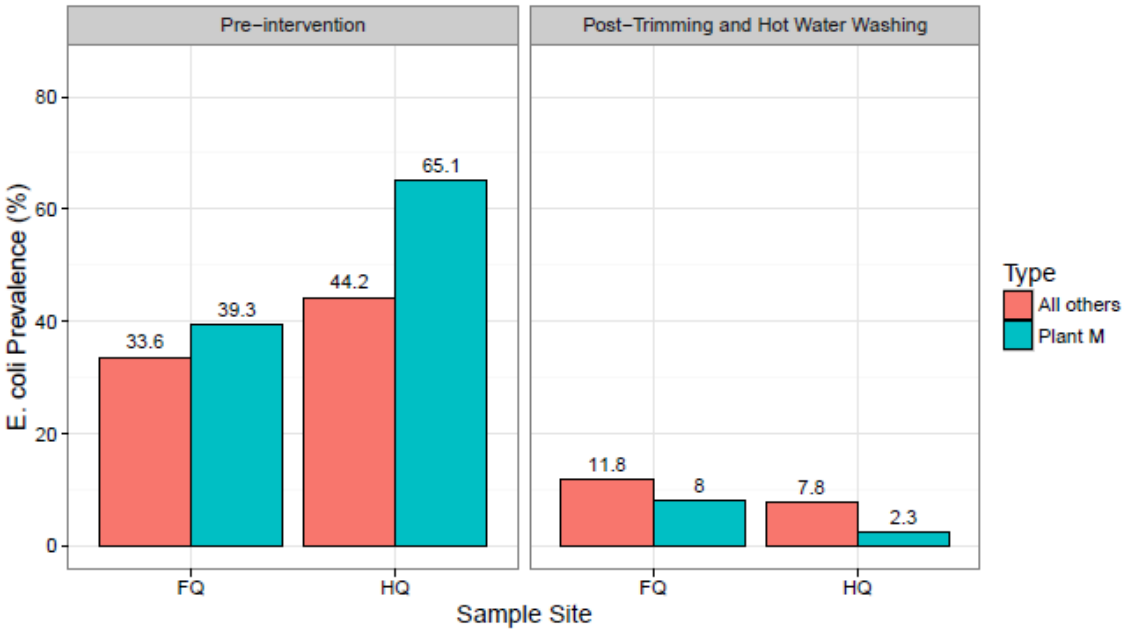
Plant M

At Plant M, a total of 170 samples were taken immediately after hide removal (84 from the FQ and 86 from the HQ); after trimming and hot water treatment, a further 174 samples were taken (88 from the FQ and 86 from the HQ).

The total bacterial loading at Plant M was slightly higher than those of other plants in this group, both at hide removal and after trimming plus hot water washing. Although there were slight reductions in TVC after trimming and hot water treatment, the TVC was higher than that of other plants, both at the FQ and HQ.



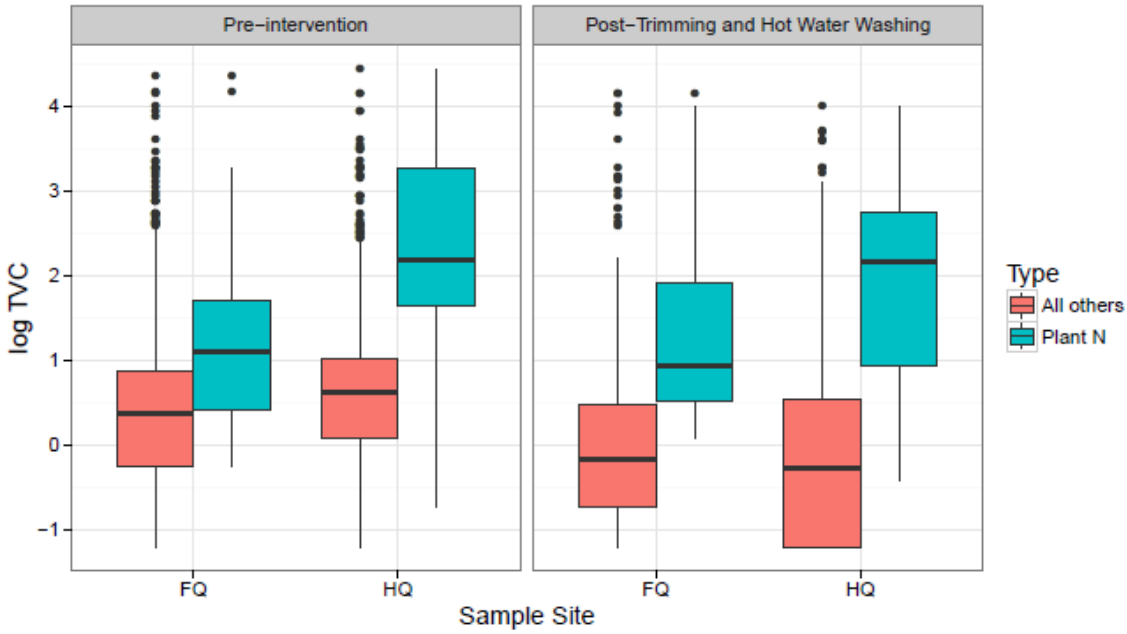
Immediately after hide removal, *E. coli* was more prevalent at Plant M than other plants in the group, particularly at the HQ. After trimming and hot water treatment, however, *E. coli* prevalence at Plant M was lower at both FQ and HQ, compared with its peers.



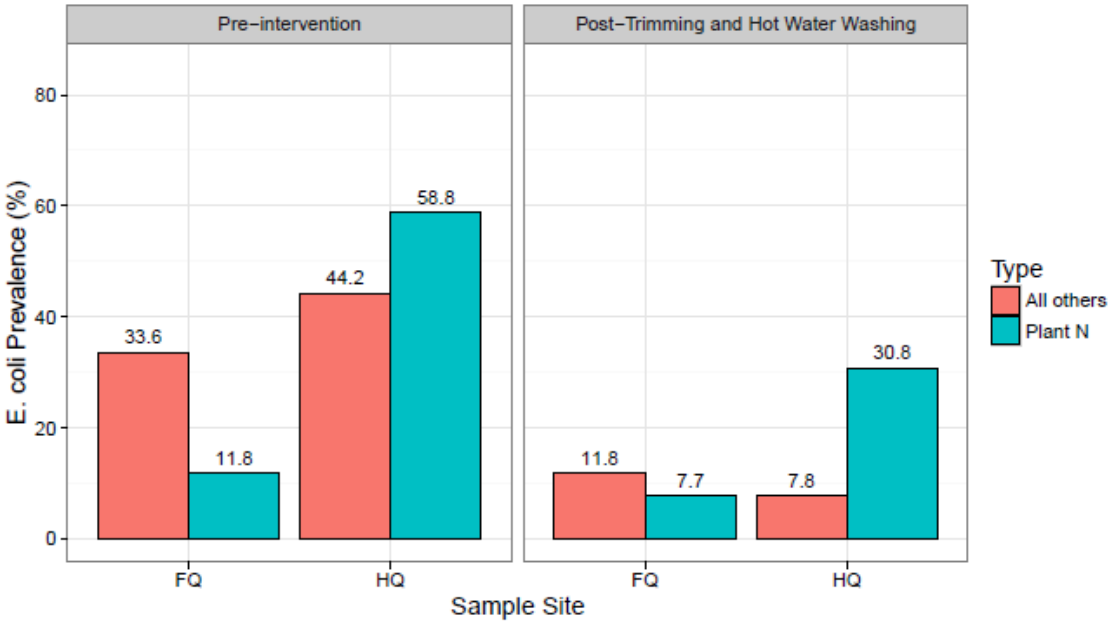
Plant N

At Plant N, a total of 34 samples were taken immediately after hide removal (17 from both FQ and HQ); after trimming and hot water treatment, a further 26 samples were taken (13 from the FQ and 13 from the HQ).

The total bacterial loading at Plant N was much higher than those of other plants in this group, both before and after trimming. Trimming and hot water treatment had little impact on the TVC.



At Plant N immediately after hide removal, *E. coli* was much less prevalent at the FQ and higher at the HQ compared with other plants in the group. After trimming and hot water treatment, *E. coli* prevalence was reduced at both FQ (slightly) and HQ compared to pre-intervention, although contamination at the HQ site was still much higher compared with other plants.

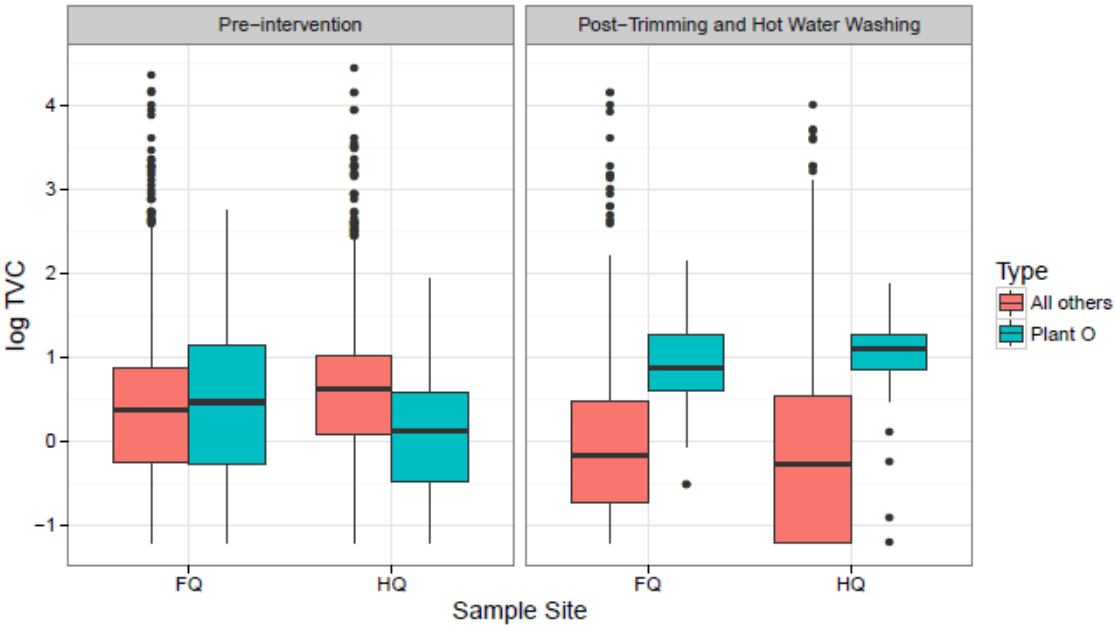


SARDI comments: TVCs are >2 log cfu/cm^2 higher at the HQ at Plant N than the mean for other plants in the group, and trimming and hot water results in no reduction. Prevalence of E. coli is also much higher at the HQ.

Plant O

At plant O, a total of 108 samples were taken immediately after hide removal and after trimming and hot water treatment (54 from FQ and HQ at each occasion).

The total bacterial loading at Plant O was similar to that of other plants in this group at the FQ and slightly lower at the HQ immediately after hide removal. After trimming plus hot water washing, the TVC increased slightly at the FQ and by around 1 log (90%) at the HQ.

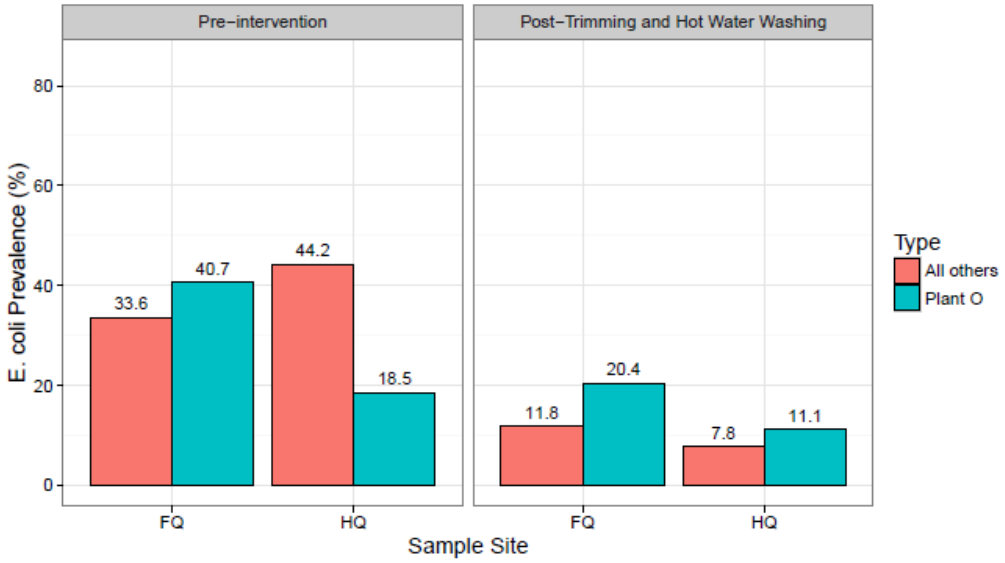


Immediately after hide removal, *E. coli* was less prevalent at Plant O than other plants in the group, particularly at the HQ. After trimming and hot water treatment, however, *E. coli* prevalence at Plant O was higher at both FQ and HQ, compared with its peers.

SARDI comments: There is obviously something not correct with the data for Plant O. The TVC at both FQ and HQ increased significantly after trimming and hot water treatment, and the latter operations did not have reductions of E. coli similar to those of other plants in this group.

In all, there were 216 samples supplied by Plant O and we speculate that perhaps sponge bags were mislabelled on some occasions.

If samples were correctly labelled, the effectiveness of the hot water treatment at Plant O comes into question. This shows the importance of correct labelling.

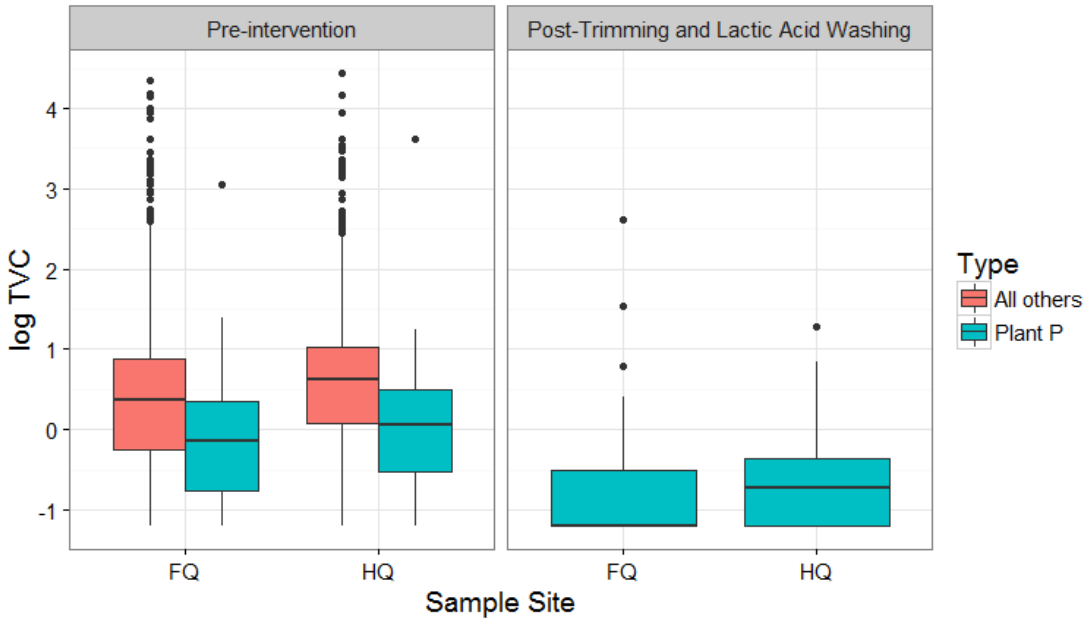


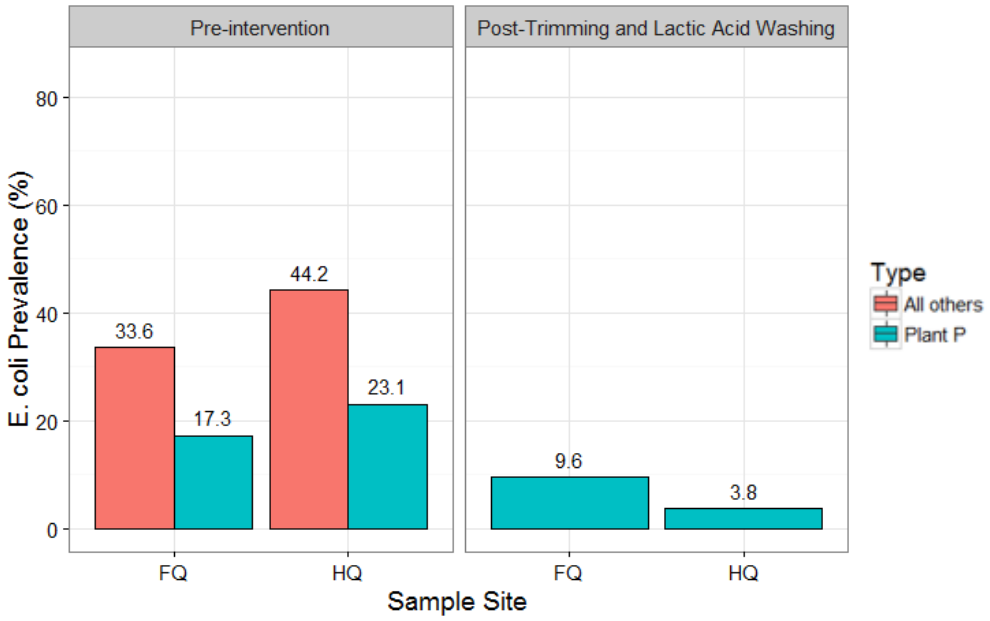
Category 3: AUS-MEAT trim plus lactic acid wash

Only one plant surveyed used lactic acid on a regular basis (Plant P), where a total of 208 samples were taken immediately after hide removal and after trimming and hot water treatment (52 from each of the FQ and HQ at each occasion).

The total bacterial loading at Plant P was lower than that of all other plants at both the FQ and the HQ immediately after hide removal. After trimming plus lactic acid treatment, the TVC decreased at the FQ by around 1 log (90%) and >0.5 log at the HQ.

Plant P





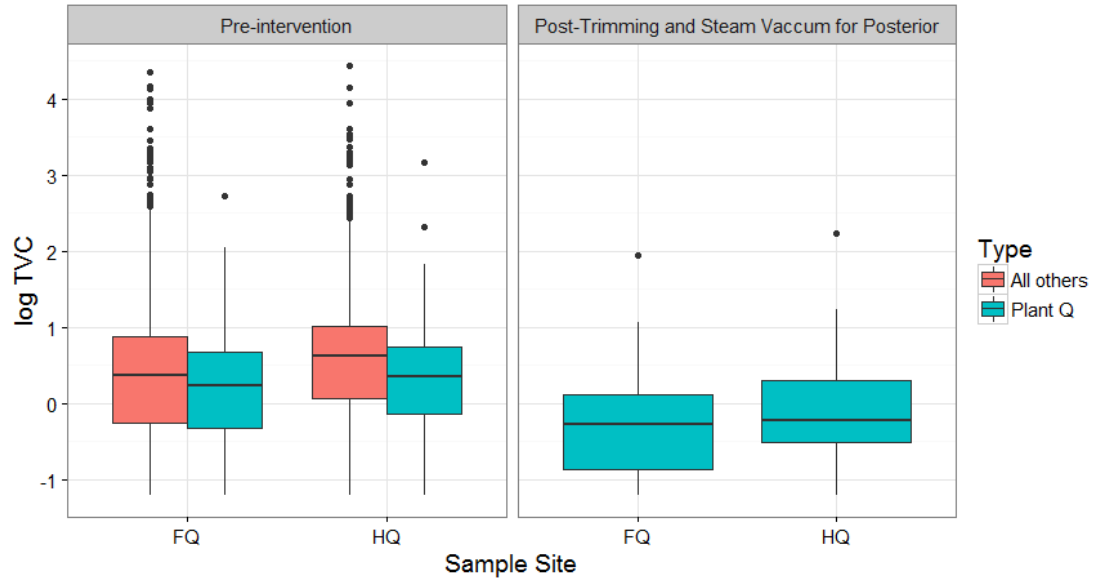
Immediately after hide removal, *E. coli* was less prevalent at Plant P than all other plants in the survey, both at the FQ and the HQ. After trimming and lactic acid treatment, there were significant reductions in prevalence at both sites.

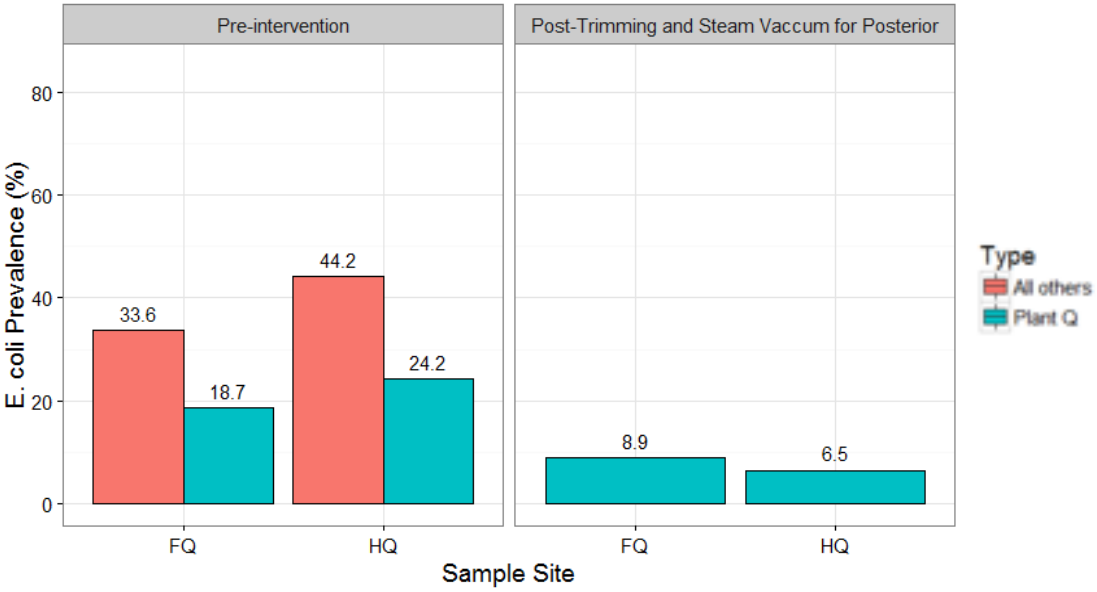
SARDI comments: Spraying carcases with lactic acid proved effective at the HQ, with E. coli being isolated from 3.8% of carcases though less so at the FQ where prevalence was 9.6%. The antimicrobial effects of lactic acid are well documented and it is widely used in the USA processing industry.

Category 4: AUS-MEAT trim plus steam vacuum

In the survey, only one plant (Plant Q) was assigned the category of trimming plus steam vacuum treatment, where a total of 182 samples were taken immediately after hide removal (91 at each of the FQ and HQ) and 182 after trimming and steam vacuum treatment (90 from the FQ and 92 from the HQ).

The total bacterial loading at Plant Q was similar to that of all other plants in the survey at both the FQ and the HQ immediately after hide removal. After trimming plus steam vacuum treatment, the TVC decreased slightly at both sites.





Plant Q

Immediately after hide removal, *E. coli* was less prevalent at Plant Q than other plants in the survey and there were further reductions after trimming and steam vacuum treatment at both FQ and HQ.

SARDI comments: *Steam vacuum was more successful at the HQ (E. coli 6.5%) than at the FQ (8.9%). It should be noted that steam vacuuming has been shown to be effective in removing visible contaminants (hair, dirt, etc.) but is not able to reliably reduce the bacterial population to an appreciable extent because of the relatively short time steam is in contact with the surface. Gill and Baker (1998)[1] comment that while vacuum cleaning may be useful in removing visible contamination "The matter of improving the microbial condition of carcases must be addressed by other means."*

[1] Gill, C. & Baker, L. 1998. Trimming, vacuum cleaning or hot water-vacuum cleaning effects of lamb hind saddles. Journal of Muscle Foods 9: 391-401

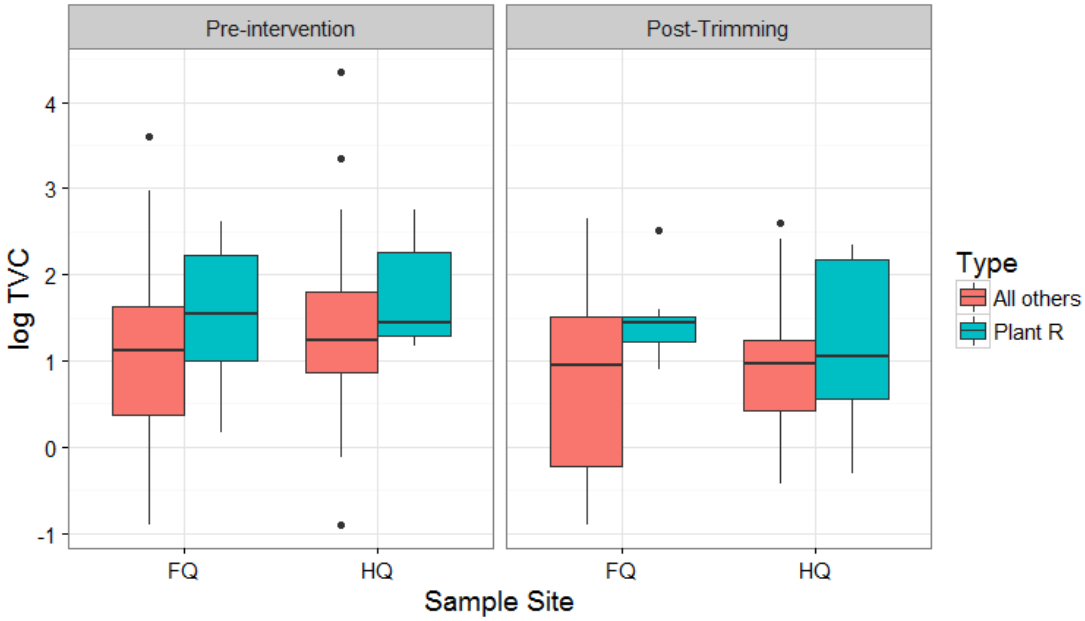
The veal baseline survey

During the Baseline survey, there were 156 samples of veal submitted of which 64 samples from two plants were made available for inclusion in this section for the Processors' Guide.

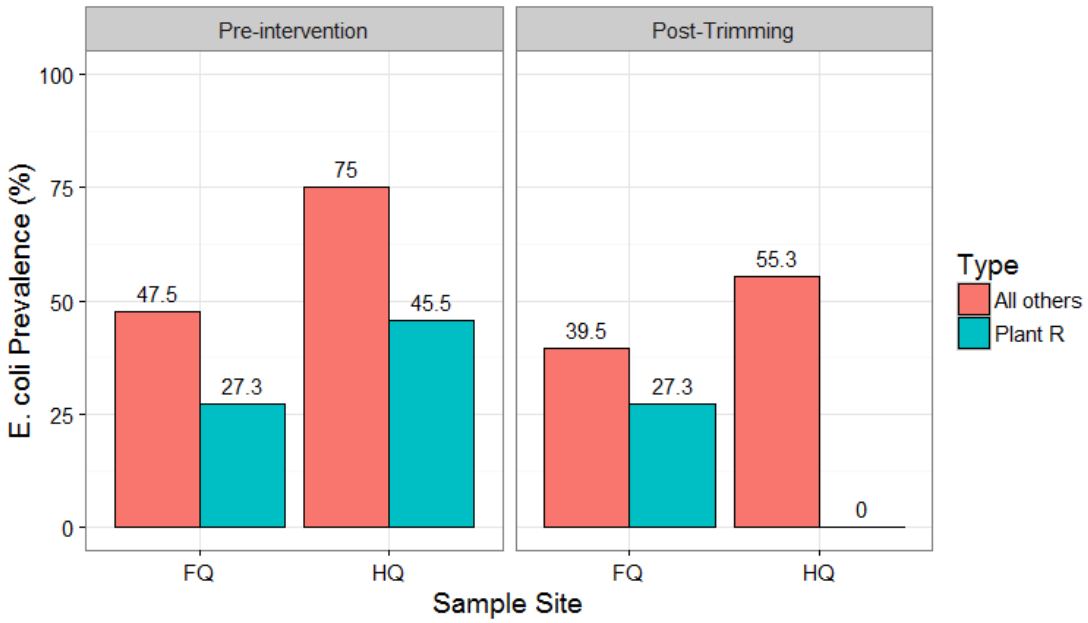
Plant R

At Plant R, a total of 22 samples were taken immediately after hide removal and after trimming (11 from both FQ and HQ on both occasions).

The total bacterial loading at Plant R was slightly higher than those of the other plant in this group (Plant S), both before and after trimming, which had little impact on the TVC.



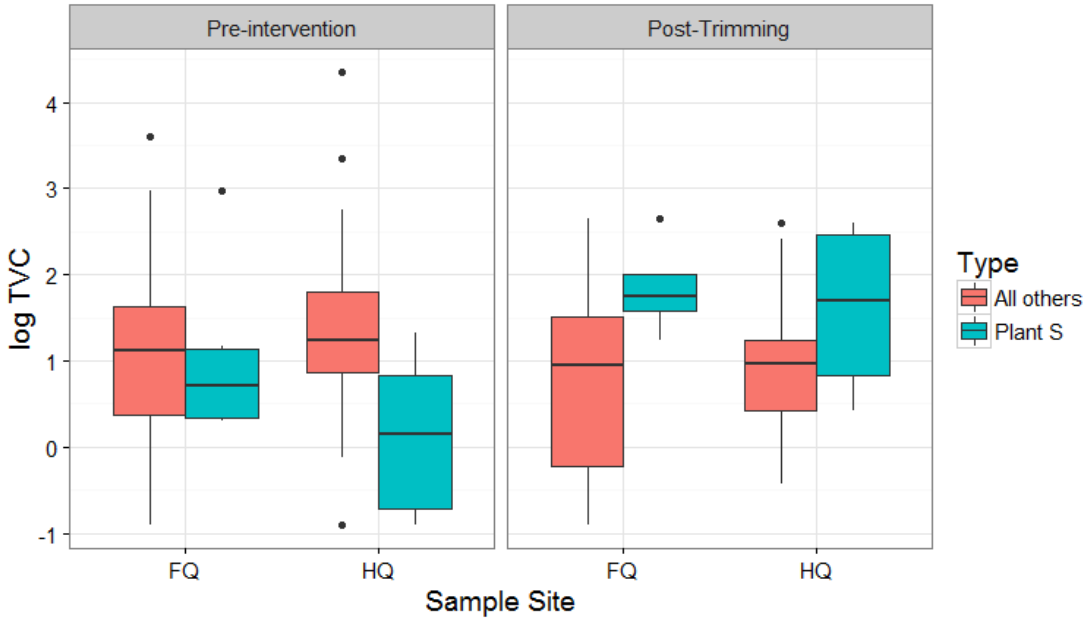
At Plant R immediately after hide removal, *E. coli* was much less prevalent at the FQ and HQ sites compared with Plant S. After processing, *E. coli* prevalence was not reduced at the FQ but was not isolated from the HQ.



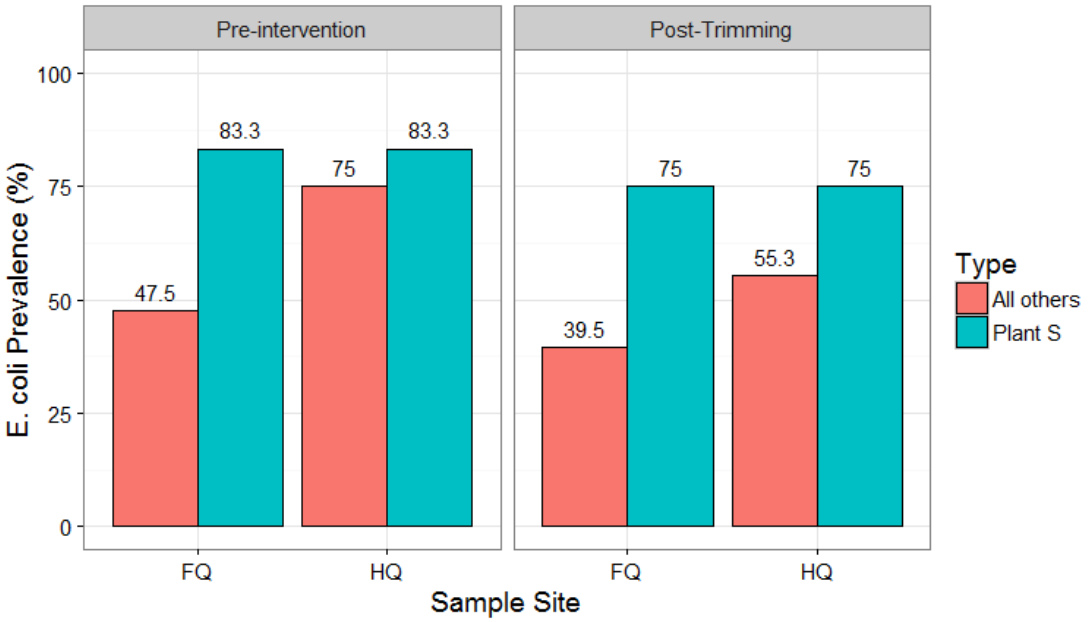
Plant S

At plant S, a total of 12 samples were taken immediately after hide removal (6 from each of the FQ and HQ); after trimming and hot water treatment, a further 8 samples were taken (4 from the FQ and 4 from the HQ).

The total bacterial loading at Plant S was higher than that at Plant R after hide removal, especially at the HQ. However after processing, the TVCs at both FQ and HQ were lower than those at Plant R.



At Plant S immediately after hide removal, *E. coli* was recovered from over 80% of samples at both HQ and FQ and was hardly reduced during the further processing on the slaughter floor.



SARDI comments: In general terms, the low numbers of samples at each site makes for the possibility of unusual results.

For example, at Plant R, it is difficult to account for the apparent absence of E. coli at the HQ on eleven veal carcases, especially since almost 50% of hind quarters were positive for the indicator organism immediately after hide removal.

Bobby calf carcases typically have much higher prevalence of E. coli than weaned and adult animals. Their hides are more likely to be contaminated with faeces because they spend much of their time lying down, and because faeces may be expressed from the anus when the animal is restrained prior to stunning. The thoracic stick operation also spreads contamination onto the carcase.

In addition, there are no interventions in calf processing which are likely to reduce the prevalence of E. coli and the position at Plant S, where 83% were positive after hide removal and 75% positive after all slaughter floor operations, reflects this.

Chilling

27. Effect of ozonation on microbial counts on a beef chiller

Introduction

Ozone is a powerful oxidizing agent, present naturally in the atmosphere and has inhibitory microbiological effects.

Objective

Determine if ozonation of a chiller over a 2h period will result in lower levels of TVC on the walls.

Methods

Processing: An empty, dirty beef chiller was used for the experiment.

Testing and analysis: A grid pattern was taped on a wall. Twenty-five sites were sampled using a press plate. After ozonation, sites adjacent to the original 25 were sampled. The 50 plates were then incubated for approx. 30 h at 25°C. Colonies were counted and data entered on a spreadsheet tool.

Results

The results are presented in Table 1 and Table 2 from which it can be seen that TVC was significantly decreased following ozonation. Boxplots of the $\log_{10}$ TVC concentrations are shown in Figure 1.

Table 1: Summary of difference in $\log_{10}$ TVC cfu/cm^2 between before and after ozonation.

Summary	Difference (log)
Mean	0.59
St. Dev.	0.70
n	9
Conf level	95%
CI Lower	0.05
CI Upper	1.13
Significance	Significant

Table 2: Summary of prevalence for $\log_{10}$ TVC cfu/cm^2 before and after ozonation.

Summary	Before Ozone	After Ozone
Detect	19	5
n	21	21
Prevalence	90.5%	23.8%
Conf level	95%	
CI Lower	69.6%	10.4%
CI Upper	98.4%	45.6%
Significance	Highly significant	

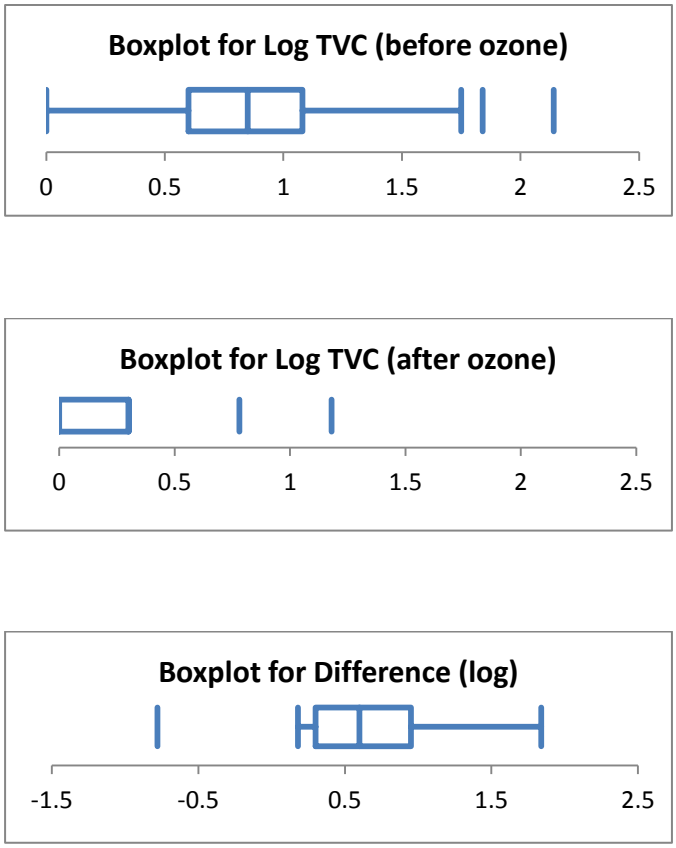


Figure 1: Boxplots of log_{10} TVC cfu/cm^2 for before and after ozonation and the difference.

Conclusion

It was concluded that ozonation is effective in reducing the TVC on chiller walls.

Boning

28. Microbial contamination of knives used for boning

Introduction

We cold-bone beef carcases in 2-hour shifts. We don't sterilise knives during production but clean them and their pouches at each break.

Objective

We want to know how the microbial loading of knives varies during a typical boning room work period.

To find out, we swabbed knives during a work period. The knives are cleaned as operators leave the boning room for a smoko break so in theory, they start the work period with a low bacterial load. We also sponged cuts of meat which had been boned at various stations in the boning room.

Methods

Sponges resuscitated with sterile peptone water were used to sponge both sides of the knife blade. Cuts of meat on the slicing tables were sponged (100cm^2). Sponges were placed in an insulated container on ice packs and bacterial counts undertaken 60 minutes later.

Appropriate dilutions were plated onto Petrifilm Aerobic Plate Count (APC) and Petrifilm *E. coli* films, which were incubated at 20-25°C for 96 hours and 37°C for 48 hours, respectively.

Colonies were counted according to the manufacturer's instruction and the count/cm^2 calculated for knives and meat. The limit of detection for APC and *E. coli* was 10 cfu/cm^2.

The profile of knives was traced on squared paper, which allowed us to calculate the surface area of the blade.

Results and conclusions

The mean log APC of meat cuts (n=15) sponged on slicing tables was 1.96 $\log_{10}$ cfu/cm^2, ranging from 0.9 to 3.3 $\log_{10}$ cfu/cm^2; *E. coli* was not detected on any samples (Table 1).

The mean log APC of cleaned knives was 0.9 $\log_{10}$ cfu/cm^2; *E. coli* was not recovered from any cleaned knife.

The mean log APC of knives after 15, 30, 45 and 60 minutes use was 1.7, 1.4, 1.5, and 1.4 $\log_{10}$ cfu/cm^2, respectively (Table 1).

The mean log APC of meat surfaces through which the knives sliced range from 0.9-3.3 $\log_{10}$ cfu/cm^2 (Table 1).

Table 1: Aerobic plate counts (APC/cm^2) of knives used for slicing

Cleaned knives	APC (log/cm^2)
	0.9
	1.0
	0.6
	1.1
	0.8
Mean	0.9
Knives in use 15 min	
Brisket	1.6
Brisket	0.6
Blade	1.7
Blade	2.2
Neck	2.2
Mean	1.7
Knives in use 30 min	
Cube roll	1.3
Cube roll	1.8
Ribs	0.6
Ribs	1.9
Topside	1.3
Mean	1.4
Knives in use 45 min	
Topside	1.5
Topside	1.3
Silverside	1.6
Silverside	1.3
Knuckle	1.7
Mean	1.5
Knives in use 60 min	
Striploin, rump	0.9
Striploin, rump	1.2
Flap	1.5
Flap	2.6
Tenderloin	0.6
Mean	1.4

Table 2: Aerobic plate counts (APC/cm^2) of meat cuts on slicing table

Meat 15 min	APC (log/cm^2)
Brisket	0.9
Blade	2.1
Blade	1.7
Neck	1.2
Neck	3.0
Mean	1.8

Meat 30 min	
Cube roll	3.3
Topside	2.2
Topside	3.0
Silverside	1.4
Knuckle	2.0
Mean	2.4

Meat 60 min	
Rump	2.4
Rump	2.0
Flap	1.7
Tenderloin	1.5
Tenderloin	1.1
Mean	1.7

SARDI comments

Blades of knives involved in slicing became contaminated as soon as they came in contact with the surface of meat, and the level of contamination is related to the contamination level of the surface being cut. Some slicing cuts pass through sterile tissue and this may remove bacteria from the blade to the meat.

Because of the boning room temperature, we expect the general contamination level of meat entering the boning room to remain similar throughout the shift.

Your study indicates "normal" contamination levels for meat surfaces, and the knife levels are consistently below that of meat, because the knife cuts through sterile and non-sterile tissue.

Your study also indicates that it is very difficult to eliminate all bacteria from the knife blade, even when it is cleaned with brush and scouring pad in hot, soapy water.

29. Chemical sanitizing of knives as an alternative to hot water

Background

In Australia, knives used during the slaughter and dressing of carcasses are sanitised in water at 82°C, after first rinsing in tepid water (~40°C). In many meat plants in Australia, the sanitising effect of hot water is increased by using a two-knife system because, while the operator uses one knife, the other remains immersed in hot water.

The scientific basis for the use of the 82°C temperature is not clear and appears to be based on convention established from previous regulatory practices rather than from empirical data.

Studies in Australia have indicated that alternatives to brief immersion in 82°C water exist. Eustace (2005) demonstrated that immersion of knives in 72°C water for 15s after a rinse in hand-wash water was as effective as momentary dipping in 82°C water. Eustace *et al*. (2007, 2008) went on to demonstrate that the use of a two-knife system with rinsing in hand-wash water then immersing in 60°C between uses was as effective as the typical 82°C system.

More recently, studies in Europe have indicated that sanitising of cutting tools used in pig slaughter and dressing could be done using a chemical sanitiser, Inspexx (Ecolab Pty Ltd, a mixture of Acetic acid, Peroxyacetic acid, Hydrogen peroxide and Octanoic acid). Testing commissioned by Ecolab indicated a reduction in Total Bacterial Count of about 1 log, or 90%, at various work stations in a pig slaughter and dressing plant when using Inspexx, compared with 82°C water.

Objective

Hot water is an expensive part of our operating costs and we were interested to see if chemical sanitising would be as effective as hot water sanitising in our beef operation.

Methodology

Setting up the experimental work presented challenges. We are an export plant and would require regulatory permissions to test the effectiveness of the sanitiser. As well, we were unsure about health and safety aspects of the chemical so we needed to test it in an area where meat was not present.

We decided to use skins, rather than meat, as our test material and were able to use a room separate from any production area and equipped with a hot water steriliser containing a 2-knife holding unit and operated at 82°C.

Our engineers made a 2-knife holder sitting in a plastic bucket and an Ecolab territory manager made up the Inspexx solution and ensured it was the correct concentration at 230 mg/kg (230 ppm).

Figure 1. Trial knife set up, hot water sterlisation set up and bottom right is the chemical sterlisation knife holder

Conduct of the investigation

We used foetal calfskins to evaluate the effect of incising the hide.

A solution of fresh faeces was made and spread as evenly as possible across the hide, which was stretched across large plastic cutting boards.

Figure 2. The skins used for the trial rather than meat

Each incision used the entire length of the blade (ca. 25-30 cm) after which the knife was rinsed in warm water before being placed in the sanitising solution (either 82°C water or Inspexx).

To estimate the bacterial loading of the hide, incisions were made and the knife blade tested prior to any rinsing or sanitising; this told us the range of bacterial loadings which were on the knife immediately after use.

The operator used a 2-knife system and the bacterial loading on the knife was tested after it had resided in the sanitising solution during the time the other knife was in use. Mean residence time was 15-20 seconds which is typical of the time many of our knives are in the steriliser during hide incision operations.

A total of 25 incisions were made for each of the sanitising solutions.

Removal of bacteria from the knife

Knife blades were sampled immediately after the operator had cleaned the knife either in 82°C water or in Inspexx solution using a sterile polyurethane sponge (Nasco Whirlpak) hydrated in 2 % (w/v) buffered peptone water. The sponge was doubled over the back of the knife and the blade wiped from handle to tip. The sponge was replaced in the Whirlpak bag and tested in our laboratory.

Microbiological testing

The sponge was squeezed firmly through the plastic bag and, from the moisture expressed, serial dilutions were prepared in 0.1% buffered peptone water blanks (9 mL) using 1mL aliquots. Aliquots (1 mL) from each dilution were spread on either Aerobic Plate Count Petrifilm (3M) to give a Total Viable Count (TVC) or *E. coli* Petrifilm (3M) and incubated at 30°C for 2 days.

Expressing the results

Colonies were identified and counted as per the manufacturer's instructions. When *E. coli* was absent from Petrifilms, the result was entered as "not detected". TVCs were converted to $\log_{10}$ colony forming units (cfu) and the mean of the $\log_{10}$ cfu was calculated. The standard deviation was determined using Microsoft Excel software.

We didn't express our results as /cm^2 of knife blade because we used the same knife throughout. So our results are the number of bacteria that remained after cleaning. In other words, how many bacteria were on the "clean" knife.

Results

Bacterial counts are presented in summary form in Tables 1-4.

Bacterial loading on the calf hides

In Tables 1 and 2 are presented the bacterial loading present on the calf hide as measured by what we were able to remove from knives immediately after incising the hide.

We were able to remove an average TVC of log 5.5 cfu (316,000) from "dirty" knives, with counts ranging up to 1.2 million cfu. We recovered *E. coli* from knives on every one of the 10 incisions we made through the calf skin, with an average of log 4.2 cfu (15,600), with counts ranging up to 58,000 cfu.

Table 1: Total Viable Count (TVC) removed from calf hides seeded with a cattle faeces solution

Number of tests	TVC (log cfu)	
	Mean	SD
10	5.5	0.5

Table 2: *E. coli* loading removed from calf hides seeded with a cattle faeces solution

Number of tests	Number of tests *E. coli* still on knife	*E. coli* (log cfu)	
		Mean	SD
10	10	4.2	0.6

* Mean of the positive tests only

This gave us the baseline for the loadings on the knives immediately after cutting through the hide.

In Tables 3 and 4, we summarise bacterial counts on knives after rinsing and sanitising either in 82°C water or in Inspexx.

The TVC (Table 3) on knives rinsed in warm water and resident in 82°C water for 15- 20 seconds averaged log 3.5 cfu (3,160) as did the TVC on knives rinsed in warm water and resident in Inspexx solution for 15- 20 seconds.

After hot water sanitising, *E. coli* persisted on 4/25 knives with counts of 20, 20, 20 and 460 cfu compared with 1/25 knives (count 20 cfu) after sanitising in Inspexx.

Table 3: TVC after sanitising

Sanitising treatment	Number of tests	TVC (log cfu)	
		Mean	SD
Rinse + 82°C	25	3.5	0.4
Rinse + Inspexx	25	3.5	0.2

Table 4: *E. coli* loading after sanitising

Sanitising treatment	Number of tests	Number of tests *E. coli* still on knife	*E. coli* (log cfu)	
			Mean	SD
Rinse + 82°C	25	4	1.6*	0.7
Rinse + Inspexx	25	1	1.3*	-

* Mean of the positive tests only

Discussion

The aim of the investigation was to replicate as nearly as possible what happens in an export meat plant when knives are cleaned using a 2-knife system.

Calf hide was chosen because it represents a worst-case in that hairs are always removed during incision, increasing the physical and microbiological loading required to be removed during knife cleaning. As well, the calf hide was low-cost compared with a full size cowhide.

The knife used, and the incision chosen, was intended to replicate what occurs when the hide is incised during a midline cut down the belly of the animal. In this investigation, the knife blade made contact with the calf hide over a length of 25-30 cm.

The present investigation indicates that treatment of knives with heavy *E. coli* and total bacterial loadings with Inspexx at a concentration of 230 mg/kg provides an equivalent reduction to that of immersion in 82°C hot water.

References

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Eustace, I., J. Midgley, C. Giarrusso, C. Laurent, I. Jenson and J. Sumner (2007). An alternative process for cleaning knives used on meat slaughter floors. Int. J. Food Microbiol. 113:23-27.

Eustace, I., Midgley, J., Small, A., Jenson, I. and Sumner, J. (2008). Knife sanitizing in abattoirs: the effectiveness of current and alternative practices. Food Protection Trends 28:712-722.

30. An alternative knife cleaning system for boning room operators

Introduction

In our boning room, the procedure is that operators clean personal equipment (knives, pouches and gloves) as they leave the boning room for every work break and also at the end of the day. Since our boning room has 70 operators and they are all in a hurry to maximise their break time, we suspect that equipment is not very well cleaned. During a break, the equipment is hung in the ante-room (boot wash and hand wash stations) at ambient temperature, which, in summer is 20-30°C on some days.

We have investigated an alternative procedure for cleaning knives and the results of the investigation are presented in this report.

Objective

We proposed an alternative procedure in which operators cleaned their equipment only at the end of shift. At work breaks, equipment was hung in the boning room near work stations. Our boning room runs between 7-9°C and we anticipate little or no growth of bacteria over the shift.

Methods

Each knife was sampled on the entire blade area (both sides) from stem (joint of blade and handle) to tip, using a sterile polyurethane sponge (Nasco Whirlpak) rehydrated with 25mL sterile Butterfield's diluent. To remove bacteria from knives, the sponge was folded over the blunt edge at the handle and run to tip of knife with constant pressure being applied.

The sponge was squeezed firmly through the plastic bag and moisture expressed from which serial dilutions were prepared in Butterfields' blanks (9 mL) using 1 mL aliquots. Aliquots (1 mL) from each dilution were plated on Aerobic Plate Count Petrifilm (3M) or *E. coli* Petrifilm (3M) and incubated at 20-25°C/2 days and 37°C/2 days, respectively. Colonies were identified and counted as per the manufacturer instructions.

The area of knife sponged varied according to type. The area of each type of knife was determined by outlining the blade area on graph paper.

The limit of detection for both TVC and *E. coli* for knives varied depending on type of knives sampled (from 0.47-0.96 cfu/cm^2).

Results

Bacterial levels on knives using the current system

Knives which had been cleaned by operators as they exited the boning room for the morning break were sampled. As indicated in Table 1, average TVCs for knives was 2.62 $\log_{10}$ /cm^2 (415/cm^2). However, the standard deviation was large, ranging between log 1.01 and 1.50 indicating that knives were not cleaned in a consistent manner. For example, the highest count on "clean" knives was 40,000/cm^2. *E. coli* was not detected on any cleaned equipment.

Table 1: *E. coli* and Total Viable Count of cleaned personal equipment

Personal equipment	Samples (n)	Mean log TVC/cm^2 (SD)	*E. coli**
Knives	60	2.62 (1.26)	0/60

* Positive/Total knives

We concluded that some operators were cleaning their equipment properly and some were not and decided to try a system where operators cleaned their equipment only at the end of the shift.

Microbiological status of knives throughout the shift

To see whether counts increased on knives through the shift, they were not cleaned at each work break but left hanging in their pouches in the boning room at the operators' workstations. From Table 2, it can be seen that average TVC of knives did not increase throughout the shift, remaining around 2.9/cm^2 until end of shift. *E. coli* was not detected on any of the knives at 09.00h and was isolated from 3/50 knives at 16.00h. On the three positive knives, the average count was low (log –0.27/cm^2 or 0.5/cm^2)

Table 2: Knives sampled at first break (approx. 9:00) and at end of shift (approx. 16:00)

Time	Samples (n)	Mean log TVC/cm^2 (SD)	*E. coli**
9:00	25	2.89 (1.02)	0/25
16:00	25	2.90 (1.05)	3/25 (-0.27)

* Positive/Total knives (mean log of positives)

Conclusions

- Our current system does not result in knives being cleaned properly.
- The total bacterial loading on knives does not increase greatly during the shift.
- The loading on "dirty" knives is not much different from that of "clean" knives in our current system.
- We will revise our end of shift cleaning so that knives are properly washed and we will validate it using the methodology we've used here.

31. Transfer belts as a potential source for cross contamination of STECs in a beef boning room

Background

Export trim meat to the USA undergoes the usual container-load testing by excising five small pieces of surface meat from each of 12 cartons (n60 testing). It is required to ensure that none of these 60 pieces has one or more STECs (Big 6 or O157), otherwise there will be a potential positive from the initial screening test, leading to substantial confirmation costs in testing and downgrading the end-use of meat in that container.

In this boning room, meat gets transferred to stations packing primals and trim intended for grinding on a total of 10 conveyor belts.

The concern is that transfer belts might increase the likelihood that STECs are detected on one of the 60 samples taken from container loads. It is thought that, if a piece of meat bearing STECs is dropped onto the primary belt, it has the potential to "stamp" other pieces of meat on its next circuit.

Objective

To assess whether STECs deposited on transfer belts could be picked up, and also any pattern of STECs being carried by belts.

Methodology

Identifying each belt

For the purposes of identifying samples, each belt was given a number as shown below:

Testing number	Name	Product transferred
1	Primal Belt 1	Primals
2	Frozen Belt (bottom)	Trim
3	Frozen Belt (top)	Trim
4	Frozen Belt 1 (incline)	Trim
5	Frozen Belt 2	Trim
6	Frozen Belt 3	Trim
7	Frozen Belt 4	Trim
8	Primal Belt 2	Primals
9	Primal Belt 3	Primals
10	Frozen Belt (bottom)	Trim

Sampling method

Each belt was sampled by pressing a plastic scraper to the moving belt and gathering meat/fat deposits as the belt circulated.

On belts with light deposits, the scraper was applied for several complete revolutions so that sufficient material was removed for testing.

On one occasion, material was removed from each belt by applying a Whirlpak sponge for at least one revolution of each belt.

Sampling frequency

Samples were removed during both work breaks and at the end of processing as follows:

Date	Time	Sample number
22/4/15	11.20	1-10
22/4/15	15.20	11-20
23/4/15	08.20	21-30
23/4/15	11.20	31-40
23/4/15	14.20	41-50
24/4/15	08.20	51-60
24/4/15	11.20	61-70
24/4/15	11.20	71-80 *

* Sponge samples after meat/fat had been scraped from the belt

Sample testing

Samples were transferred to the laboratory for testing as follows:

- Total Viable Count (TVC)
- *E. coli*/Coliforms
- *Enterobacteriaceae*
- STECs by GDS
- STECs by BAX

Counts were expressed as cfu per gram of meat/fat scraped from the belt or cfu/ml for sponged samples. The limit of detection for *E. coli*, Coliforms, Enterobacteriaceae and TVC was 10cfu/g or ml.

Results

In total, belts were scraped at 7 work breaks over the period Wednesday to Friday and 79 samples were taken for analysis, in effect providing 79 snapshots of the level of contamination (both visible and microbial).

Appearance of the belts

The degree with which belts were encrusted with meat/fat deposits varied between runs and sometimes several revolutions of the belt were needed to generate 1g of meat/fat.

Counts of indicator organisms on belts

All counts are summarised in Table 1.

- The mean TVC was log 3.8 cfu/g (6,300 cfu/g), which is much higher than counts on product, and TVCs ranged above log 5 cfu/g (100,000 cfu/g).
- *Enterobacteriaceae* were present on almost all belts and averaged log 2.9 (800 cfu/g) and ranged up to log 4.1 (12,600 cfu)/g.
- *E. coli* was present on 21.5% of samples from belts.
- Concentration of *E. coli* ranged up to 50 cfu/g of meat/fat scraped from belts.

STECs on belts

Samples were tested by GDS for presence of genes associated with an STEC (*eae* and either stx_1 and/or stx_2) and for "O" antigens by BAX.

GDS results

- *E. coli* O157 was not detected in any sample, but genes associated with the Big 6 were.
- From the 79 samples, 16 had one or more indicator genes (*eae*, stx_1 and/or stx_2).
- 5/79 samples had both virulence factors (*eae* and stx_1 and/or stx_2).
- Only one sample (Sample 67) was close to being potential positive for STEC and it had the *eae* gene and a weak signal for stx_2
- Interestingly, generic *E. coli* was not detected in that sample.

Table 1: Microbial profiles of transfer belts over 3 working days (colours used to break samples into time slots)

Sample #	Date	Time	Location	*E. coli*	Coliform	Entero	TVC
				cfu/g or /ml		log cfu/g or /ml	
1	22-Apr	11.30	Primal belt 1	1.0	2.1	2.5	3.2
2	22-Apr	11.30	Trim belt (under)	nd	nd	1.3	2.6
3	22-Apr	11.30	Trim belt (top)	nd	1.5	1.7	2.9
4	22-Apr	11.30	Missing	-	-	-	-
5	22-Apr	11.30	FF belt 2	nd	1.3	1.0	2.8
6	22-Apr	11.30	FF belt 3a	nd	1.0	1.5	2.4
7	22-Apr	11.30	FF belt 3b	nd	1.3	1.8	2.8
8	22-Apr	11.30	Primal belt 2	1.0	1.3	1.5	3.5
9	22-Apr	11.30	Primal belt 3	nd	nd	nd	2.7
10	22-Apr	11.30	Underbelt trim	nd	nd	nd	1.6
11	22-Apr	14.30	Primal belt 1	nd	1.6	2.4	3.3
12	22-Apr	14.30	Trim belt (under)	1.5	2.1	2.8	3.5
13	22-Apr	14.30	Trim belt (top)	nd	2.6	3.5	4.6
14	22-Apr	14.30	Incline	nd	2.0	3.0	3.9
15	22-Apr	14.30	FF belt 2	nd	2.0	2.4	3.9
16	22-Apr	14.30	FF belt 3a	nd	1.8	2.3	3.5
17	22-Apr	14.30	FF belt 3b	nd	1.0	2.0	3.2
18	22-Apr	14.30	Primal belt 2	1.3	1.7	2.3	3.6
19	22-Apr	14.30	Primal belt 3	nd	1.5	2.1	3.6
20	22-Apr	14.30	Underbelt trim	nd	1.5	2.1	3.6
21	23-Apr	8.30	Primal belt 1	1.0	2.1	2.4	3.6
22	23-Apr	8.30	Trim belt (under)	nd	1.3	1.7	4.3
23	23-Apr	8.30	Trim belt (top)	nd	1.7	2.1	3.9
24	23-Apr	8.30	Incline	1.0	1.3	1.6	3.3
25	23-Apr	8.30	FF belt 2	nd	1.0	1.3	3.4
26	23-Apr	8.30	FF belt 3a	nd	1.9	2.3	3.9

Sample #	Date	Time	Location	*E. coli*	Coliform	Entero	TVC
				cfu/g or /ml		log cfu/g or /ml	
27	23-Apr	8.30	FF belt 3b	1.0	1.8	3.0	3.6
28	23-Apr	8.30	Primal belt 2	nd	1.9	2.3	4.9
29	23-Apr	8.30	Primal belt 3	nd	2.2	2.4	4.7
30	23-Apr	8.30	Underbelt trim	nd	nd	1.3	2.9
31	23-Apr	11.30	Primal belt 1	1.0	2.0	2.5	3.8
32	23-Apr	11.30	Trim belt (under)	nd	1.0	0.0	4.0
33	23-Apr	11.30	Trim belt (top)	nd	1.8	2.4	4.7
34	23-Apr	11.30	Incline	nd	2.1	2.6	3.9
35	23-Apr	11.30	FF belt 2	1.0	1.8	2.2	4.1
36	23-Apr	11.30	FF belt 3a	nd	1.6	1.6	3.3
37	23-Apr	11.30	FF belt 3b	nd	1.0	1.6	3.7
38	23-Apr	11.30	Primal belt 2	1.0	1.6	1.9	4.9
39	23-Apr	11.30	Primal belt 3	nd	1.5	2.2	4.7
40	23-Apr	11.30	Underbelt trim	nd	1.0	1.7	3.4
41	23-Apr	14.30	Primal belt 1	nd	2.8	3.3	3.9
42	23-Apr	14.30	Trim belt (under)	nd	1.8	2.0	4.9
43	23-Apr	14.30	Trim belt (top)	nd	2.0	2.3	4.0
44	23-Apr	14.30	Incline	1.7	2.8	3.8	5.1
45	23-Apr	14.30	FF belt 2	nd	2.1	2.5	4.5
46	23-Apr	14.30	FF belt 3a	nd	3.9	4.1	5.3
47	23-Apr	14.30	FF belt 3b	nd	1.7	2.2	4.1
48	23-Apr	14.30	Primal belt 2	nd	nd	1.7	5.1
49	23-Apr	14.30	Primal belt 3	nd	1.5	1.8	4.4
50	23-Apr	14.30	Underbelt trim	nd	1.0	1.3	3.7
51	24-Apr	8.30	Primal belt 1	1.3	1.9	3.3	3.7
52	24-Apr	8.30	Trim belt (under)	nd	nd	1.3	3.7
53	24-Apr	8.30	Trim belt (top)	1.5	2.1	1.8	4.1

Sample #	Date	Time	Location	*E. coli*	Coliform	Entero	TVC
				cfu/g or /ml		log cfu/g or /ml	
54	24-Apr	8.30	Incline	nd	1.8	2.1	3.6
55	24-Apr	8.30	FF belt 2	nd	1.5	2.0	4.1
56	24-Apr	8.30	FF belt 3a	nd	nd	0.0	3.3
57	24-Apr	8.30	FF belt 3b	nd	nd	1.0	4.2
58	24-Apr	8.30	Primal belt 2	nd	1.0	1.3	3.9
59	24-Apr	8.30	Primal belt 3	nd	1.7	1.8	4.2
60	24-Apr	8.30	Underbelt trim	nd	nd	nd	3.1
61	24-Apr	11.30	Primal belt 1	nd	2.1	2.6	3.5
62	24-Apr	11.30	Trim belt (under)	1.3	2.4	3.4	3.7
63	24-Apr	11.30	Trim belt (top)	1.0	1.3	2.3	3.9
64	24-Apr	11.30	Incline	nd	1.0	2.1	3.4
65	24-Apr	11.30	FF belt 2	nd	1.0	1.5	3.4
66	24-Apr	11.30	FF belt 3a	nd	nd	1.0	2.6
67	24-Apr	11.30	FF belt 3b	nd	1.5	2.0	3.8
68	24-Apr	11.30	Primal belt 2	1.3	1.8	2.1	4.6
69	24-Apr	11.30	Primal belt 3	nd	1.3	1.8	4.5
70	24-Apr	11.30	Underbelt trim	nd	nd	0.0	3.0
71	24-Apr	11.30*	Primal belt 1	nd	nd	1.8	2.5
72	24-Apr	11.30*	Trim belt (under)	1.0	1.0	1.5	3.2
73	24-Apr	11.30*	Trim belt (top)	nd	1.5	1.5	2.8
74	24-Apr	11.30*	Incline	nd	1.8	2.5	3.7
75	24-Apr	11.30*	FF belt 2	nd	nd	nd	2.9
76	24-Apr	11.30*	FF belt 3a	nd	nd	nd	2.0
77	24-Apr	11.30*	FF belt 3b	nd	nd	nd	2.8
78	24-Apr	11.30*	Primal belt 2	nd	nd	nd	3.8
79	24-Apr	11.30*	Primal belt 3	nd	nd	nd	2.8
80	24-Apr	11.30*	Underbelt trim	nd	nd	nd	1.8

* Sponge samples after belt had been scraped

BAX results

- From the 60 samples screened for *eae* and *stx* genes using BAX, 15 samples had either or both virulence factors (*eae* and/or *stx*).
- Of these 15 samples, 5 were Potential Positive for one or more pSTECs (see Table 2).
- Antigens for several Big 6 STECs were frequently detected by BAX.
- STEC suspected Number of suspect samples
    - *E. coli* O45 68
    - *E. coli* O121 7
    - *E. coli* O103 6
    - *E. coli* O111 1
    - *E. coli* O26 1

It is interesting that a cluster of STECs was isolated from sequential trim belts during the same sampling on 24 April at 11.30 (Samples 62 to 67) with serotypes O45, O103 and O121 implicated. Note that, while generic *E. coli* was present in samples 62 and 63, the indicator was below the limit of detection in samples 64-67.

This is consistent with the pattern that can be expected if STEC had been ‘stamped’ onto the primary trim belts and then transferred by further pieces of trim contaminating downstream belts.

Table 2: Samples of meat/fat from transfer belts showing both attachment/effacing (eae) and toxin (stx_1 and/or stx_2)

Sample	Date	Time	Location	O157	STEC	GDS	BAX		
						eae:stx2:stx1	eae:stx	Panel 1	Panel 2
49	23-Apr	14.30	Primal belt 3	-ve	-ve	000	11 (+ve)	-	O45
62	24-Apr	11.30	Trim belt (under)	-ve	-ve	010	11 (+ve)	O121	O45, O103
63	24-Apr	11.30	Trim belt (top)	-ve	-ve	100	11 (+ve)	-	O45, O103
64	24-Apr	11.30	Incline	-ve	-ve	100	11 (+ve)	O121	O45, O103
65	24-Apr	11.30	FF belt 2	-ve	-ve	100	10	O121	O45
66	24-Apr	11.30	FF belt 3a	-ve	-ve	100	10	O121	O45
67	24-Apr	11.30	FF belt 3b	-ve	(+ve)	(110)	11 (+ve)	O121	O45

What has been learned?

1- It was found “O” antigens for STECs were present in a large majority of samples of meat/fat removed from our transfer belts.

2- Often one or more of the three virulence genes associated with STECs were found.

3- At one sampling occasion, a cluster of STECs was found on six transfer belts that move trim meat to packing stations.

4- This project supports the idea that transfer belts can amplify the chance that one STEC is found on one or more of the 60 pieces of meat tested from each container.

5- Further work needs to be done to improving the conveyor system and better belt sanitation.

32. Sanitising boning room belts by Ultra Violet irradiation

Introduction

Conveyor belts in the boning room are a possible source of cross contamination. UV light is known to be an anti-microbial agent and we investigated UV light treatment of a boning room belt as a decontamination measure.

Objective

Determine if the application of a UV light on a boning room belt will result in lower TVC and *E. coli* contamination.

Methods

The UV light unit was installed underneath a boning room conveyor belt carrying primals, and was run continually throughout the day. Each pass of the boning room belt results in irradiation of the belt with UV light and reduction of contamination. It is a safe method of decontamination for staff as it involves no chemicals. It is unsuitable for use on trim or primals as the radiation does not penetrate the meat, so we placed it underneath the belt to sanitise the surface of the belt. The belt completes a rotation in about 2 minutes, and so each section of the belt passed over the light 200-250 times in a shift.

Sampling: Fifty samples were gathered over two consecutive weeks, by swabbing the boning room belt at 5 times during the day:

- Start of the day
- After the first, second and third production runs
- At the end of the day.

The belt is cleaned and disinfected before the start of each day.

25 samples were taken with the light on, and 25 the following week with the light switched off.

Testing and analysis: Sponge samples were plated on *E. coli* and Aerobic Plate Count (APC) Petrifilm and incubated at 35°C. After 48 hours, bacterial colonies were counted and data entered into a spreadsheet tool.

Results

The results are presented in the tables below from which it can be seen that TVC was lower after UV treatment by 0.7 $\log_{10}$ cfu/cm^2 on average, which is considered marginally significant in practical terms.

There was no significant reduction in *E. coli* prevalence from the UV light.

Table 1: Summary of difference in log_{10} TVC cfu/cm^2 between normal and UV light treatment.

Summary	Normal	UV
Mean	3.13	2.43
St. Dev.	0.64	0.70
n	25	25
Conf level	95%	
CI Lower	2.86	2.14
CI Upper	3.39	2.72
Significance	Highly significant	

Table 2: Summary of *E. coli* prevalence for normal and UV treatment.

Summary	Normal	UV
Detect	4	2
n	25	25
Prev	16.0%	8.0%
Conf level	95%	
CI Lower	5.9%	1.2%
CI Upper	35.4%	26.3%
Significance	Not significant	

Boxplots of the log_{10} TVC concentrations are presented below.

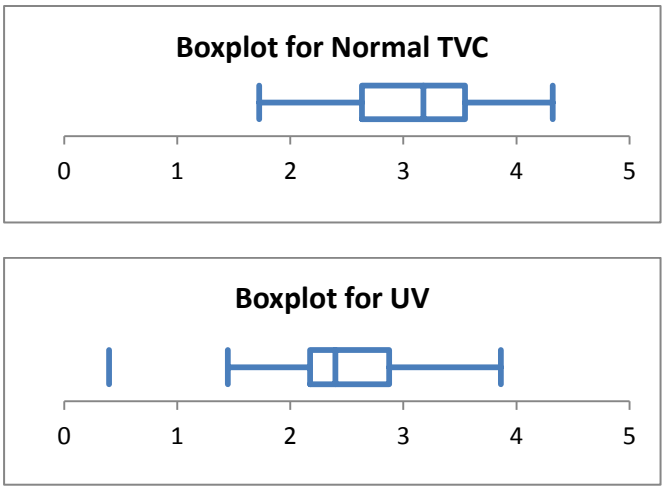


Figure 1: Boxplots showing log_{10} TVC cfu/cm^2 before and after UV application.

Conclusion

We concluded that the application of UV light to the boning room belt was not effective enough in reducing the TVC concentrations and *E. coli* prevalence to justify the cost of the units. We will continue to monitor the technology and its potential for future use in our plant.

SARDI Comments

UV light could be investigated further, looking at different parameters of the light such as exposure time and intensity.

33. Effect of turning cutting boards in the boning room

Introduction

Cutting boards potentially act as sources of contamination for meat surfaces following boning. *E. coli* is used as the target organism for this study given the significance of Shigatoxigenic *E. coli* in manufacturing beef within international trade.

Objective

Determine the difference in *E. coli* count from the process of turning cutting boards used in the boning room half way through the production day.

Methods

20 samples were gathered by actively sponging the centre of cutting boards (~200cm^2) at each of five separate points in the boning room on each of two days. The boards were swabbed immediately prior to flipping and then immediately following flipping.

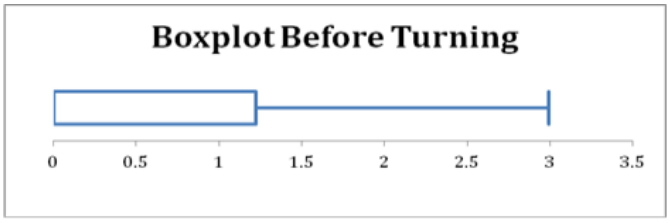
Testing and analysis: Sponge samples were plated on *E. coli* Petrifilm and incubated at 35°C (reference to method). After 48 hours, colonies were counted and data entered on a spreadsheet tool.

Results

The results presented in Table 1 and Figure 1 show that there was not a significant difference in the *E. coli* levels before and after turning.

Table 1: Summary of $\log_{10}$ *E. coli* cfu/cm^2 on Cutting Boards.

Summary	Before turning	After turning
Mean	1.88	1.10
St. Dev.	0.91	0.17
n	4	3
Conf level	95%	
CI Lower	0.44	0.67
CI Upper	3.32	1.53
Significance	Not significant	



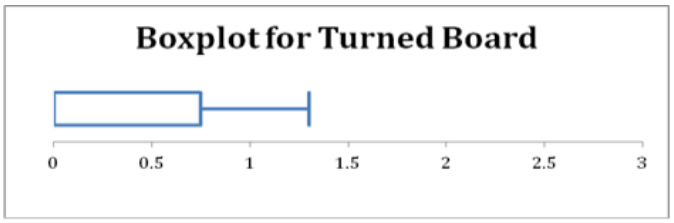


Figure 1: Boxplots of the $\log_{10}$ *E. coli* cfu/cm^2 from before and after the turning of cutting boards.

Conclusion

The analysis indicates no significant difference between *E. coli* levels before and following turning of cutting boards in the boning room. However, the analysis is limited by a lack of data.

34. Hygiene status of mesh and Kevlar gloves in the boning room

Introduction

The company needs to establish a benchmark to allow assessment for hygiene practice improvements regarding personal protective equipment (PPE).

Objective

Measure hygiene status of steel mesh gloves and Kevlar gloves during production.

Methods

During the first production break of consecutive days, mesh gloves (n=20) were sampled on day one in 50mL of peptone water and this was repeated for 20 Kevlar gloves on day 2. The gloves were randomly picked and placed into the bag with 50mL of peptone water, the bag was shaken, the glove removed and the bag sealed. Aliquots from the bag were plated on Aerobic Plate Count (APC) and *E. coli* Petrifilm.

Figure 1: Sampling and testing equipment.

The plates were incubated for 48 h, TVC at 28°C and *E. coli* at 35°C.

Results

As seen from Tables 1 and 2, mesh had a higher mean *E. coli* count than Kevlar gloves during production although this was not significant. There was one high *E. coli* count on one mesh glove.

Table 1: Summary of log_{10} TVC cfu/cm^2 for Kevlar and Mesh gloves.

Summary	Kevlar TVC	Mesh TVC
Mean	2.91	2.90
St. Dev.	0.41	0.28
n	20	20
Conf level	95%	
CI Lower	2.72	2.77
CI Upper	3.10	3.04
Significance	Not significant	

Table 2: Summary of log_{10} *E. coli* cfu/cm^2 for Kevlar and Mesh gloves.

Summary	Kevlar *E. coli*	Mesh *E. coli*
Mean	0.59	0.94
St. Dev.	0.50	0.68
n	5	7
Conf level	95%	
CI Lower	-0.03	0.31
CI Upper	1.22	1.57
Significance	Not significant	

Box Plots

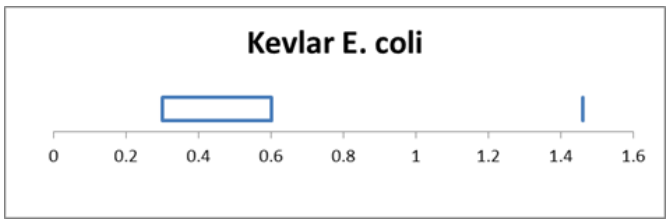


Figure 2: Boxplot of log_{10} *E. coli* cfu/cm^2 for Kevlar.

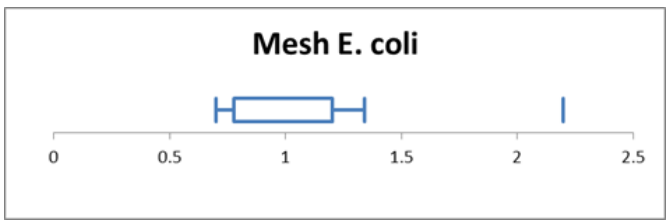


Figure 3: Boxplot of log_{10} *E. coli* cfu/cm^2 for Mesh.

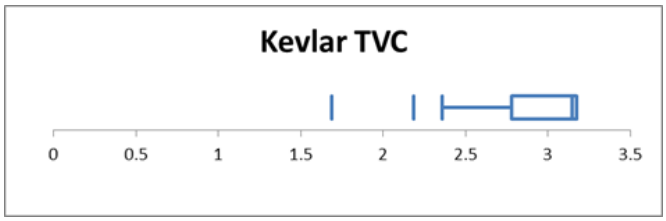


Figure 4: Boxplot of log_{10} TVC cfu/cm^2 for Kevlar.

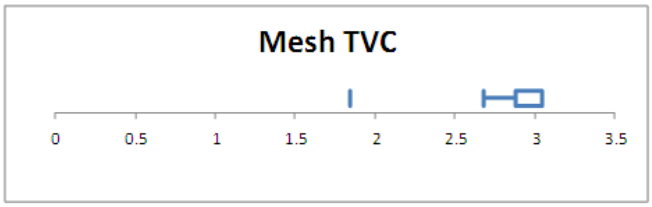


Figure 5: Boxplot of log_{10} TVC cfu/cm^2 for Mesh.

Conclusion

The testing demonstrated there was no real difference in micro results between either type of personal equipment and this leads to further developments in the facility and cleaning process.

35. Using the massage technique to estimate bacterial loading of primal cuts

Background

In order to know the shelf life of a range of primal cuts, the industry's latest advice on setting up shelf life trials is the *"Guidelines for developing a method for estimating shelf life of chilled raw vacuumed meat products"* (Appendix 1) published by AMPC and MLA.

Some primals have both lean and fat surfaces – CSIRO have used a non-destructive technique in shelf life trials in which the whole primal is placed in a plastic bag and then a sterile solution is added and massaged over the primal surface to release bacteria.

A preliminary investigation was set up where the massage technique was carried out on three primals: cube rolls, striploins and point end briskets.

Five massages were also undertaken on each primal and then an area was excised for stomaching, to determine how many bacteria had failed to be recovered during the five-massage sequence.

Objective

The objective was to determine the percentage of bacteria which are released by the massaging technique.

Methodology

Each chilled primal was placed in a sterile Stomacher bag, 225mL of 0.1% buffered peptone water (BPW) was added and massaged around the primal for 2 minutes.

Aliquots (1mL) were diluted with BPW and plated on Aerobic Plate Count Petrifilm, incubated at 25°C for 96 hours and colonies counted.

The surface area of each primal was calculated by placing it on squared (1 cm^2) paper and tracing the outline to estimate the count/cm^2.

After five massage cycles, surface tissue (100cm^2) was excised, placed in a Stomacher bag with BPW and stomached for 2 minutes, after which aliquots were removed and plated as above.

For each primal cut, either five or six replicates were used.

Results

The results of the investigation are presented in Figures 1-2 and Tables 1-3, and are the averages of five or six replicates of the primals.

The first massage recovered 39% (cube roll), 55% (striploin) and 59% (point end brisket) and after five massages, recovery from the excised tissue sample was 42%, 21% and 2% (cube roll, striploin and brisket, respectively).

There was considerable variability in first massage recoveries between replicates of all three primal cuts: cube rolls (29.4-50.7%), striploins (40.9-64.3%) and briskets (38.4-71.3%).

When massages 1 and 2 were combined (massage 1 + massage 2), total recoveries were 50% for cube rolls, 69% for striploins and 80% for briskets.

There was similar variability between excision recoveries of cube rolls (21.9-51.4%), and striploins (9.1-44.2%), while recoveries of briskets varied only between 0.7-5.2%.

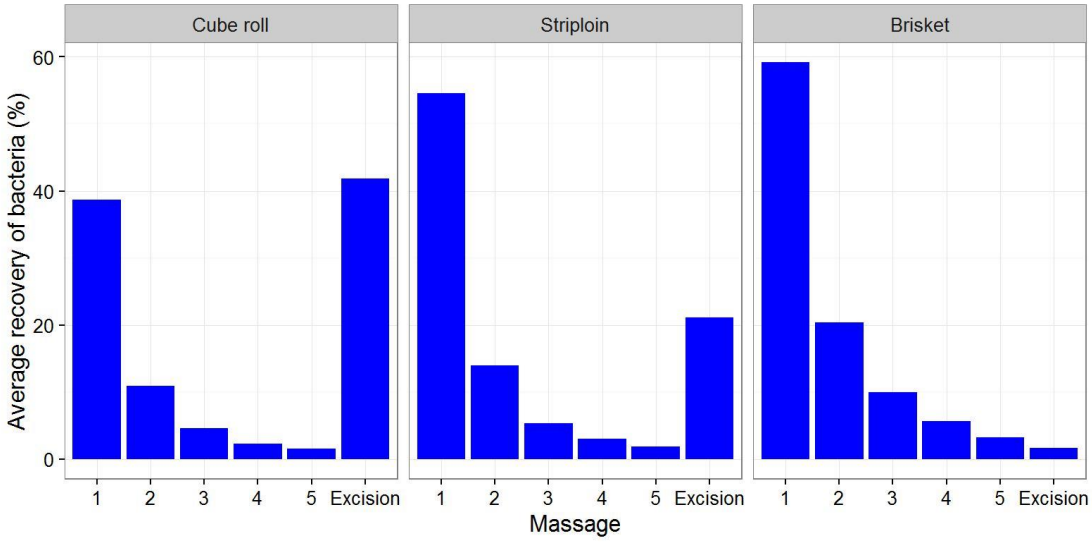


Figure 1: Average recovery of bacteria from the surface of cube rolls (n=5), striploins (n=5) and briskets (n=6) by sequential massaging and excision.

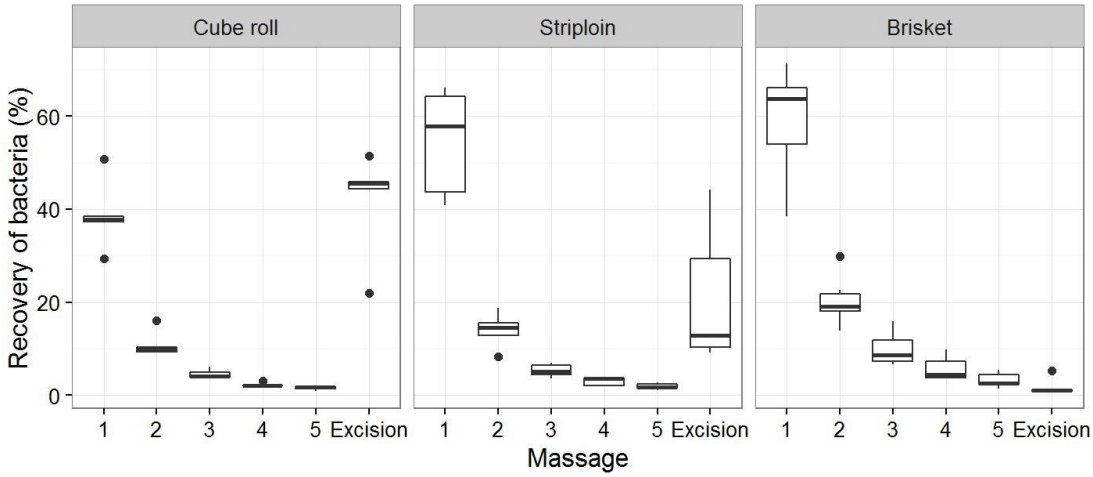


Figure 2: Recovery of bacteria from the surface of cube rolls (n=5), striploins (n=5) and briskets (n=6) by sequential massaging and excision.

Conclusions

There was variability in recovery via the massage technique both between primals and between replicates of each primal.

Cube rolls had the lowest recovery and this may reflect the degree of knife work involved in boning out an inside cut, and the subsequent cut surfaces, which may allow deeper attachment of bacteria.

In contrast, striploins and brisket point ends had some fat and selvedge cover, which may have resulted in greater recovery of bacteria.

The between-replicate variability is more difficult to account for, but may reflect operator variability.

Table 1: Recovery of bacteria from the surface of cube rolls (n=5) by sequential massaging and excision

	Replicates					
Massage	1	2	3	4	5	Mean
1	29.4	50.7	37.3	37.8	38.4	38.7
2	10.3	16.1	9.9	9.1	9.3	10.9
3	4.9	6.1	3.9	4.0	4.1	4.6
4	2.1	3.1	1.9	2.3	2.1	2.3
5	1.9	2.1	1.4	0.9	1.7	1.6
Excision	51.4	21.9	45.6	45.9	44.4	41.8

Table 2: Recovery of bacteria from the surface of striploins (n=5) by sequential massaging and excision

	Replicates					
Massage	1	2	3	4	5	Mean
1	64.3	43.7	57.8	40.9	66.2	54.6
2	14.6	15.6	18.7	8.3	12.8	14.0
3	6.5	5.1	7.0	3.6	4.5	5.3
4	3.9	3.5	3.8	1.9	2.1	3.0
5	1.7	2.8	2.4	1.1	1.5	1.9
Excision	9.1	29.3	10.3	44.2	12.9	21.2

Table 3: Recovery of bacteria from the surface of point end briskets (n=6) by sequential massaging and excision

	Replicates						
Massage	1	2	3	4	5	6	Mean
1	50.9	71.3	66.7	63.0	38.4	64.6	59.2
2	17.9	13.8	18.7	22.6	29.9	19.5	20.4
3	12.4	6.6	7.3	7.3	15.9	10.1	9.9
4	8.2	4.6	4.2	3.5	9.8	3.5	5.6
5	5.4	2.4	2.3	2.9	4.9	1.4	3.2
Excision	5.2	1.3	0.9	0.7	1.2	1.0	1.7

36. Using the massage technique to estimate bacterial loading of primal cuts – take 2

Background

In Case study 33, we looked at the impact of the massaging technique on three primals: cube rolls, striploins and point end briskets. In this study tenderloins, navel end briskets and backstraps were assessed, with some differences in the methodology.

This non-destructive technique involves a whole primal being placed in a plastic bag, sterile solution added and the primal surface massaged to release bacteria into a suspending fluid. Five massages were undertaken in sequence on each primal, after which an area was excised for stomaching, the latter to determine how many bacteria were not recovered during the five-massage sequence.

Objective

The objective was to determine the percentage of bacteria which are released by the massaging technique.

Methodology

In this study, primals were stored at 0-2°C for 30-40 days prior to sampling.

Each chilled primal was placed in a large sterile Stomacher bag (38cm x 51cm) and 500mL of 0.1% buffered peptone water (BPW) added and massaged around the primal for 2 minutes.

Aliquots (1mL) were diluted with BPW and plated on Aerobic Plate Count Petrifilm, incubated at 25°C for 96 hours and colonies counted.

The surface area of each primal was calculated using squared (1 cm^2) paper to calculate the count/cm^2. The formula for the surface area of a cone was used for the tenderloins and for a rectangular prism for the navel end briskets and backstraps.

After five massage cycles, surface tissue (100cm^2) was excised, placed in a Stomacher bag with BPW and stomached for 2 minutes, after which aliquots were removed and plated as above.

The replicates were three, five and six for the tenderloins, navel end briskets and backstraps, respectively.

Results

The results of the study are presented in Figures 1-2 and Tables 1-3, and are the averages of the replicates of the primals.

The first massage recovered 69.4% (tenderloin), 63.5% (navel end brisket) and 65.7 (backstrap) on average and after five massages, the average recovery from the excised tissue sample was 0.5%, 1.5% and 3.3% (tenderloin, navel end brisket and backstrap, respectively).

There was considerable variability in first massage recoveries between replicates of all three primal cuts: tenderloin (51.4-81.2%), navel end brisket (57.9-71.7%) and backstrap (54.0-77.6%).

When massages 1 and 2 were combined (massage 1 + massage 2), total recoveries were 87% for tenderloins, 85% for navel end briskets and 84% for backstraps.

Table 1: Recovery of bacteria from the surface of tenderloins (n=3) by sequential massaging and excision

	Replicates			
Massage	1	2	3	Mean
1	75.7	81.2	51.4	69.4
2	16.1	11.5	24.5	17.4
3	4.1	3.9	13.6	7.2
4	1.4	1.7	5.8	3.0
5	2.8	1.4	3.5	2.6
Excision	0.0	0.3	1.1	0.5

Table 2: Recovery of bacteria from the surface of navel end briskets (n=5) by sequential massaging and excision

	Replicates					
Massage	1	2	3	4	5	Mean
1	60.5	58.2	69.2	71.7	57.9	63.5
2	25.2	25.5	18.3	15.9	24.3	21.8
3	8.2	7.3	6.1	6.2	9.2	7.4
4	2.4	3.8	3.1	3.1	4.1	3.3
5	1.6	3.3	2.4	1.6	3.2	2.4
Excision	2.1	2.0	0.8	1.5	1.2	1.5

Table 3: Recovery of bacteria from the surface of backstraps (n=6) by sequential massaging and excision

	Replicates						
Massage	1	2	3	4	5	6	Mean
1	77.6	77.4	54.0	63.5	64.3	57.2	65.7
2	13.8	14.1	28.8	17.8	17.1	17.8	18.2
3	4.0	4.5	8.2	7.9	9.0	11.3	7.5
4	2.4	1.6	2.9	4.7	3.7	5.0	3.4
5	0.7	0.9	2.9	2.3	2.3	2.4	1.9
Excision	1.4	1.4	3.2	3.7	3.6	6.3	3.3

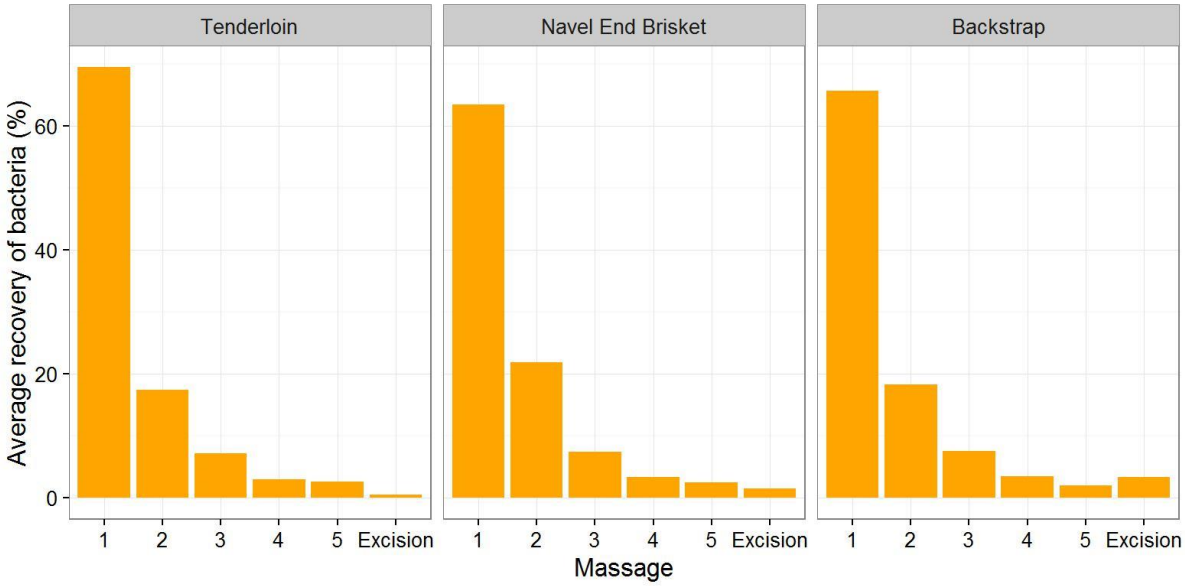


Figure 1: Average recovery of bacteria from the surface of tenderloins (n=3), navel end briskets (n=5) and backstraps (n=6) by sequential massaging and excision

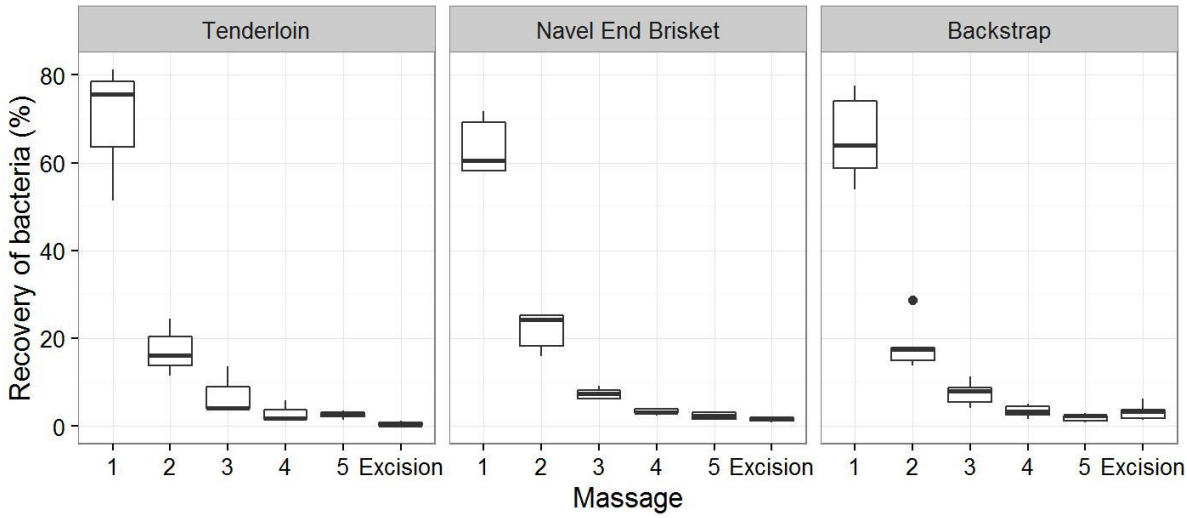


Figure 2: Recovery of bacteria from the surface of tenderloins (n=3), navel end briskets (n=5) and backstraps (n=6) by sequential massaging and excision

Conclusions

The recovery of bacteria from this trial was greater than from the first trial, indicating that the massaging technique was more effective. However, there were key differences between the two studies:

- Different primals (tenderloin/navel end brisket/backstrap versus cube roll/striploin/point end brisket)
- More diluent (500mL versus 225mL)
- Older product (30-40 days versus 0 days)

As before, there was variability in recovery via the massage technique between primals and between replicates of each primal, but in all cases, each massage iteration removed bacteria and the maximum % left on the excision samples was 3.3% (backstraps).

Packing

37. How does our carton meat compare with the national profile?

Introduction

Our plant exports beef trim to USA for grinding and we are concerned with faecal contamination. Since 2007, we have routinely sampled carcases hot by sponging at the ESAM sites. This is an in-house procedure to inform our operators. Of course, we also undertake ESAM sponging of chilled carcases and carton testing of boned-out trim. So, over time we've accumulated a huge amount of in-house data.

SARDI statisticians said they could look at our data in a number of ways and they've helped us make better use of the data, so we have agreed to publish the work in this book.

Objective

To have a long-term look at our carton testing data to find out how we cope with any seasonal trends and how we compare with the national profile for carton meat.

Methods

We take excision samples of approximately 25g from 12 cartons per day, which are tested in our onsite laboratory by stomaching for 2 minutes, plating on *E. coli* and Aerobic count Petrifilm and incubating at 35°C. After 48 hours, colonies are counted and data entered into an Excel spreadsheet.

We have a great deal of data (from 2007, a total of more than 22,000 tests) and SARDI analysed the data to give graphs and tables, which are presented in the results.

Results

Figure 1 shows us that, over the period 2007-2014, our carton meat typically has a TVC between log 1-1.5 cfu/g and it appears to be slightly lower over recent years.

Since 2011, comparable national data are available. The TVCs are generally 1 log higher (log 2.0-2.5 cfu/g) than our levels.

SARDI comments

Nationally, the TVC of carton meat is about 1 log higher than that of carcases.

Your carton meat TVCs are much lower than the national average and is difficult to explain.

By contrast, your E. coli prevalence is about the same as the national average.

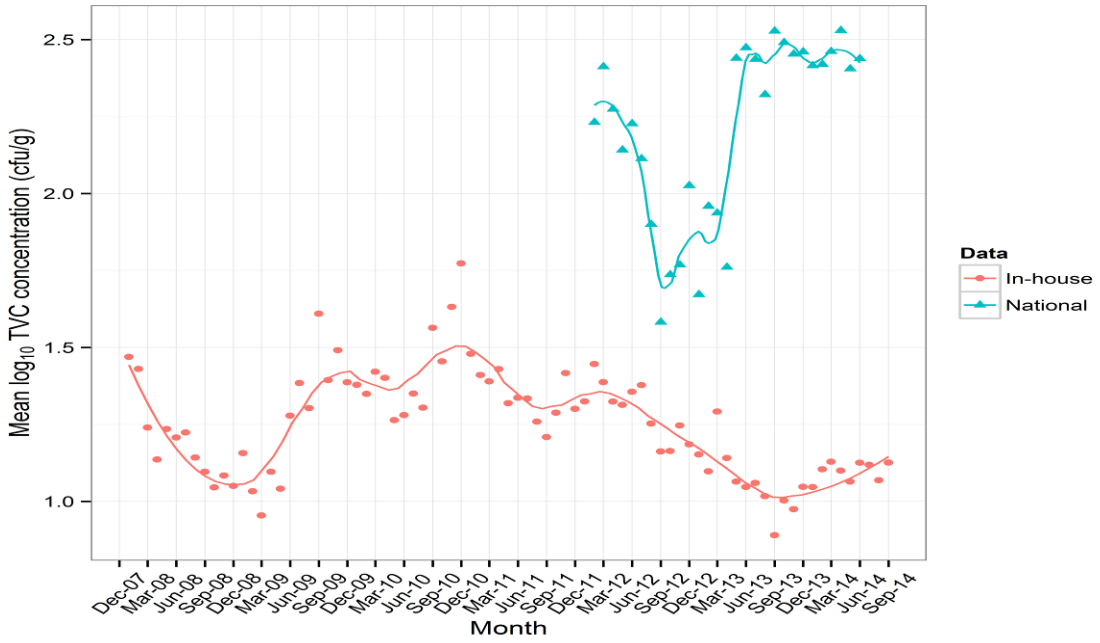


Figure 1: Mean log_{10} TVC cfu/g for in-house carton samples from Plant A compared with national carton samples.

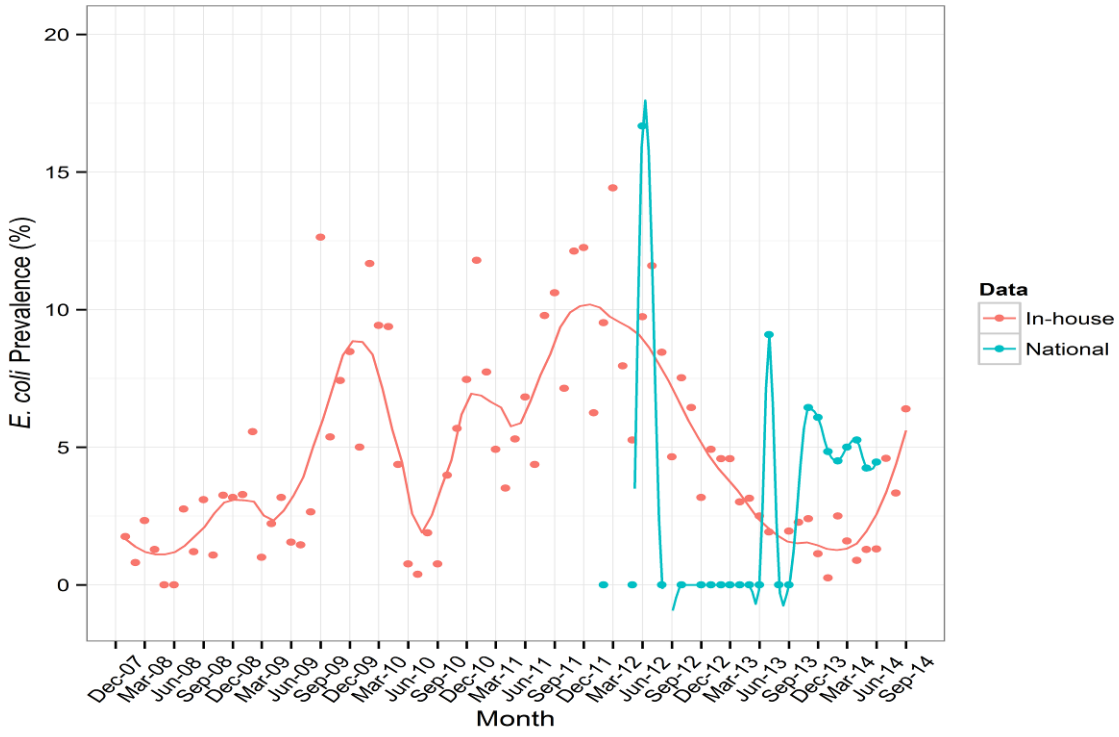


Figure 2: *E. coli* prevalence for in-house carton samples at Plant A compared with national carton samples.

38. Carton testing for processed product (Shift 1 versus Shift 2)

Introduction

Determine the level of contamination of products processed during both the shifts, thereby providing objective evidence for hygiene levels.

Objective

Monitor bacterial counts on carton meat manufactured by two shifts.

Methods

25 grams were collected from random cartons removed from the production line (5 pieces of 5g each). These samples were transported to the lab and 225 mL of buffered peptone water added. This mixture was stomached for 30 seconds and aliquots plated onto aerobic plate count Petrifilms. Petrifilms were incubated at 26°C for 48 hours. A total of 44 sanples were taken from each shift.

Results

The results are presented in Table 1 and Figures 1 and 2 from which it can be seen that there is a difference in mean counts between meat manufactured on each shift. The difference is statistically significant, though only 0.3 log.

Table 1: Summary of log_{10} TVC cfu/cm^2 for Shift 1 and Shift 2.

Summary	Shift 1	Shift 2
Mean	2.14	2.57
St. Dev.	0.67	0.80
n	39	39
Conf level	95%	
CI Lower	1.92	2.31
CI Upper	2.36	2.83
Significance	Significant	

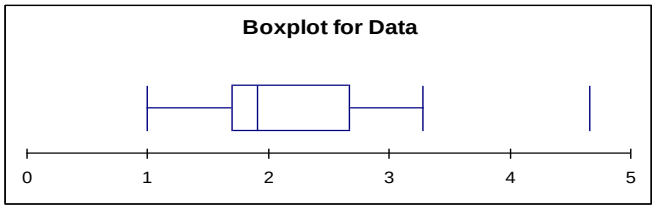


Figure 1: Boxplot of log_{10} TVC cfu/cm^2 for carton meat from Shift 1.

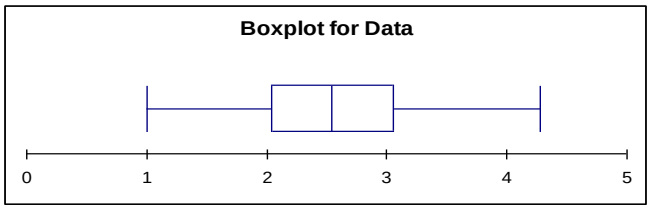


Figure 2: Boxplot of log_{10} TVC cfu/cm^2 for carton meat from Shift 2.

Conclusion

It was concluded that there wasn't a relevant difference between shifts 1 and 2 procedures. The difference between the mean APCs was 0.3 log, and 0.5 log is considered a 'real' or important difference. Although the difference was statistically significant, it wasn't practically significant enough to change the processing procedures. This investigation will continue to monitor manufacturing hygiene of each shift.

Case studies on sheep processing

Fleece Removal

1. Can you please explain our long-term *E. coli* data?

Introduction

We are an export sheep establishment in South-Eastern Australia. At the MINTRAC conference in 2014, SARDI spoke about trend analysis and said they could help with data analysis.

We were interested to know how we have controlled *E. coli* on our sheep carcases over the last seven years since we began ESAM testing.

Objective

We have made changes to our slaughter floor over the past seven years e.g. introduced the use of gloves for all operators (not that we expect that to affect control of faecal contamination). But we have introduced policies on presenting sheep with long, dirty fleeces and in recent years, we have crutched animals with heavy contamination.

The objective of this investigation was to get a general profile of *E. coli* levels on ovine carcases over a seven-year period. We also asked for a comparison with other plants in our region.

Methods

SARDI Comments: *We made a monthly average of Plant A's ESAM data (represented by a black dot on Figure 1) plus a band within which E. coli usually fell (a grey band which represents the consistency of their operation).*

We also amalgamated all the data of 12 other export plants in the S-E region of Australia (South Australian and Victorian plants) and made a similar profile over the same period. This allows Plant A to compare themselves with other plants in their region.

Results

In Figure 1 are our ESAM data for *E. coli* from 2007-2014. These are our take-homes:

- We generally get a winter "high" and a summer "low" of *E. coli.*
- We didn't get a "low" in the summer of 2010-2011.
- From 2007 until 2011, our average monthly *E. coli* rotated between about 15% in summer and 35% in winter.
- From 2012 onwards, it was lower, around 15%.

Looking at these results, we seem to not cope well with stock in winter, which are usually wetter and dirtier, though we seem to have lifted our game since we started our fleece length and crutching policies in 2012.

But we're interested to know how we measure up against other sheep plants and SARDI accumulated data from 12 other export plants in S-E Australia which source livestock from the same regions that we do.

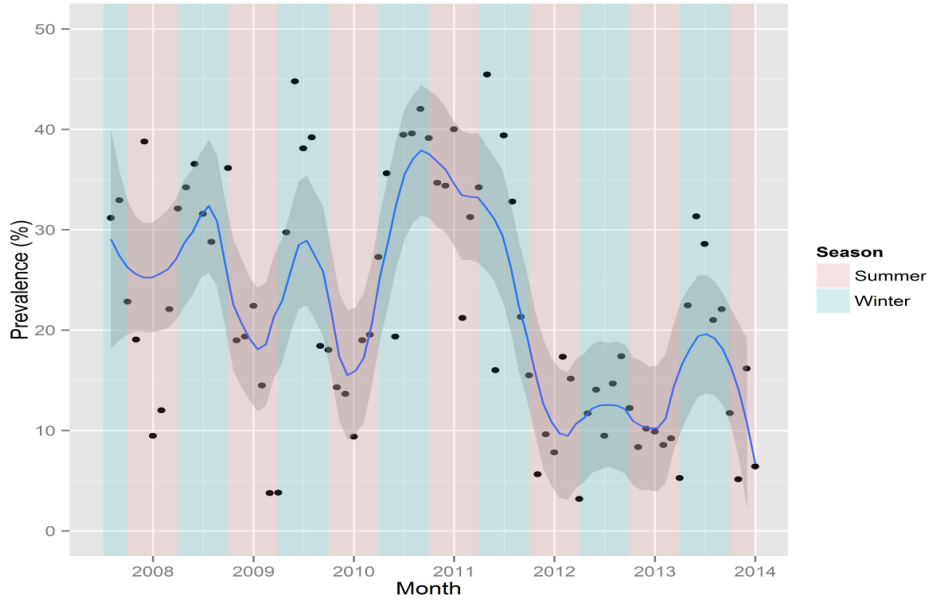


Figure 1: Monthly prevalence of *E. coli* on sheep carcases at S-E Australian plant A during 2007-2014

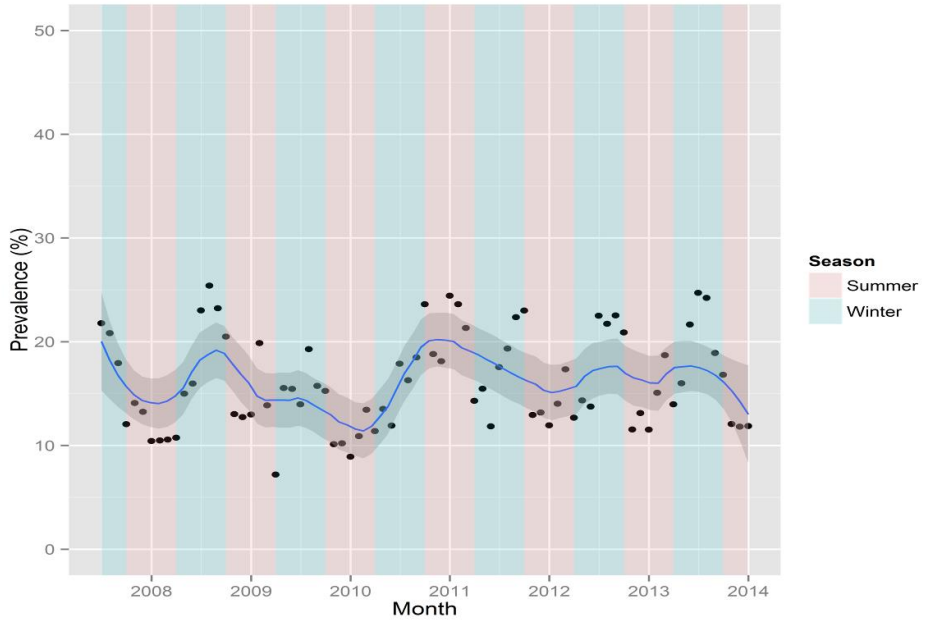


Figure 2: Average monthly prevalence of *E. coli* on sheep carcases from twelve other S-E Australian plant during 2007-2014

SARDI comments

The E. coli monthly average E. coli prevalence for twelve S-E Australian plants is presented in Figure 2. There are some similarities with Plant A in that there is usually a winter "high" and a summer "low"; also like Plant A there was no summer low in 2010-11.

However, Plant A has generally higher levels of E. coli in the early part of their profile (2007-11) with the 12 plants cycling between about 10% in summer and 20% in winter. After 2007, Plant A's E. coli is more like the regional average.

We were interested in why E. coli levels stayed high during the summer of 2010-11 and we think it was all due to the end of the Millennium Drought that affected S-E Australia for 1997-2009. The drought broke in late-2010 when S-E Australia recorded its largest annual rainfall on record.

The region experienced a strong La Niña, with widespread rainfall in the Murray Darling Basin, a region with a high density of sheep.

Spring rainfall was 60% above, and summer rainfall was 150% above the 20th century average (Bureau of Meteorology data).

Tropical cyclone Yasi, which crossed the North Queensland coast in early February 2011 also had a significant effect in south-eastern Australia.

Extreme rain events occurred in each month from September 2010 to February 2011 (Australia's spring and summer, respectively) in S-E Australia with the Bureau of Meteorology (BoM) issuing more than 1500 flood watches and warnings.

The rainfall formed an inland sea approximately 90 km long by 40 km wide in north-eastern Victoria which moved progressively through that state and neighbouring South Australia towards the mouth of the Murray River.

Many properties remained flooded for several weeks with significant stock losses, particularly of sheep, with more than 11,000 killed and more than 14,000 injured/missing (Comrie, 2011[1]).

We think the unusual rainfall and flooding conditions were the cause of the high E. coli prevalence in the summer of 2010-11.

[1] Comrie, N. Review of the 2010-11 flood warnings and response. Government of Victoria. (2011).

2. Microbiological impact of conventional knife vs. air knife during dropping of socks

Introduction

The current on-plant sock dropping technique approved by DA is to use a conventional knife after the air knife operation to drop the socks. The company could reduce the labour requirement by one person if allowed to use the air knife operator to perform this task.

Objective

Determine if dropping socks with air knife compared with normal knife would have the same result when it comes to contamination by performing a microbiological assessment of both techniques.

Methods

Our current work instruction requires the dropping of socks to be performed by an operator using a conventional knife after the air knife operation as shown below in Figure 1.

Figure 1: Current procedure using conventional knife.

To help the company to reduce labour cost, we would like to add to the current work instruction for 'Air Knife Inside Legs' a variation that would utilize the air knife operator to perform the task of dropping of socks, as shown in Figure 2.

Sampling: Samples were gathered by sponging the foreshank area (~25cm^2) using the same technique as for ESAM sampling. Twenty-five samples were taken after the sock was dropped using a conventional knife and 25 samples were taken after the sock was dropped using the air-knife.

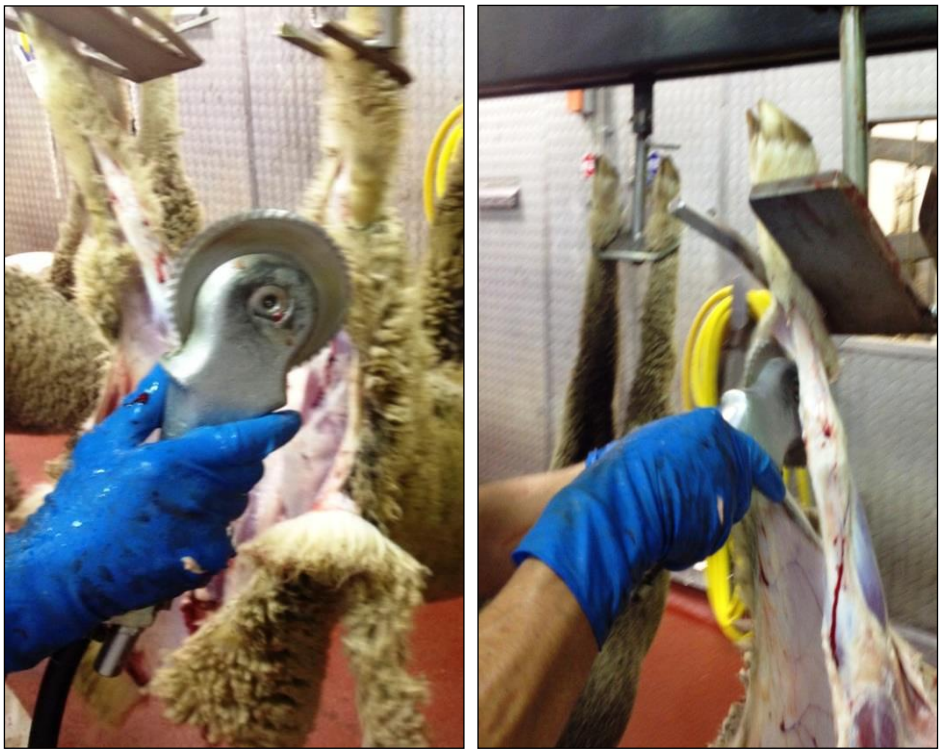


Figure 2: New procedure using air knife.

Testing and analysis: Sponge samples were plated on *E. coli* Petrifilm and TVC Petrifilm, plates were then incubated at 35°C (*E. coli*) and 25°C (TVC) as per work instructions. After 48 hours, colonies were counted and data entered onto a spreadsheet, where we have been able to apply the following tables and boxplots to show our final results.

Results

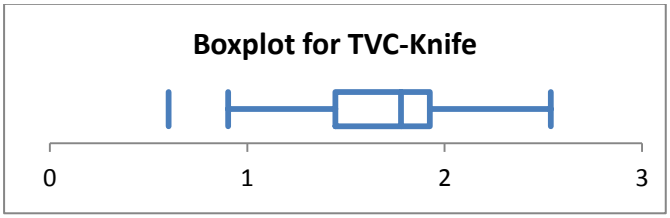
The results are presented in Tables 1 and 2 and Figure 3 from which there is only a 0.02 difference in the average $\log_{10}$ TVC/cm^2 (P-value > 0.1) and no difference in the prevalence of *E. coli*.

Table 1: Summary of $\log_{10}$ TVC cfu/cm^2 for conventional knife and air knife.

Summary	KNIFE(log)	AIR KNIFE(log)
Mean	1.70	1.72
St. Dev.	0.45	0.43
N	25	25
Conf level	95%	
CI Lower	1.51	1.54
CI Upper	1.88	1.90
Significance	Not significant	

Table 2: Summary of *E. coli* prevalence for conventional knife and air knife.

Summary	KNIFE	AIR KNIFE
Detect	3	3
N	25	25
Prev	12.0%	12.0%
Conf level	95%	
CI Lower	3.5%	3.5%
CI Upper	31.0%	31.0%
Significance	Not significant	



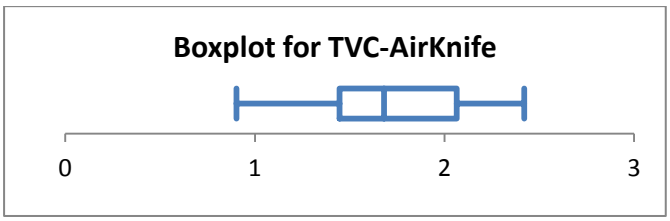


Figure 3: Boxplots of the $\log_{10}$ TVC results showing the slight difference between Conventional Knife and the Air Knife.

Conclusion

It was concluded that there is no significant microbiological difference between the use of a conventional knife compared to an air knife for the operation of dropping socks.

3. Comparison of contamination between Dorpers and Crossbred sheep

Introduction

We process Dorpers and Crossbred sheep on an inverted system and are concerned that the Dorpers may have a higher level of contamination than Crossbreds.

Objective

Determine if Dorpers have a higher level of contamination at the forequarter area compared to Crossbreds.

Methods

Sampling: Twenty five samples were gathered from Dorpers and Crossbreds, 50 in total, by sponging the forequarter cutting line on the left side of the carcases (100 cm^2).

Testing and analysis: Tested on plant. Sponge samples were plated on *E. coli* and TVC Petrifilm and incubated at 35°C and 30°C respectively. After 48 hours, colonies were counted and data entered.

Results

Results are presented in the table below from which it can be seen that the prevalence of *E. coli* on legs from the two breeds was not significantly different. As shown in Tables 1, 2 and Figures 1 and 2, total bacteria were much higher on Dorpers, the mean TVC count was 1.2 log higher (mob sampled appeared to be dirtier than usual). Boxplots of the TVC are both fairly compact with 2 Dorper results sitting outside on the lower end of the scale. The boxplots show a highly significant TVC level on the Dorpers.

Table 1: Summary of *E. coli* prevalence for Dorpers and Crossbreds

Summary	Dorper	Cross
Detect	9	6
n	25	25
Prev	36.0%	24.0%
Conf level	95%	
CI Lower	20.3%	11.3%
CI Upper	55.6%	43.9%
Significance	Not significant	

Table 2: Summary of log_{10} TVC cfu/cm^2 for Dorpers and Crossbreds.

Summary	Dorper	Cross
Mean	4.02	2.83
St. Dev.	0.23	0.19
n	25	25
Conf level	95%	
CI Lower	3.92	2.75
CI Upper	4.11	2.91
Significance	Highly significant	

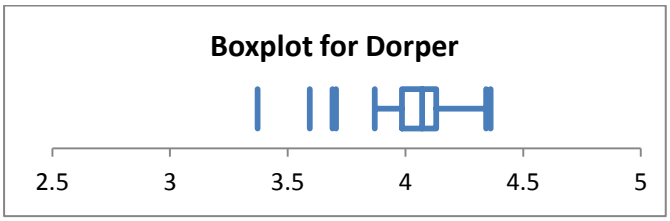


Figure 1: Boxplot of log_{10} TVC cfu/cm^2 for Dorper.

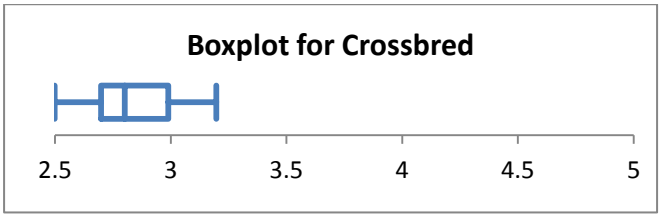


Figure 2: Boxplot of log_{10} TVC cfu/cm^2 for Crossbred.

Conclusion

It was concluded that the Dorpers have a significantly higher TVC than crossbreds (by about 1.2 log_{10} cfu/cm^2) and that we need to look into methods to reduce the count.

Final Inspection

4. Impact of meat inspection on the microbiological status of sheep carcases[1]

Introduction

Traditional meat inspection of adult sheep carcasses in Australia involves extensive palpation and incision. The process emphasises detection of lesions in lymph nodes due to caseous lymphadenitis (CLA). CLA does not have any food safety implications. However, the process of manual inspection to detect CLA is likely to spread microbial contamination on and between carcasses.

Objective

To assess the extent to which traditional meat inspection of adult sheep affects the microbiological characteristics of selected areas of the carcass surface.

Methods

A total of 96 sheep carcasses were sampled (48 before, 48 after). Half of the carcasses were assessed prior to any meat inspection and the remaining half assessed immediately after inspection of the superficial lymph nodes for evidence of CLA. Half the carcasses were assessed at a site near the shoulder (prescapular); the others were assessed near the tail/bung.

Sampling:

- Carcasses were "systematically selected" for sampling from the processing chain. For example, if the sampling interval is 10 then every 10th carcass will be selected for sampling.
- There were two groups of carcasses: "Pre-inspection" carcasses are assessed for microbial load immediately before normal inspection.
- "Post-inspection" carcasses are assessed immediately after inspection.
- Post-inspection carcasses were sampled as soon as possible following the completion of the inspection.
- One individual performed the sampling (carcass swabbing) with additional support as required for handling of swabs and recording data. It was important for only one individual at any one plant to perform swabbing to minimise the effect of individual samplers on the data.
- There were two "standard swabbing sites" on carcasses, tail/bung and pre-scapular.
- Swabbing alternated between tail and shoulder sites. i.e. first sheep will be tail, second bung, third tail, fourth bung etc.

[1] Jordan, D., Sentence, C., Spooncer, W., Balan, J. and Morris, S. (2012). Inspection of lymph nodes for caseous lymphadenitis and its effect on the density of microbes on sheep carcasses. Meat Science, 92:837-840.

- A single swab was used to collect from both the a. and b. site with the swab being inverted when changing from the a. to b. location.
- Each swabbing site consisted of two 25cm^2 area of carcass sampled in a fashion identical to that normally used for all smallstock.

Storage and transport of samples

- Plastic bags holding swabs were stored on ice in insulated containers until the completion of each sampling session.
- Insulated containers holding the specimens and ice packs were sealed and sent by air courier to the laboratory as soon as possible after completion of sampling.

`Testing and analysis

Specimens were sent to a laboratory to be analysed using NATA accredited methods within 24 hours of collection.

Results

The results are presented in Tables 1-4 and Figures 1 and 2. *E. coli* was isolated more frequently from the tail area prior to palpation. This is not the case for swabs which were taken from the pre-scapular area, where *E. coli* was isolated less frequently after palpation.

Table 1: Summary of *E. coli* prevalence for investigation of microbial contamination at the tail before and after palpation.

Summary	Tail Before	Tail after
Detect	15	28
n	48	48
Prev	31.3%	58.3%
Conf level	95%	
CI Lower	19.9%	44.3%
CI Upper	45.4%	71.1%
Significance	Highly significant	

Table 2: Summary of *E. coli* concentration for investigation of microbial contamination at the tail before and after palpation.

Summary	Tail Before	Tail after
Mean*	-1.08	-0.63
St. Dev.*	0.61	0.58
n	5	8
Conf level	95%	
CI Lower	-1.84	-1.11
CI Upper	-0.32	-0.15
Significance	Not significant	

* includes only samples with detectable levels of *E. coli*

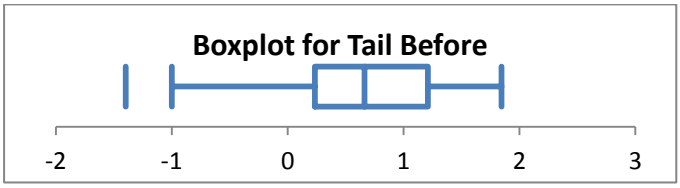
Table 3: Summary of *E. coli* prevalence for investigation of microbial contamination at the pre-scapular before and after palpation.

Summary	Prescapular before	Prescapular after
Detect	24	1
n	48	25
Prev	50.0%	4.0%
Conf level	95%	
CI Lower	36.4%	0.0%
CI Upper	63.6%	21.4%
Significance	Highly significant	

Table 4: Summary of *E. coli* concentration for investigation of microbial contamination at the pre-scapular before and after palpation.

Summary	Prescapular before	Prescapular after
Mean*	-1.06	-0.64
St. Dev.*	0.63	0.69
n	5	8
Conf level	95%	
CI Lower	-1.84	-1.22
CI Upper	-0.28	-0.06
Significance	Not significant	

* includes only samples with detectable levels of *E. coli*



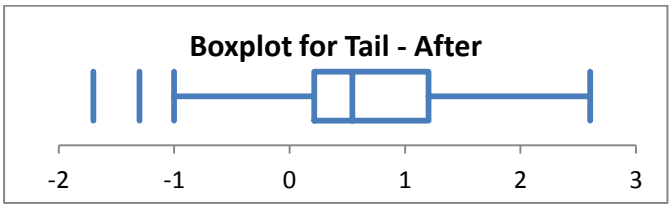


Figure 1: Boxplots of log_{10} TVC cfu/cm^2 for Tail before and after palpation.

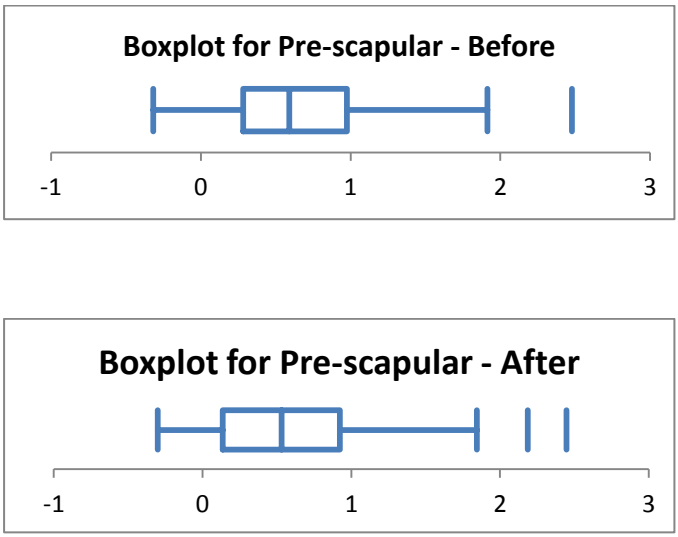


Figure 2: Boxplots of $\log_{10}$ TVC cfu/cm^2 for Pre-scapular before and after palpation.

Conclusion

It was concluded that the current procedures for manual inspection of lymph nodes for CLA do not have a significant impact on the microbial concentration of sheep carcasses at either of the two selected sites. There is a significant difference in *E. coli* prevalence before and after palpation. *E. coli* was isolated more frequently from the tail area prior to palpation. This is not the case for swabs which were taken from the pre-scapular area, where *E. coli* was isolated less frequently after palpation.

5. Effect of carcass wash on hygienic status of ovine carcases

Introduction

TVC results taken from the slaughter floor at the MHA stand, in conjunction with the ESAM program, are consistently much lower than the TVC results that are taken from carton meat samples in the Boning Room. These differences in results are much more noticeable in hot-boned mutton products compared to cold-boned lamb products.

Because the carcass wash is performed on lamb but not mutton, it is thought that this may be the reason behind these differences.

Objective

To determine if washing the carcasses will result in a lower TVC.

Methods

Processing: Our current processing method does not require the washing of mutton carcasses. All lamb carcasses are washed manually by an operator using a high-pressure hose. The hindquarters are not washed in this process, only the mid sections and the forequarters.

Sampling: Forty samples were collected by sponging three different sites on the carcass (75cm^2). Twenty were taken at the MHA station prior to the carcass wash, and 20 were taken immediately after the carcass wash. The three sampling sites were located on the mid or forequarter section of the carcasses. The sampling site used after the carcass wash was immediately adjacent to the site used prior to washing.

Testing and analysis: Sponge samples were plated on Aerobic Plate Count (APC) Petrifilm and incubated at 35°C using the AOAC official method 990 12 at an external NATA accredited laboratory. After 48 hours, colonies were counted and data entered on a spreadsheet tool.

Results

The results are presented in Table 1, 2 and Figures 1-3 from which it can be seen that the difference in TVC is not significant.

Table 1: Summary of $\log_{10}$ TVC cfu/cm^2 before and after carcass wash.

TVC	Before Wash	After Wash
Mean ($\log_{10}$ cfu/cm^2)*	1.04	1.14
SD ($\log_{10}$ cfu/cm^2)*	0.65	0.59

Table 2: Summary of difference in log_{10} TVC cfu/cm^2 before and after carcass wash.

Summary	Difference (log)
Mean	-0.11
St. Dev.	0.57
n	12
Conf level	95%
CI Lower	-0.47
CI Upper	0.26
Significance	Not significant

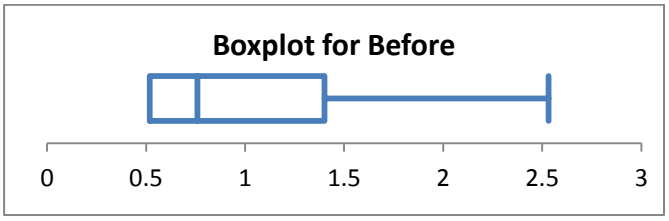


Figure 1: Boxplot of the log_{10} TVC/cm^2 from before the carcass wash.

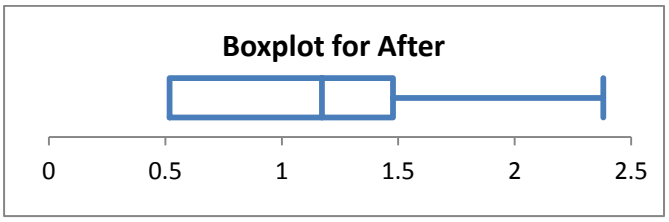


Figure 2: Boxplot of the log_{10} TVC/cm^2 from after the carcass wash.

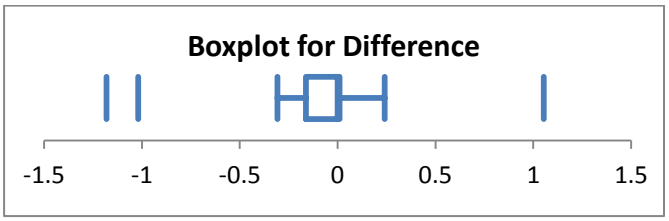


Figure 3: Boxplot of the difference between the results in log_{10} TVC/cm^2.

Conclusion

It was concluded that the carcass wash is not a significant process when trying to reduce microbial results.

6. Contamination sites for *E. coli* on sheep carcases

Introduction

We recently completed a study looking at swabbing non-ESAM sites on beef carcases to identify highly *E. coli* prevalent carcase sites (Case Study 16). One follow-up question is: do sheep also have high-risk contamination sites? And if they do, can knowledge of these sites could be used to inform changes in processing steps that may help to reduce overall bacterial contamination.

Objective

- To sample alternative sites on sheep/mutton carcases and determine which sites have the highest prevalence and concentration of *E. coli*.
- To see whether there are differences in results between establishments.

Methods

Four sheep abattoirs in South Australia, Victoria and New South Wales were selected for sampling. At each establishment, sampling was conducted over four days and each day, eight carcases, spread over the shift, were selected prior to entry to the chillers and hot swabbed. Carcases at all four plants were inverted during dressing. Aseptic sponge sampling was carried out on four sites individually (100cm^2 each) – rump, belly, neck and shank (Figure 1) on alternating left and right sides of successive carcases.

Samples were transported to the SARDI Food Safety and Innovation laboratories for microbiological testing within 24 hours and the temperature of samples in transit did not exceed 4°C. Sponges were tested for Total Viable Count (TVC), *Enterobacteriaceae*, coliforms and *E. coli* using Petrifilm™.

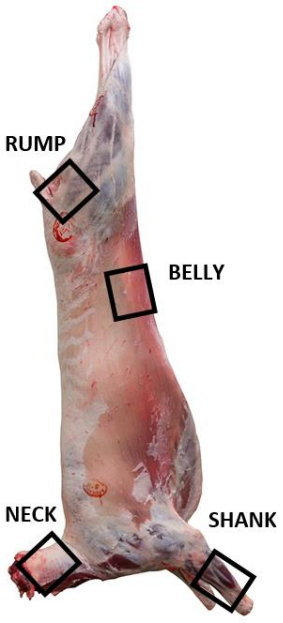


Figure 1: Swab sites on sheep carcase.

Results

In total, across the four establishments, 512 swab samples were collected from 128 sheep/mutton carcases.

Statistical analysis of TVC (Table 1, Figure 2) indicates average counts at the neck at Plants 1 and 4 were significantly lower than those at Plants 2 and 3 ($p<0.001$). Plant 4 also had significantly lower TVCs at the shank compared to the other three plants ($p<0.001$).

Table 1: Summary of mean $\log_{10}$ TVC (cfu/cm^2) of carcases at four Australian sheep abattoirs.

	Rump	Belly	Neck	Shank
Plant 1	2.61	1.91	2.67	3.11
Plant 2	3.26	2.70	3.41	3.57
Plant 3	3.00	3.14	3.67	3.57
Plant 4	2.80	2.48	2.60	2.30

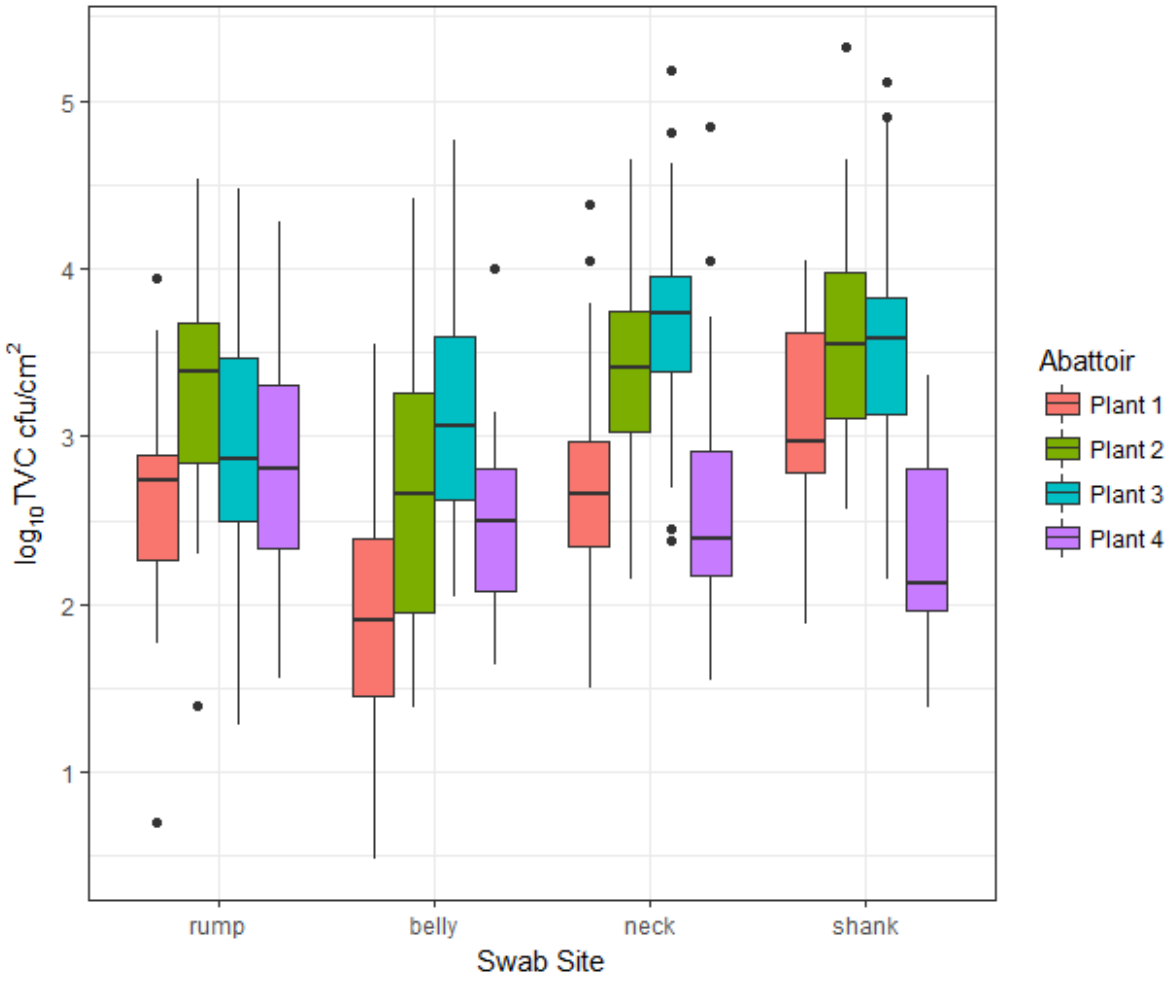


Figure 2: Comparison of TVC of carcases at four Australian sheep abattoirs.

E. coli prevalence at the neck, belly and shank sites varied widely at all plants while the rump had consistently high *E. coli* prevalence at all four abattoirs (Table 2).

Table 2: Prevalence of generic *E. coli* detections on carcases from four Australian sheep abattoirs.

	Rump	Belly	Neck	Shank
Plant 1	27/32 (84.4%)	8/32 (25%)	18/32 (56.3%)	14/32 (43.8%)
Plant 2	32/32 (100%)	25/32 (78.1%)	30/32 (93.8%)	29/32 (90.6%)
Plant 3	30/32 (93.8%)	27/32 (84.4%)	21/32 (65.6%)	21/32 (65.6%)
Plant 4	26/32 (81.3%)	17/32 (53.1%)	18/32 (56.3%)	13/32 (40.6%)
Prevalence	**115/128 (89.8%)**	**77/128 (60.2%)**	**87/128 (68%)**	**77/128 (60.2%)**

Mean counts of the *E. coli* positive samples are presented in Table 3 and Figure 4, with highest counts at the rump at all four plants. Plant 2 had *E. coli* counts at the rump, belly and neck which were 0.72-1.34 $\log_{10}$ cfu/cm^2 higher than at other plants.

Table 3: Summary of mean $\log_{10}$ *E. coli* (cfu/cm^2) of *E. coli* prevalent carcases at four Australian sheep abattoirs.

	Rump	Belly	Neck	Shank
Plant 1	1.36	0.27	0.80	0.66
Plant 2	2.08	1.38	1.12	0.57
Plant 3	0.92	0.51	0.57	0.69
Plant 4	0.74	0.52	0.59	0.19

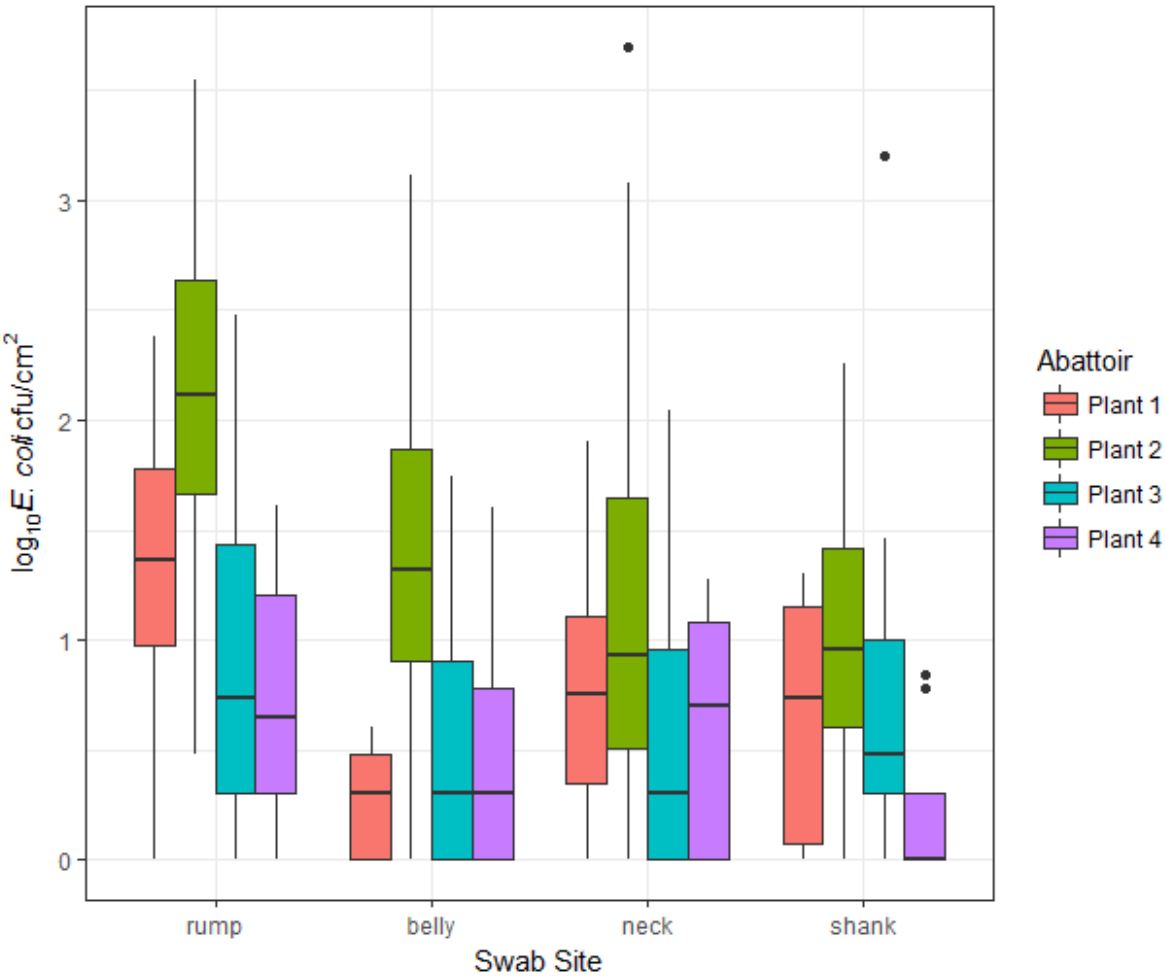


Figure 4: Comparison of *E. coli* counts on carcases at four Australian sheep abattoirs.

Conclusion

The first objective of this study was to sample alternative carcase sites and identify sites with a high prevalence and concentration of *E. coli*. Results showed that the neck and shank were peak sites for TVC, while *E. coli* was more prevalent at the rump. This is not surprising given that *E. coli* is a faecal indicator.

The second objective related to differences between abattoirs. Results showed differences both in prevalence and concentration of *E. coli*. For example, Plant 2 was observed to have a very high prevalence of *E. coli* at all carcase sites (>78%) whilst the other three plants had prevalence of *E. coli* in the range of 25%-60%.

While all plants practiced inverted dressing of carcases, there are many factors that may influence inter-plant bacterial counts on finished carcases. For this reason, plant comparisons should be used only as a guideline.

7. Comparing *Enterobacteriaceae* and *E. coli* as indicator organisms

Introduction

We recently completed a study which looked at high risk *E. coli* contamination sites on sheep carcases, the results of which you can find in Case Study 6. In this study, all the samples were tested for both *Enterobacteriaceae* and generic *E. coli*. Because *E. coli* is detected only infrequently, it can be difficult to observe trends or assess the efficacy of interventions. *Enterobacteriaceae* is a large family of bacteria, of which *Escherichia* is one genus, so it is more likely to be present at detectable concentrations, and may be suitable as an indicator organism.

Objective

To see if there is a relationship between *Enterobacteriaceae* and *E. coli* counts at the rump, belly, neck and shank sites on sheep carcases.

Methods

Four sheep abattoirs in South Australia, Victoria and New South Wales were selected for sampling. At each establishment, sampling was conducted over four days and each day, eight carcases, spread over the shift, were selected prior to entry to the chillers and hot swabbed. Carcases at all four plants were inverted during dressing. Aseptic sponge sampling was carried out on four sites individually (100cm^2 each) – rump, belly, neck and shank (Figure 1) on alternating left and right sides of successive carcases.

Samples were transported to the SARDI Food Safety and Innovation laboratories for microbiological testing within 24 hours and the temperature of samples in transit did not exceed 4°C. Sponges were tested for Total Viable Counts (TVC), *Enterobacteriaceae*, coliforms and *E. coli* using Petrifilm™.

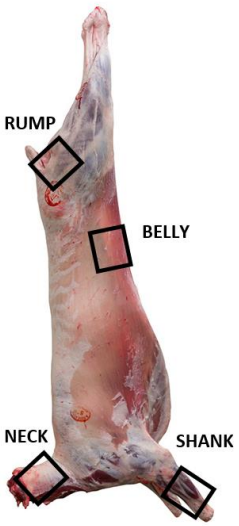


Figure 1: Swab sites on sheep carcase.

Results

In total, across the four establishments, 512 swab samples were collected from 128 sheep/mutton carcases.

E. coli prevalence varied greatest at the belly, ranging from 25% at Plant 1 to 84.4% at Plant 3. The rump was the peak site for *E. coli* prevalence consistently across all four abattoirs (Table 1).

Table 1: Prevalence of *E. coli* on sheep carcases from four Australian sheep abattoirs.

	Rump	Belly	Neck	Shank
Plant 1	27/32 (84.4%)	8/32 (25%)	18/32 (56.3%)	14/32 (43.8%)
Plant 2	32/32 (100%)	25/32 (78.1%)	30/32 (93.8%)	29/32 (90.6%)
Plant 3	30/32 (93.8%)	27/32 (84.4%)	21/32 (65.6%)	21/32 (65.6%)
Plant 4	26/32 (81.3%)	17/32 (53.1%)	18/32 (56.3%)	13/32 (40.6%)

For all sites and across all plants, *Enterobacteriaceae* prevalence was higher than *E. coli* prevalence, though it followed the same trend, varying greatest at the belly from 50% at Plant 1 to 93.8% at Plant 2. The rump was also the peak site for *Enterobacteriaceae* at Plants 1, 2 and 3 while Plant 4 recorded peak *Enterobacteriaceae* at the neck site (Table 2).

Table 2: Prevalence of Enterobacteriaceae on sheep carcases from four Australian sheep abattoirs.

	Rump	Belly	Neck	Shank
Plant 1	29/32 (90.6%)	16/32 (50%)	26/32 (81.3%)	19/32 (59.4%)
Plant 2	32/32 (100%)	30/32 (93.8%)	30/32 (93.8%)	29/32 (90.6%)
Plant 3	30/32 (93.8%)	27/32 (84.4%)	23/32 (71.9%)	29/32 (90.6%)
Plant 4	26/32 (81.3%)	24/32 (75%)	27/32 (84.4%)	18/32 (56.3%)

The rump was the site with the highest mean *E. coli* and *Enterobacteriaceae* counts across all four plants. Plant 2 also had *E. coli* and *Enterobacteriaceae* counts that were 0.72-1.34 $\log_{10}$ cfu/cm^2 higher than the other plants at the rump and belly carcase sites.

At Plant 2, *E. coli* counts (Figure 2) from both the rump and belly were significantly higher on average than those at the same carcase site at the other three plants ($p<0.005$). Neck and shank *E. coli* counts were not significantly different across the four abattoirs.

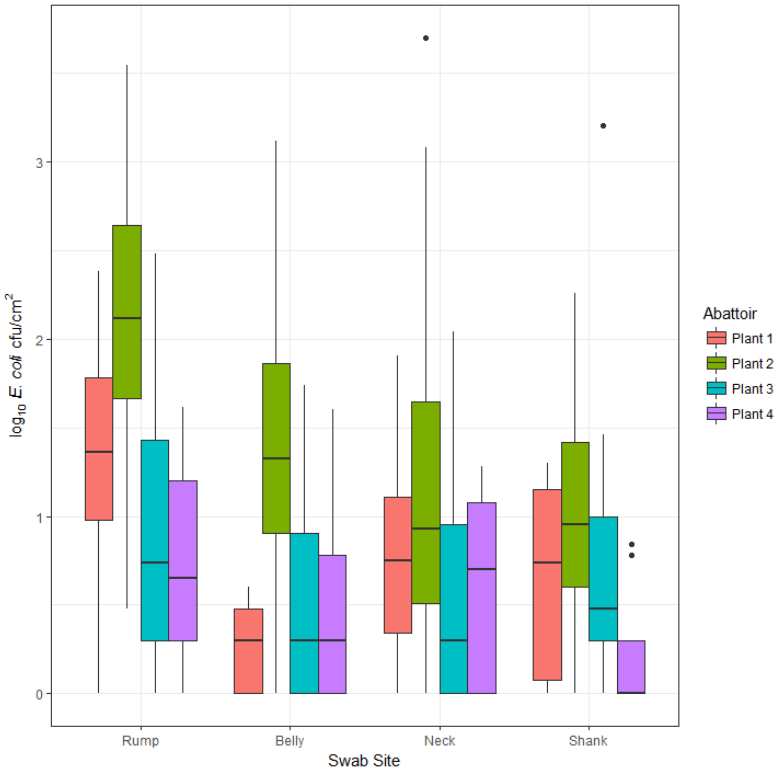


Figure 2: *E. coli* counts (cfu/cm^2) on positive samples at four Australian sheep abattoirs.

Similarly for *Enterobacteriaceae* (Figure 3), Plant 2 registered significantly higher average counts at the rump when compared to the other three plants ($p<0.005$). At the belly, Plant 2 registered significantly higher *Enterobacteriaceae* counts than Plant 1 ($p=0.007$) and Plant 4 ($p=0.01$), although no significant difference was detected when compared with Plant 3. As with *E. coli*, *Enterobacteriaceae* at the neck and shank were not significantly different across the four abattoirs.

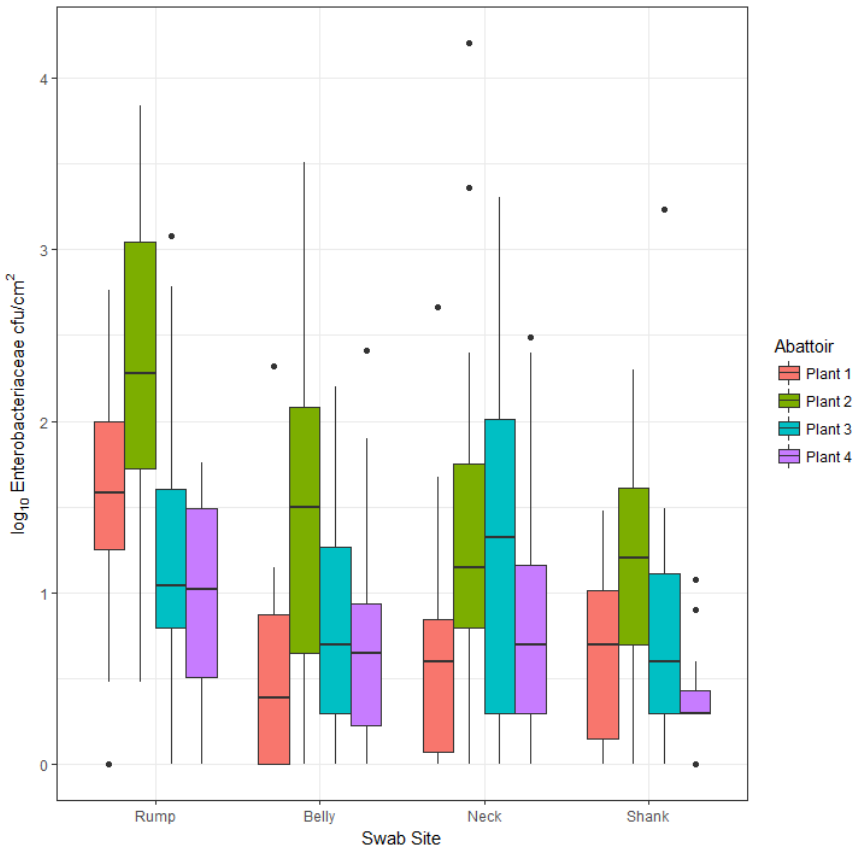


Figure 3: *Enterobacteriaceae* counts (cfu/cm^2) on positive samples at four Australian sheep abattoirs.

Results from all four sheep abattoirs were pooled together and linear regression analysis was undertaken. Figure 4 shows a strong positive correlation between *E. coli* and *Enterobacteriaceae* counts at the rump (r^2=0.80), belly (r^2=0.82) and shank (r^2=0.78), indicating that there is a statistical link between the two organisms at these sites. While at the neck, a weaker correlation was found (r^2=0.49).

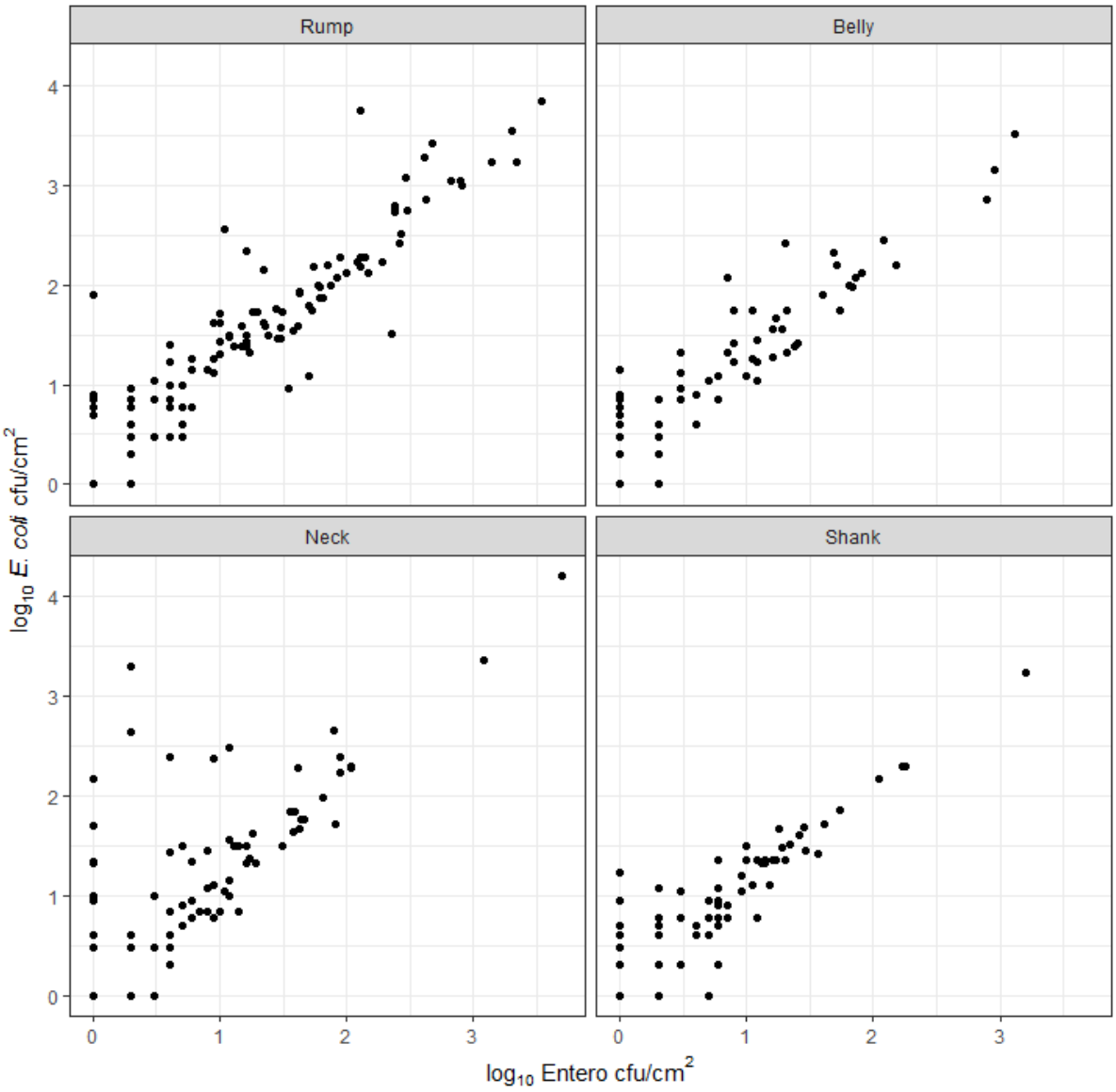


Figure 4: Comparison of *E. coli* and *Enterobacteriaceae* counts (cfu/cm^2).

Conclusion

The objective of this study was to compare *Enterobacteriaceae* and *E. coli* results at the rump, belly, neck and shank of sheep carcases from four Australian abattoirs. Not unexpectedly, *Enterobacteriaceae* was more prevalent than *E. coli* at all carcase sites and at all four abattoirs. In positive samples, *Enterobacteriaceae* was present at higher concentration than *E. coli* at the majority of carcase sites at all four abattoirs. The rump was the peak site in terms of *E. coli* and *Enterobacteriaceae* prevalence and concentration (which is not surprising given that *E. coli* is a faecal indicator), while the belly, neck and shank were comparable. Further statistical analysis of the data from all of the abattoirs revealed a positive linear correlation between *E. coli* and *Enterobacteriaceae* counts at the rump, belly and shank. This study has shown that *Enterobacteriaceae*, due to its higher prevalence and concentration on surface sites of carcases has potential as an indicator of the presence of *E. coli* on sheep carcases.

Boning

8. Investigation of contamination on the band saw on microbiology of lamb primals

Introduction

We have attached a glycol heat plate to a bandsaw so we can eliminate the use of water on the saw.

Objective

Determine the effect of using a glycol heat plate *versus* water on the band saw on contamination of product.

Methods

Sampling: Twenty samples were gathered by sponging the forequarter area (~25cm^2) using the same technique as for ESAM sampling. Ten samples were taken before the saw and ten after running through the saw. This was repeated for both use of water and heat glycol plate.

Testing and analysis: Sponge samples were plated on APC Petrifilm and incubated at 35°C. After 48 hours, colonies were counted and data entered on a spreadsheet tool.

Results

Table 1 and Figures 1 and 2 show the difference in contamination on the forequarter between using water and using the glycol heat plate after running through the saw.

Use of water on the band saw table had an average value of 4.03 $\log_{10}$ cfu/cm^2 while use of the glycol heat plate had an average value of 2.93 $\log_{10}$ cfu/cm^2.

There was a highly significant difference in contamination of the forequarter in using the glycol heat plate compared to water.

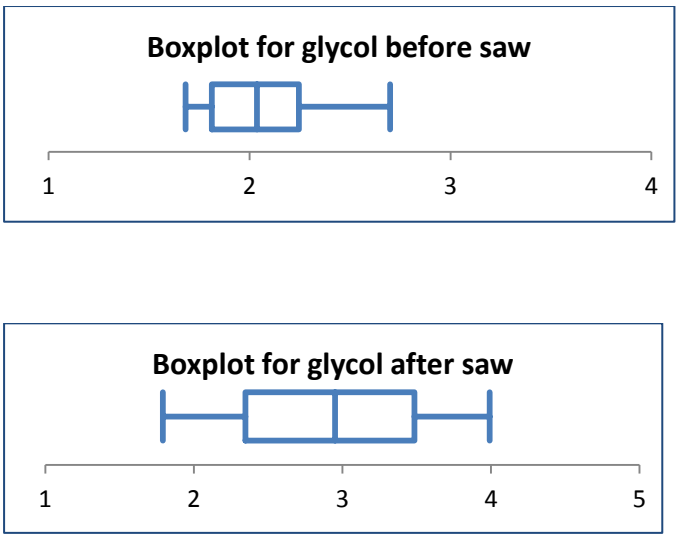


Figure 1: Boxplots of $\log_{10}$ TVC cfu/cm^2 for lamb forequarters when glycol was used on the bandsaw.

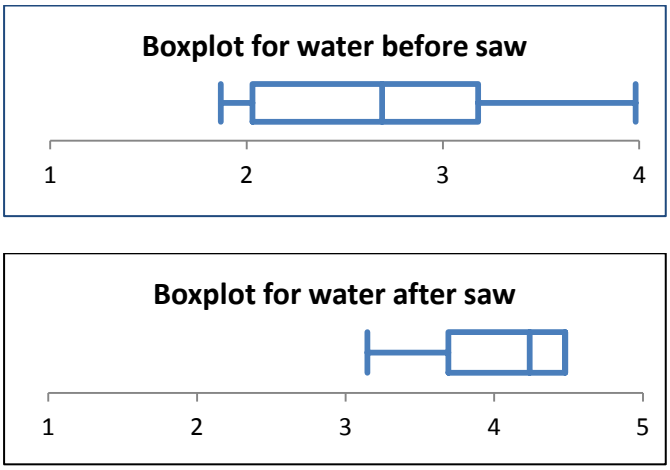


Figure 2: Boxplots of log_{10} TVC cfu/cm^2 for lamb forequarters when water was used on the bandsaw.

Table 1: Summary of log_{10} TVC cfu/cm^2 for Glycol and water.

Summary	Glycol	Water
Mean	2.93	4.03
St. Dev.	0.77	0.53
n	10	10
Conf level	95%	
CI Lower	2.38	3.64
CI Upper	3.47	4.41
Significance	Highly significant	

Conclusion

The use of the glycol heat plate results in lower contamination of the forequarter than when using water on the bandsaw table and this is more effective in reducing bacteria counts on the end product.

9. Lamb leg microbiological status before and after boning

Introduction

We are conducting this investigation to assess the impact boning operations have on TVC and *E. coli* counts.

Objective

Determine bacterial counts before and after boning.

Methods

Processing: Our current work instructions were checked for compliance throughout the swabbing process and were assessed as acceptable.

Sampling: The sampling for this investigation was conducted using the technique as for ESAM sampling. A total of fifty samples were gathered from 25 carcases. This was conducted by sponging the chump area (100cm^2) of the leg on entry to the boning room (prior to pre-trim). The other leg on the carcase was then tagged and the tagged leg was swabbed as above on the leg boning table, the leg was swabbed after the completion of all operations including pre-trim, boning and trimming to specification.

Testing and analysis: Sponge samples were sent to a NATA-accredited Laboratory. Samples sent were tested within 24 h of sampling by plating on Aerobic Plate Count (APC) and *E. coli* Petrifilm and were incubated at 35°C. After 48 hours, colonies were counted and data entered on a spreadsheet tool.

Results

The results are presented in two separate examples from which it can be seen that APC (Table 1 and Figure 1) & *E. coli* (Table 2 and Figure 2) were isolated with higher counts significantly more frequently from the legs swabbed after processing operations were completed than the swabs taken prior to operations (TVC & *E. coli* P-value = <0.001).

Table 1: Summary of difference in log_{10} TVC cfu/cm^2 before and after boning.

Summary	Difference (log)
Mean (Before)	1.06
Mean (After)	2.46
Mean (Diff)	-1.40
SD (Diff)	0.98
n	25
Conf level	95%
CI Lower	-1.80
CI Upper	-1.00
Significance	Highly significant

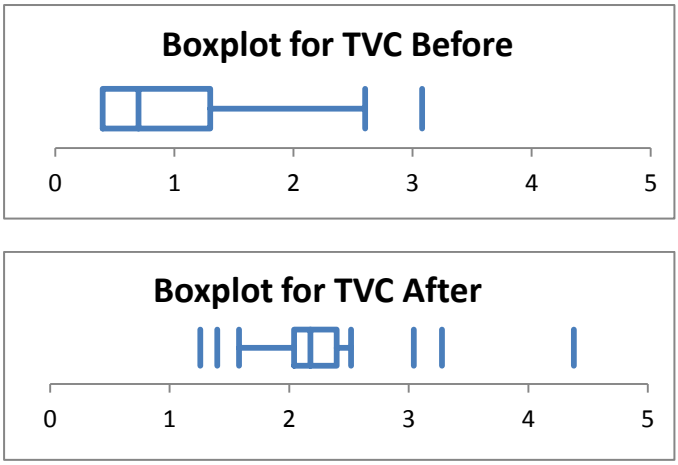


Figure 1: Boxplots of $\log_{10}$ TVC cfu/cm^2 for before and after boning.

Table 2: Summary of *E. coli* prevalence for before and after boning.

Summary	Before	After
Detect	2	16
n	25	25
Prev	8.0%	64.0%
Conf level	95%	
CI Lower	1.2%	44.4%
CI Upper	26.3%	79.7%
Significance	Highly significant	

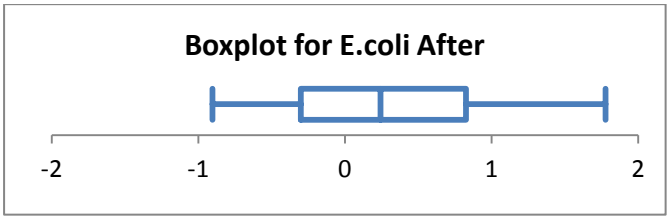


Figure 2: Boxplot of $\log_{10}$ *E. coli* cfu/cm^2 after boning.

Conclusion

It was concluded that current procedures are not acceptable as the rise in contamination is not only in TVC counts but also in *E. coli* counts and prevalence; this indicates there is a significant issue in our process in regards to personnel and/or equipment in regards to cross contamination.

10. Microbiological condition of boning room conveyor belts through a 2-shift working day, and impact on bacterial loading of lamb legs

Introduction

We bone lamb carcases over two shifts, the first beginning at 06:00 and the second ending around 01:00. At end of processing, a team of contract cleaners remove the build-up of soils from belts, bandsaws, cutting boards and other equipment before undertaking detergent and sanitiser application. Turnaround must be achieved in around 4 hours. Quality assurance staff perform a visual check (pre-op) before processing is allowed to begin.

Objective

Determine if running the boning operation for 19 hours without stopping for cleaning affects the bacterial loading on final products.

Methods

Processing: During further processing, each carcase is divided into six portions at the band saw: four legs and two half-torsos (6-way cut), after which primals pass on plastic, jointed belts to boners who work on cutting boards before transferring finished cuts back onto transfer belts for packing.

Sampling: In our study, we sampled at three times during the working day:

- 08:00 (2 hours production)
- 13:00 (7 hours production)
- 23:00 (17 hours production)

At each sampling, we tested the band saw, cutting boards, transfer belts, hind legs on the carcase and the fully-boned leg.

Sponge sampling was carried out on product and contact surfaces using Whirlpak sponges resuscitated with Butterfield's solution (25mL).

Areas sponged were:

- Product (100cm^2) at a hind leg site on carcases and on finished legs; the site was on the outside of the leg, away from the bung and at the margin of the bandsaw cut (next to the strip brand).
- Surfaces in-process (100cm^2).
- Clean surfaces were sponged over 5000cm^2.

Testing and analysis: Serial dilutions were prepared using Butterfield's solution and plated onto Aerobic Plate Count Petrifilm and Coliform/*E. coli* Petrifilm. After incubation at 25°C/72 hours for Total Viable Count (TVC) and 37°C/48 hours for *E. coli*, plates were counted according to the manufacturer's instructions. Countable plates were obtained for TVC using 10x and 100x dilutions and for *E. coli* using a 1mL aliquot from the sponge bag.

Results

Tables 1 to 5 below show the mean, standard deviation (SD) and *E. coli* prevalence for each of the different testing locations and times.

There was a significant difference at the 8:00 and 13:00 samplings for the Bandsaw, with the TVC concentration at 13:00 being almost 0.9 log higher than that at 8:00. There were no significant differences between 13:00 and 23:00 or 8:00 and 23:00.

Table 1: Summary of bandsaw hygiene status.

Bandsaw	8:00 (2 hours)	13:00 (7 hours)	23:00 (17 hours)
TVC Mean (log_{10} cfu/cm^2)	0.98	1.84	1.28
TVC SD (log_{10} cfu/cm^2)	0.76	0.76	0.08
E. coli Detections/n (%)	0/5 (0%)	0/5 (0%)	1/5 (20%)
E. coli Mean (log_{10} cfu/cm^2)*	NA	NA	0.5
E. coli SD (log_{10} cfu/cm^2)*	NA	NA	NA

* includes only samples with detectable levels of *E. coli*

There were no significant differences in TVC concentration for any of the sampling times for the cutting board.

Table 2: Summary of cutting board hygiene status.

Cutting Board	8:00 (2 hours)	13:00 (7 hours)	23:00 (17 hours)
TVC Mean (log_{10} cfu/cm^2)	1.64	2.68	2.34
TVC SD (log_{10} cfu/cm^2)	0.83	0.77	0.57
E. coli Detections/n (%)	1/5 (20%)	1/5 (20%)	1/5 (20%)
E. coli Mean (log_{10} cfu/cm^2)*	0.25	0.5	0.5
E. coli SD (log_{10} cfu/cm^2)*	NA	NA	NA

* includes only samples with detectable levels of *E. coli*

Samples taken from the belt show a significant difference in the TVC concentration between 8:00 and 13:00 with the samples taken at 8:00 being 0.6 log higher than those taken at 13:00. There was no statistically significant difference between the other time combinations.

Table 3: Summary of belt hygiene status.

Belt	8:00 (2 hours)	13:00 (7 hours)	23:00 (17 hours)
TVC Mean (log_{10} cfu/cm^2)	2.80	2.18	2.48
TVC SD (log_{10} cfu/cm^2)	0.14	0.29	0.44
E. coli Detections/n (%)	2/5 (40%)	3/5 (60%)	2/5 (40%)
E. coli Mean (log_{10} cfu/cm^2)*	0.5	0.58	1.38
E. coli SD (log_{10} cfu/cm^2)*	0.35	0.38	1.59

* includes only samples with detectable levels of *E. coli*

There were no significant differences in TVC concentration for any of the sampling times for the carcase.

Table 4: Summary of carcase hygiene status at the hind leg.

Carcase	8:00 (2 hours)	13:00 (7 hours)	23:00 (17 hours)
TVC Mean (log_{10} cfu/cm^2)	1.86	1.42	1.82
TVC SD (log_{10} cfu/cm^2)	0.42	0.43	1.03
E. coli Detections/n (%)	1/5 (20%)	3/5 (60%)	1/5 (20%)
E. coli Mean (log_{10} cfu/cm^2)*	11.25	0.92	0.25
E. coli SD (log_{10} cfu/cm^2)*	NA	0.63	NA

* includes only samples with detectable levels of *E. coli*

Table 5: Summary of Finished legs hygiene status

Finished Legs	8:00 (2 hours)	13:00 (7 hours)	23:00 (17 hours)
TVC Mean (log_{10} cfu/cm^2)	1.48	1.94	1.82
TVC SD (log_{10} cfu/cm^2)	0.43	0.38	0.89
E. coli Detections/n (%)	0/5 (0%)	1/5 (20%)	0/5 (0%)
E. coli Mean (log_{10} cfu/cm^2)*	NA	0.25	NA
E. coli SD (log_{10} cfu/cm^2)*	NA	NA	NA

* includes only samples with detectable levels of *E. coli*

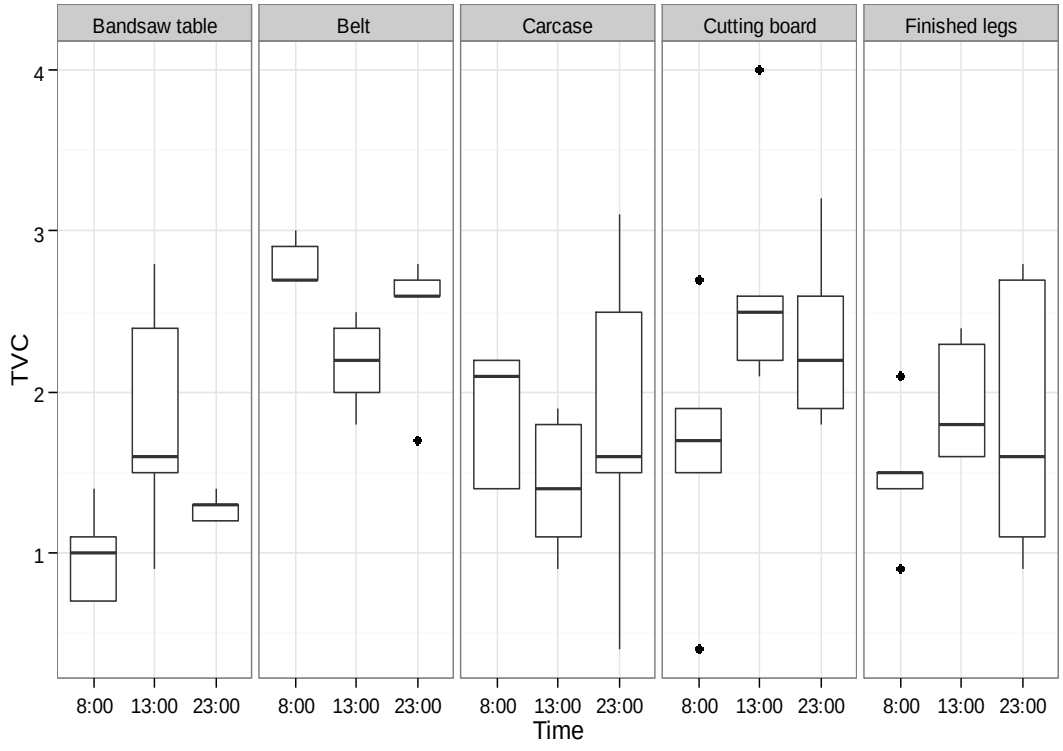


Figure 1: Boxplots showing log_{10} TVC cfu/cm^2 for each sampling site and time.

Conclusion

It was concluded that there was no practically significant differences in *E. coli* prevalence or TVC concentration at the different times throughout the production period.

11. Effectiveness of cleaning program for cleaning conveyor belts

Introduction

We run two shifts boning ovine carcases, the first beginning at 06:00 and the second ending around 01:00. At end of processing, the cleaning team must turn the boning room around in approximately 4 hours to the satisfaction of QA staff who undertake a pre-op inspection.

Objective

Determine whether the cleaning regime results in equipment which is not only visually clean but also of acceptable microbiological status as defined by criteria in the Microbiological Guidelines which accompany the Australian Standard (AS 4696:2002) where a Total Count of no more than 5 cfu/cm^2 is considered satisfactory.

Methods

At the end of processing, there is considerable build-up of soils from belts, bandsaws, cutting boards and other equipment, which is dismantled and dry-cleaned by removing as much soil (meat scraps, fat and blood) as possible. All surfaces are foamed with a chlorinated alkali detergent for a contact time of at least 15 minutes before rinsing and sanitizing (at present a QUAT is used).

Sampling

Food contact surfaces were tested at two stages:

- Before cleaning (at 23:00) after 17hours of processing
- After cleaning (at 05:00) after equipment had been re-assembled and dried.

Testing and analysis

Food contact surfaces were sponged using Whirlpak sponges resuscitated with Butterfield's solution (25mL). Areas sponged were 5000cm^2 for conveyor belts and 2000cm^2 for other surfaces.

Serial dilutions were prepared using Butterfield's solution and plated onto Aerobic Plate Count Petrifilm and Coliform/*E. coli* Petrifilm. After incubation at 25°C/72 hours for Total Viable Count (TVC) and 37°C/48 hours for *E. coli*, plates were counted according to the manufacturer's instructions. Countable plates were obtained for TVC using 10x and 100x dilutions and for *E. coli* using a 1mL aliquot from the sponge bag.

Counts on the Petrifilm were converted to APC/cm^2 and *E. coli*/cm^2.

Results

Bacterial loading at the end of production

Towards the end of the processing day (23:00), counts were undertaken on selected surfaces listed in Tables 1 and 2. The results give an indication of the bacterial loading which must be removed, together with visible soil, during the clean down process. The APC loading varied from 1 log/cm^2 to 4 log/cm^2 and *E. coli* was isolated from 4/25 surfaces tested.

Table 1: APCs and *E. coli* counts on food contact surfaces at 23:00 hours.

Sampling time	23:00	
	E. coli/cm^2	Log_{10} APC/cm^2
Bandsaw table	nd	1.4
Bandsaw table	nd	1.3
Bandsaw table	nd	1.2
Bandsaw table	0.5	1.3
Bandsaw table	nd	1.2
Mean		**1.3**
Cutting board	nd	2.5
Cutting board	nd	2.2
Cutting board	nd	2.6
Cutting board	0.5	4.0
Cutting board	nd	2.1
Mean		**2.7**
Transfer belt	2.5	2.6
Transfer belt	nd	2.8
Transfer belt	nd	1.7
Transfer belt	0.25	2.7
Transfer belt	nd	2.6
Mean		**2.5**

nd = not detected

Table 2: APCs and *E. coli* counts on food contact surfaces at 23:00 hours.

	***E. coli*/cm^2**	**Log_{10} APC/cm^2**
Transfer belt square cut shoulders	nd	2.4
Square cut shoulder belt for trimming	nd	1.0
Square cut bandsaw table	nd	1.4
Rack bandsaw 1 table	nd	1.9
Rack bandsaw 2 table	nd	1.9

nd = not detected

Bacterial loading after cleaning

On the day of testing, production ceased around 01:15 and recommenced at 06:00. By 05:00, cleaning of the boning room had been completed and a pre-op check began, involving a member of the company's QA team and the supervisor of the cleaning team. The department generally appeared clean, except for some scale deposits on some stainless surfaces e.g. the guard of the square cut transfer belt, bandsaw tables and supports for cutting boards.

Microbiological testing of cleaned surfaces was undertaken between 05:00 and 05:30, and the bacterial loading presented in Table 3 reflect the effectiveness of clean down.

When the results of the sampling are assessed against the criteria in the microbiological guidelines which accompany the Australian Standard, it can be seen that almost all tests were either Unsatisfactory (>5 cfu/cm^2) or were almost at that level.

- The two plastic belts which are used in association with the square cut shoulder operation had high counts (20 and 25 cfu/cm^2) as did the stainless steel guard on both sides of the transfer belt (9.6 cfu/cm^2).
- The main bandsaw table was 14 cfu/cm^2.
- Supports for cutting boards were 0.7 and 9.5 cfu/cm^2.
- Cutting boards were 1.2, 9 and 12 cfu/cm^2.

Table 3: APCs of cleaned surfaces.

Cleaned surface	Log_{10} APC/cm^2
Transfer belt	0.7
Transfer belt	0.8
Transfer belt	0.7
Transfer belt	0.7
Transfer belt	0.8
Transfer belt	0.7
Transfer belt	0.8
Transfer belt	1.0
Transfer belt	0.8
Transfer belt	0.9
Transfer belt	1.3
Transfer belt	1.0
Shoulder trimmer belt	1.4
Main band saw table	1.1
Guard on transfer belt	1.0
Support for cutting board	-0.2
Support for cutting board	1.0
Cutting board	1.1
Cutting board	1.0
Cutting board	0.1

Conclusions

Our survey involved only surfaces which are easy to clean, so it is surprising that we had counts which were almost always >5/cm^2 (>0.7 log_{10} cfu/cm^2). The surfaces were visually clean and we suspect that, in order to have the room ready for pre-op, the cleaning team did not sanitise the surfaces.

12. Processing sheep heads – batch collection versus individually processing

Introduction

Extraction of sheep brains involves opening of the skull, removal of the brain, batching of brains for inspection, sending brains to the offal room, rinsing with water, and vacuum packing. For operational reasons, it would be beneficial to collect heads for processing, instead of processing each head individually as it becomes available.

Objective

To compare the microbiological quality of sheep brains extracted from skulls that were collected in a tub with skulls individually processed (no batching).

Methods

Thirty sheep skulls were collected either in a tub (batched) or processed individually (without batching). Brains from either treatment were processed as usual. Brains from the two treatments were removed aseptically from the vacuum packs for microbiological testing. Each brain was swabbed on the ventral and the dorsal sides using a sterile 5x5 cm template, and one swab per side. The swabs were combined and 25ml Buffered Peptone Water was added for testing. A 1ml aliquot of diluent was extracted and plated on APC Petrifilm, or similarly on *E. coli* Petrifilm. Petrifilm plates were incubated at 35°C for 48hrs at which time, colonies were counted and a per cm^2 concentration was calculated.

All counts were log_{10} transformed for data analysis, which included graphical comparison using box plots, and testing for difference in the mean log_{10} concentration using a two-sample t-test.

Results

A summary of the microbiological results of both treatment groups, batch and single, are shown in Table 1. From these, it can be seen that there were no coliform or *E. coli* detections in either treatment group, and the mean Total Viable Counts (TVC) concentrations were also very similar. This is also evident when comparing the TVC box plots for the two treatment groups (Figure 1).

Table 1: Summary statistics of sheep brains processed in batch or single.

		Prevalence	
Treatment Group	**Mean log_{10} TVC/cm^2**	**Coliforms**	***E. coli***
Batch	2.44	0/30	0/30
Single	2.53	0/30	0/30

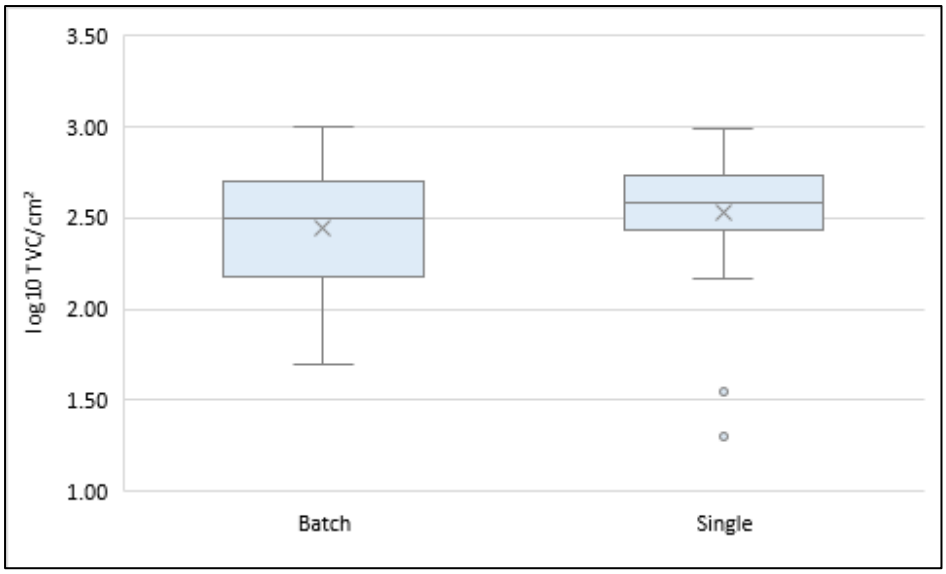


Figure 1: Box plots of log_{10} TVC concentrations on sheep brains processed in batch or single.

The statistical comparison of the means log_{10} concentrations, using a two-sample t-test, is shown in Table 2, with an associated P-value of 0.38.

Table 2: Two-sample statistical summary

Summary	log(Batch)	log(Single)
Mean	2.44	2.53
St. Dev.	0.34	0.38
n	30	30
Conf level	95%	
CI Lower	2.32	2.39
CI Upper	2.57	2.67
Significance	Not significant	

Conclusions

There is no evidence to indicate that the microbiological quality of sheep brains from tub-batched skulls is different from those processed individually.

Case studies on calf processing

Chemical decontamination

1. Effect of chemical decontaminants on TVC of calf carcases

Introduction

We currently use hot water treatment in order to reduce bacterial contamination of our calf carcases, but it is quite expensive. We are considering chemical decontamination as a cheaper alternative and designed this study to examine the effect of chemical sprays at different concentrations on reducing Total Viable Counts (TVC) of calf carcases.

The USA has approved the use of ClO_2 and PAA as a direct food additive for decontamination of red meat carcases and could be applied as a spray or dip at a level not to exceed 3 ppm residual chlorine dioxide; PAA could be used as a spray not to exceed 220 ppm PAA. However, other overseas markets such as Korea and Japan do not accept the use of ClO_2 and PAA as an antimicrobial agent for red meat.

Objective

- To assess the effect of chemical decontamination on the TVC of calf carcases.

Methods

Calf carcases (n=30) were divided into 6 groups each of 5 units and sprayed as follows:

- PAA at 150ppm and 350ppm
- Lactic acid at 2% and 5%
- Twin Oxide at 100 ppm
- Acidified sodium chlorite at 1000ppm

Sponge sampling was carried out on hot carcases at the three ESAM sites (25cm^2 at flank, brisket and midline). One side of the carcase was sampled prior to treatment with chemical (pre spray), and the opposing side sampled 30 minutes after treatment (post spray). Samples were plated onto TVC Petrifilm™ and incubated at 35°C for 48 hours, colonies were counted and entered into a spreadsheet tool for analysis.

Results

As seen from Figure 1 & Table 1, there was a reduction in log_{10} TVC (cfu/cm^2) of carcases post spray after all decontamination treatments.

Lactic acid at 5% yielded the greatest reduction in TVC (log_{10} 0.85 cfu/cm^2) but resulted in discolouration of the carcase while Twin Oxide (100ppm), PAA (350 ppm) and sodium chlorite (1000ppm) were all similarly effective, with reductions of log_{10} 0.54, log_{10} 0.55 and log_{10} 0.55, respectively. PAA (150ppm) resulted in the smallest TVC reduction (log_{10} 0.02 cfu/cm^2).

A paired t-test to compare log_{10} TVC (cfu/cm^2) of calf carcases before and after the application of a particular chemical treatment found acidified sodium chlorite

(1000ppm) caused the greatest reduction (p = 0.04) while no significant difference was detected following the application of any of the other five chemical sprays.

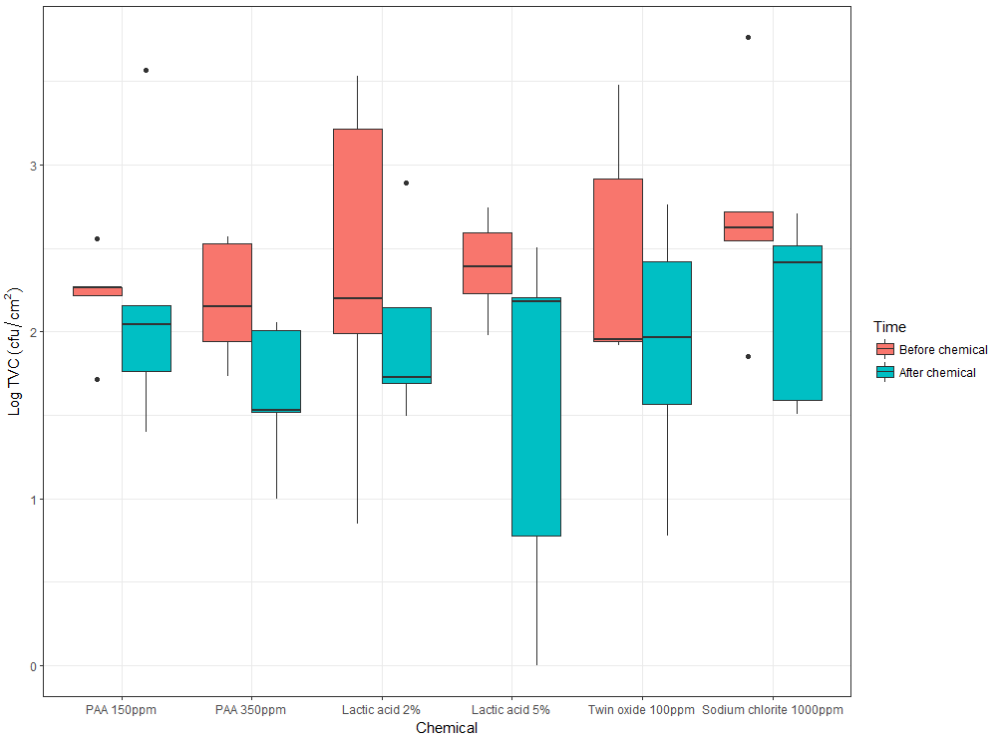


Figure 1: Side by side boxplots of log_{10} TVC (cfu/cm^2) before and after chemical spray treatment.

Table 1: Comparison of mean log_{10} TVC (cfu/cm^2) of calf carcases before and after chemical spray treatment.

Spray treatment	TVC		Change in TVC
	Before spray	After spray	
PAA 150ppm	2.20	2.19	0.02
PAA 350ppm	2.18	1.62	0.56
Lactic acid 2%	2.36	1.99	0.37
Lactic acid 5%	2.39	1.53	0.85
Twin oxide 100ppm	2.44	1.90	0.54
Sodium chlorite 1000ppm	2.70	2.15	0.55

Conclusion

We observed solid reductions in mean log_{10} TVC of 0.54, 0.55, 0.56 and 0.85 cfu/cm^2 for Twin Oxide, acidified sodium chlorite, PAA (350ppm) and lactic acid (5%) respectively. However, only the acidified sodium chlorite was found to cause a statistically significant reduction. The sample size used in this study (n=5 per chemical treatment) is too small to make any further conclusions. We would require 20-25 swab samples before and after for each chemical treatment to answer our research question with greater confidence.

Guide to working with shelf life of chilled vacuum-packed product

As well as food safety, shelf life (or storage life) has always been important in the meat trade, and is becoming increasingly so, as Australian retailers and importers in overseas countries request information to substantiate company claims about how long their products will remain saleable.

The MLA publication, *Shelf life of Australian red meat (2nd edition[6])* provides background information on the science of shelf life and the requirements of many export markets. AMPC/MLA have also produced *Guidelines for developing a method for estimating shelf life of chilled raw vacuumed meat products*, which describes how to set up storage trials and determine product shelf life. The guidelines are updated here based on the experience we have had since they were first published.

MLA and AMPC have also been working with groups at the University of Tasmania and CSIRO, among others, on aspects of shelf life, and we are taking the opportunity to bring all of that information together here.

We also add information on:

1. Setting up storage trials in your plant – static trials
2. Setting up a cold chain trial with an international partner
3. Using the correct data logger for the job
4. Interpreting cold chain data
5. Using the predictive model for the shelf life of chilled vacuum-packed beef and sheep meat

We give examples of monitoring cold chains to give an idea of what careful temperature monitoring and using the shelf life predictor will do for you. Most of the focus will be on storage life of chilled meats, with some information on frozen meats.

For further information or advice in planning, running a trail please contact UTAS or MLA.

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[6] https://www.mla.com.au/globalassets/mla-corporate/research-and-development/program-areas/food-safety/pdfs/shelf-life-of-australian-red-meat-2nd-edition.pdf

1. Cold chains then and now

Australia's first cold chain was established in February 1880 when the *SS Strathleven* arrived in London with a 34-tonne cargo of frozen beef and mutton. The mere achievement of getting meat from one side of the globe to the mother country was rightly hailed – but would the quality stack up?

The world's first taste panel was set up on a grand scale on the *Strathleven* where one hundred and fifty notables dined and at Buckingham Palace where Queen Victoria gave the royal seal of approval. The venture also proved commercially viable, with auction prices close to those of fresh beef and a global frozen meat trade was born.

Early problems with freezer burn and loss of shelf life during thawing were quickly solved and Australia continues to ship large quantities of frozen meat to many destinations – in 2016 it was over 900,000 tonnes.

Australia's early domination of the global meat trade was short-lived - South American countries such as Argentina and Paraguay could land chilled meat in London after a 14-day voyage. The product was markedly superior to Australian frozen meat because there was no 'drip', and it attracted a price premium.

It wasn't until the late 1960s and the advent of vacuum packaging that Australia was able to compete successfully on the chilled meat trade when. With advances in packaging films and technology, it is now possible to reach distant markets.

Australia now exports chilled meats to more than one hundred countries and in 2016, more than 350,000 tonnes being exported

1.1 Storage life of frozen meats

Most countries which import frozen meat either impose no expiry limit or allow manufacturers to set their own shelf-life. Most of Australia's larger trading partners allow 24-months for consumption from the date of slaughter, though some Middle Eastern countries require storage lives for frozen meat of 12 months or shorter.

The CSIRO summarised the science underpinning the freezing and frozen storage of meat: *During frozen storage microbiological growth is arrested, but meat will slowly deteriorate due to oxidative and other changes. Frozen storage life is normally limited by the development of adverse flavours caused by oxidative rancidity of fat.*

The temperature of storage, method of packaging and degree of saturation of the fat all affect the onset of these changes (CSIRO, 2002).

In recent years the packaging of frozen primals has improved significantly due to the use of films with low oxygen and moisture transmission rates, which optimise storage life by reducing fat oxidation and moisture loss.

The International Institute of Refrigeration (IIR) comments on the effect of packaging on storage life:

Unprotected (not packaged) frozen meat carcasses and cuts will continue losing moisture and weight through sublimation of ice from the meat surface during storage. The loss of

weight during storage will increase with increasing and fluctuating storage temperatures and increasing storage time.

The IIR recommends: *Packaging meat with strong, moisture impermeable plastic film that is in close contact with the meat surface, e.g. by shrink-wrapping, can reduce the weight loss to insignificant levels, not only during freezing, but also more importantly during frozen storage* (IIR, 2006).

Such recommendations align with current packaging formats for frozen meat with product generally wrapped in films with low oxygen and moisture transmission rates then shrink-wrapped or vacuumed packed to facilitate freezing and extend frozen storage life.

1.2 Storage life of chilled meats

The chilled meat trade is based on two packaging and transport modes:

1. Carcases and vacuum packed primals air freighted to destinations
2. Vacuum packed primal cuts transported by sea freight

For many years there was anecdotal evidence from the trade that Australian vacuum packaged primals had longer storage lives in commerce compared with product from competing countries, with 100 days (~14 weeks) at -1°C quoted. The evidence for achieving the long shelf life of Australian beef and sheep meats is discussed in MLA publication, *Shelf life of Australian red meat (2nd edition)*[7].

MLA commissioned CSIRO to undertake a series of storage trials on vacuum-packed beef including primals (striploins and cube rolls). Samples were withdrawn after intervals from CSIRO's chiller and microbiological and sensory testing carried out. Cube rolls and striploins from six abattoirs located from Tasmania to far-north Queensland, were stored at -0.5°C for up to 30 weeks, with sensory panels finding product acceptable for at least 27 weeks (Small *et al*. 2012).

2. Setting up storage trials in your plant – static trials

Establishments which supply major supermarkets or fast food chains need to provide shelf life information on a regular basis. These customers require information on relatively short-term storage (up to 6-7 weeks) and microbiological criteria are of primary importance to them. There is up-to-date information on the microbiological limits required by supermarkets in MLA's *Shelf life of Australian red meat (2nd edition)*, which also contains information of what a realistic retail microbiological specification looks like. These shelf life studies can usually be performed at a single site, without the need for transporting product (static trials). We provide an updated version of *Guidelines for developing a method for estimating shelf life of chilled raw vacuumed meat products* (see page 193).

[7] https://www.mla.com.au/globalassets/mla-corporate/research-and-development/program-areas/food-safety/pdfs/shelf-life-of-australian-red-meat-2nd-edition.pdf

3. Setting up a cold chain trial with an international partner

Establishments also need to set up trials for international customers and these can be based on microbiological criteria and/or expiry dates from date of slaughter. Information is provided in *Shelf life of Australian red meat (2nd edition)* and updates in importing country requirements that are supplied by the Department of Agriculture and Water Resources (DAWR).

Sometimes your sales staff will see a market opportunity by exporting a product which is new to a particular country, posing the question: *how will the product fare in the cold chain and how acceptable will it be to wholesalers, retailers and consumers?* For instance, the opening of the Chinese market to chilled meats requires establishments to guarantee a storage life of 120 days (~17 weeks) through the cold chain.

The only way to assess if your product will survive the marketing and retailing chain of the importing country is to set up a trial with an importer. Recently an Australian company wanted to evaluate how a range of products would remain acceptable in a Middle Eastern country and took a number of steps to manage the trial at both ends:

- Personal contacts were made between sales and QA staff in the supplier and importing establishments
- The methodology for the trial was settled well in advance
- The supplier provided a detailed micro profile of all product types at the time of packing, so that advantage could be taken of the UTas predictor tool
- Six data loggers were inserted in well-marked cartons so that a time:temperature profile could be established door-to-door and during storage with the importer
- The importer set up a sensory panel with a range of experience in the Middle Eastern wholesale and retail trade
- After each sensory panel the information was circulated for immediate evaluation
- The panel tested each product type to end of shelf life

Despite all these precautions there were lessons to be learned from this and other trials.

3.1 Recovering the loggers themselves

It sounds simple, but recovering a data logger at its destination is sometimes difficult. In the mid-1990s first-generation loggers were large and expensive, around $1,000. The forerunner of MLA, the Meat Research Corporation, invested in 30 loggers for a mega experiment to Asia and managed to recover not one of them.

Recently an Australian company placed thirteen loggers in a consignment, but recovered only six for downloading. From the images below you can see the company did their best by labelling each carton containing a logger.

Recovering Loggers:

Have a dedicated person on the other end and keep in close communication when the containers are delivered for the best chance of recovering the data logger

They also:

- Sent a full list of logger numbers and container numbers to the importer
- Identified the position of each logger carton in the container
- Only 6 were recovered out of 13

Lesson to be learned: Have a dedicated person the other end and keep in close communication with him/her when the containers are delivered to the importer's cold store.

3.2 Controlling their downloading

During the Middle Eastern trial it was arranged that cartons would be withdrawn at intervals and samples taken for sensory analysis. The suppliers put six data loggers in six cartons and arranged for the importer to download one logger when the consignment was received, and then to download one every two weeks. This would provide the temperature profile across the whole life of the product and would be key information into the shelf life prediction tool.

As shown in the images above, the Australian establishment labelled each carton containing a logger and identified where all six cartons were located in the container. The good news is that the receival team recovered all six loggers. The bad news is that the cold store team did what they always did - downloaded all loggers straight away.

Lesson to be learned: In the words of the famous line from the 1967 film Cool Hand Luke: *What we've got here is a failure to communicate*.

3.3 Managing your sensory panel – lessons learned

Organising sensory panels sounds straightforward: get your team in the room, provide the samples and evaluation forms and they'll do the job – or will they?

Examples of where panels go awry include:

1. **Not enough panellists**. In one trial the QA Manager was involved in a car accident and the Sales Manager became ill. A five-person panel suddenly became three.
2. **Lack of focus on what the market will accept**. The five-person panel above all had different backgrounds and different ideas of what the market wanted.
3. **Panel composition and management**. The composition of a panel is important. Amongst the sensory panel members, there were large discrepancies in scores on which is the end of shelf life.

Sensory Panel:

Select plenty of panellists, screen them on their backgrounds and train them on the task

Lesson to be learned: Select plenty of panellists; screen them on their backgrounds; "train" them on the task – what does the market want?; keep the panel on track so no-one disrupts it.

4. Using the correct data logger for the job

Data loggers are used in two ways:

1. In commerce, primarily as an independent source of information on temperature during shipping
2. By researchers and companies wishing to establish the storage life based on using the UTas predictor tool.

The need for accuracy differs between the two uses, being much more important in the latter case, as even a small discrepancy in temperature can have a large effect on predicted storage life.

4.1 Commercial use

In the international meat trade the most commonly used data loggers are single-use, launched when the container is being loaded and downloaded by the recipient at the end of the supply chain. Single-use loggers have an accuracy range around +/- 0.5°C from -10°C to +30°C and are calibrated during manufacture.

It is not unusual when duplicate data loggers are placed in cartons of meat in a container for slightly different temperature profiles to emerge during downloading raising the question of whether the temperature variation is due to differences in logger accuracy or variable temperatures in the container.

In the example below, the six data loggers were inserted in different cartons as part of a storage life trial along the supply chain. It was intended to download loggers progressively over the trial but staff at the importer's cold store downloaded them all at the same time.

As can be seen below both mean temperature and standard deviation of loggers in the same container varied during the 30 days during the journey from Australia to the destination.

Logger #	Mean (°C)	SD (°C)
1	-1.0	0.2
2	-1.1	0.5
3	-1.1	0.3
4	-1.1	0.8
5	-1.2	0.9
6	-1.4	0.9

Temperature in a container:

Temperatures vary within containers. loggers should be placed in the front, middle and end of the container

Temperatures vary within cartons across a container, which is why savvy companies place loggers in cartons in the front row (first to be loaded), in the middle of the container and in the final row next to the doors.

4.2 Research use

Data loggers used for research studies have either an inbuilt probe, or have an external probe attachment which can be inserted just below the meat surface to inform on temperatures at the site of microbiological concern.

It has been suggested that it would be wise to calibrate data loggers which are going to be used as part of shelf life trials, particularly when the data will be entered into the shelf life prediction tool.

Even small differences in temperature around 0°C over a long storage time will result in very different predictions for storage life. It is possible when purchasing data loggers to have them calibrated by an accredited authority. Alternatively, the data logger can be calibrated against a set point, either melting ice or a certified reference thermometer, which is detailed in the *Data logger calibration section* (page 202).

5. Interpreting cold chain data

When data loggers are used to monitor the cold chain for commercial purposes, the exporter usually just wants to know whether everything looks OK. Most of the time everything is OK, as demonstrated by a survey conducted on more than 100 consignments to distant markets (Sumner, 2016)[8].

When single use loggers are downloaded, two files are usually available one of which provides a graphical record over the period when the logger was operating and the other a time:temperature file which can be entered into the shelf life prediction tool.,

5.1 A Normal shipment

An example of the summary file (below) provides information, together with a chart on which temperature 'blips' indicate key events such as loading aboard the vessel, trans-shipment and final unloading at the destination.

[8] Sumner, J. (2016). The impact of transport to Australia's distant markets on the shelf –life of beef and sheep primals. AMPC project report 2016.1075. http://www.ampc.com.au/2016/07/Impact-of-transport-to-Australias-distant-markets-on-the-shelf-life-of-beef-and-sheep-primals-with-special-reference-to-the-Chinese-market

Alarm Status

Low Alarm:	✓
High Alarm:	✓

Monitor Read

On: 5/18/2015 10:09:44 AM By: TempTale4 USB

Note: All Times Shown Are In Greenwich Mean Time(GMT)

Monitor Configuration

Low Alarm:	Enabled	High Alarm:	Enabled
Limit:	-30.0 °C	Limit:	70.0 °C
Type:	Time-Single Event	Type:	Time-Single Event
Threshold:	0 mins	Threshold:	0 mins
Startup Delay:	1 hrs 30 min	Interval:	10 min

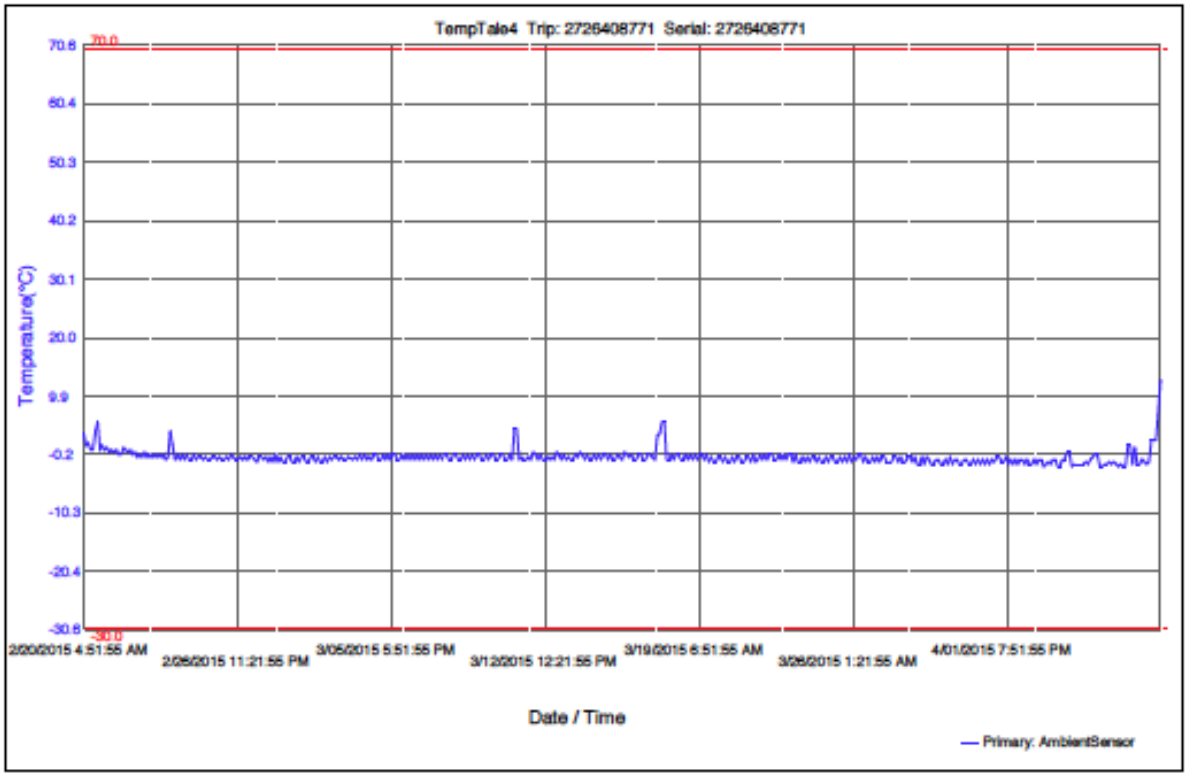
Recorded Data

First Point:	2/20/2015 4:51:55 AM
Stop Time:	4/06/2015 2:27:06 PM
Number of Points:	6826

Alarms

		Low Alarm		High Alarm	
Low Extreme:	-2.2 °C @ 4/04/2015 6:41:55 PM	Time Below:	0 sec	Time Above:	0 sec
High Extreme:	12.7 °C @ 4/06/2015 12:41:55 PM	Longest Time Below:	0 sec	Longest Time Above:	0 sec
Mean ± Standard Deviation:	-0.6 °C ± 1.0 °C	Degree Minutes Below:	0.0 °C min	Degree Minutes Above:	0.0 °C min
Mean Kinetic Temperature:	-0.5 °C	Number Events Below:	0	Number Events Above:	0
		Triggered:		Triggered:	

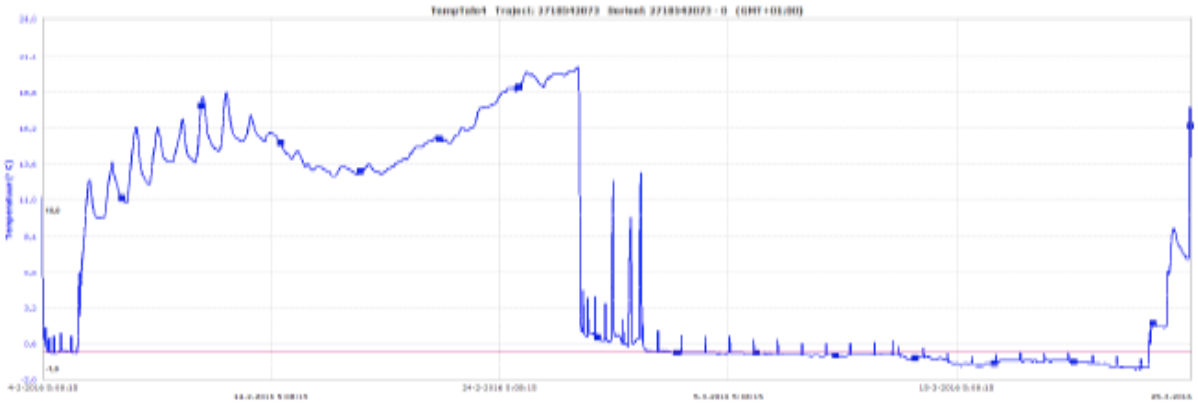
Originator Notes



In the above example the mean temperature was -0.6°C with no damaging rises in temperature during loading, trans shipping and unloading.

5.2 A serious problem

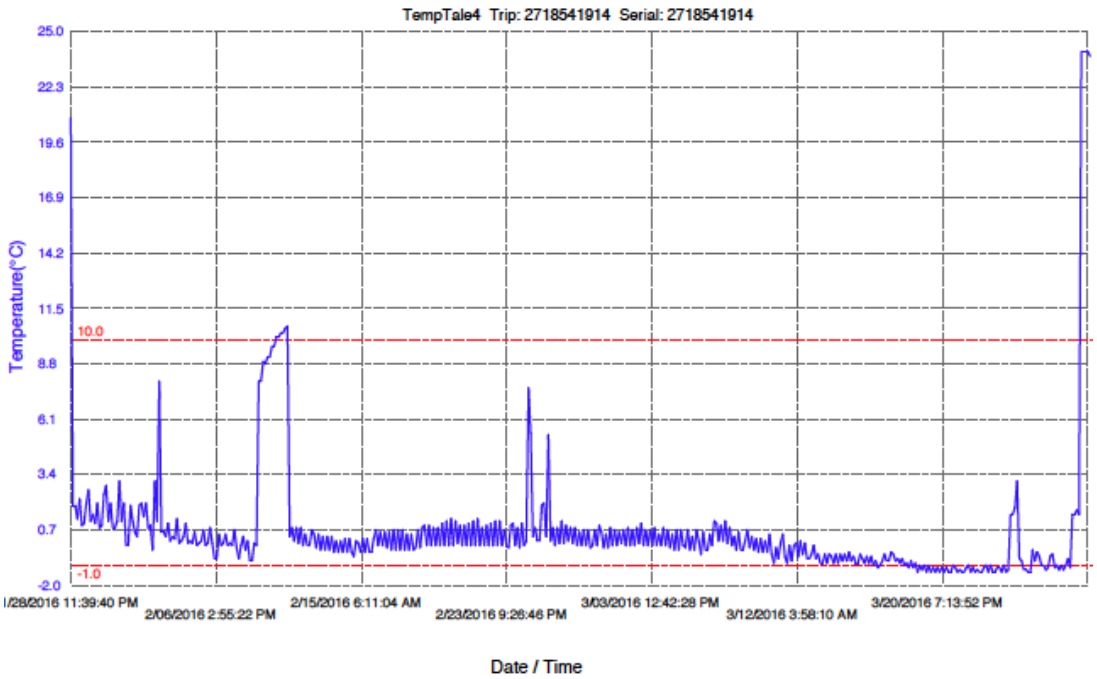
By contrast, in the example below the entire consignment from Australia to the UK was spoiled on arrival. Fortunately the company had used a data logger and it became obvious that the container had not been connected to power from Australia to Singapore so that product cycled between 16°C and 19°C for over 20 days; the container was not faulty and product chilled when the container was ‘plugged in’ during trans-shipment for the Singapore-UK leg.



5.3 Temperature variation with uncertain consequences

As well as recording catastrophic loss of temperature control (above example), loggers also assist in decisions on how to dispose of product which receives a degree of temperature abuse. In the example below, a major spike occurred over three days, when the temperature rose to 10°C early in a voyage from Australia to UK.

When the time:temperature data were entered into the shelf life prediction tool it was found that the consignment of beef primals had effectively lost three days storage life because of the spike, enabling the importer to market product as usual.



5.4 A confusing temperature history

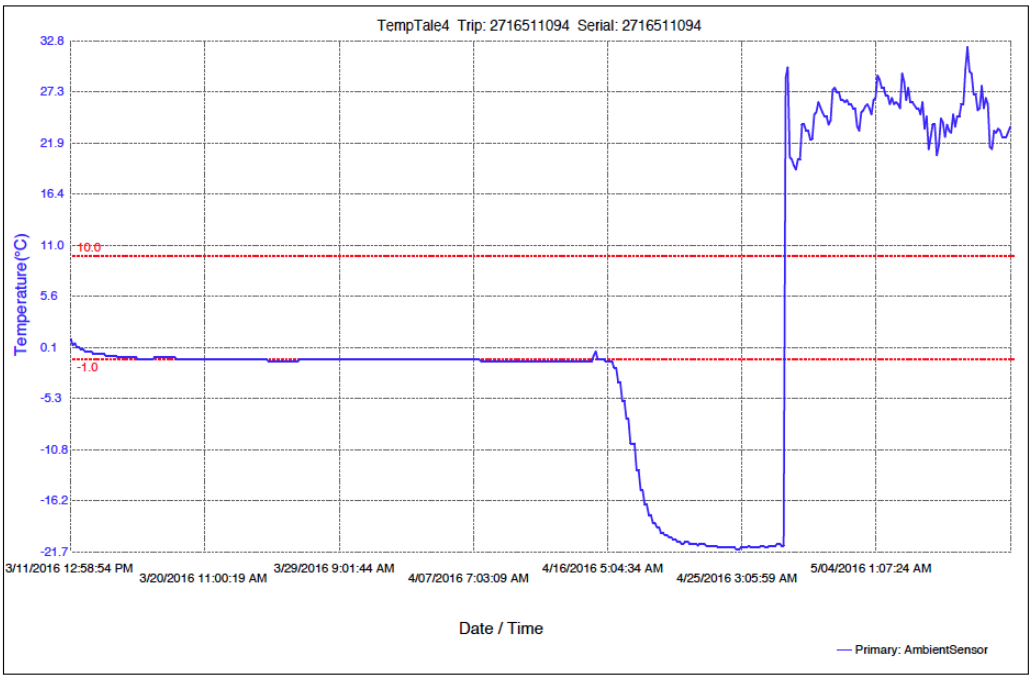
At first sight the example below appears confusing with three phases:

1. A stable phase at -1.0°C for 36 days
2. Freezing and frozen storage for a further 12 days
3. Temperatures between 20-30°C for a final 20 days

Deciphering what has happened to the consignment involves obtaining the transport plan for the voyage, which indicated that on day 35 the consignment was unloaded at the destination port (small blip).

The importer immediately placed the consignment in a freezer store and, over 3 days, the temperature was brought to -21°C.

After almost two weeks in the freezer store the data logger was recovered from the carton and placed in someone's top drawer until it was downloaded and the data sent to the supplier in Australia. Usually, when you see a rapid change in temperature it's a good indication that the logger has been removed from storage.



6. Predicting shelf life with the Shelf life prediction tool

University of Tasmania have developed a predictive tool which estimates shelf life of vacuum packed beef and sheep primals. The tool is described in *Shelf life of Australian red meat* and can be used to predict remaining shelf life providing you know the TVC at packing and the time:temperature record during storage. Once these parameters are entered into the model and either the lamb or beef is selected, predictions for TVCs and days remaining until detection of off odour can be predicted.

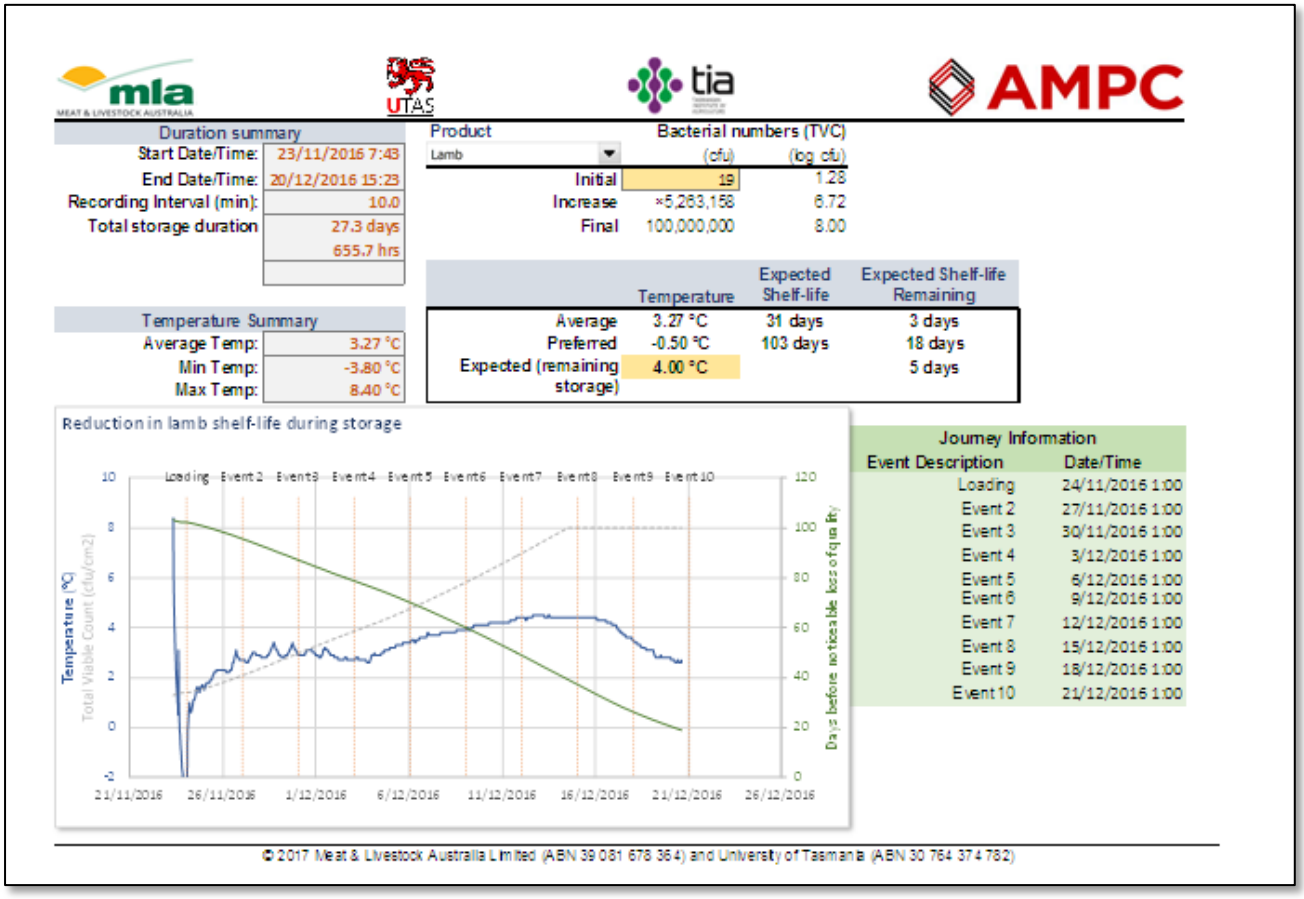
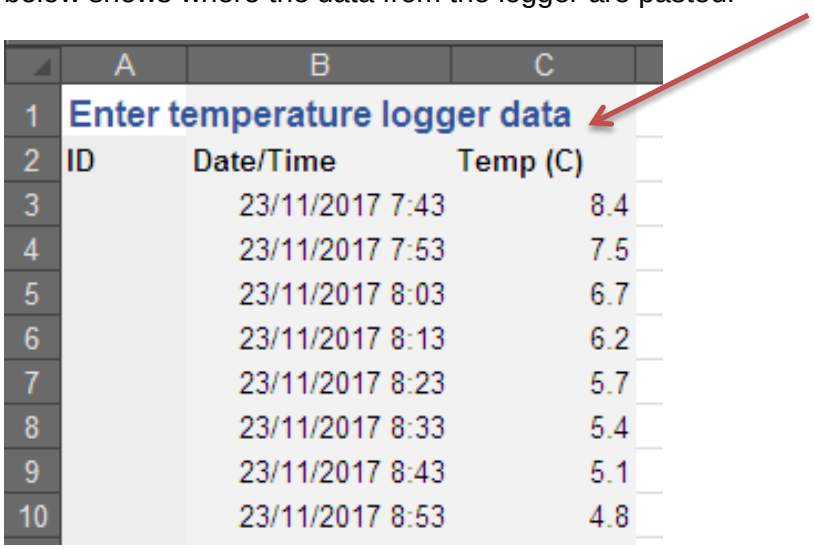


Figure 6.1 – A print out of the shelf life prediction tool.

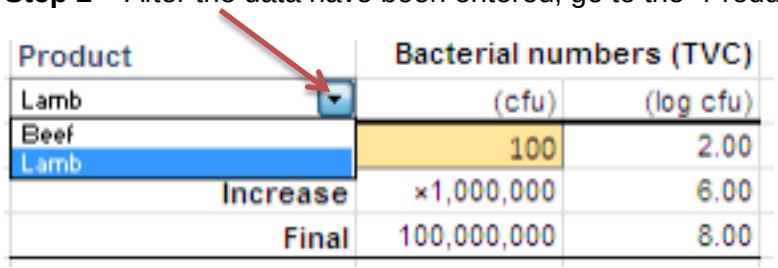
The predictor tool has instructions on the first page when you open it however, below is simple guide on how to use the tool:

Step 1 - Open up the predictor excel sheet and go to the “Temperature log” tab. The image below shows where the data from the logger are pasted.



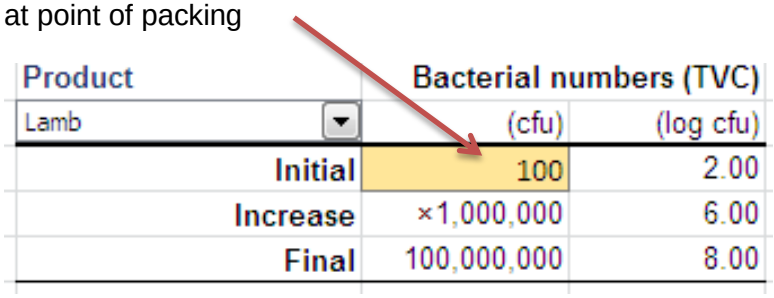
	A	B	C
1	Enter temperature logger data		
2	ID	Date/Time	Temp (C)
3		23/11/2017 7:43	8.4
4		23/11/2017 7:53	7.5
5		23/11/2017 8:03	6.7
6		23/11/2017 8:13	6.2
7		23/11/2017 8:23	5.7
8		23/11/2017 8:33	5.4
9		23/11/2017 8:43	5.1
10		23/11/2017 8:53	4.8

Step 2 – After the data have been entered, go to the “Product” tab to select Beef or Lamb



Product	Bacterial numbers (TVC)	
Lamb	(cfu)	(log cfu)
Beef		
Lamb	100	2.00
Increase	×1,000,000	6.00
Final	100,000,000	8.00

Step 3 – Enter the starting micro count in Colony Forming Units (cfu) the product usually has at point of packing



Product	Bacterial numbers (TVC)	
Lamb	(cfu)	(log cfu)
Initial	100	2.00
Increase	×1,000,000	6.00
Final	100,000,000	8.00

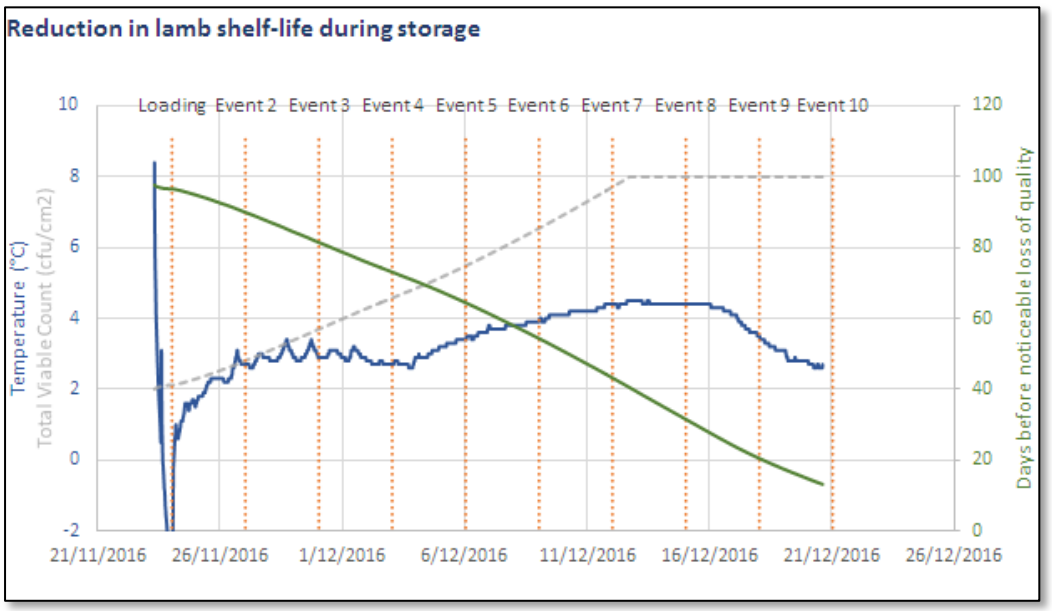
Step 4 – You can also enter specific dates and an event has happen during the data logger journey such as loading a container, transhipping, unloading etc. This will show up on the graph. Up to 10 events can be recorded.

Journey Information	
Event Description	Date/Time
Loading	24/11/2017 1:00
Event 2	27/11/2017 1:00
Event 3	30/11/2017 1:00
Event 4	3/12/2017 1:00
Event 5	6/12/2017 1:00
Event 6	9/12/2017 1:00
Event 7	12/12/2017 1:00
Event 8	15/12/2017 1:00
Event 9	18/12/2017 1:00
Event 10	21/12/2017 1:00

Step 5 – You can also enter the expected temperature the product will be stored to predict how much shelf life will remain after the journey

	Temperature	Expected Shelf-life	Expected Shelf-life Remaining
Average	3.27 °C	31 days	3 days
Preferred	-0.50 °C	103 days	18 days
Expected (remaining storage)	4.00 °C		5 days

Step 6 – A graph will be generated, showing the logger temperature (blue line), the prediction of microbrial growth (grey dotted lines), and days left on shelf life (green line). The events are displayed at the top of the graph (orange dotted lines).



The predictive model which underpins the tool has been validated by data obtained in a number of shelf life studies, including long-haul consignments making it a robust, functional tool. To find out more about the tools development, plus read the supporting data go to http://blogs.utas.edu.au/promep/shelf-life-prediction-service/ .

7. Supply chain monitoring in action - Lamb to the Middle East

In 2017, the United Arab Emirates (UAE) lifted the accepted shelf life for VP sheep meat from 70 days to 90 days, a decision of importance to the Australian meat industry, for which the Emirates is the major Middle Eastern destination. Long shelf life of sheep meat has been reported in laboratory trials at controlled temperatures. To validate these results in commercial cold chain MLA commissioned a trial involving an Australian lamb exporter and a Middle Eastern importer. Vacuum packed racks, boneless legs and bone-in legs in cartons containing temperature data loggers were shipped from an Australia to the Middle East.

Initial bacterial counts were enumerated on all three cuts at the time of packing, with initial microbial counts around $\log_{10}$ 2 cfu/cm^2 (100 cfu/cm^2)

On arrival in the Middle East product was stored under commercial storage conditions. The temperature profile of the product over time from slaughter, vacuum packing, shipping and cold storage was monitored and shown in Table 1.

Table 1: Temperature profile of stored lamb during trial

Phase	Average temperature (°C)
Slaughter to Packing	2
Sea freight	-1.4
Storage in Gulf	0.01

At intervals between 88 – 112 days after slaughter, samples were withdrawn from the cold store for sensory analysis by an experienced sensory panel. The panellists assessed three replicates of each vacuum packed lamb product for various sensory attributes on a five point scale as presented in *Guidelines for developing a protocol for estimating shelf life of chilled meat products* (page 193). Product was considered unacceptable when any attribute scored <3. The sensory panel observed shelf lives of 94 days for lamb racks, 97 days for bone-in legs and 103 days for boneless legs.

The UTas shelf life model for vacuum packed lamb was used to predict the shelf life of meat cuts used in this trial and there was close agreement between the observed and predicted shelf lives of vacuum packed lamb cuts (Table 2).

Table 2: Observed and predicted shelf lives of vacuum packed lamb cuts

Vacuum-packed lamb product	Observed shelf life* (days)	Predicted shelf life** (days)
Racks	94	92
Boneless legs	103	88
Bone-in legs	97	92

*Last day when panellists considered product is acceptable

** Shelf life predicted by the model

Guidelines for developing a protocol for estimating shelf life of chilled meat products

Scope

These guidelines contain information designed to assist in the development of a protocol for undertaking shelf life estimation studies which will satisfy customer requirements.

The guidelines are based on information from publications and reports available in the literature and intended primarily for use with chilled, vacuum packed meat cuts, both ovine and bovine exported to distant markets.

Elements required in a shelf life protocol

Elements of a credible protocol include:

1. Design of a shelf life trial
2. Defining end of shelf life
3. Sensory testing
4. Microbiological testing
5. Chemical testing

Note that, where unique customer requirements are specified, they will need to be incorporated.

1. Defining end of shelf life

Shelf life ends when meat becomes unfit for use, human consumption, or sale; this may occur because of sensory reasons (appearance or smell) or microbiological reasons (a customer specification is exceeded).

2. Design of a shelf life trial

The aim of a shelf life trial is to estimate with reasonable accuracy the number of days that sensory and microbiological criteria of the meat product under test remain acceptable.

To do that meat samples need to be stored under conditions as close as possible to those to which they will be subjected in the marketplace, and samples withdrawn at key intervals to define the time when the product will meet customer requirements and expectations.

The shelf life trial should challenge the product until spoilage occurs, and this requires sufficient samples to allow testing to proceed past the expected shelf life of the product.

a. Sampling days

If the last day when the shelf life is still acceptable is defined as 100%, sampling may be focused around this expected day:

Sampling day	1	2	3	4	5	6
Shelf life used	0%	90%	95%	100%	105%	110%

If a customer requests more frequent sampling intervals these will need to be added to the above.

b. Number of samples

Shelf life trials are a cost of doing business and can be expensive particularly when high-value cuts such as striploins, cube rolls or rumps are used; laboratory and sensory testing also have their costs.

Key considerations are that:

- There are sufficient samples to go to the end of shelf life and beyond.
- Replicate samples at each sampling day – if only one sample is used and the pack turns out to be a leaker no result will be possible for this sampling day.

Three replicate samples is a good number and was used in trials on chilled meat in Australia (Holdhus Small *et al*. 2012; Kiermeier *et al*. 2013) and in Canada (Youssef *et al*. 2014).

If you're setting the shelf life for a vacuum packed (VP) beef primal your historical data may indicate around 160 days in your holding chiller.

In the trial below a total of 18 packs are used and samples are withdrawn at times around the expected shelf life and beyond.

Sampling day	1	2	3	4	5	6
Shelf life used	0%	90%	95%	100%	105%	110%
Days after storage	0	144	152	160	168	176

If we trial the shelf life of VP lamb primals, again we use a total of 18 packs and we focus on the time around the expected shelf life (around 90 days) and beyond.

Sampling day	1	2	3	4	5	6
Shelf life used	0%	90%	95%	100%	105%	110%
Days after storage	0	80	85	90	95	100

Note that the above are suggested sampling times - actual schedules should be based on historical product knowledge and also on customer requirements.

It is also wise to include 2-3 'spares' in case you find a 'leaker' pack among the stored samples.

c. Type of cut and packaging

Ideally you use cuts taken from the boning room immediately before they are packed into their cartons as was done in the study by Holhus-Small *et al*. (2012) where strip loins and cube rolls from six abattoir in Australia were tested.

To minimise expense cuts are sometimes divided before packaging e.g. Canadian researchers divided strip loins into two before packaging (Youssef *et al*. 2014). For this particular cut the procedure probably has no influence on the shelf life since the bag has the appropriate dimensions for the cut and sealing can be done without any impact on the heat seal and there should be no creases to trap air.

It is tempting to divide a primal cut into as many small pieces as possible e.g. cutting a strip loin into 10-12 steaks. This should be avoided because the surface area:volume ratio of the steak is very different from that of an entire strip loin and the shelf life of a steak may not represent the shelf life of an entire cut.

d. Storage temperature

The choice of storage temperature depends on customer requirements or the storage temperature recommended to the customer by the establishment.

For a domestic retailer a good temperature is 5°C since that will be their retail display temperature; the retailer may also require shelf life to be established at 8°C – this is termed an “abusive temperature”.

For an overseas customer a good temperature is close to 0°C since this approximates what is achieved in a refrigerated container.

Since temperature has such a large influence on shelf life at least one data logger should be included in your trial, located securely between two individual cuts and maintained *in situ* for the entire trial. The data file from the logger should be kept with other documentation from the trial

3. Sensory testing

a. Training a sensory panel

When meat is assessed senses are used: eyes, nose and mouth and, unlike machines or instruments, individuals don’t all assess the same product in a uniform manner.

For this reason it’s necessary to assemble a sensory panel/team; the number panellists can vary but three is the minimum number required.

Panels are more effective if each individual receives some training on how to interpret what they are seeing, smelling or tasting. This can be achieved by exposing panellists to products with a range of attributes so they become experienced in what each descriptor means e.g. “Moderate odour” (see section 3.5 for more detail).

The panel will also need training in how to fill in the assessment sheets and on the golden rule of doing the assessment without communicating their thoughts to any other panellists, at least in the first instance.

If sample packs are sent for evaluation to an off-site laboratory it is necessary to establish the necessary skills are in place and that an acceptable protocol will be followed.

b. Creating a suitable area for assessment

Product should be assessed in an area which is quiet, well-lit and not cramped.

A laboratory is a good location as benches can be cleaned after the panel has finished, and any spilled liquids removed.

c. Assessing appearance of the pack

When a carton is opened the first criterion to note is whether a pack has leaked, in which case there will be a putrid odour.

The leaker must be discarded from your test and removed from the testing area.

It's prudent to remove other packs from the carton and wipe them clean with a damp paper towel, and to remove the plastic liner and replace it before putting packs back in the carton.

The next criterion to note is the amount of drip/purge/weep.

A 'normal' amount of drip is 1-2% of the weight of the cut, with seam-boned primals losing less than pieces subjected to trimming/cutting e.g. denuded knuckle (CSIRO, 2002).

Drip may also increase if individual cuts have been packed into the carton so that those at the bottom are under pressure.

In commerce, excessive drip is not equated with end of shelf life, though it may become a compensation claim.

d. Bag integrity

Before opening the pack the seams should be examined to check whether there is any 'doubling-up' caused by the bag not being laid correctly on the heat seal bar.

Assess also whether there are folds in the bag. Air becomes trapped if the bag doesn't fit closely over the meat and allow aerobic growth; folds also facilitate production of drip.

e. Opening the pack

The pack is opened by slitting just beneath and along the line of the seam.

There is almost always an odour detectable on opening the pack, usually slightly sour, which dissipates after a few minutes. It is called confinement odour, is a normal occurrence as the meat ages and should not be considered as part of the odour assessment.

The odour which must be assessed is that which persists around the meat when it has been removed from its packaging for a few minutes.

f. Assessing odour

Training will involve exposing your team/panel to various odours, which involves guiding them to assessing terms such as sour, acidic, cheesy, sweet, sickly, putrid and to other descriptors such as slight, moderate and extreme.

Experienced team members with good industry and product experience are best suited to perform product sensory assessments; sales staff can be especially useful because they deal with customers and their perceptions

The essential feature of odour assessment is that each panellist is “grounded” in identifying unacceptable odours and this may take continuous coaching/guidance from the leader of the sensory team.

g. Meat colour and bloom

When meat is removed from the vacuum pack it should quickly regain its bright, red colour (bloom).

h. Scoring the assessments

Panellists score their observations against a set of criteria for which they have received training on a score sheet.

All score sheets have a scale in which criteria gradually change from acceptable to unacceptable.

The number of points on the scale can vary from 9-point to 4-point.

Sensory score sheet with 9-point scale

Attribute		
Vacuum	**Appearance**	**Odour**
8 = complete, tight package adhesion	8 = very fresh, no discolouration	8 = fresh, no off odour
6 = good vacuum	6 = fresh, slight discolouration	6 = slight off odour
4 = moderate vacuum	4 = good, acceptable	4 = medium odour
2 = poor vacuum	2 = poor	2 = strong off odour
0 = no vacuum, probable leaker	0 = severe discolouration	0 = extreme off odour

The point when shelf life expires on the 9-point scale, above, is the time when either the appearance or odour reaches a score of 2.

Note that a score of 2 for vacuum leads to a consideration of whether the pack is a leaker before rating that the shelf life has expired; only intact packs should be used for shelf life assessments.

Sensory score sheet with 5-point scale

Score	Drip	Vacuum	Appearance	Odour
4	None	Complete adhesion	Deep red colour	Fresh
3	Slight	Good	Light red colour	Slight sour/dairy
2	Acceptable	Moderate	Slight discolouration	Sour/dairy
1	Heavy	Poor	Poor colour	Strong sour/dairy
0	Extreme	None/blown	Severe discolouration	Off odours

The point when shelf life expires on the 5-point scale, above, is the time when either the vacuum, appearance or odour reaches a score of 1; note the remarks above re whether the pack is a leaker.

Similar criteria are covered in a 4-point sensory scale, where the cut-off point for acceptability is a score of 1 for either colour or odour.

Sensory score sheet with 4-point scale

Score	Vacuum	Colour	Odour
3	Seal intact, minimal drip	Purple/red	Fresh
2	Seal intact, normal drip	Purple/red	Slight stale
1	Broken seal, slack pack, excess drip	Two toning, browning	Strong stale/dairy
0	Broken seal, copious drip	Brown, grey colour	Putrid

i. Reaching a consensus

When the panel has completed its assessment the scores are compared and evaluated.

There will be occasions when discrepancies occur e.g. sometimes a panellist scores differently from other team members and the discrepancy will need to be resolved by re-training the panel.

On occasion, all panellists may agree that one of the three packs sampled is unacceptable and two are acceptable. In this case it is advisable to open three more packs to assist the decision on whether end of shelf life has been reached.

When the panel determines that all three packs are unacceptable terminate the end of shelf life has been reached. A safe shelf life is therefore the previous sampling day when all three packs were acceptable.

4. Microbiological testing

While some customers impose only sensory specifications or proportion of shelf life remaining when the consignment is accepted, others set a microbiological criterion and we describe some criteria set by importing countries and Australian supermarkets in Chapter 9 of MLA's *Shelf life of Australian Red Meat*.

a. Sampling meat for microbiological testing

Methods for removing bacteria from meat surfaces fall into two categories:

- Destructive sampling, where tissue is removed
- Non-destructive sampling, where the meat surface is swabbed, sponged or palpated to remove bacteria into a surrounding medium (so-called 'meat-in-bag' technique).

Destructive sampling

It is generally agreed that excising surface tissue, then blending or stomaching it, will result in greater recovery of bacteria than will non-destructive sampling methods (Capita *et al*. 2004).

For those establishments which have laboratory staff skilled in excising tissue and blending it using aseptic technique, excision sampling is considered the 'gold standard'.

Non-destructive sampling

The numbers removed by non-destructive sampling vary widely according to the vigour with which the tissue is rubbed and to the abrasiveness of the sponge or swab.

Gill and Jones (2000) found that recovery was lower when cotton wool swabs were used, compared with excision samples and with samples obtained using a sponge or abrasive gauze pads, with the difference in TVC/cm^2 when chilled carcases where sampled was around 0.5 log.

In Australia, Seager *et al*. (2010) monitored the recovery of bacteria from beef carcases using ten experienced samplers. On average, about 40% of the total bacteria on the meat surface was removed by using a Whirlpak sponge but the standard deviation at each site was high, reflecting the wide variation of recovery among operators (2.3 - 93.1%).

Using a Whirlpak sponge for shelf life testing may be a favoured method given that all establishments use this technique for ESAM testing.

An alternative non-destructive method was used by Holdhus Small *et al*. (2012) in which rinse samples were collected from the primal by placing it in a sterile bag with 500mL of sterile saline and massaging its surfaces for 2 minutes.

This technique is widely used in the poultry industry and has the advantage that bacteria are removed from all surfaces of the meat, and the disadvantage that converting the count on the plate to a count/cm^2 requires a mathematical formula (Holdhus Small *et al*. 2012 show how to do this for strip loins and cube rolls).

b. Media used in estimating microbial counts

In research studies such as those quoted previously (Holdhus Small *et al*. 2012; Kiermeier *et al*. 2013; Youssef *et al*. 2014) a range of culture media are used to enumerate bacteria which dominate the population during the various stages of shelf life. Information on these bacteria is presented in Chapters 5, 6 and 7 of MLA's *Shelf life of Australian Red Meat*.

In general, for the purpose of gathering microbiological information to accompany an Aerobic Plate Count (APC) is sufficient. Of course, if customers specify a suite of organisms their requirements must be met.

c. Incubation temperatures

Because shelf life is assessed by storing meat under refrigeration for many weeks the dominant microflora is composed of psychrotrophic bacteria.

Psychrotrophs generally have a temperature optimum of 15-25°C and a maximum growth temperature of 30-35°C (ICMSF, 1980) and it is logical to incubate cultures near their

optimum (25°C) for sufficient time (4 days) so the colonies are clearly visible and therefore countable on the culture plate.

This was captured by an Australian Standard *(AS 1766.3.1-1991) Food microbiology Method 3.1: Examination of specific products - Meat and meat products other than poultry: Standard plate count. Incubate at 25 ±1°C for 96 ±2 h. Examine the plates after 72 ± 2 h, and record the counts for those plates that are likely to be overgrown before the full incubation period has elapsed.*

While this Standard has been replaced by less prescriptive standards it is recommended that cultures are incubated for 4 days at 25°C.

The importance of using the correct incubation temperature in monitoring shelf life studies is captured by Pothakos *et al*. (2012) who incubated plate counts of stored food samples at either 22°C/5 days or 30°C/3 days and found that counts from the former temperature were 0.5 – 3 log cfu/g higher. The authors concluded: *"This study highlights the potential fallacy of the total aerobic mesophilic count as a reference shelf life parameter for chilled food products as it can often underestimate the contamination levels at the end of the shelf life."*

d. Interpreting micro counts in shelf life studies

Early work by CSIRO during the 1980s showed that APCs reach very high levels – between log 7 and log 8/cm^2 (10,000,000 – 100,000,000/cm^2) when VP beef is stored around 0°C (Egan, 1983).

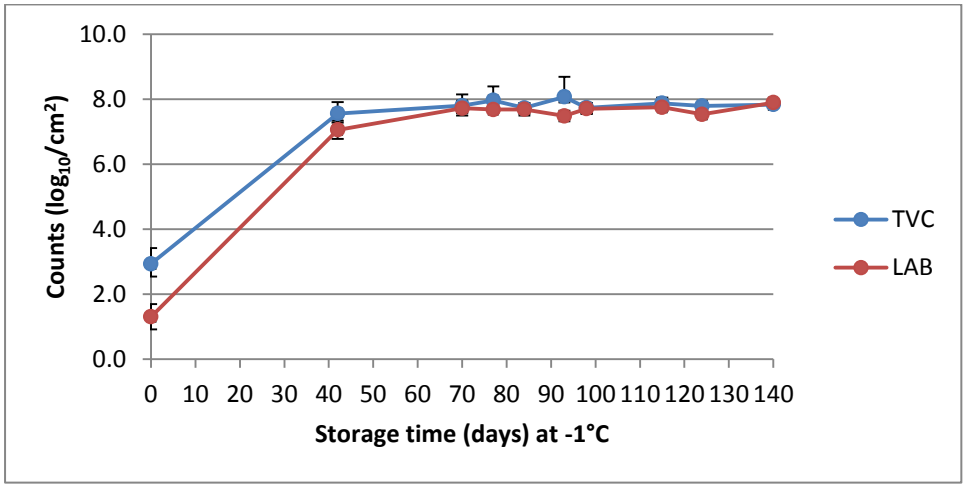
The meat is still acceptable when counts reach a maximum; only after several more weeks of storage do spoilage criteria become apparent.

The figure below is important because it shows that:

- A very high count is 'normal' in VP meat storage
- The dominant bacteria are lactic acid bacteria (LAB)

High counts towards the end of storage should not be of concern and customers should be reassured that such counts are not only normal but are the reason why vacuum packed primals have such long chilled storage lives.

Growth of total viable counts (TVC) and lactic acid bacteria (LAB) on vacuum-packed lamb meat stored at -1°C



5. Chemical testing

Chemical tests are not usually necessary as part of shelf life testing in the commercial setting, though knowing the initial pH may prove valuable.

6. References

Capita, R., Prieto, M. and Alonso-Calleja, C. (2004). Sampling methods for microbiological analysis of red meat and poultry carcasses. Journal of Food Protection 67:1303-1308.

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Data logger calibration

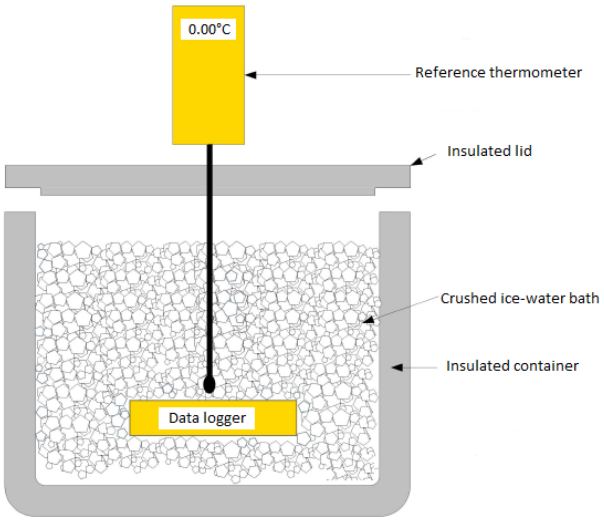
Material required:

- Reference/certified thermometer (NATA) - reference thermometer should be of higher accuracy than the logger to be calibrated e.g. a thermometer with a rated accuracy of ±0.1°C should be used to calibrate a logger with a rated accuracy of ±0.2°C.
- Distilled water to prepare ice and ice water bath
- Thermally insulated container with lid such as an esky
- Zip lock bag
- Data logger
- Computer

Calibration procedure:

- Make enough ice with distilled water in advance
- Finely crush the ice, and fill the clean thermally insulated container/esky with the ice, add cold distilled water (avoid using too much water), mix thoroughly to form an ice slurry and avoid any air and water pockets (think of slightly runny slushy)

Figure 1: Ice water bath for data logger calibration (adapted from Hafner, 2013 [9])



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- Launch the data logger by plugging into computer and set the logger to record at one-minute intervals. Pre cool the data logger (in a zip lock bag) in a fridge before immersing it in the ice water bath. Precooling reduces the time to reach equilibrium at the ice point; it also helps to preserve the bath at the ice point for a prolonged time
- Form a well in the ice water bath, put the data logger with built-in probe in a zip lock bag, and place it in the ice water bath as shown in the Figure 1 (above) without touching the sides and bottom of the container. Make sure that as little air as possible is trapped inside the zip lock bag in order to avoid any false result either by floating and/or lack of contact between logger and the ice water. Data logger with external probe can be placed on the lid of the container and its probe can be inserted in the ice water bath (in or without zip lock bag) through a hole in the lid like reference thermometer below
- Replace the lid on the container. Make a small hole in the lid of the insulated container and place the reference thermometer into the ice bath without touching the sides or bottom of the container to a specified depth. The depth of reference thermometer immersion should be checked from the accompanied instruction manual/certification documents. The thermometer must be immersed to the required depth however; liquid-in-glass thermometer must also be readable with the top of the liquid column at eye level to avoid parallax error. Do not lift the thermometer out of the water bath to read it. Allow the bath, data logger and reference thermometer to come to equilibrium ensuring that a temperature of 0°C is reached (taking into account any correction factor associated with the reference thermometer). A steady reading is reached when there is no difference between two readings taken one minute apart
- Record the start time of the calibration and the start temperature of the ice water bath on reference thermometer (0.00°C). Continue to monitor temperature (every one minute) and record any changes throughout the calibration process
- Allow enough time ~1 hours for monitoring/calibration. On completion, remove the data logger from the ice water bath along with reference thermometer. Record the finish time and the temperature of the ice water bath
- Plug data logger into the computer and down load the time-temperature data (both graph and numeric data). Take an average of all the data points recorded by the data logger during calibration, compare it with corrected reference thermometer reading and calculate a correction factor to be applied to the temperature data of that specific data logger in future trials. The correction factor refers to the difference between corrected reading of the reference thermometer and the data logger reading (Table 1).

Table 1: Example of data logger calibration

Temperature data logger ID	Data logger 1	Data logger 2
Reference thermometer reading (°C)	-0.10	-0.10
Corrected reading of reference thermometer (°C)	0.00	0.00
Data logger reading (°C)	0.20	-0.20
Correction factor to be applied to data logger (°C)	-0.20 (subtract 0.20)	+ 0.20 (add 0.20)

- Save this information in an excel spreadsheet or notebook along with the data logger serial number, calibration time, calibration temperature and correction factor. Otherwise you could also note the correction factor on the data logger directly as well.

Shelf life enquiry form

An electronic copy can be found here (http://blogs.utas.edu.au/promep/shelf-life-prediction-service/), once completed please send this form and relevant information to:

Dr Mandeep Kaur

University of Tasmania

(03) 6226 2871

mandeepk@utas.edu.au

Contact Information

The information you provide below will be confidential.

Name:

Establishment:

Email:

Phone:

Enquiry is about:

☐ Predicting shelf life **or** ☐ Product in transport / market **or** ☐ Shelf life study

Question (as clear as you can about the question you have):

Available information:

☐ Beef **or** ☐ Lamb/Sheep meat

What is the usual microbiological quality at the time of packing (a single test result, usual range of data from carton meat assessment or other tests) in cfu/g -

Time and Temperature (actual or expected - include a data logger file, if available, date/time removal of logger, logger placement in container) -

Optional information to improve accuracy:

The type of cut and packaging type:

Consignment size:

Date/time of container loading:

Departure date and product arrival date/time:

Shipping duration and transit route:

Date/time of major events during transport such as loading, transhipping, breakdown etc. this will be included in the graph.

UTAS RESPONSE