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Prepared by: Dr Joe Liu
Symbio Laboratories

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Locked Bag 1961
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Beef and veal baseline survey 2016 – Final report

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Abstract

MLA has conducted a survey of beef and veal carcases from Australian export meat processing establishments to demonstrate the level of process control in slaughter operations and the resulting hygienic quality of beef/veal carcases. This survey was initiated in response to the announcement that the Food Safety and Inspection Service (FSIS) was conducting a nationwide beef and veal microbiological baseline data collection program in the USA which commenced in August 2014. Five thousand four hundred and fifty two beef and veal carcase sponge samples were collected from different meat processing establishments throughout Australia. Samples were collected immediately after the hide was removed and again at the end of processing prior to entering the chiller. The samples were tested for *Salmonella* and indicator microorganisms including *E.coli*, coliforms and Total Viable Count (TVC). Results showed that immediately after hide removal the *Salmonella* prevalence was 1.33% and 3.75% on beef and veal carcases respectively. *Salmonella* prevalence was reduced to 0.34% and 1.30% on beef and veal carcases respectively at the end of processing. The indicator microorganism counts and detection rates were also reduced after processing.

This survey showed there is a low prevalence of *Salmonella* on beef and veal carcases processed in Australian export meat processing establishments.

Furthermore, the results demonstrated the effectiveness of Australian dressing procedure in export processing establishments in terms of reducing *Salmonella* detection and the microbiological load on the carcases.

Executive Summary

In the USA, the Food Safety and Inspection Service (FSIS) has completed a baseline survey of beef and veal carcases (B-VCBS), collecting 2612 and 576 sponge samples from 184 beef establishments and 16 veal establishments respectively. The FSIS have indicated that the results of this work will form the basis for the development of compliance guidelines for their industry, and, as in the past, will expect importing countries to demonstrate process control which is at least the equivalent to that of their own (the US) industry.

As a potential response to the B-VCBS, MLA has commissioned a survey of beef and veal carcases from Australian export meat processing establishments to demonstrate the level of process control and the resulting hygienic quality of beef/veal carcases. Five thousand four hundred and fifty two carcases sponge samples were collected from different beef and veal processing establishments throughout Australia. The objectives of this survey were to estimate the prevalence of *Salmonella* and prevalence and concentration of indicator organisms on beef and veal carcases immediately after hide removal and at the end of all slaughter floor operations after any processing interventions, and to establish Australian beef and veal baseline data.

The samples were tested for *Salmonella* and indicator microorganisms including *E.coli*, coliforms and TVC. Results showed that immediately after hide removal the *Salmonella* prevalence was 1.33% and 3.75% on beef and veal carcases respectively. The *Salmonella* prevalence was reduced to 0.34% and 1.30% at the end of processing prior to entry to the chillers. Indicator microorganism detection rates and counts were also reduced after processing. This study supports the claim that Australian beef and veal carcases have a low prevalence of *Salmonella*.

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1. Background

In the USA, the Food Safety and Inspection Service (FSIS) has completed a baseline survey of beef and veal carcases (B-VCBS), collecting 2612 and 576 sponge samples from 184 beef establishments and 16 veal establishments respectively. The samples consisted of large area (4000cm^2) sponging of the hind- and the forequarters at two points along the processing chain

1. Immediately after hide removal (post-hide removal)
2. At the end of the slaughter floor - after all operations have been completed and any interventions applied.

The FSIS have indicated that the results will be used to develop compliance guidelines for their industry and, as in the past, will expect importing countries to demonstrate process control which is at least the equivalent to that achieved by their own (the US) industry.

The FSIS initiative has prompted the Australian industry to generate comparable data so that the industry is in a strong position to respond to any changes in US requirements. Accordingly, over the period June 2015 to October 2016 5296 and 156 sponge samples were collected from 24 beef and 4 veal establishments, respectively.

2. Objectives

Using methodology similar to that used in the FSIS study, the objectives of this project were to:

1. Estimate the prevalence *Salmonella* and concentration and prevalence of indicator organisms on beef and veal carcases immediately after hide removal and at the end of all slaughter floor operations.
2. Establish Australian beef and veal baseline data

3. Methodology

A total of 24 establishments participated in this survey, taking 1-2 sample sets each week over the duration of the study. A sample set consisted of sponge samples from both the hindquarter (HQ) and forequarter (FQ) of one side of the carcase after hide removal and similar sites on the matching side from the same carcase after completion of all processing steps, just prior to chilling.

The FSIS sampling methodology [1] was followed as closely as possible without slowing or stopping the chain for sampling. Post-hide removal samples were collected as soon as it was safe and practicable to do so after hide removal. In the majority of cases samples were collected before evisceration while carcases were on a moving rail.

Pre-chilling samples were collected close to the meat hygiene assessment (MHA) stand after trimming to AusMeat specifications [2] and after the application of any intervention, at the end of the slaughter process.

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[1] http://www.youtube.com/watch?v=SP0t9raTLCw&feature=youtu.be
[2] http://www.ausmeat.com.au/custom-content/cdrom/Handbook-7th-edition/English/9959BFAE-F68A-11DA-AA4B-000A95D14B6E.html

Samples were collected from the Hindquarter (HQ) and Forequarter (FQ) of beef and veal carcases by sponging designated areas using both sides of a single Whirlpak sponge. Beef HQ and FQ has an assumed surface area of 4000 cm^2, whereas Veal HQ and FQ have an assumed are of 2000 cm^2 per carcase quarter (see Appendix 1).

Carcase sponge samples were collected from different meat processing establishments throughout Australia (n=5452). Samples were distributed among establishments according to the sample type as shown in Table 1. All samples received by the testing laboratory in good condition were analysed. In some cases, a full set of samples was not available for testing.

Table 1: Collection of samples from various types of establishments

		Number of Establishments	Sample sets (4 sponges in a set)
Cattle age	beef	24	1324
	veal	4	39
Cattle origin	grass	20	628
	grain	16	582
	not known*	-	153
Intervention (cattle)	none	12	570
	steam vac	1	91
	lactic acid	1	52
	hot water	10	611
Intervention (veal)	none	4	39

* information was not recorded for individual carcases

Sponge samples were chilled and shipped to a laboratory accredited to ISO 17025 for analysis. Samples were processed no later than on the day following collection at the establishment. After incubation of enumeration tests, typical colonies were counted and results expressed as log cfu/cm^2. For Beef carcases, the limit of detection was -1.2 $\log_{10}$ (0.063 cfu/cm^2) for the total viable count (TVC), -2.2 $\log_{10}$ (0.0063 cfu/cm^2) for *Escherichia coli* and -2.2 $\log_{10}$ (0.0063 cfu/cm^2) for *Salmonella*. For Veal, the limits of detection were; TVC (-0.9 $\log_{10}$ = 0.12 cfu/cm^2), *E. coli* (-1.9 $\log_{10}$ = 0.012 cfu/cm^2) and *Salmonella* (-1.9 $\log_{10}$ = 0.012 cfu/cm^2). A complete description of the microbiological methodology is provided in Appendix 2.

Descriptive statistics were calculated using $\log_{10}$ transformed data. Medians and values for box plots were calculated only on the samples that gave a result above the limit of detection on a quantitative test (*E. coli*, TVC).

4. Results

4.1. Beef carcases

Summary statistics for *Salmonella* and indicator bacteria recovered from beef carcases after hide removal and after completion of all dressing procedures are provided in Table 2. The range and distribution of TVC is shown in Figure 1.

Table 2: Summary statistics for results from all beef sponge samples collected from Australian export establishments

	Post-hide Removal		Pre-chilling	
	FQ	HQ	FQ	HQ
TVC Median (cfu/cm^2)	2.29 (0.36)*	4.17 (0.60)*	0.81 (-0.09)*	1.00 (0.00)*
E. coli prevalence	32.9%	43.7%	15.6%	14.0%
E. coli Median (cfu/cm^2)	0.01 (-2.00)*	0.01 (-2.00)*	0.01 (-2.00)*	0.01 (-2.00)*
Salmonella prevalence	17/1318 (1.29%)	18/1317 (1.37%)	6/1329 (0.45%)	3/1331 (0.23%)

*$\log_{10}$ cfu/cm^2

The following conclusions can be made in relation to the information in Table 2 and other sources of data collected from the industry:

1. The concentrations of total bacteria on the HQ and FQ soon after hide removal were much lower than those obtained from the national ESAM sampling program (Sumner et al., 2011). This is probably a reflection of the large area sampled in the current study (4,000 cm^2 compared with 300 cm^2 in ESAM). Increasing sampling area generally reduces the total count as areas with low bacterial load are included or reflecting the brief time tissues were exposed before sponging. Whereas the ESAM sites are generally considered to be the sites of highest bacterial load on the carcase and sampling at least 12 hours of active chilling.
2. Prevalence of *E. coli* immediately after hide removal was much higher than obtained by ESAM sampling, reflecting the large area (4000 cm^2) sponged. While there is generally a reduction in total count associated with large area sampling the likelihood of detecting specific bacteria increases as more of the carcase is sampled.
3. There were reductions in TVC and in prevalence of *E. coli* by the end of processing.
4. Prevalence of *Salmonella* immediately after hide removal was higher than normally seen in ESAM or other published baseline studies (Phillips et al., 2006) *Salmonella* was isolated from 35 of 2635 samples (1.3%) immediately after hide removal and from 9/2660 (0.3%) samples at the completion of processing. Again the larger area sampled in this study compared to ESAM is a likely contributor to the relatively high detection rate.

5. Salmonella serovars isolated from beef carcases in this survey were:
    - **Post-hide Removal***: S.* Hvittingfoss*, S.* Bredeney*, S.*Muenster*, S.* Adelaide*, S.* Infantis*, S. subspecies II serotype: 42:g,t-*, *S.* Poona*, S.* Bovismorbificans*, S.* Typhimurium*, S.* Senftenberg*, S.* Havana*, S.* Anatum*, S.* Oranienburg*, S.* Chester*, S.* Cerro*.*
    - **Pre-chilling***: S. subspecies 1 serotype: 16:l,v:-*, *S.* Tennessee*, S.* Zanzibar*, S.* Mbandaka*, S.* Havana*, S.* Dublin*.*

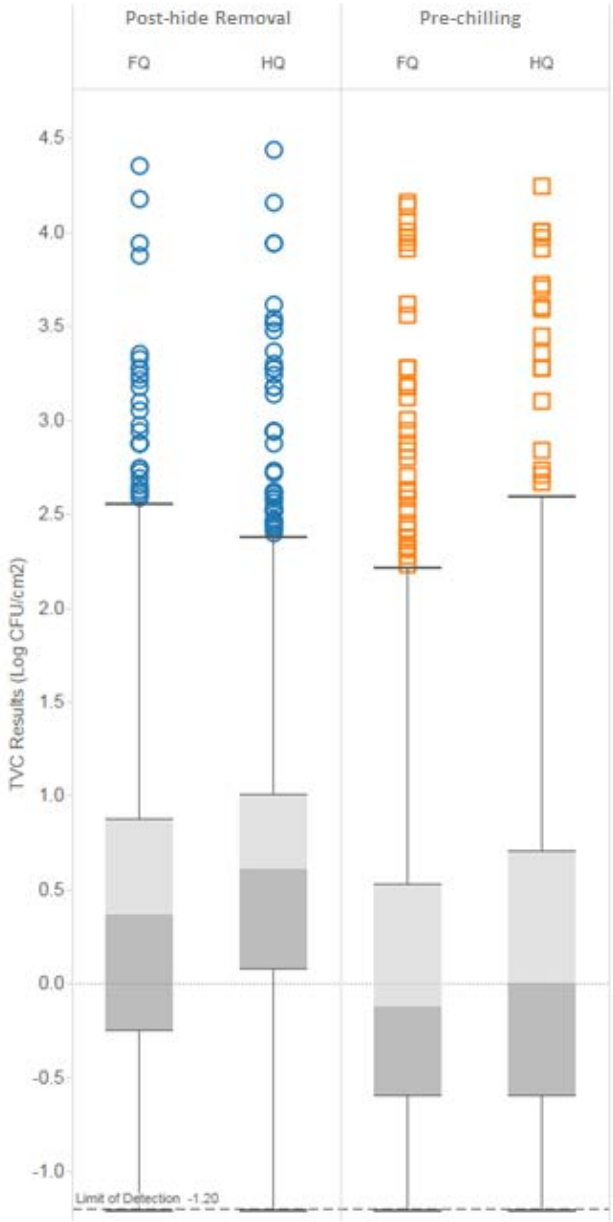


Figure 1: Box plots for Total Viable Count (TVC) of forequarter (FQ) and hindquarter (HQ) beef carcases samples after hide removal and at the end of processing (pre-chilling). The junction of the two shaded areas represents the median, the bottom and top of the box represent the 25th and 75th percentiles, respectively (i.e. 50% of the data falls within the shaded area) and points outside the 'whiskers' represent statistical outliers. The limit of detection is also indicated.

The potential for differences to exist between cattle coming from extensive grazing operations and those that have been consigned to slaughter from feedlots was investigated (Figure 2). There was little if any effect of cattle origin on the TVC for either FQ and HQ samples collected post hide removal or pre-chilling.

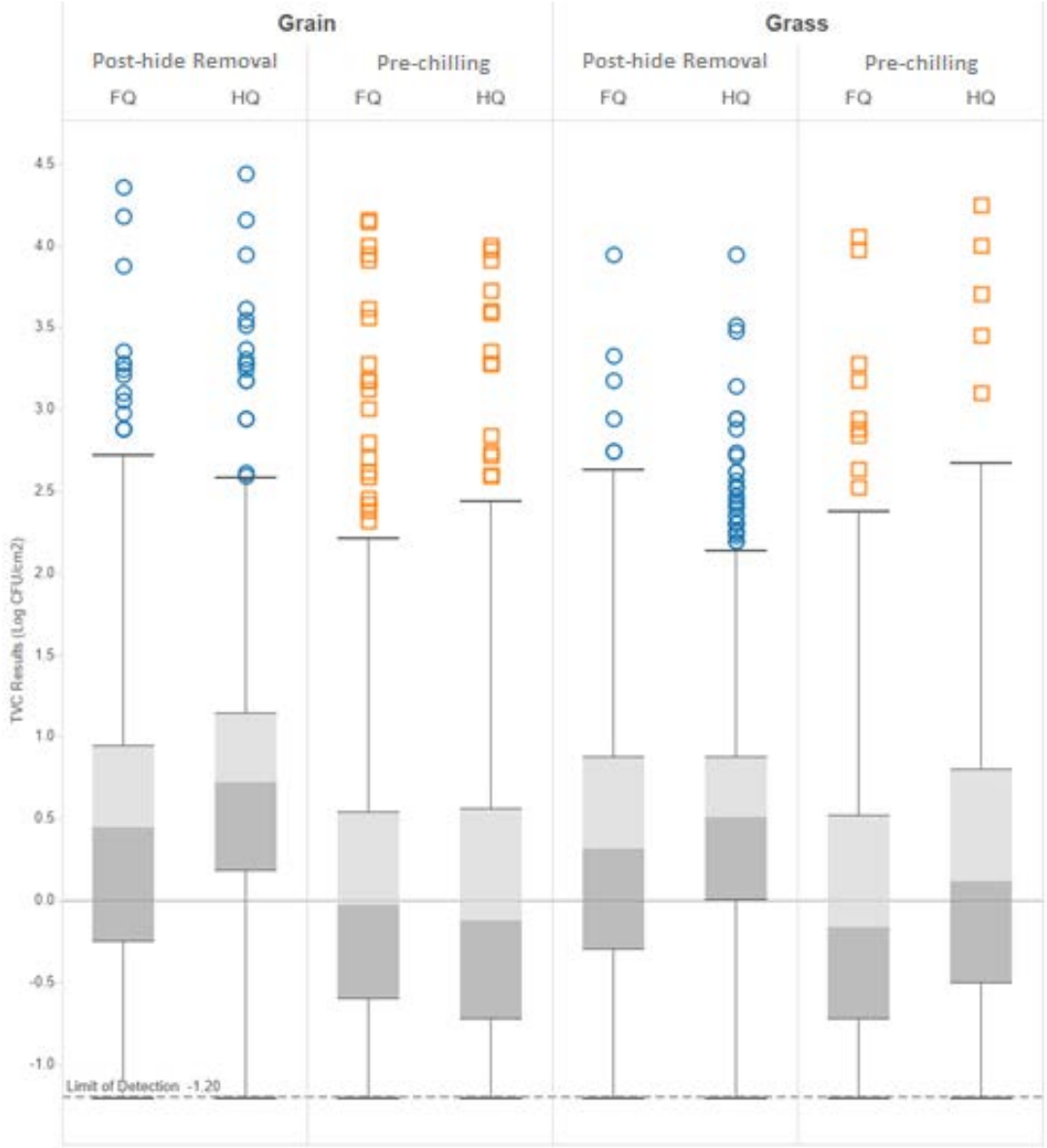


Figure 2: Box plots for Total Viable Count (TVC) of beef carcases – Feed type Grain vs Grass. See Figure 1 for an explanation of box plots.

All 24 establishments participating in this survey trimmed carcases to AusMeat specifications and to remove visible contamination. In the case of 12 establishments, trimmed sides were transferred to active chilling without any decontamination intervention step. The microbiological condition of Post-hide removal carcases and Pre-chilling trimmed carcase sides at these 12 establishments is summarised in Table 3. It can be seen that there was a small reduction in TVC and a reduction in *E. coli* prevalence from almost 40% to 20.1%; *Salmonella* was recovered from 1.7% of Post-hide removal carcases and 0.3% of Pre-chilling trimmed carcase sides.

Table 3: Summary statistics for large area sampling of beef carcases at establishments without any decontamination intervention

	TVC		*E. coli*		*Salmonella*	
	Post-hide removal	Pre-chilling	Post-hide removal	Pre-chilling	Post-hide removal	Pre-chilling
n	1131	1149	1131	1149	1127	1145
Detection	96.7%	95.7%	39.8%	20.1%	1.7%	0.3%
Median (cfu/cm^2)	4.90 (0.69)*	1.62 (0.21)*	0.01 (-1.90)*	0.01 (-1.90)*	-	-

*Log_{10} cfu/cm^2

One establishment employed steam vacuum on the hindquarters of carcases after hide removal. There was a slightly greater reduction in *E. coli* prevalence after treatment with steam vacuum compared to reductions noted for establishments employing trimming alone (Table 4), but as only one plant was utilising steam vacuum no conclusions can be drawn as to the efficacy of this intervention. The prevalence of *Salmonella* was 0% on carcases both before and after treatment (Table 4). Steam vacuuming has been shown to be effective in removing visible contaminants (hair, dirt) but was not able to reliably reduce the bacterial population to any appreciable extent (Gill and Baker, 1998).

Table 4: Summary statistics for large area sampling of beef carcases after hide removal and utilisation of **steam vacuum** on hindquarters

	TVC		*E. coli*		*Salmonella*	
	Post-hide removal	Pre-chilling	Post-hide removal	Pre-chilling	Post-hide removal	Pre-chilling
n	182	182	182	182	182	182
Detections	96.2%	94.0%	21.4%	7.7%	0%	0%
Median(cfu/cm^2)	2.00 (0.30)*	0.56 (-0.25)*	0.01 (-2.20)*	0.01 (-2.20)*	-	-

*log_{10} cfu/cm^2

Eleven of the participating establishments employed what are considered as interventions in the US context: one utilised lactic acid to manually treat carcases sides (Table5) while 10 establishments had installed a commercial hot water wash cabinet (Table 6).

In the case of the establishment using lactic acid, recovery of bacteria from the carcase surface after hide removal was already low (median - 0.07 log_{10} cfu/^{cm2}, Table 5) and it is therefore difficult to judge the effect of the manual application of lactic acid. Reduction in the prevalence of *E. coli* was similar to that observed for treatment using steam vacuum (Table 4). There was a notable reduction in the number of samples with detectable levels of TVC at this establishment, This was not generally observed at other establishments in the study. Further investigations at this establishment may be warranted.
In the case of the 10 establishments using commercial hot water decontamination (Table 6), there was a slightly greater reduction in TVC and a significant reduction in *E. coli* prevalence compared to result for plants without any interventions (Table 3). The *E. coli* prevalence after treatment was 11.4% compared to 20.1% at plants without any interventions. *Salmonella* was recovered from 1.3% of Post-hide removal carcases and 0.5% of Pre-chilling trimmed carcase sides, this was similar to detection rates from samples collected at establishments without any interventions.

Trimming plus hot water washing had little effect on the TVC although there was a significant reduction in the *E. coli* prevalence at plants utilising hot water. It is not clear if this effect is as a result of decontamination or other practices at those establishments utilising hot water interventions..

Table 5: Summary statistics for large area sampling of beef carcases after hide removal and manual application of **lactic acid**

	TVC		*E. coli*		*Salmonella*	
	Post-hide removal	Pre-chilling	Post-hide removal	Pre-chilling	Post-hide removal	Pre-chilling
n	104	104	104	104	106	104
Detections	87.5%	58.7%	20.2%	6.7%	0%	0%
Median(cfu/cm^2)	0.85 (-0.07)*	0.06 (-1.20)*	0.01 (-2.20)*	0.01 (-2.20)*	-	-

*log_{10} cfu/cm^2

Table 6: Summary statistics for large area sampling of beef carcases after hide removal and after **hot water** decontamination

	TVC		*E. coli*		*Salmonella*	
	Post-hide removal	Pre-chilling	Post-hide removal	Pre-chilling	Post-hide removal	Pre-chilling
n	1217	1227	1218	1226	1222	1229
Detections	95.7%	85.3%	40.9%	11.4%	1.3%	0.5%
Median (cfu/cm^2)	2.63 (0.42)*	0.63 (-0.20)*	0.02 (-1.73)*	0.01 (-1.90)*	-	-

*$\log_{10}$ cfu/cm^2

4.2. Veal carcases

Summary statistics for *Salmonella* and indicator bacteria on veal carcases immediately after hide removal and after completion of all dressing procedures on the slaughter floor (pre-chilling) are provided in Table 7. The range and distribution of TVCs are presented in Figure 3. None of the establishments participating in the survey utilised any decontamination intervention other than normal trimming and washing.

Table 7: Summary statistics for the veal sponge samples from Australian establishments

	Post-hide Removal		Pre-chilling	
	FQ	HQ	FQ	HQ
n	40	40	37	39
TVC Median (cfu/cm^2)	12.88 (1.11)*	17.38 (1.24)*	8.91 (0.95)*	9.12 (0.96)*
E. coli detected	47.0%	75.0%	39.5%	53.9%
E.coli Median (cfu/cm^2)	0.09 (-1.06)*	0.13 (-0.90)*	0.01 (-1.90)*	0.04 (-1.43)*
Salmonella detected	0/40 (0%)	3/40 (7.5%)	1/37 (2.7%)	0/39 (0%)

*$\log_{10}$ cfu/cm^2

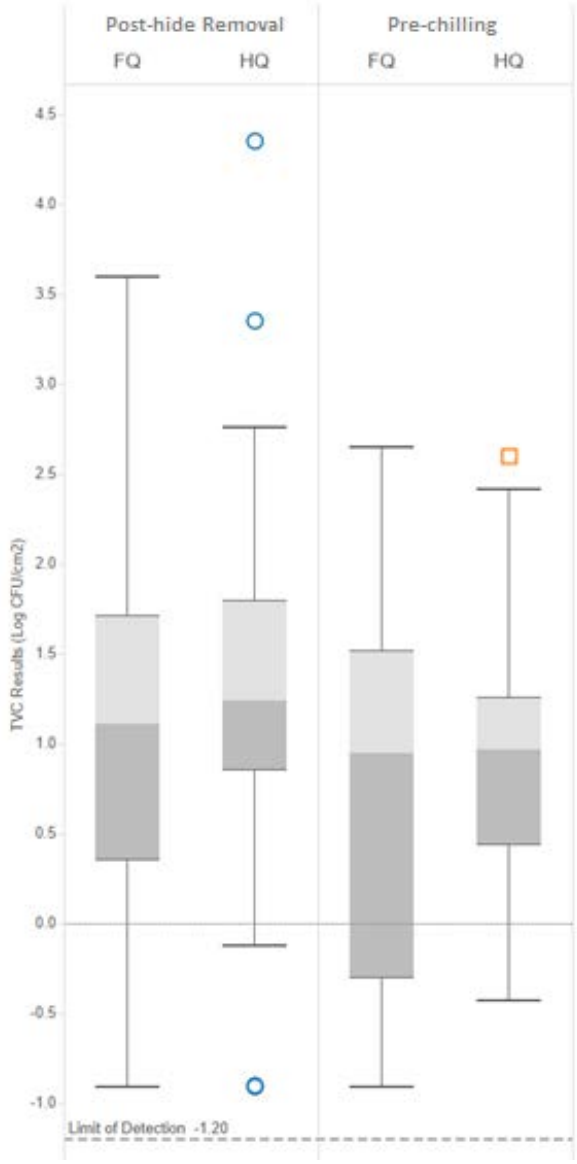


Figure 3: Box plots for Total Viable Count (TVC) on the forequarter (FQ) and hindquarter (HQ) of veal carcases after hide removal and at the end of the processing chain (pre-chilling)

The following conclusions can be drawn in relation to the information in Table 6 and other sources of data collected from the industry:

1. Similar to beef, the concentrations of total bacteria on the HQ and FQ soon after hide removal were much lower for veal than those obtained during ESAM sampling. Again this is likely a reflection of large area sampling.
2. Prevalence of *E. coli* immediately after hide removal was much higher than that reported under the ESAM program, which again reflects the larger area (2000cm^2) sponged resulting in recovery of more of the targeted bacteria than ESAM sampling.
3. As with beef, there were small reductions in TVC and in prevalence of *E. coli* by the end of the processing line.
4. Prevalence of *Salmonella* immediately after hide removal was higher than normally seen in ESAM or baseline studies, reflecting the increased sensitivity resulting from the larger area sampled.

5. *Salmonella* was isolated on 3/80 (3.7%) of samples immediately after hide removal and from 1/76 (1.3%) after processing.
6. *Salmonella* serovars isolated from veal carcases in this survey included:
    - **Post-hide Removal***: S.* Chailey*, S.* Havana and *S.* St Paul;
    - **Pre-chilling**: *S.* Chailey

5. Conclusions

The survey show interventions have some positive effect in reducing the prevalence of *E. coli* there is little effect on *Salmonella* prevalence. The results demonstrates the effectiveness of Australian dressing procedure in export processing establishments in terms of reducing Salmonella detection and the microbiological load on carcases without the application of multiple interventions.

6. References

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

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

Sumner, J., A. Kiermeier and I. Jenson (2011). "Verification of hygiene in Australian manufacturing beef processing - Focus on Escherichia coli O157." Food Protection Trends 31(8): 514-520.

Youssef, M., Gill, C. & Yang, X. 2014. Storage life at 2°C or -1.5°C of vacuum-packaged boneless and bone-in cuts from decontaminated beef carcases. Journal of the Science of Food and Agriculture, 94: 3118-3124.

7. Appendix

Appendix 1 - Sampling based on FSIS methodology

A detailed training video from the FSIS on sampling methodology is available on: http://www.youtube.com/watch?v=SP0t9raTLCw&feature=youtu.be

Carcase sampling area:

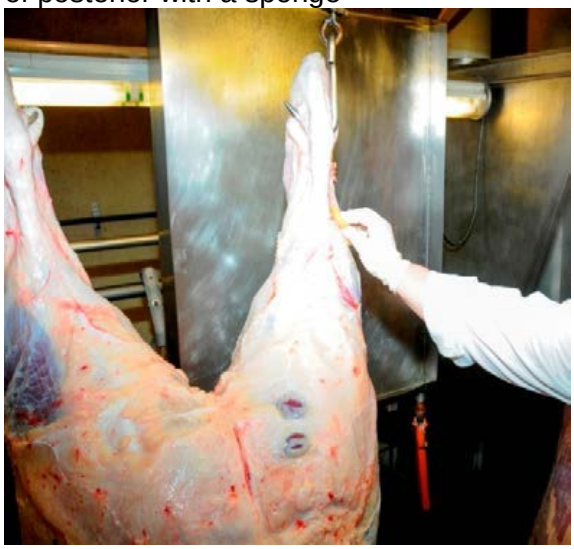
- Beef – Total area of 8,000cm^2 (2 approx equal sized parts each 4000cm^2)
- Veal – Total area of 4,000cm^2 (2 approx equal sized parts each 2000cm^2)

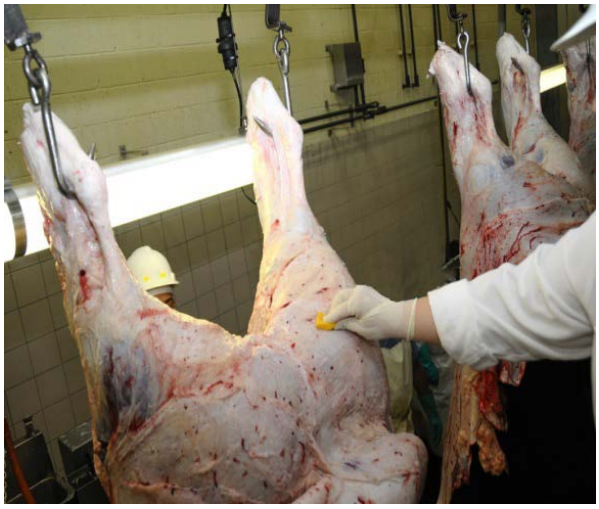
Carcase sampling method:

By using 2 sponges at each sampling point:

- **To swab the posterior (inside and outside round):**
  - Using back and forth strokes and applying sufficient pressure, swab the inside round.
  - Flip the sponge and use the other side of the sponge to swab the outside round making sure to apply sufficient pressure.

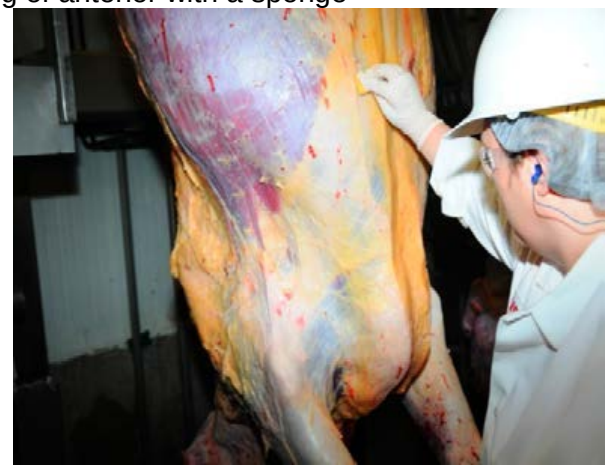
Figure 1. Swabbing of posterior with a sponge





- **To swab the anterior (navel-plate-brisket-foreshank) :**
    - Using back and forth strokes and applying sufficient pressure, swab the navel-plate area.
    - Flip the sponge and use the other side of the sponge to swab the brisket and foreshank area making sure to apply sufficient pressure.

Figure 2. Swabbing of anterior with a sponge



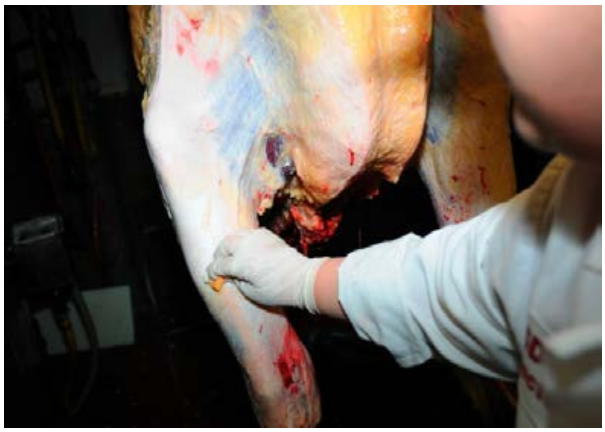


Figure 3. The 4000 cm^2 anterior (lateral brisket and short plate) and 4000 cm^2 posterior (lateral hock, round, and rump) sampling sites for post-hide removal/pre-evisceration and pre-chill carcase swabs from adult cattle carcases. The swabbing size is 2000 cm^2 for each sampling site on veal carcases.

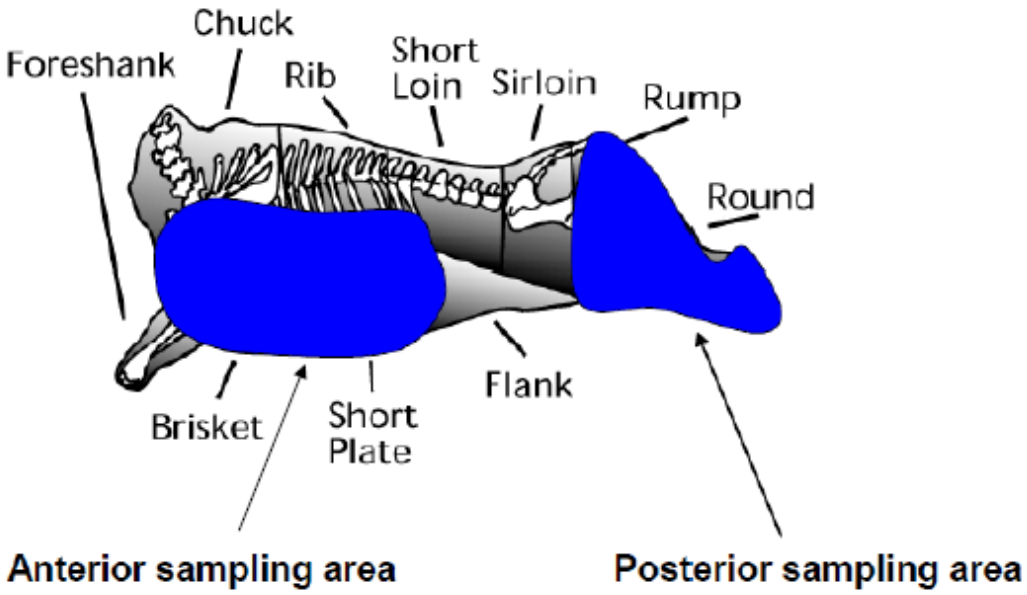


Figure 4. 4000 cm^2 anterior (lateral brisket and short plate) and 4000 cm^2 posterior (lateral hock, round, and rump) sampling sites for post-hide removal/pre-evisceration and pre-chill carcase swabs from adult cattle carcases. The swabbing size is 2000 cm^2 for each sampling site in veal carcases.

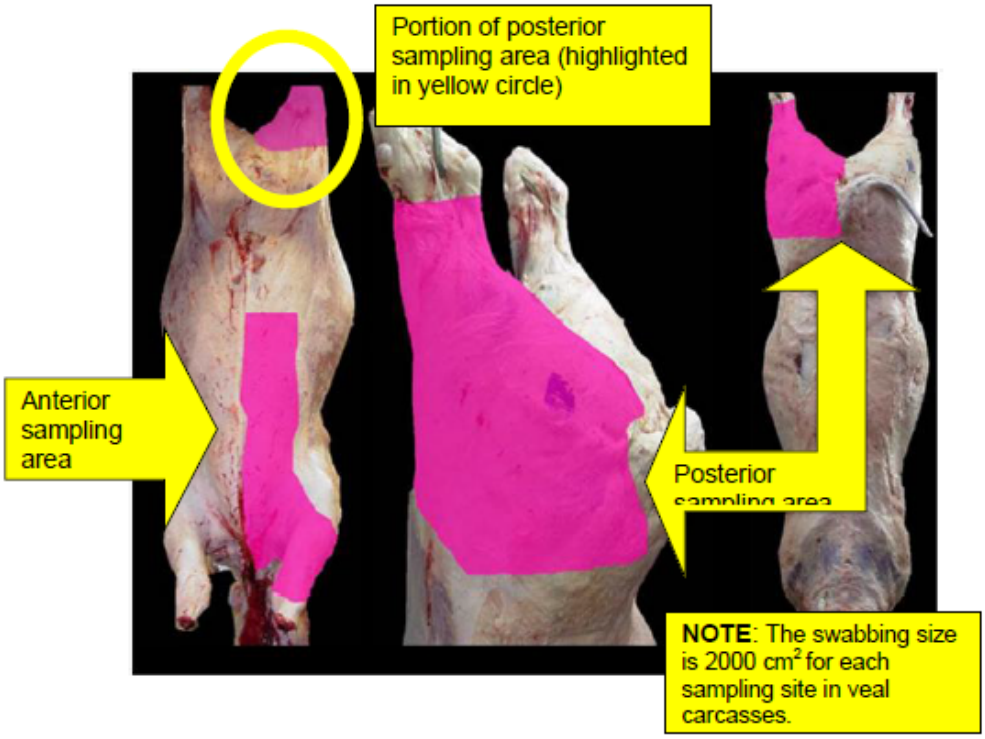


Figure 5. 4000 cm^2 anterior (lateral brisket and short plate) and 4000 cm^2 posterior (lateral hock, round, and rump) sampling sites for post-hide removal/pre-evisceration and pre-chill carcase swabs from adult cattle carcases. The swabbing size is 2000 cm^2 for each sampling site in veal carcases.

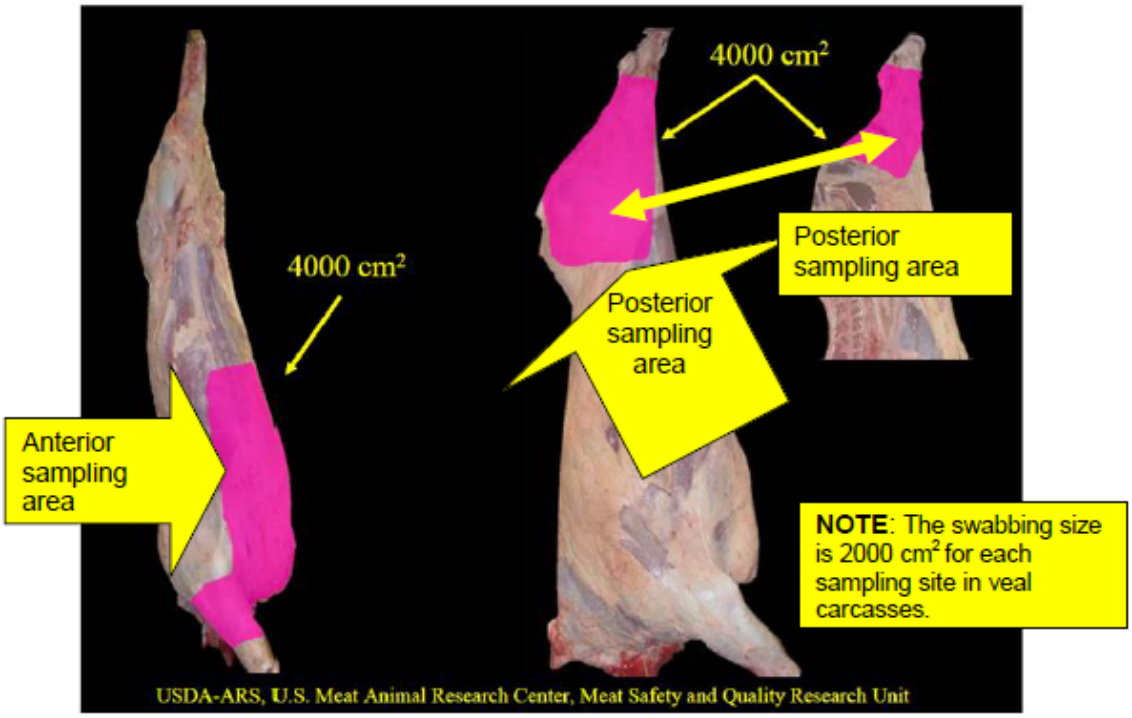
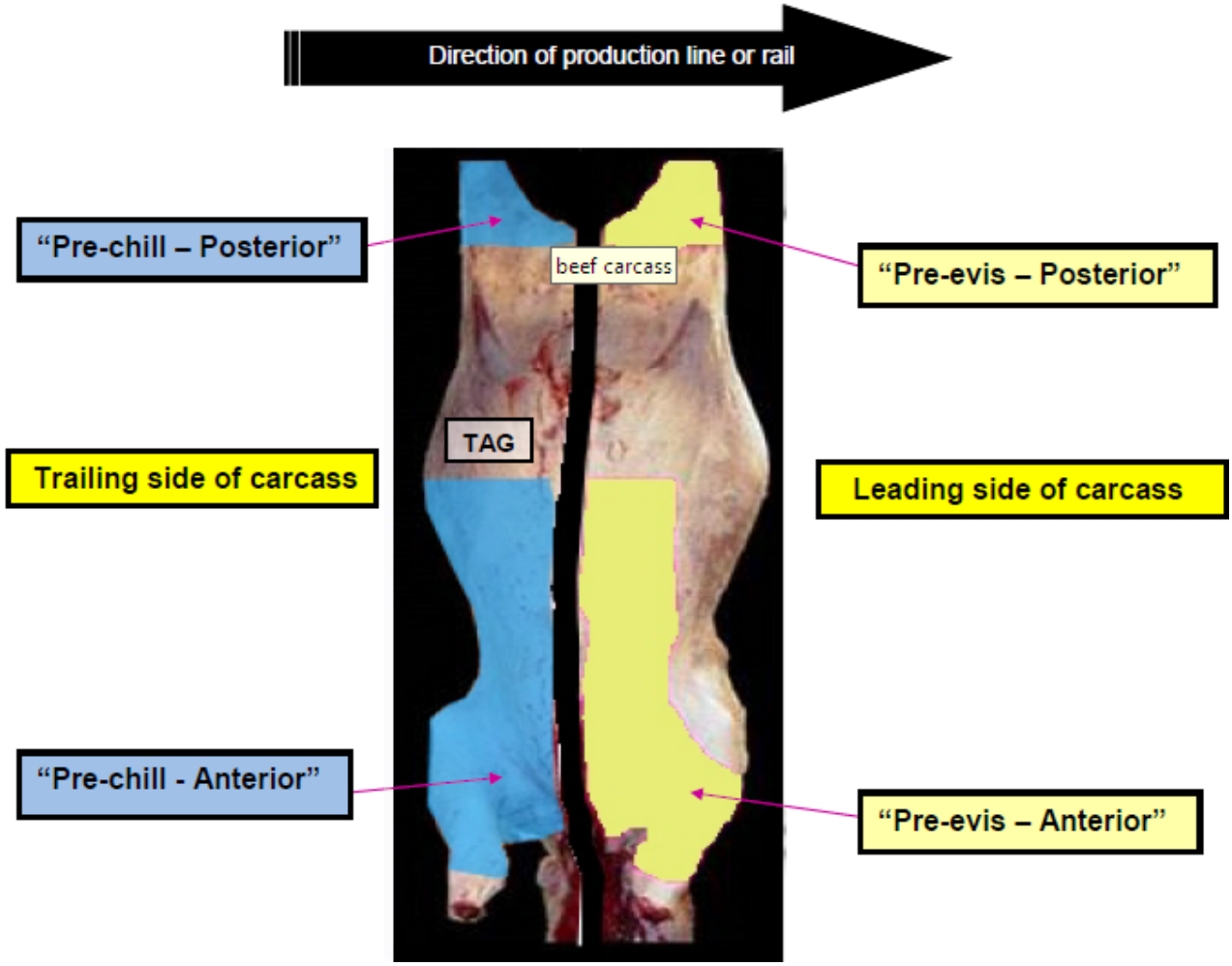


Figure 6. Determining the Leading and Trailing Sides of the Carcase



Appendix 2 – Testing Method SOP

Total viable count

Total Viable Count (AOAC 990.12): Carcass sponge samples (hydrated with 25mL Butterfield's solution) are diluted 1:10 in Butterfield's solution and one-ml plated onto Petrifilm (3M Petrifilm). Petrifilm plates are incubated at 35 ± 1 ºC for 48 ± 3 h. All red colonies are counted and results are expressed in CFU/ cm^2.

E. coli

E.coli (AOAC 991.14): Carcass sponge samples (hydrated with 25mL Butterfield's solution) are diluted 1:10 in Butterfield's solution and one-ml plated onto Petrifilm (3M Petrifilm). Petrifilm plates are incubated at 35 ± 1 ºC for 48 ± 3 h. All blue colonies associated with gas are counted as *E.coli*. Red and blue colonies with gas are coliforms.

Salmonella

Salmonella (MLG 4.07): Carcass sponge samples (hydrated with 25mL Butterfield's solution) are diluted with 125mL mTSB enrichment broth to bring total volume to 150 mL. Broths are incubated at 42 ± 2 ºC for 15 - 24 h. After incubation, PCR Assay is carried out on each ssample. Presumptive positive samples are confirmed by Australian Standard method (AS 5013.10-2009) by streaking sample to XLD and BGA plates and incubating at 37 ± 1 ºC for 21 to 27 h. Suspect colonies are picked and streaked to nutrient agar plates for purification. Oxidase test, urease test and *Salmonella* agglutination tests (O & H) are performed for confirmation. Confirmed *Salmonella*-positive isolates areis sent to Queensland Health Scientific Services for serotyping.

Appendix 3 - Acknowledgement of Participant Establishments

E C THROSBY PTY LTD
GREENHAM HW & SONS PTY LTD TASMANIA
GREENHAM HW & SONS PTY LTD TONGALA
GREENMOUNTAIN FOOD PROCESSING PTY LTD
JBS AUSTRALIA PTY LTD BEEF CITY
JBS AUSTRALIA PTY LTD BROOKLYN
JBS AUSTRALIA PTY LTD DINMORE
JBS AUSTRALIA PTY LTD PRIMO SCONE ABATTOIR
JBS AUSTRALIA PTY LTD RIVERINA
JBS AUSTRALIA PTY LTD ROCKHAMPTON
JBS AUSTRALIA PTY LTD TAMSMANIA
JBS AUSTRALIA PTY LTD TOWNSVILLE
JOHN DEE WARWICK PTY LTD
KILCOY PASTORAL COMPANY
NOLAN MEATS PTY LTD
OAKEY ABATTOIR PTY LTD
O'CONNOR G & K PTY LTD
TEYS AUSTRALIA PTY LTD BILOELA
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