Processor's Guide to Improving Microbiological Quality 2nd Edition

Government of South Australia
Primary Industries and Regions SA

SARDI
SOUTH AUSTRALIAN RESEARCH AND DEVELOPMENT INSTITUTE

Processor's Guide to Improving Microbiological Quality 2nd Edition

Contents

Evisceration and Trimming

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Meat & Livestock Australia acknowledge the matching funds provided by the Australian Government to support the research and development detailed in this publication.

What's new in this edition?

Our first edition was published in 2014 and distributed at AMPC Meat Inspection and Quality Assurance Network meetings in each state and at the MINTRAC Meat Inspection and Quality Assurance Conference. At these meetings, SARDI extended the opportunity to establishments to have their in-house data analysed. A number of requests were made and these are now presented in this edition. It's clear that some establishments do more investigative work in addition to their ESAM requirements.

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 they rate, not just on a month-to-month basis but over long time periods.

Edition 1: Origin and Contents

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 tell you whether your unit operations are effective.
6. Writing up a report that documented important aspects of the investigation.

The reports were published 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 in this booklet 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).

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.

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

- Is there room?
- How much time do I have?
- Is there room to store my kit safely (sponges etc.)?
- Can I keep my hands sterile?
- Can I do it on my own or do I need a mate to prepare the sponges 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.

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 booklet 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.

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 (arithmetic count) or = log (cell number).

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.

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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.

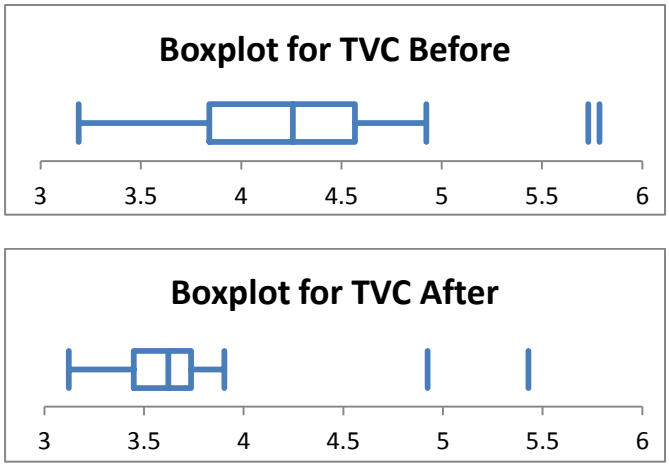


Figure 1: Boxplots showing log_{10} TVC cfu/cm^2 before and after Twin Oxide application.

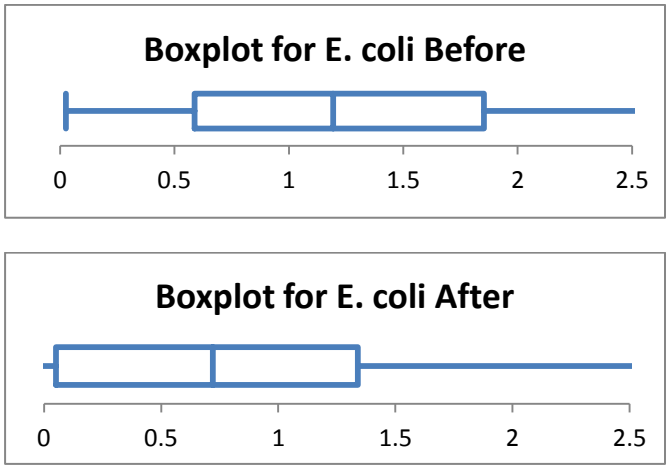


Figure 2: Boxplots showing log_{10} *E. coli* cfu/cm^2 before and after Twin Oxide application.

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.

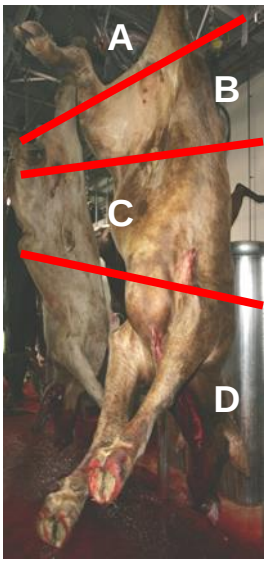


Figure 1: Sampling sites

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

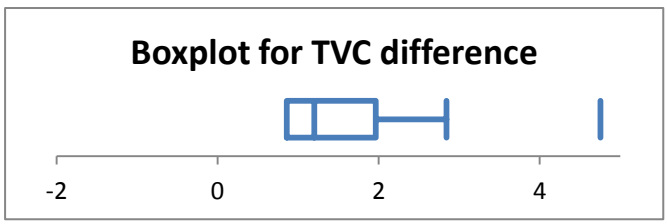


Figure 2: Boxplot showing difference in $\log_{10}$ TVC cfu/cm^2 before and after Twin Oxide application.

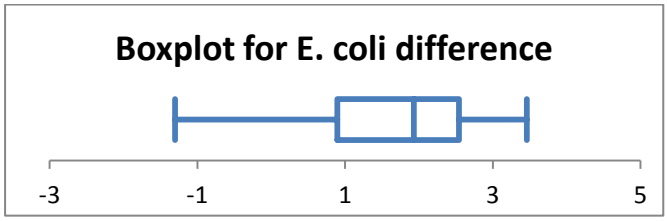


Figure 3: Boxplot showing difference in log_{10} *E. coli* cfu/cm^2 before and after Twin Oxide application.

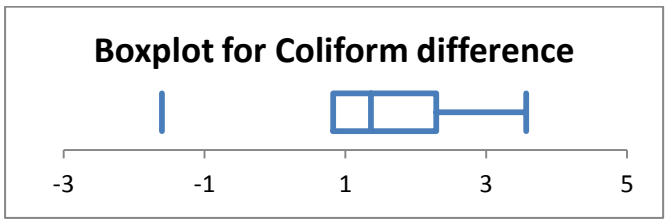


Figure 4: Boxplot showing difference in log_{10} Coliforms cfu/cm^2 before and after Twin Oxide application.

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

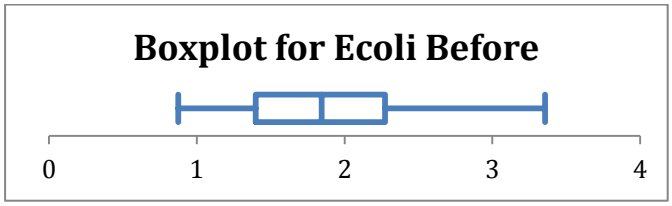


Figure 1: Boxplot showing $\log_{10}$ *E. coli* cfu/cm^2 concentration before Twin Oxide application.

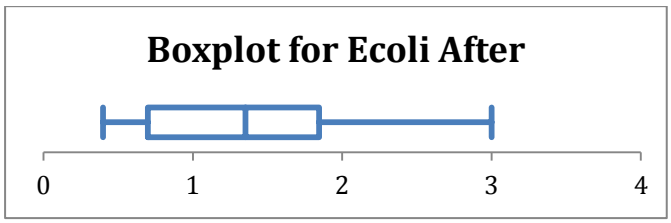


Figure 2: Boxplot showing $\log_{10}$ *E. coli* cfu/cm^2 concentration after Twin Oxide application.

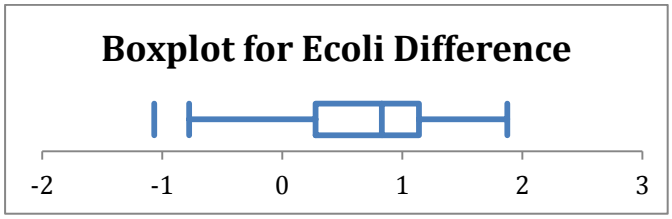


Figure 3: Boxplot showing the difference in $\log_{10}$ *E. coli* cfu/cm^2 concentration before and after Twin Oxide application.

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

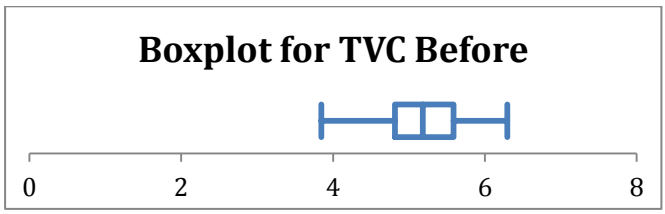


Figure 4: Boxplot showing $\log_{10}$ TVC cfu/cm^2 concentration before Twin Oxide application.

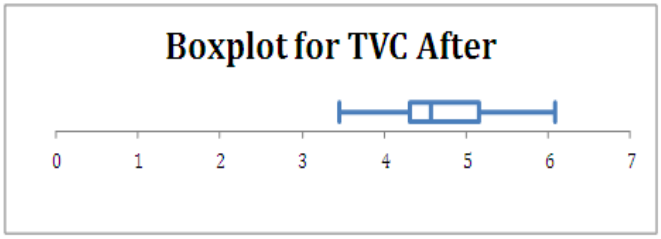


Figure 5: Boxplot showing log_{10} TVC cfu/cm^2 concentration after Twin Oxide application.

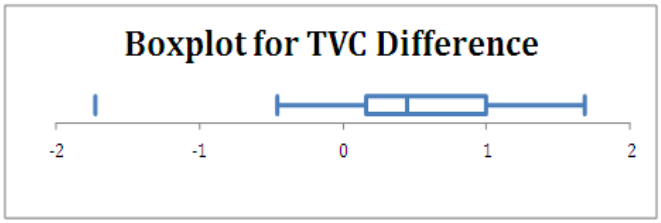


Figure 6: Boxplot showing difference in log_{10} TVC cfu/cm^2 concentration before and after Twin Oxide application.

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*

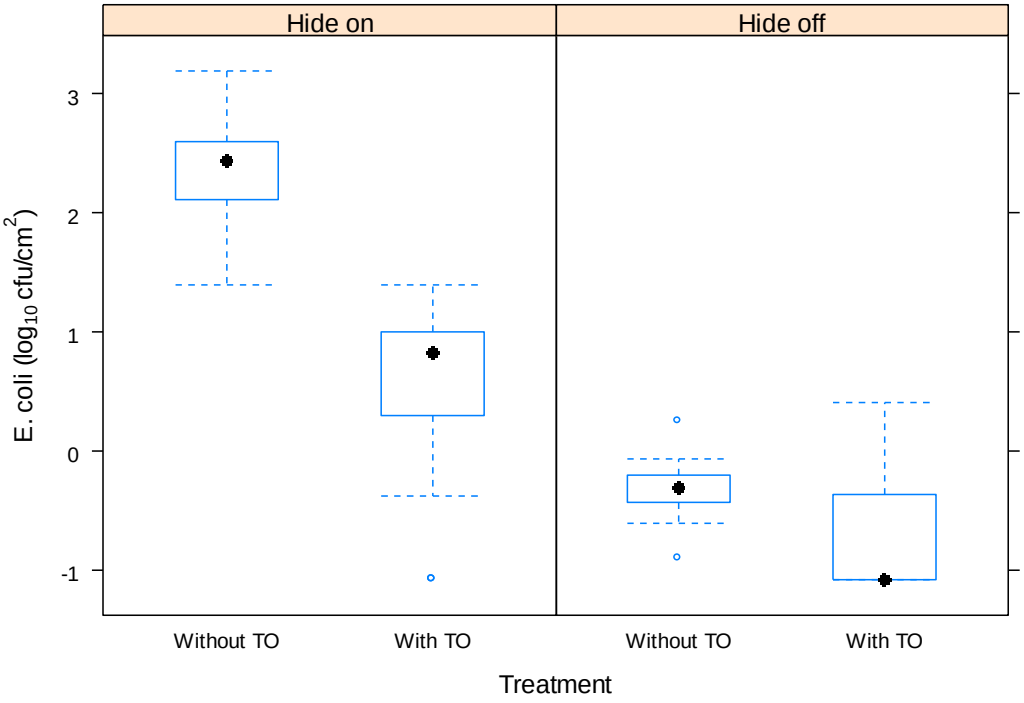


Figure 1: Boxplots of log_{10} *E. coli* cfu/cm^2 collected from brisket hide (left panel) and freshly exposed brisket carcase area (right panel) with and without the application of Twin Oxide on the hide.

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.

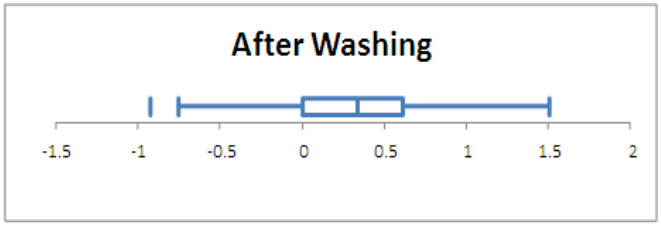


Figure 1: Boxplot of $\log_{10}$ TVC cfu/cm^2 after washing hands.

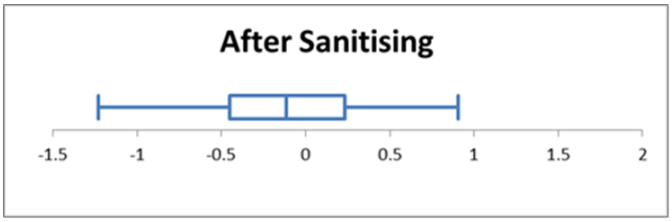


Figure 2: Boxplot of $\log_{10}$ TVC cfu/cm^2 after using hand sanitiser.

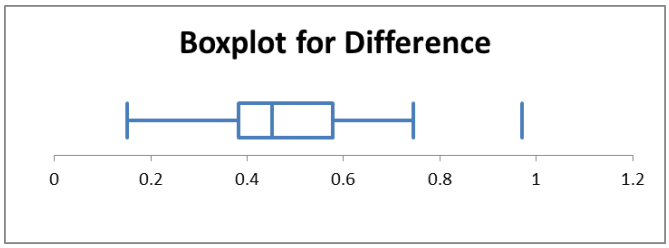


Figure 3: Boxplot of difference in $\log_{10}$ TVC cfu/cm^2 after washing hands compared to after using hand sanitiser.

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. 10 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.

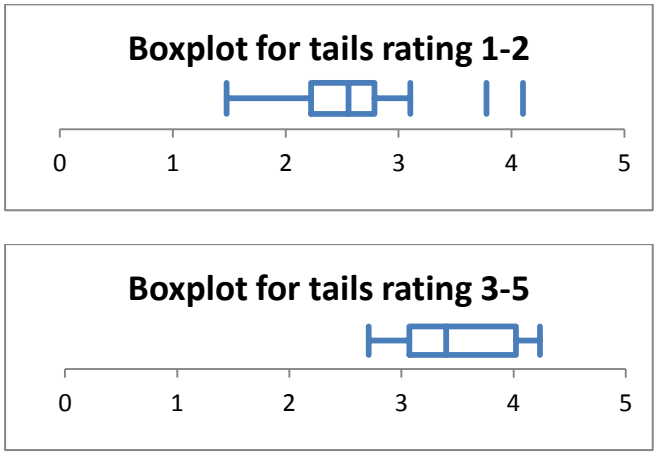


Figure 1: Boxplots of the log_{10} TVC cfu/cm^2 for Tail ratings 1-2 and ratings 3-5.

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.

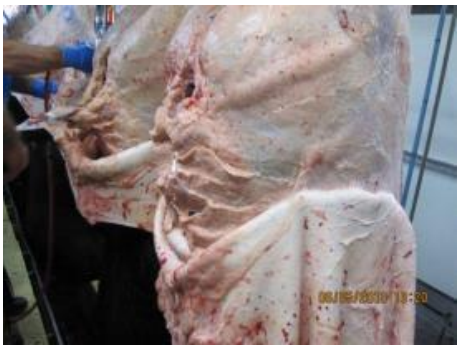


Figure 2: Tail Off.

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.

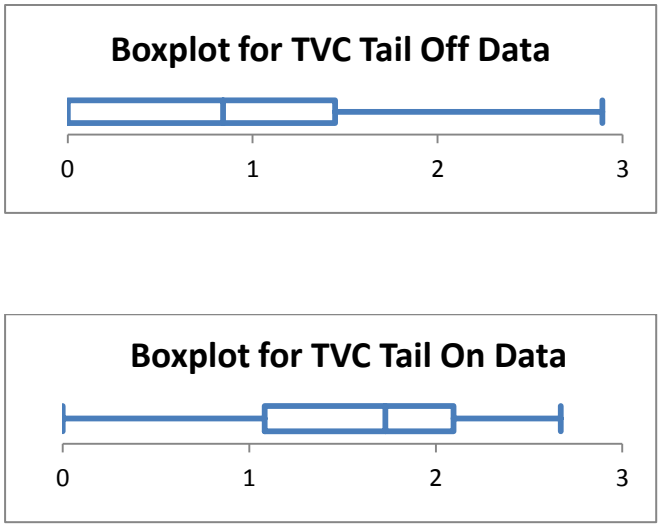


Figure 4: Boxplots of the $\log_{10}$ TVC cfu/cm^2 from Tail Off and Tail On.

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).

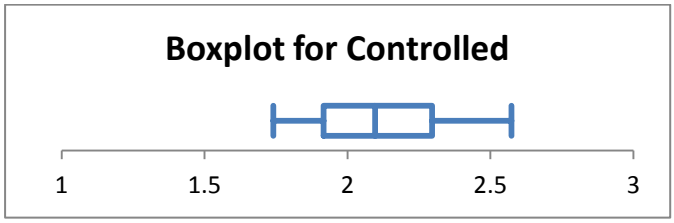


Figure 1: Boxplot for $\log_{10}$ TVC cfu/cm^2 for controlled tail pull.

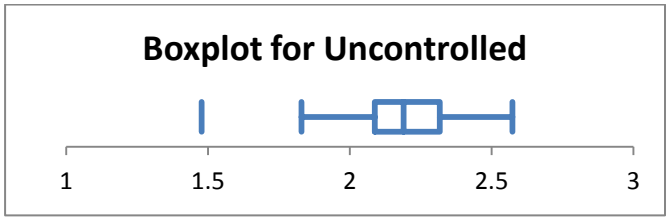


Figure 2: Boxplot of log_{10} TVC cfu/cm^2 for uncontrolled tail pull.

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	

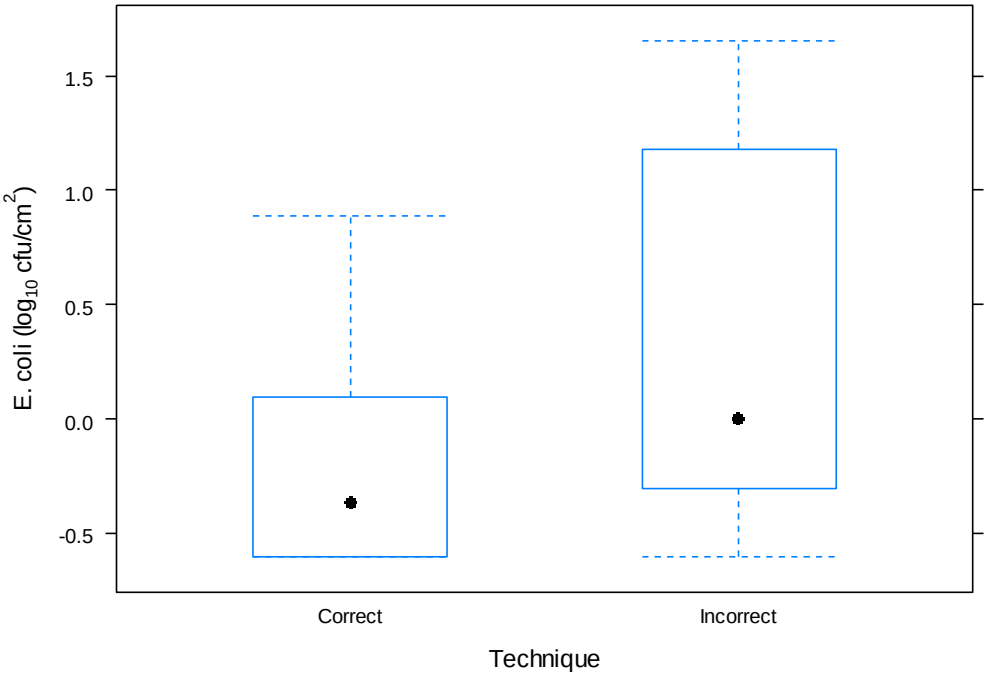


Figure 1: Boxplots of log_{10} *E. coli* cfu/cm^2 for correct and incorrect bunging technique. Includes only samples with detectable levels of *E. coli*.

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.

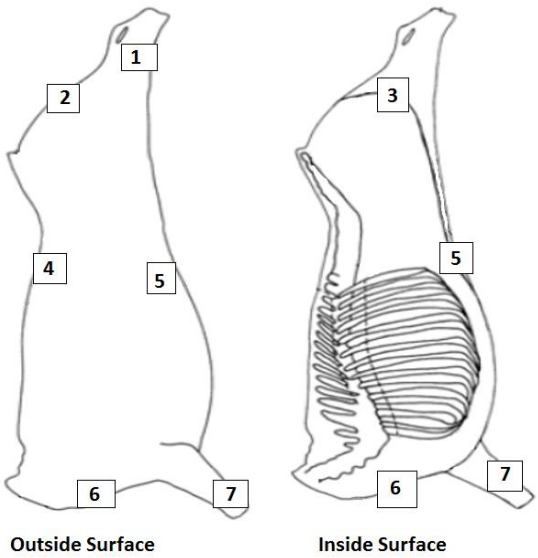


Figure 1: Sampling sites

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.

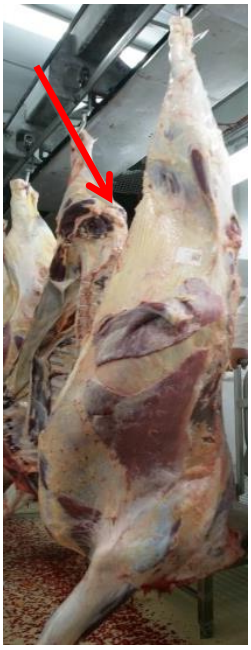


Figure 1: Sampling location near opening cut

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	

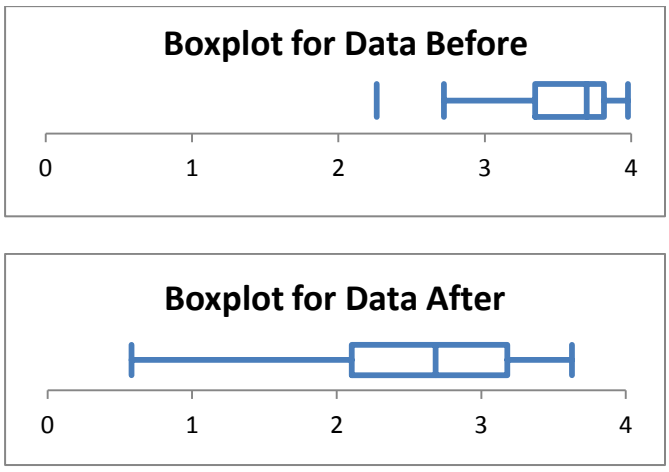


Figure 2: Boxplots of the log_{10} TVC cfu/cm^2 for before and after trimming.

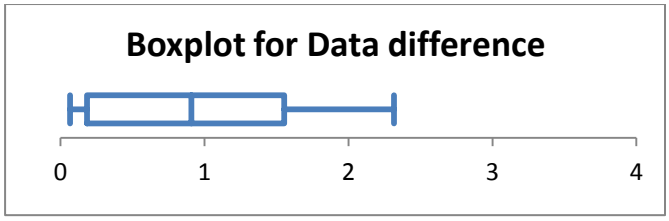
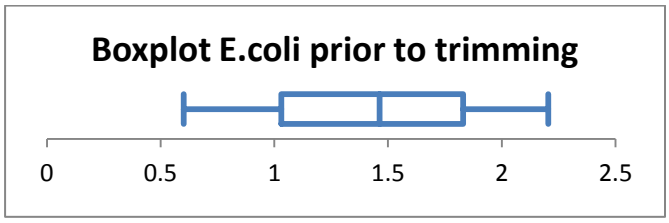


Figure 3: Boxplot of the difference in log_{10} TVC cfu/cm^2 before and after trimming.



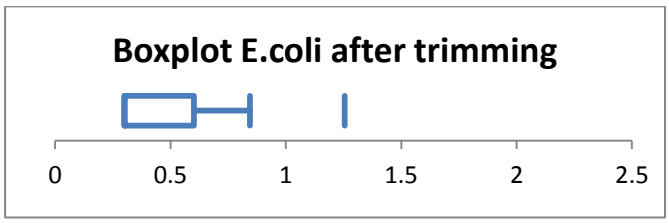


Figure 4: Boxplots of log_{10} *E. coli* cfu/cm^2 for before and after trimming. Includes only samples with detectable levels of *E. coli*.

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	

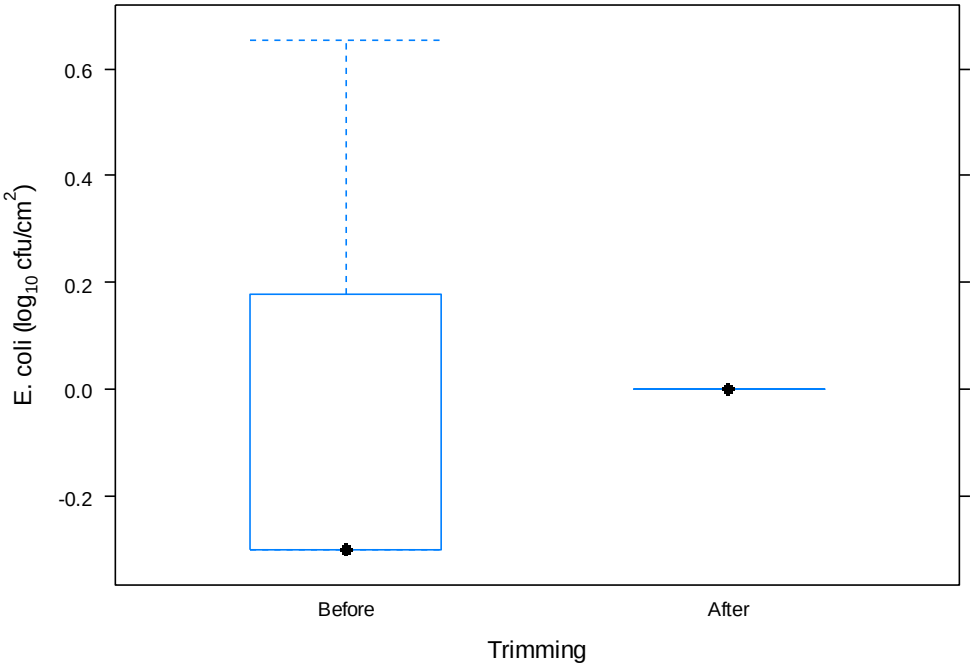


Figure 1: Boxplots of the $\log_{10}$ *E. coli* cfu/cm^2 from before and after Halal neck trimming. Includes only samples with detectable levels of *E. coli* – only one detection was made from the samples collected after trimming.

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*

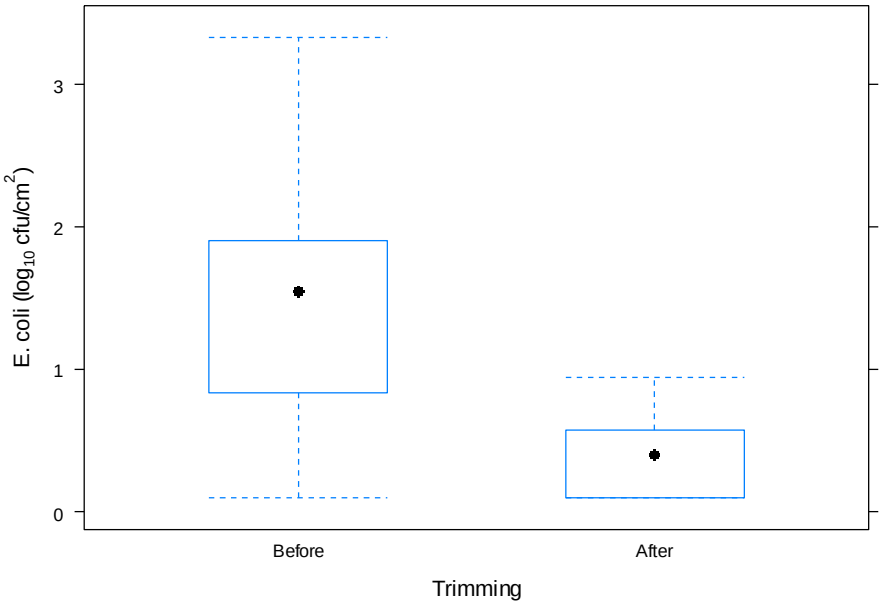


Figure 1: Boxplots of $\log_{10}$ *E. coli* cfu/cm^2 from samples collected before and after retain rail trimming. Includes only samples with detectable levels of *E. coli*.

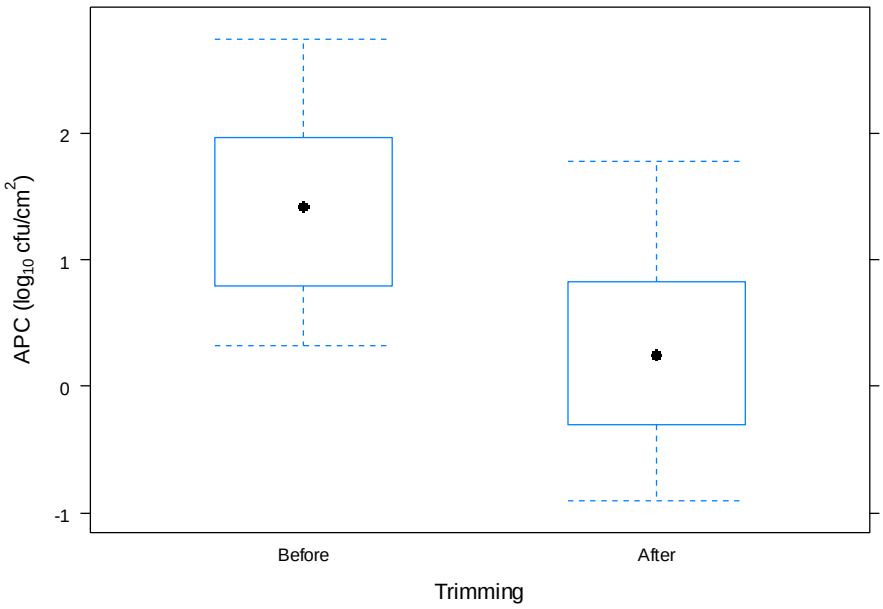


Figure 2: Boxplots of $\log_{10}$ APC cfu/cm^2 from samples collected before and after retain rail trimming.

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 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	

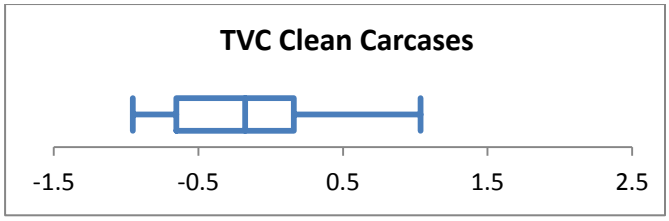


Figure 2: Boxplot of the log_{10} TVC cfu/cm^2 from clean carcases.

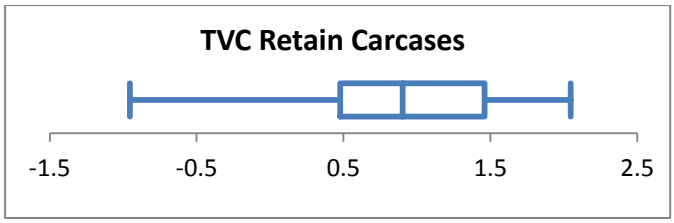


Figure 3: Boxplot of the log_{10} TVC cfu/cm^2 from retain carcases after hygiene and retain carcase trimming.

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.

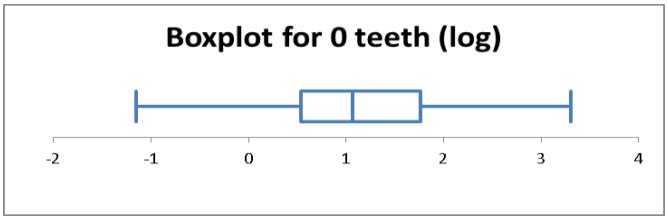


Figure 2: Boxplot of the overall log_{10} TVC cfu/cm^2 for 0 teeth animals.

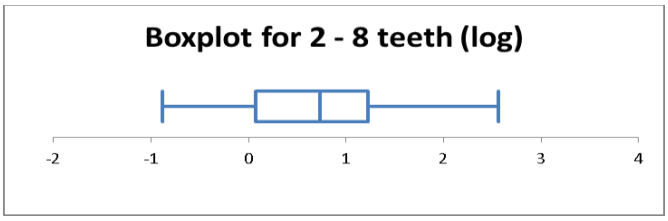
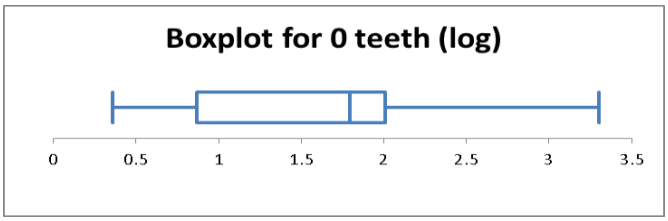


Figure 3: Boxplot of the overall log_{10} TVC cfu/cm^2 for 2-8 teeth animals.

Specific location swabbing results



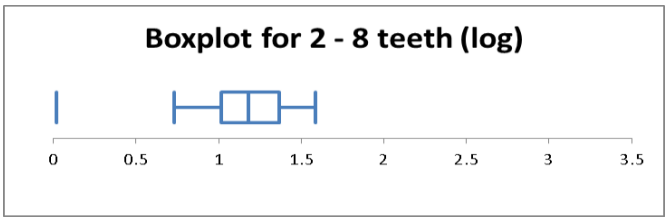
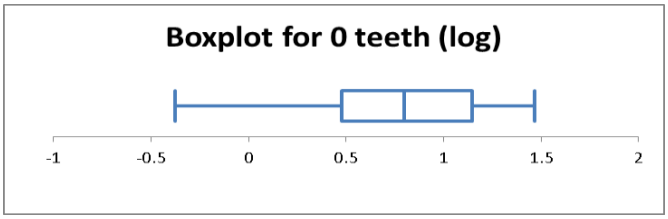


Figure 4: Boxplots for log_{10} TVC cfu/cm^2 for Butt. Marginal difference.



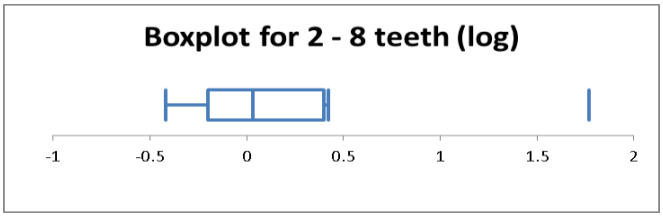
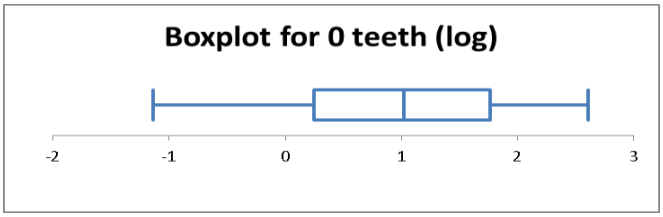


Figure 5: Boxplots of log_{10} TVC cfu/cm^2 for Loin. Marginal difference.



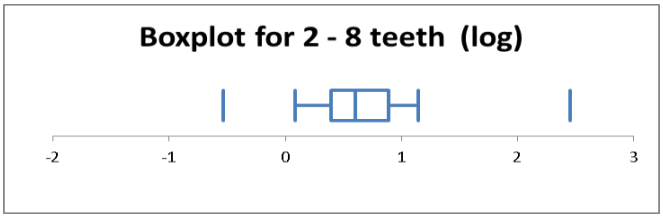
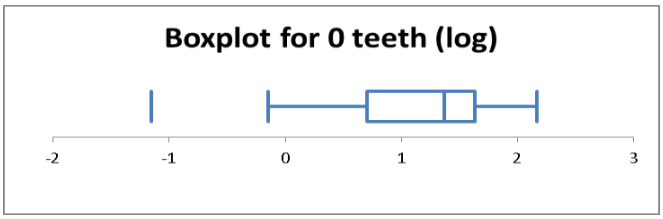


Figure 6: Boxplots of log_{10} TVC cfu/cm^2 for Blade.



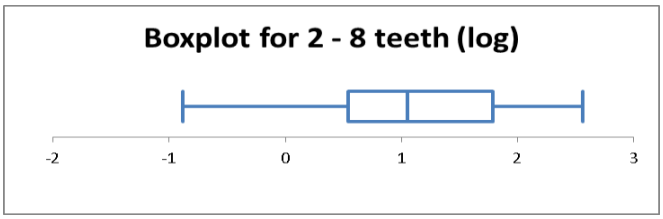


Figure 7: Boxplots of log_{10} TVC cfu/cm^2 for Chuck.

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. 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.

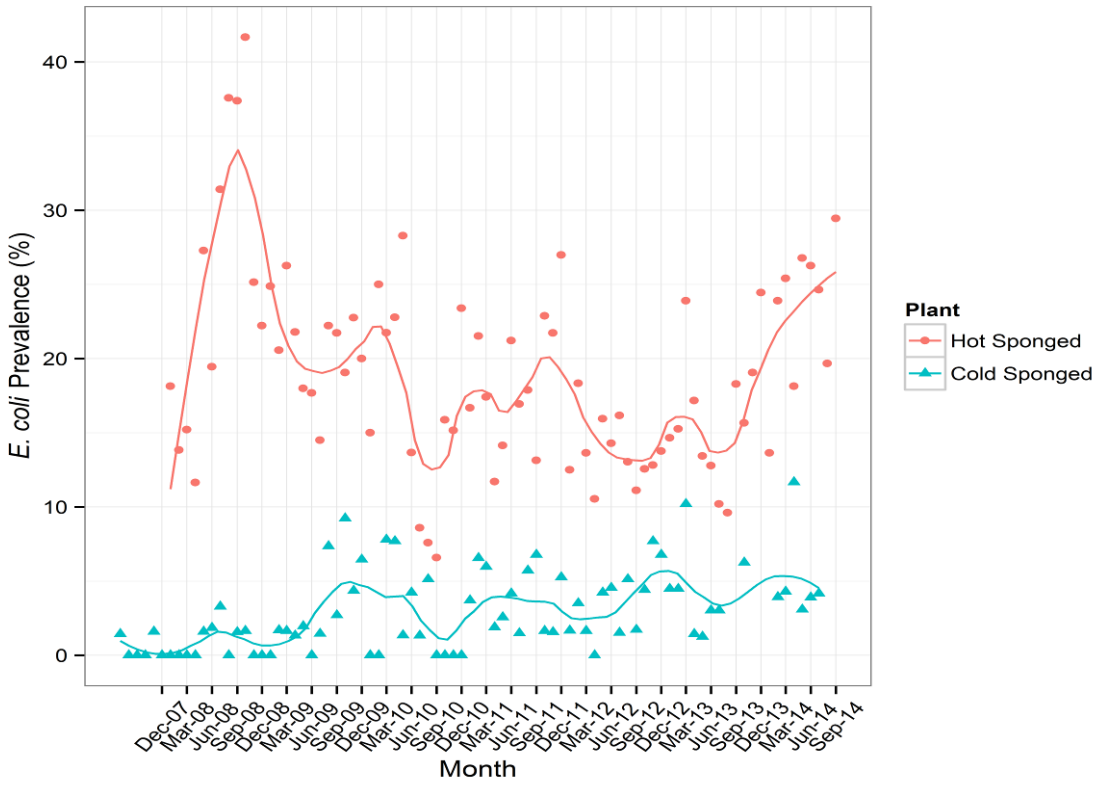


Figure 1: Prevalence of *E. coli* on hot-sponged carcases compared with cold-sponged carcases

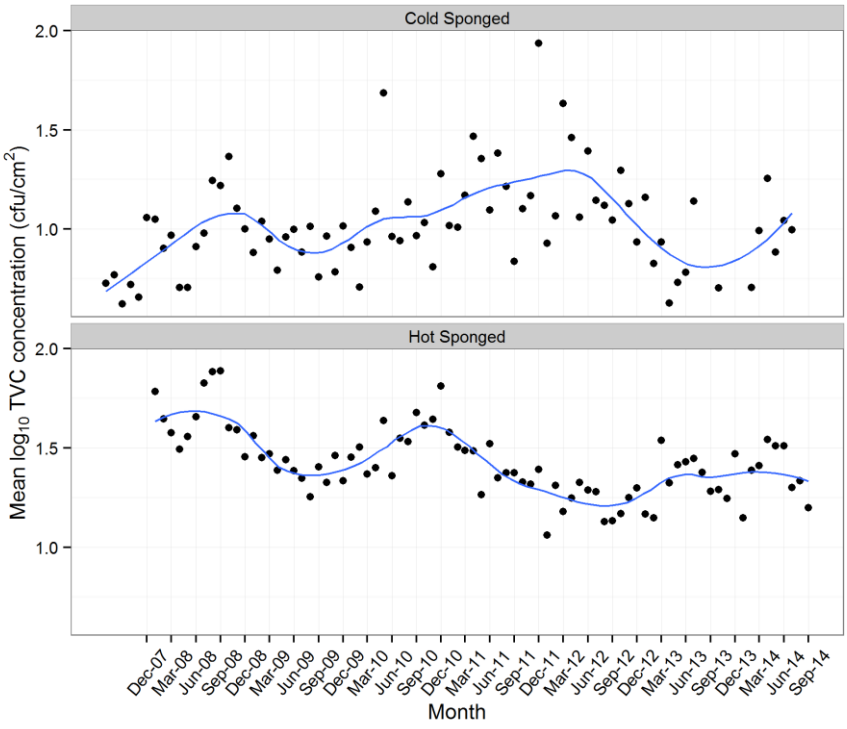


Figure 2: Mean log_{10} TVC cfu/cm^2 for hot-sponged carcases compared with cold (ESAM) carcases

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.

17. 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.

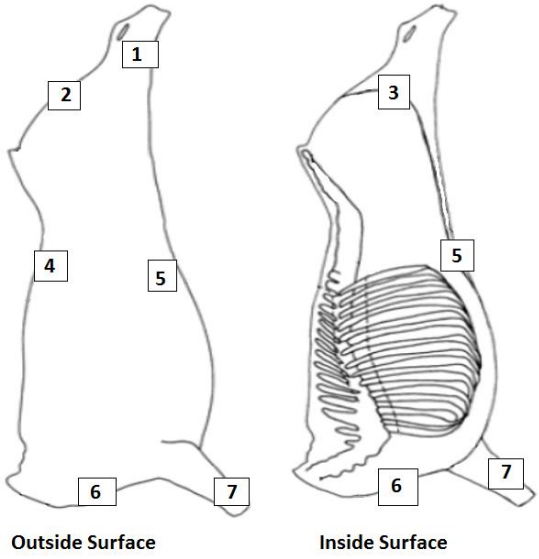


Figure 1: Sampling sites

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.

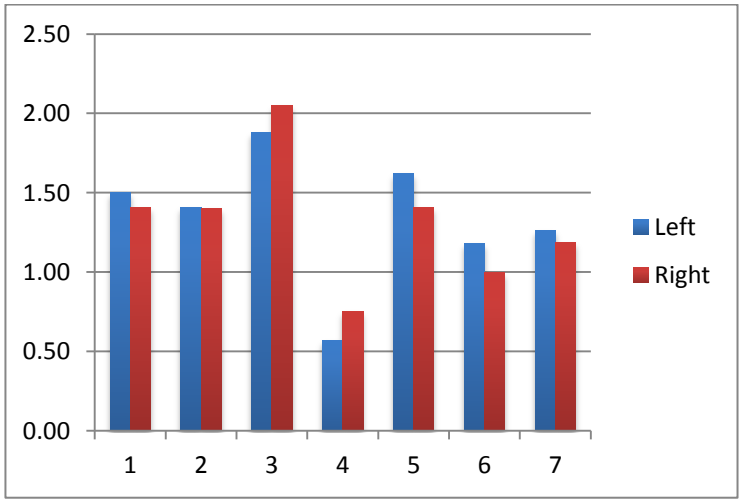


Figure 2: Mean log TVC cfu/cm^2 (vertical axis) at seven sites on the right and left sides of each carcase

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).

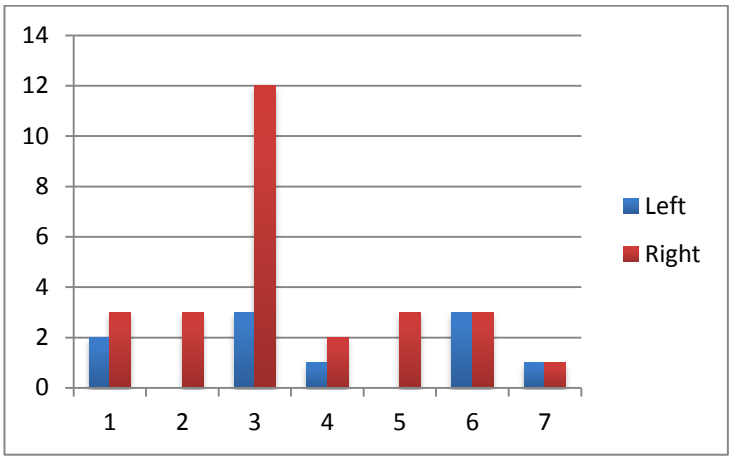


Figure 3: Detections of *E. coli* at seven sites on the right and left sides

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.

18. 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 are 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 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 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.

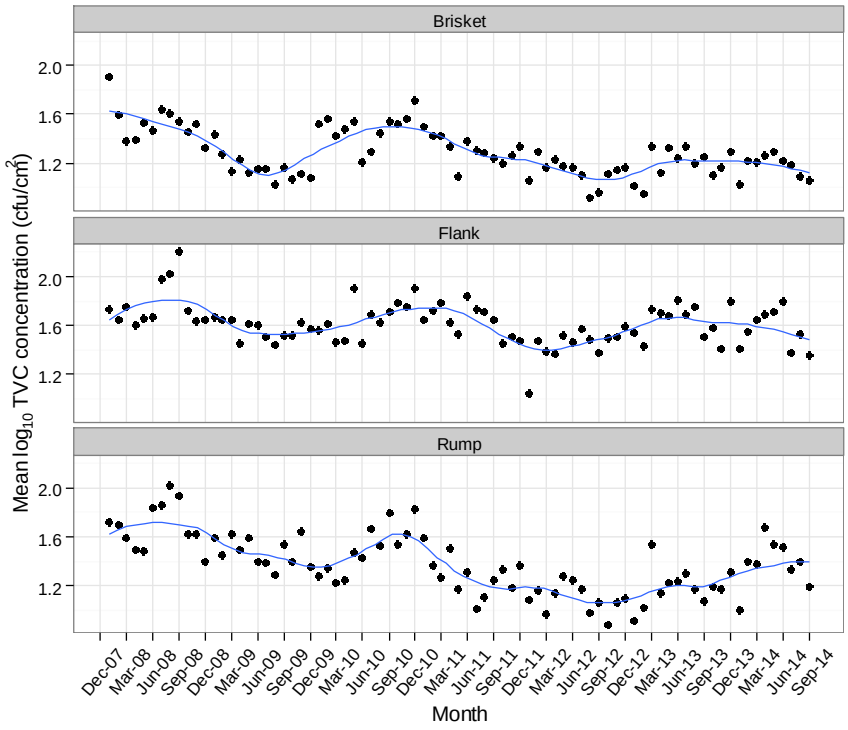


Figure 1: Mean monthly log_{10} TVC cfu/cm^2 for hot carcase sites

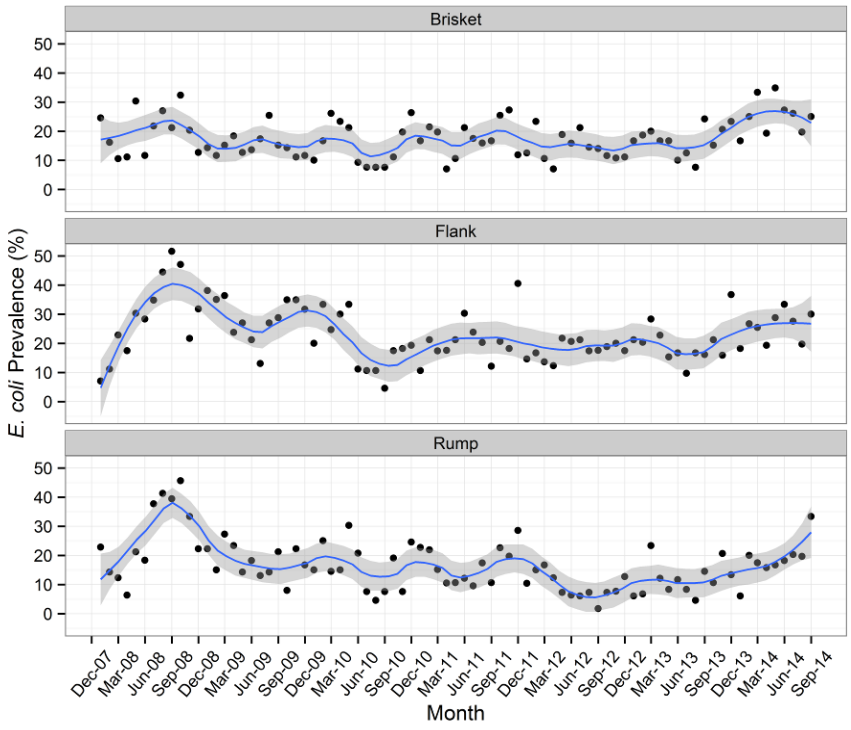


Figure 2: Monthly *E. coli* prevalence for hot carcase sites

19. 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

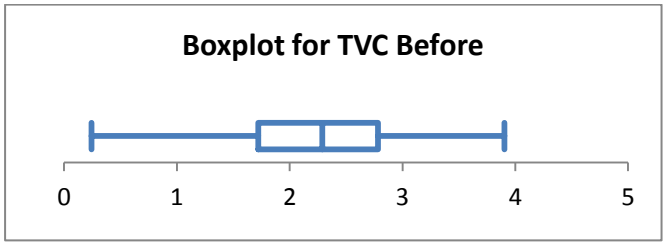


Figure 1: Boxplot of log_{10} TVC cfu/cm^2 for before steam vacuum and 82°C wash.

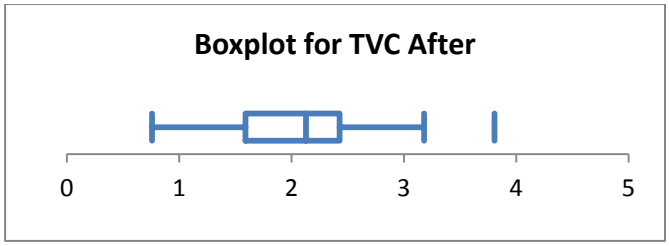


Figure 2: Boxplot of log_{10} TVC cfu/cm^2 for after steam vacuum and 82°C wash.

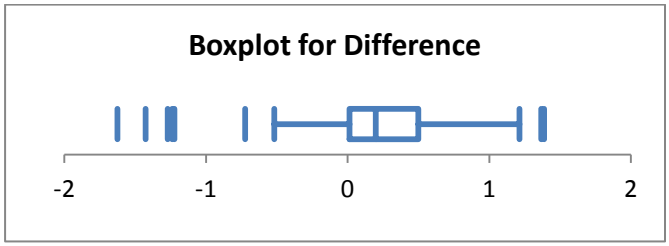


Figure 3: Boxplot of difference in log_{10} TVC cfu/cm^2 for before and after steam vacuum and 82°C wash.

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.

20. 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 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

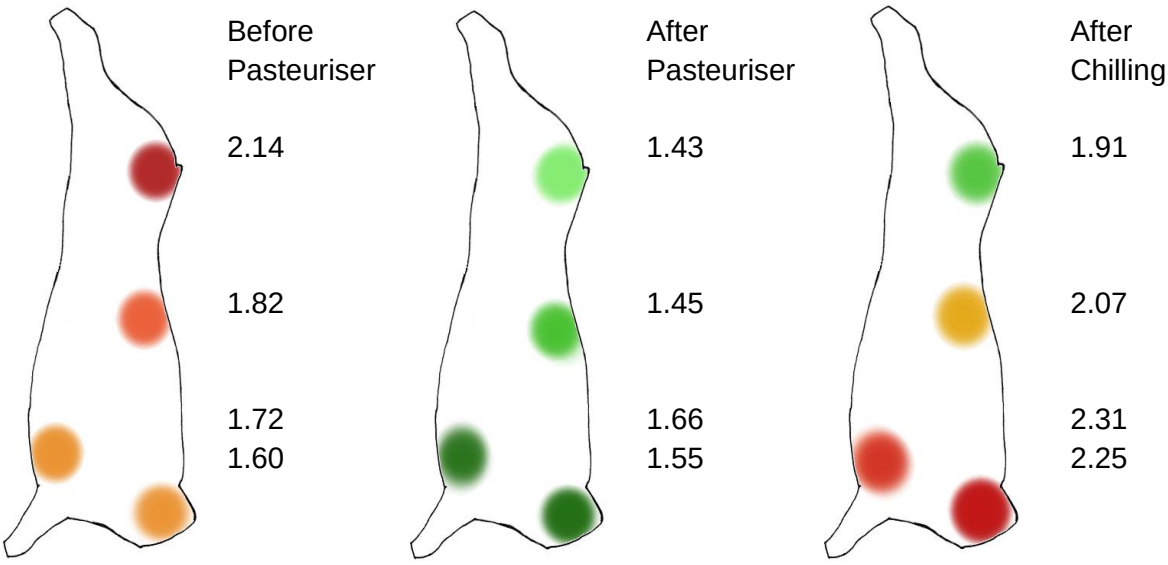


Figure 1: Mean TVCs (log_{10} cfu/g) on carcases before and after pasteurising and after overnight chilling

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

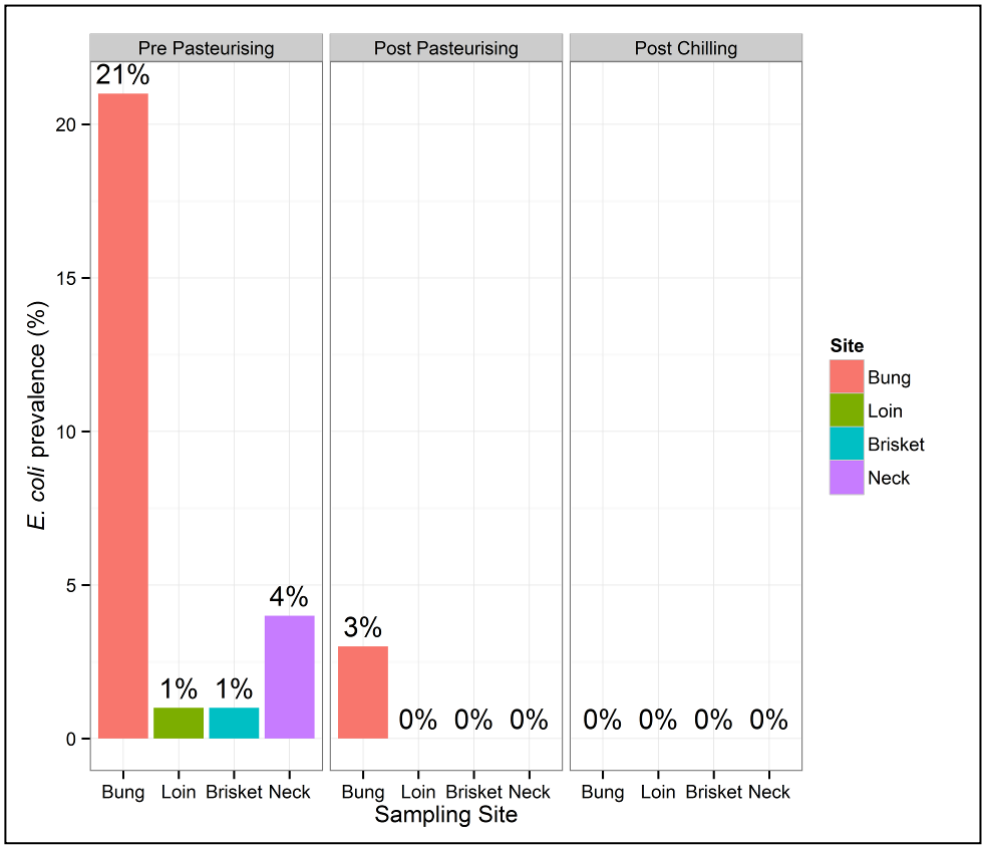


Figure 2: *E. coli* prevalence (%) at the three carcase locations before and after pasteurising and after chilling

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.

Chilling

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

Introduction

Ozone is a powerful oxidizing agent which is 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	

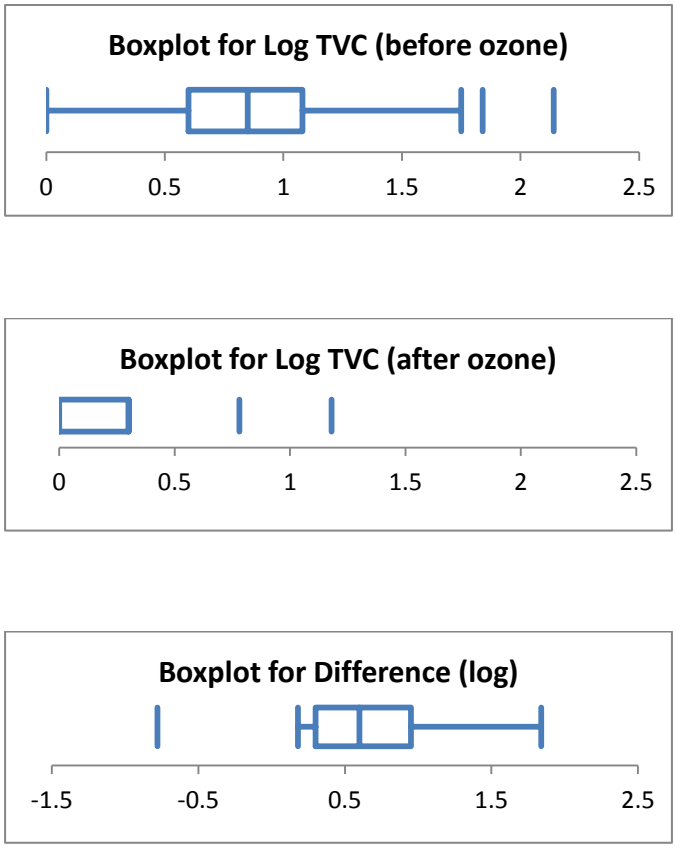


Figure 1: Boxplots of log_{10} TVC cfu/cm^2 for before and after ozonation and the difference.

Conclusion

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

Boning

22. 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.

23. 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 before and 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 before and 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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24. 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.

25. 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 amplified 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.

26. Sanitisation of 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.

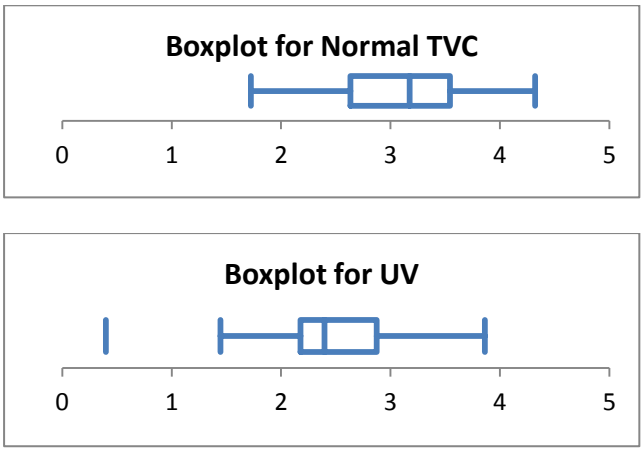


Figure 1: Boxplots showing log_{10} TVC cfu/cm^2 before and after UV application.

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.

27. 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 Shigatoxic *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 below 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	

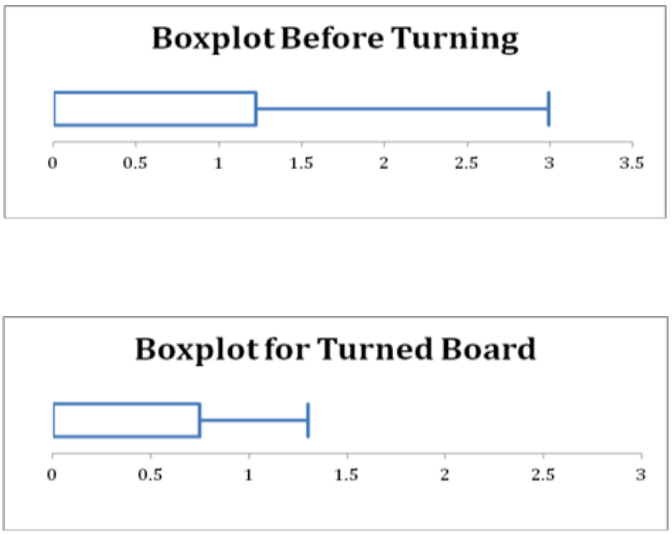


Figure 1: Boxplots of the $\log_{10}$ *E. coli* cfu/cm^2 from before and after the turning of cutting boards.

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.

28. 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

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

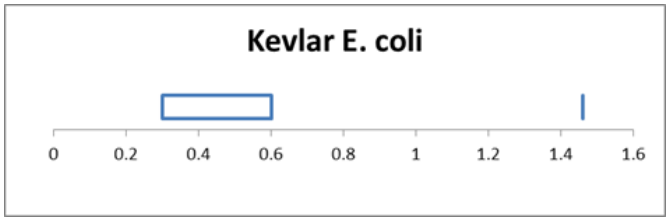


Figure 2: Boxplot of log_{10} *E. coli* cfu/cm^2 for Kevlar.

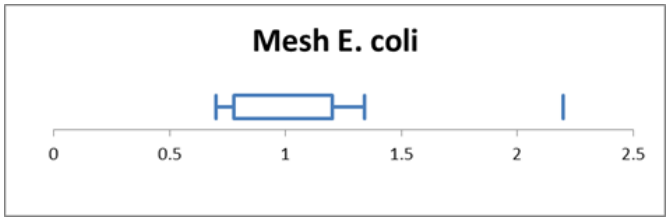


Figure 3: Boxplot of log_{10} *E. coli* cfu/cm^2 for Mesh.

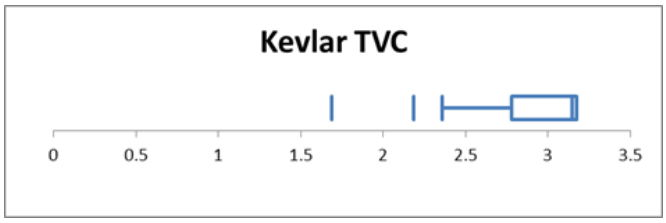


Figure 4: Boxplot of log_{10} TVC cfu/cm^2 for Kevlar.

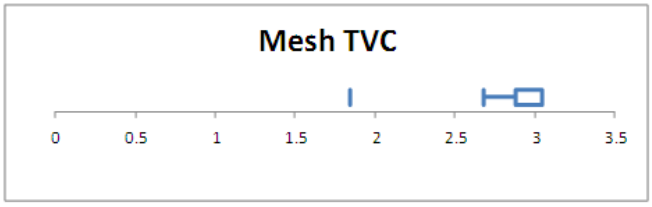


Figure 5: Boxplot of log_{10} TVC cfu/cm^2 for Mesh.

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.

Packing

29. 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.

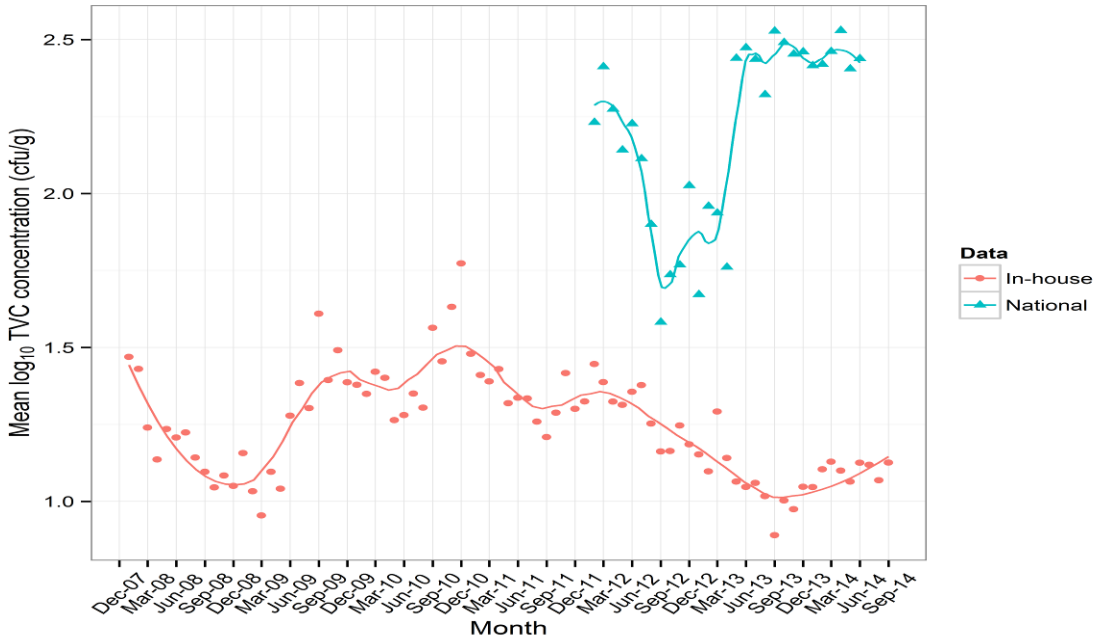


Figure 1: Mean $\log_{10}$ TVC cfu/g for in-house carton samples from Plant A compared with national carton samples.

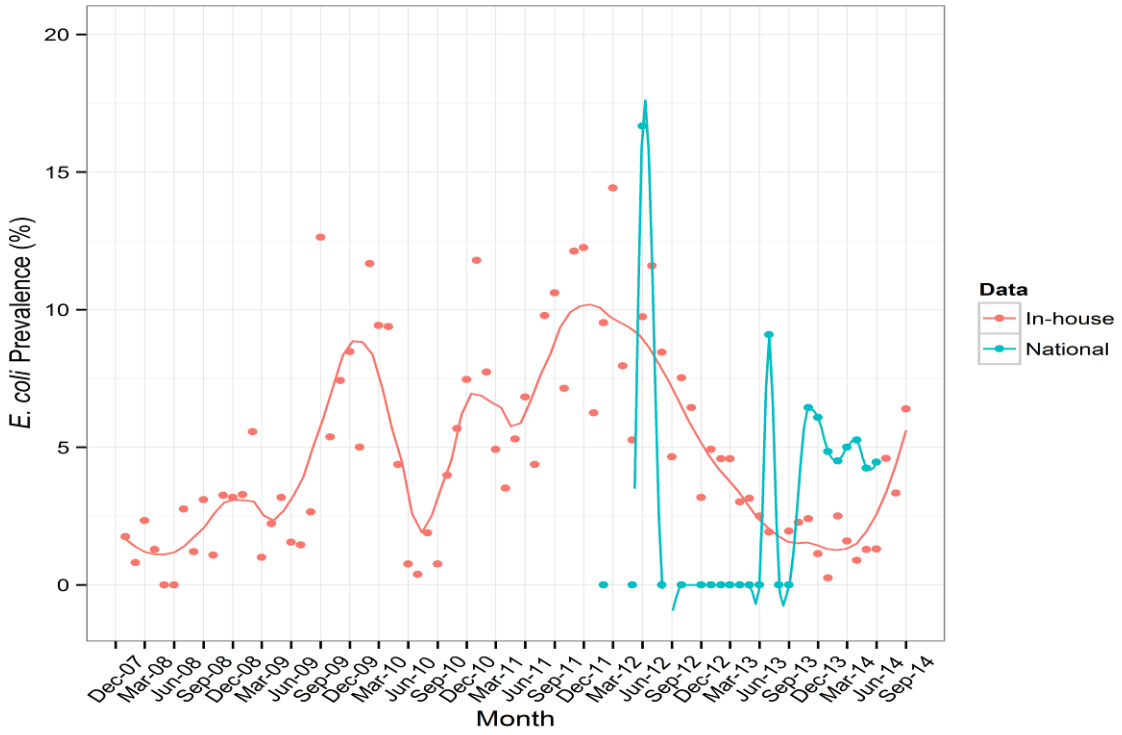


Figure 2: *E. coli* prevalence for in-house carton samples at Plant A compared with national carton samples.

30. 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 the table below 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	

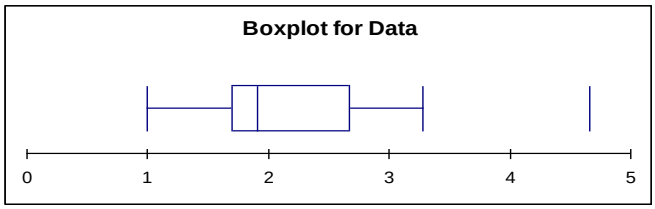


Figure 1: Boxplot of log_{10} TVC cfu/cm^2 for carton meat from Shift 1.

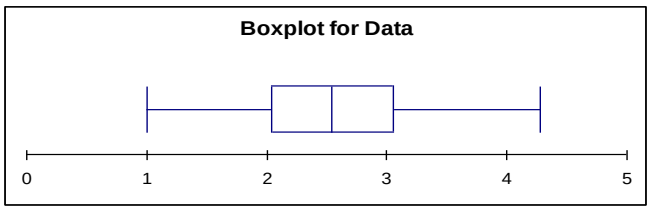


Figure 2: Boxplot of log_{10} TVC cfu/cm^2 for carton meat from Shift 2.

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.

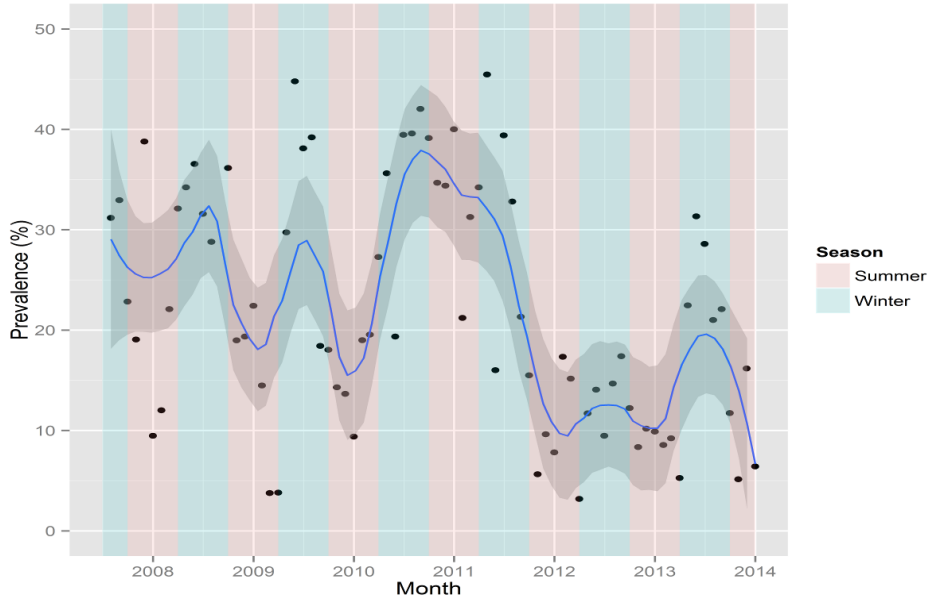


Figure 1: Monthly prevalence of *E. coli* on sheep carcases at S-E Australian plant A during 2007-2014

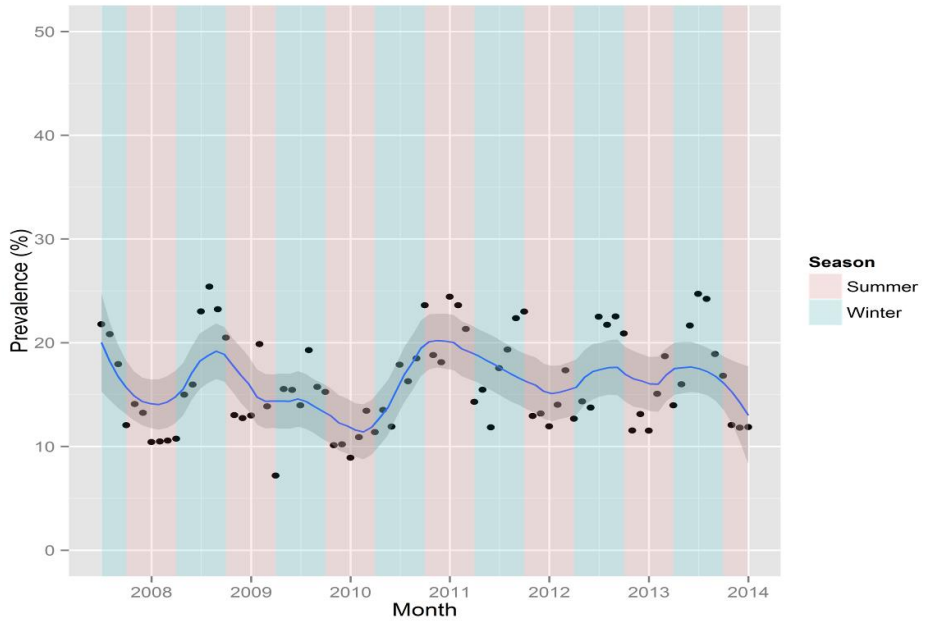


Figure 2: Average monthly prevalence of *E. coli* on sheep carcases from twelve other S-E Australian plant during 2007-2014

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).

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

Reference

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.

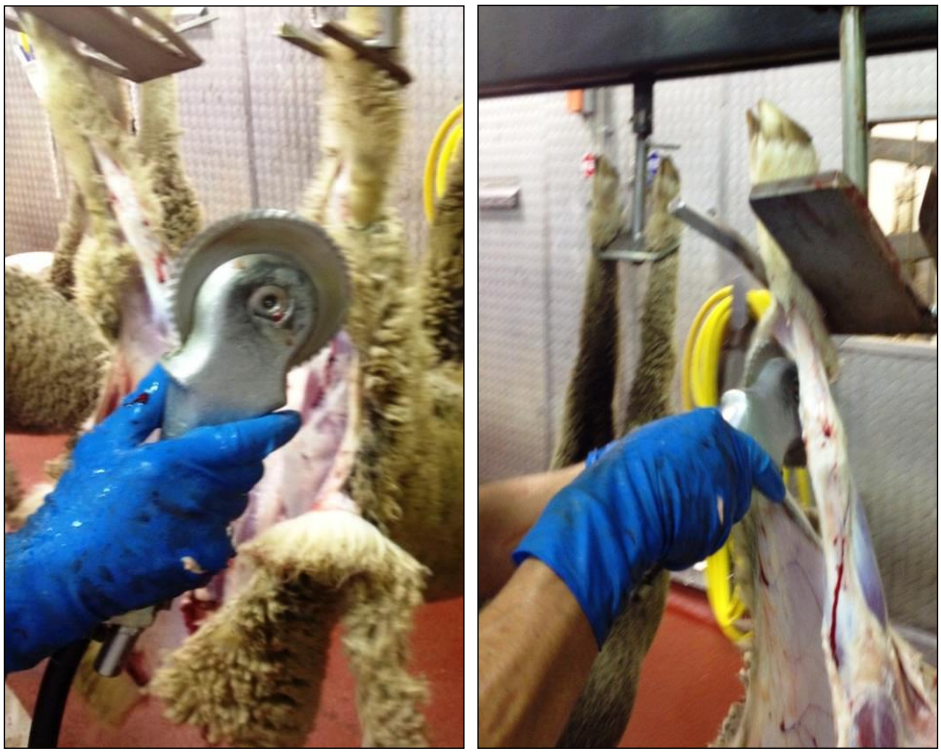


Figure 2: New procedure using air knife.

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 the Tables below from which you can see in Table 1, there is only a 0.02 difference in the average $\log_{10}$ TVC/cm^2 (P-value > 0.1) and from Table 2 it can be seen that there is 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	

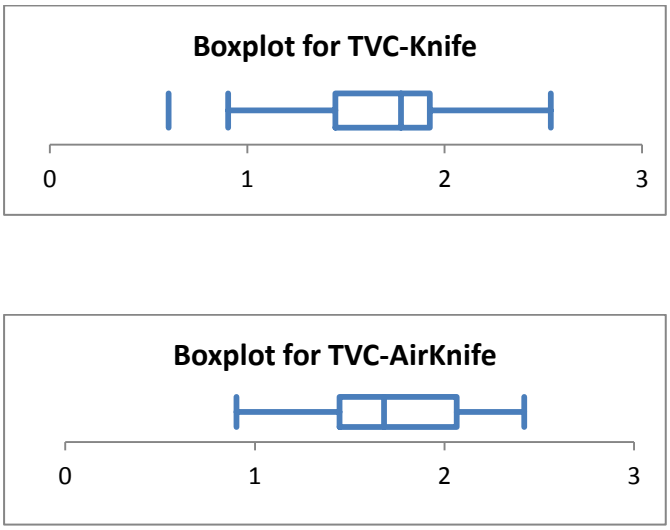


Figure 3: Boxplots of the $\log_{10}$ TVC results showing the slight difference between Conventional Knife and the Air Knife.

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 the table (below), 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	

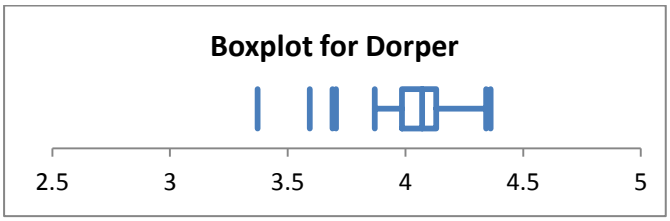


Figure 1: Boxplot of log_{10} TVC cfu/cm^2 for Dorper.

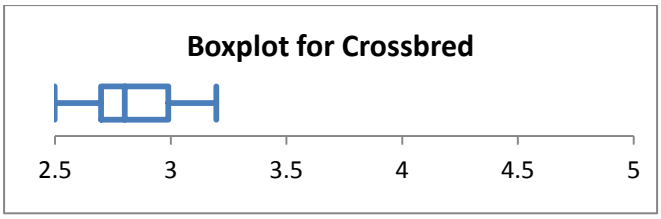


Figure 2: Boxplot of log_{10} TVC cfu/cm^2 for Crossbred.

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

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.
- 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 the table below. *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*

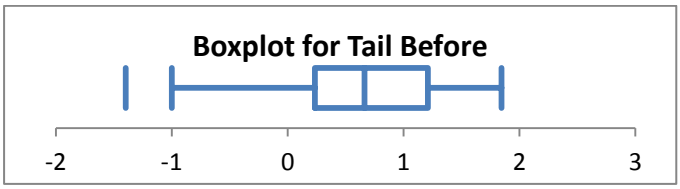
Table 3: Summary of *E. coli* prevalence for investigation of microbial contamination at the pre-scapular before and after palpation.

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*



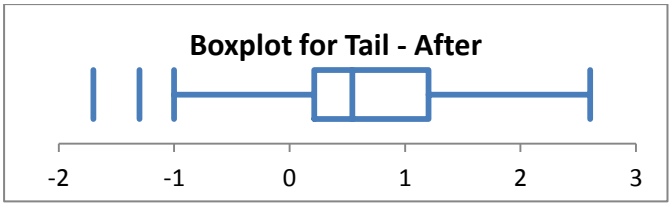


Figure 1: Boxplots of $\log_{10}$ TVC cfu/cm^2 for Tail before and after palpation.

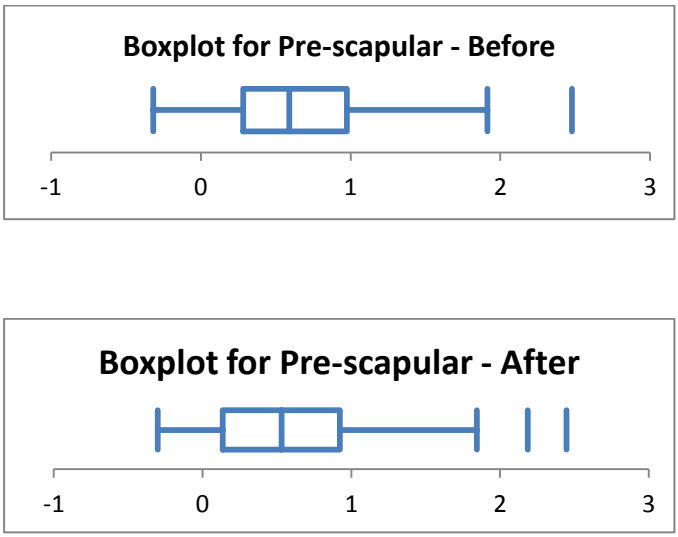


Figure 2: Boxplots of log_{10} TVC cfu/cm^2 for Pre-scapular before and after palpation.

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 the table below from which it can be seen that the difference in TVC results 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

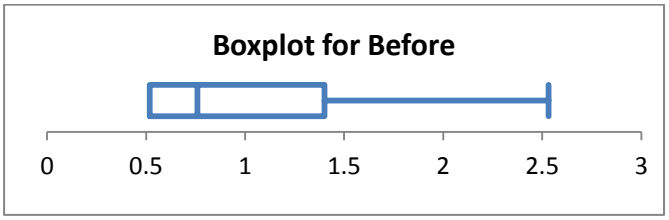


Figure 1: Boxplot of the log_{10} TVC/cm^2 from before the carcass wash.

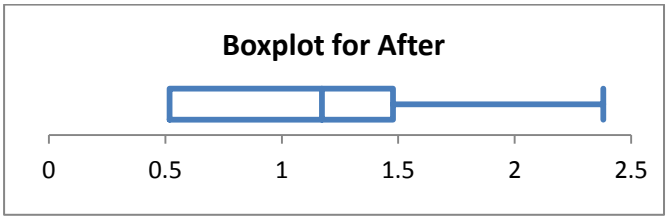


Figure 2: Boxplot of the log_{10} TVC/cm^2 from after the carcass wash.

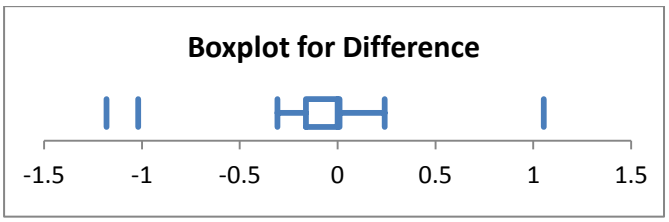


Figure 3: Boxplot of the difference between the results in log_{10} TVC/cm^2.

Conclusion

It was concluded that the carcass wash is not a significant process when trying to reduce microbial results.

Boning

6. 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

The table below shows 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.

Table 1, below, shows that there was a highly significant difference in contamination of the forequarter in using the glycol heat plate compared to water.

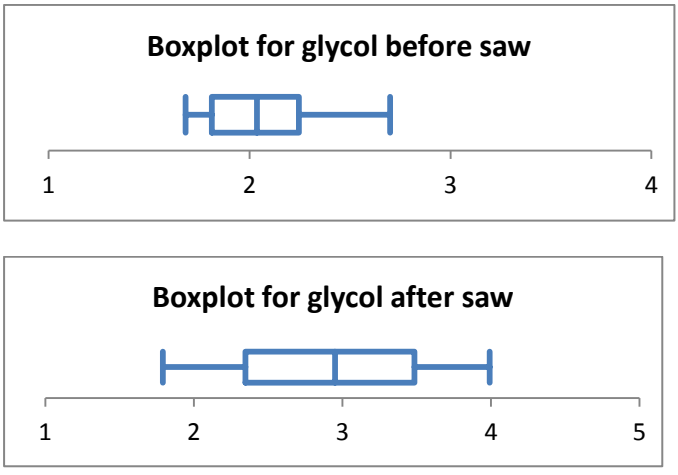
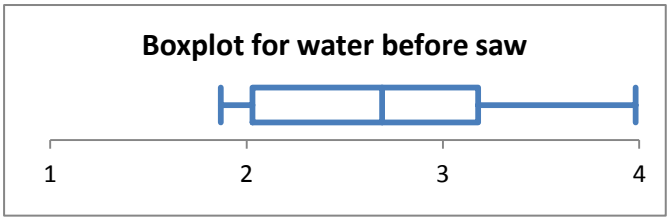


Figure 1: Boxplots of $\log_{10}$ TVC cfu/cm^2 for lamb forequarters when glycol was used on the bandsaw.



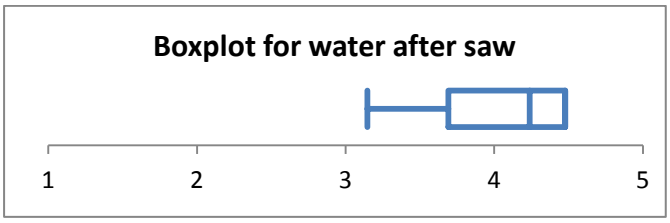


Figure 2: Boxplots of log_{10} TVC cfu/cm^2 for lamb forequarters when water was used on the bandsaw.

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.

7. 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 Symbio Alliance, which is 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) & *E. coli* (Table 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

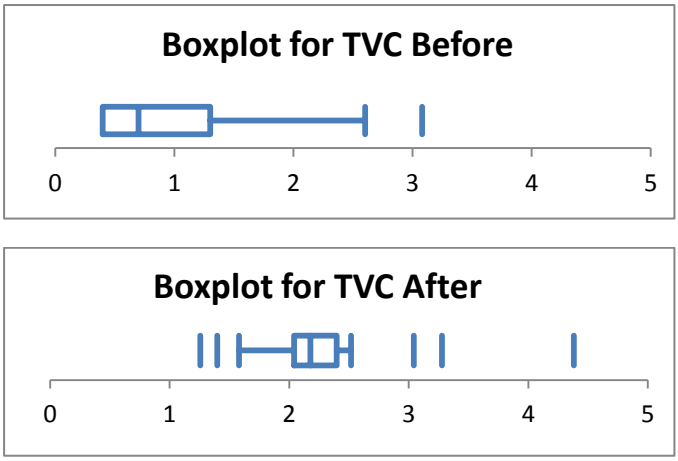


Figure 1: Boxplots of log_{10} TVC cfu/cm^2 for before and after boning.

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	

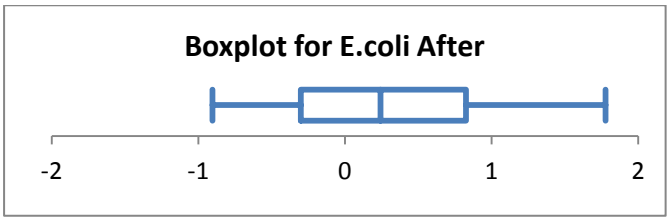


Figure 2: Boxplot of log_{10} *E. coli* cfu/cm^2 after boning.

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.

8. 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*

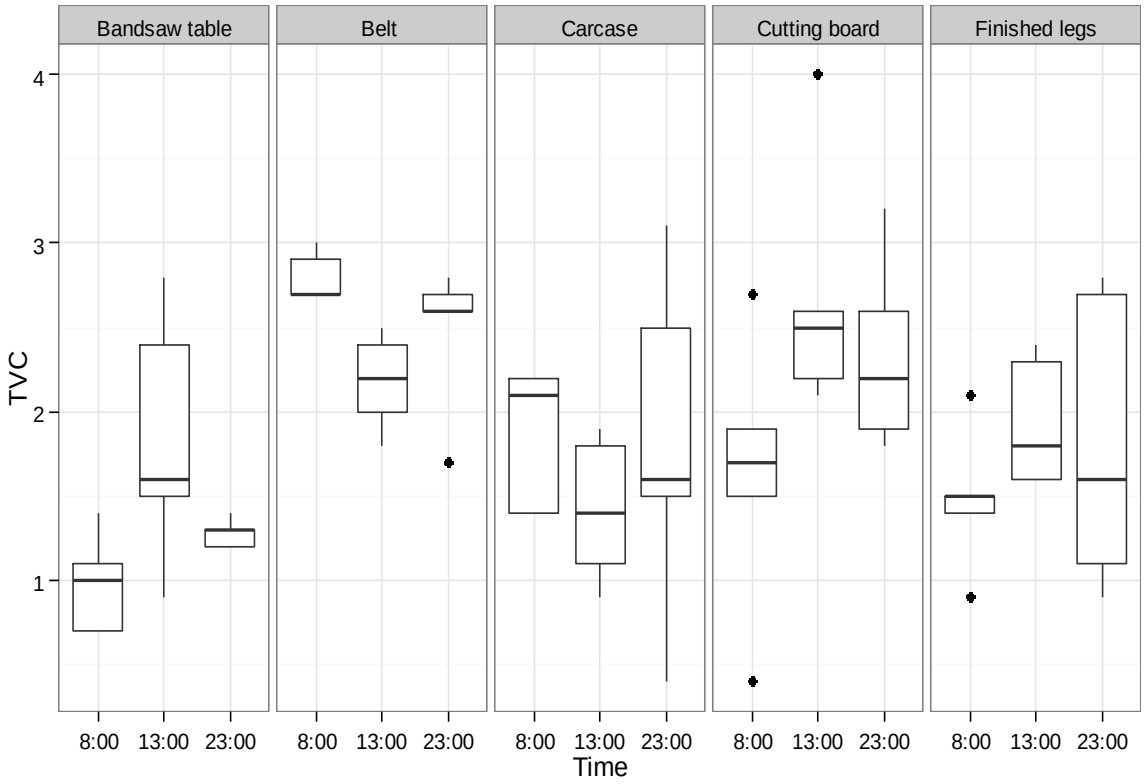


Figure 1: Boxplots showing log_{10} TVC cfu/cm^2 for each sampling site and time.

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.

9. 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.