

Microbiological quality of Australian beef and sheepmeat

Results of the industry's fourth national abattoir study

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Executive Summary

The fourth national baseline microbiological survey of Australian beef and sheep meat quality was conducted in 2011.

How the survey was conducted

Samples of frozen boxed beef and sheep meat and beef and sheep primals were collected during January-March and June-August, 2011 from meat processing establishments (MPEs) selected on the basis that they collectively accounted for at least 80% of either beef or sheep meat processed in Australia. In all, samples were taken from 29 beef and 12 sheep MPEs, all under the jurisdiction of the Australian Quarantine and Inspection Service (AQIS) (now DAFF Biosecurity). The number of samples taken at each MPE reflected its share of processing capacity with an upper limit of 30 samples of any one type (eg: frozen trim, primal cuts) taken on any one day. Where samples were taken from a single MPE on more than one day, the duration between visits was at least 5 weeks.

Testing was conducted in an ISO 17025-accredited laboratory following internationally acceptable methods.

Results of Beef Testing

Beef primals were found to have an average Total Viable Count (TVC, 25°C) of 18 and 32 cfu/cm² respectively for striploins and outsides. *E. coli* was isolated from 10.7 and 25.2% respectively for striploins and outsides with average counts of 0.3 and 0.5 cfu/cm² on positive samples. The average TVC for boneless beef was 166 cfu/g and the average count for the 2.1% of samples with detectable *E. coli* was 21 cfu/g. *E. coli* O157:H7 and *Campylobacter* were not detected in any of the primal samples. *Salmonella* was not detected in any of the primal or boneless product samples. *Listeria* spp. were not detected in any of the boneless product or outsides but was detected in one striploin (1 cfu/cm²) sample during Winter. Coagulase positive staphylococci were isolated from 7.7 and 8.4% of striploin and outside samples respectively, and, 3.4% of boneless beef samples with positive samples having an average count of 2 cfu/cm² for both striploins and outsides and 85 cfu/g for boneless product.

Results of Sheepmeat Testing

Sheep primals were found to have an average total viable count (TVC, 25°C) of 105 and 195 cfu/cm² respectively for legs and shoulders. *E. coli* was isolated from 42.9 and 34.6% respectively for legs and shoulders with average counts of 0.4 and 0.2 cfu/cm² respectively on positive samples. The average TVC for frozen boneless sheep meat was 231 cfu/g. The average *E. coli* count for the 12.5% of positive samples was 32 cfu/g. *E. coli* O157:H7 was isolated from 0.3 and 0.1% respectively of leg and shoulder samples. *Salmonella* was isolated from 2.8% of leg and 0.8% of shoulder samples and from 3.1% samples of boneless product. *Campylobacter* spp. were isolated from one (0.2%) of shoulder sample. *Listeria* spp. were not detected in any of the boneless sheep meat samples, but were detected in one (0.2%) leg sample. Coagulase positive staphylococci were isolated from 4.2% and 5.2% of leg and shoulder samples respectively, and, from 1.8% of boneless samples. Positive samples had average counts of 0.6 cfu/cm² and 2 cfu/cm² respectively for legs and shoulders, and, 46 cfu/g for boneless sheep meat.

What do these results tell us?

The latest baseline study provides benchmark data of the microbiological quality of beef and sheep meat from Australian meat processing establishments during the Summer and Winter periods of 2011. Together with data collected from similar surveys conducted in 1993/94, 1998 and 2003/04 it provides valuable reference material demonstrating the hygienic quality of meat processed in Australia.

In the present baseline study, pathogens such as *Salmonella*, *E. coli* O157, *Listeria* and *Campylobacter* were either not detected or were at extremely low levels. However, levels of indicator bacteria such as TVC and generic *E. coli* were higher than in the previous (2004) survey. The most likely explanation involves gross anomalies in rainfall in the eastern states in early 2011 when transport and processing of both cattle and sheep were disrupted by extreme flooding events.

Introduction

This report provides summary data from a survey of the microbiological quality of beef and sheep meat conducted at Australian meat processing establishments during January-March, and, June-August 2011.

For the Australian meat industry, data generated in this and previous baseline studies can be used to provide confidence in the state and federal regulatory systems. It also provides a valuable scientific reference for comparison to be made to the respective industries of our global trading partners and competitors.

The Australian red meat industry has supported three previous surveys of the microbiological quality of red meat through MRC's/MLA's Food Safety Research and Development Program. The 'baseline' surveys were conducted in 1993/1994, 1998 and 2003/2004. The results have been published in the scientific literature and reported to industry.

Unlike earlier baseline surveys, this survey has assessed the microbiological quality of chilled primals. There is a shortage of information on these products and the data reported here comprise a 'starting point' for shelf-life models being developed in other projects. As in previous studies the microbiological qualities of frozen boxed meat (beef and sheep meat) was also assessed.

Background – the industry's previous national baselines studies

The industry's three previous baselines studies focused on the microbiological status of beef and sheep carcasses, plus manufacturing meat taken from them. The results are reported in Vanderlinde et al., (1998, 1999); Phillips et al. (2001a, 2001b, 2006a, 2006b).

Carcasses were not surveyed in the present survey because:

- Carcasses are tested via the *E. coli* *Salmonella* Monitoring (ESAM) program
- Monthly updates of carcass microbiology are provided both at the individual plant and the national level by the South Australian research and Development Institute (SARDI)

Accordingly, the present baseline study examined the microbiological status of beef and sheep primal just prior to vacuum packing, plus frozen manufacturing beef and sheep meat.

How the study was done

The overall aim of this study was to define the microbiological attributes of red meats at specified stages of processing or storage within medium and large production facilities.

Sampling was done at two times of the year: a Summer sampling in January-March, 2011 and a Winter sampling June-August, 2011. Meat processing establishments (MPEs) were distributed throughout each of the five mainland states of Australia were selected on the basis that they collectively accounted for at least 80% of either beef or sheep meat production in Australia. All establishments in the survey were under the jurisdiction of the Australian Quarantine and Inspection Service (AQIS) (now DAFF Biosecurity).

Samples were taken from 29 MPEs processing beef and 12 processing sheep meat and each was sampled at least once in each of the Summer and Winter periods. The number of samples taken at each MPE reflected its share of processing volume with an upper limit of 30 samples of any one type (e.g. frozen trim, primal cuts) taken on any one day. Where samples were taken from a single MPE on more than one day, the duration between visits was at least 5 weeks.

Samples were collected by a team of trained technicians. A polyurethane sponge (Whirlpak, USA) moistened with buffered peptone water (10mL) was used to sample primal cuts by sponging a 300cm² area. The primal cuts selected for sampling were beef striploins and outsides, and sheep bone-in legs and shoulders; at some sheep MPEs only boneless primals were available and these were sampled. At each establishment primals were sampled just prior to final packaging and were taken during the course of normal production typically over a period of approximately one hour.

Samples of boneless meat trim were collected from frozen cartons which had been in the freezer store usually no longer than one month, but typically less than one week. Approximately 300g of meat drilled from 8-9 different locations in each carton using a sterile drill bit were transferred into sterile plastic bags by a technician wearing sterile gloves.

Wherever possible samples were taken from cartons that covered a range of production dates and/or chemical lean (CL) contents. The range of product presented for sampling was constrained by the degree of access to cartons within the freezer store at each participating establishment.

All samples were packed in insulated containers with chiller packs for transportation to an ISO 17025-accredited laboratory for testing. Upon arrival at the laboratory, samples were held at 2-4°C until examination. To standardise the time between sample collection and analysis samples were analysed at least 18 hours after collection. In most cases this was on the day of arrival at the laboratory. All samples were <10°C upon receipt at the laboratory.

Microbiological analysis

Three sponge samples were collected from each primal one of which was used for detection of *E. coli* O157:H7, one for *Salmonella* and *Listeria* enumeration and one for all other analyses (TVC, *E. coli*, coliforms, coagulase positive staphylococci and *Campylobacter*). Results for TVC, *E. coli*, coliforms and coagulase positive staphylococci were reported with a limit of detection of 0.08 cfu/cm².

For frozen boneless meat, 25g was examined for *Salmonella* spp. and *Listeria monocytogenes* and reported as detected/not detected in 25g. Aliquots (1mL) of a 10g subsample of the boneless meat sample were plated for TVC, Coliforms/*E. coli* and coagulase-positive staphylococci. The limit of detection was 10 cfu/g.

Tests were conducted in an ISO 17025-accredited laboratory. Test methods were usually Australian Standard methods, which are aligned to internationally accepted methods. More detail on the tests performed, how to interpret them and their significance is provided in Appendix 1.

How we are reporting results

Some tests yielded qualitative (detected/not detected) results and others yielded quantitative (numerical) results, which were treated in different ways.

Tests for *Salmonella*, *Campylobacter*, and *E. coli* O157:H7 provided qualitative results for individual samples. We refer to these types of results as prevalence of detection, as a percentage. Some tests estimate the numbers of a specific bacterium or group of bacteria present in the sample e.g. generic *E. coli*, coliforms, and coagulase positive staphylococci. In this case we report the prevalence of the organism and the concentration in which it was found in positive samples.

For each test there is a Limit of Detection (LOD). For tests where areas were sponged the LOD is 0.83 cfu/cm² (colony-forming units/cm²) and for tests where we took pieces of meat the LOD is 10 cfu/g.

It is common practice for microbiologists to convert actual counts to a log count, which is easier to work with and to interpret. These transformed data are referred to as 'log₁₀'. The results for log₁₀ data can be represented as a 'box-plot'. Box-plots are a convenient graphical device for summarizing data for each establishment or other subgroup. The box-plots used here have the features illustrated in Figure 1.

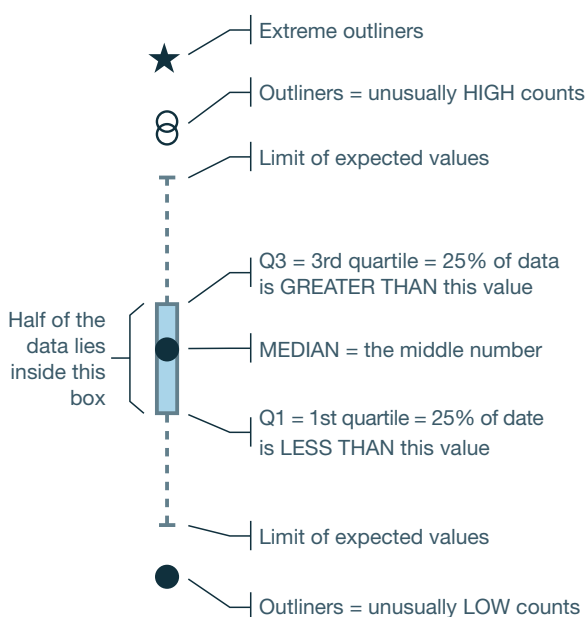


Figure 1. Explanation of the features of box-plots used for describing the bacterial counts in the baseline survey.

Results – microbiology of frozen boneless beef and beef primals

Primal cuts

A total of 572 striploins and 572 outsides were sampled at 29 abattoirs (see Appendix 2 for detailed results). The mean log₁₀ TVC was 1.25 cfu/cm² (equivalent to 18 cfu/cm²) for striploins and mean log₁₀ TVC 1.51 cfu/cm² (32 cfu/cm²) for outsides.

E. coli was detected on 10.7% of striploins and 25.2% of outsides with positive samples having a mean log₁₀ count of -0.49 cfu/cm² (0.3 cfu/cm²) and mean log₁₀ count of -0.26 cfu/cm² (0.5 cfu/cm²) respectively.

Coagulase positive staphylococci were isolated from 7.7% and 8.4% respectively of striploin and outside samples with positive samples having a mean log₁₀ count of 0.19 cfu/cm² (2 cfu/cm²) and mean log₁₀ count of 0.18 cfu/cm² (2 cfu/cm²) respectively.

Boneless beef

For frozen boneless beef 1165 cartons were tested from 29 boning rooms (see Appendix 3 for detailed results). The mean log₁₀ TVC/g was 2.22 cfu/g (166 cfu/g).

E. coli was detected on 2.1% of samples with positive samples having an average count of log₁₀ 1.32 cfu/g (21 cfu/g).

Coagulase positive staphylococci were isolated from 3.4% of samples with positive samples having an average count of log₁₀ 1.93 cfu/g (85 cfu/g).

Pathogens

Salmonella was not detected on any of the beef samples. *Listeria* spp. were detected on one beef striploin (1 cfu/cm²). There were no detections of *Campylobacter* or *E. coli* O157:H7 on beef primal samples; these pathogens were not tested in boneless beef trim because there is a substantial industry testing program for O157 and *Campylobacter* is sensitive to freezing.

Establishment TVCs

Figures 2 and 3 show the variation observed within and between plants sampled in this study for TVCs of beef primals and boneless beef respectively. Although there are differences in the median count between

plants, the size of the differences are small and, for most comparisons, not significant. The box plots help us to understand the process control at various plants. Shorter boxes and lines indicate that process control is more consistent, while a longer box and line indicates less uniformity in process control.

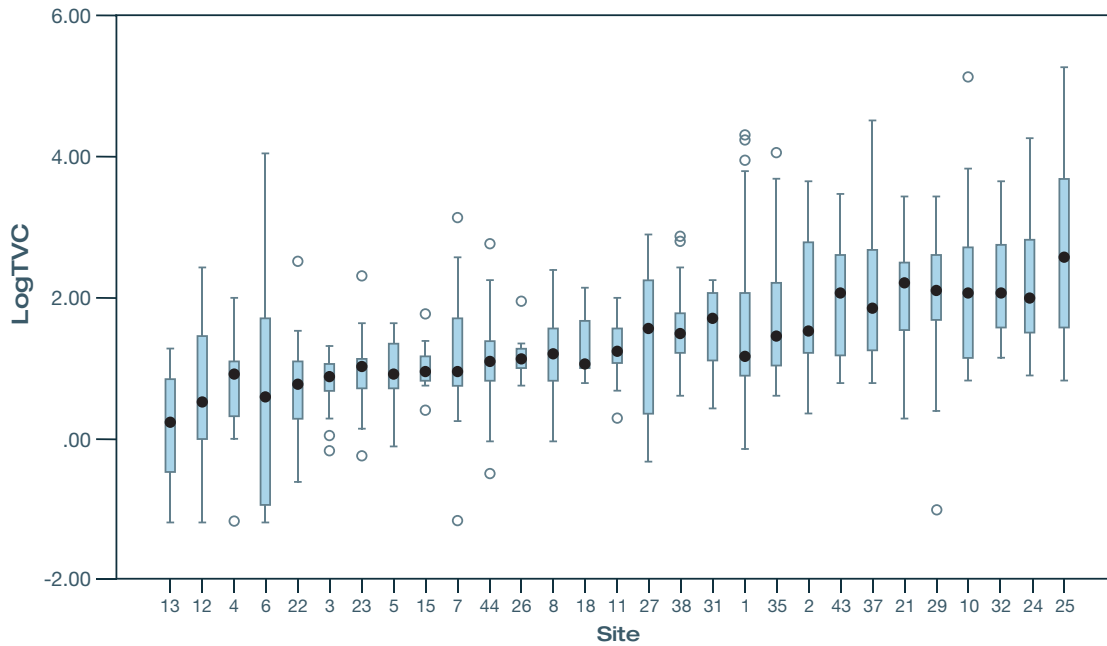


Figure 2: Variation in Total Viable Count ($\log \text{TVC}/\text{cm}^2$) of beef primals within and between establishments (arranged from the lowest to the highest median)

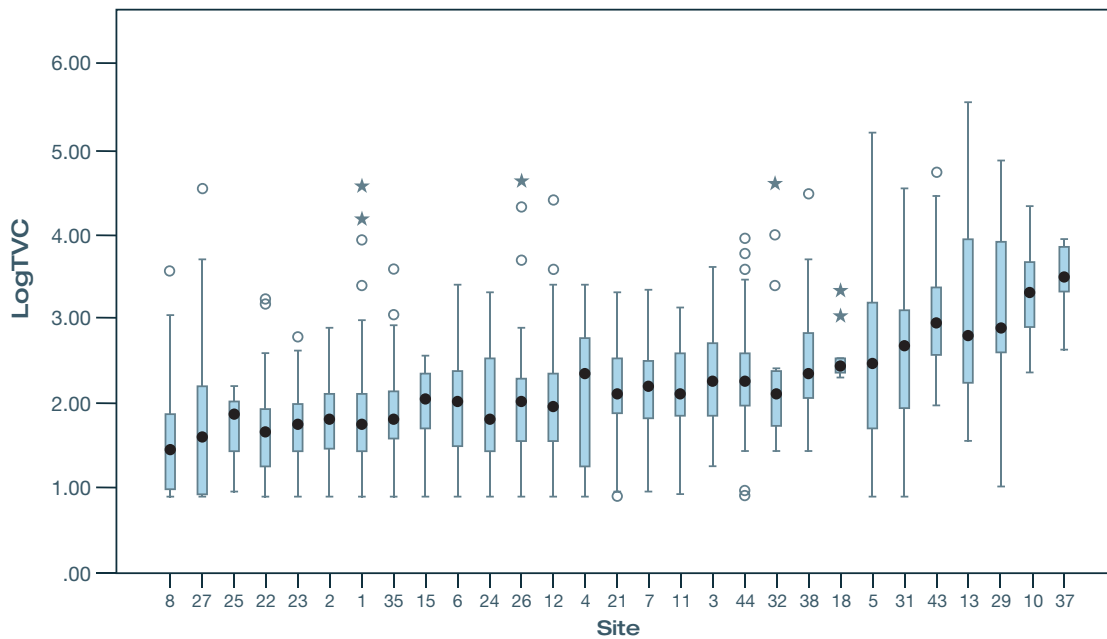


Figure 3: Variation in Total Viable Count ($\log \text{TVC}/\text{g}$) of boneless beef within and between establishments (arranged from the lowest to the highest median)

Results – microbiology of frozen boneless sheep meat and sheep primals

Primal cuts

A total of 1226 sheep primals processed at 12 abattoirs were tested (for detailed results see Appendix 4). The mean TVC was \log_{10} 2.02 cfu/cm² (equivalent to 105 cfu/cm²) for legs, and \log_{10} 2.29 cfu/cm² (195 cfu/cm²) for shoulders.

E. coli was detected on 42.3% of legs and 34.6% of shoulders with positive samples having an average count of \log_{10} -0.44 cfu/cm² (0.4 cfu/cm²) for legs and -0.63 \log_{10} cfu/cm² (0.2 cfu/cm²) for shoulders.

Coagulase positive staphylococci were isolated from 4.2% of legs and 5.2% of shoulders with positive samples having an average count of -0.21 \log_{10} cfu/cm² (0.6 cfu/cm²) for legs and \log_{10} 0.34 cfu/cm² (2 cfu/cm²) for shoulders.

Boneless sheepmeat

For frozen boneless sheepmeat, 551 samples from 11 boning rooms were tested (for detailed results see Appendix 5). The mean TVC was \log_{10} 2.80 cfu/g (631 cfu/g).

E. coli was detected on 12.5% of samples with positive samples having an average count of 1.51 \log_{10} cfu/g (32 cfu/g).

Coagulase positive staphylococci were isolated from 1.8% of samples with positive samples having an average count of \log_{10} 1.66 cfu/g (equivalent to 46 cfu/g).

Pathogens

Salmonella was detected on 2.8% of legs, 0.8% of shoulders and 3.1% of boneless sheep meat samples.

Listeria spp. were not detected in any boneless sheep meat samples but was detected on one leg sample (2 cfu/cm²).

E. coli O157:H7 was recovered from 2/613 (0.3%) leg samples and 1/613 (0.2%) shoulder samples.

Campylobacter was detected from 1/613 (0.2%) shoulder samples.

Establishment TVCs

Figures 4 and 5 show the variation observed within and between plants sampled in this study for TVCs of sheep primals and boneless sheepmeat, respectively. Although there are differences in the median count between plants, these are not considered significant.

Box plots help us to understand the process control at various plants. Shorter boxes and lines indicate uniform process control, while longer boxes and lines indicates less uniformity in process control.

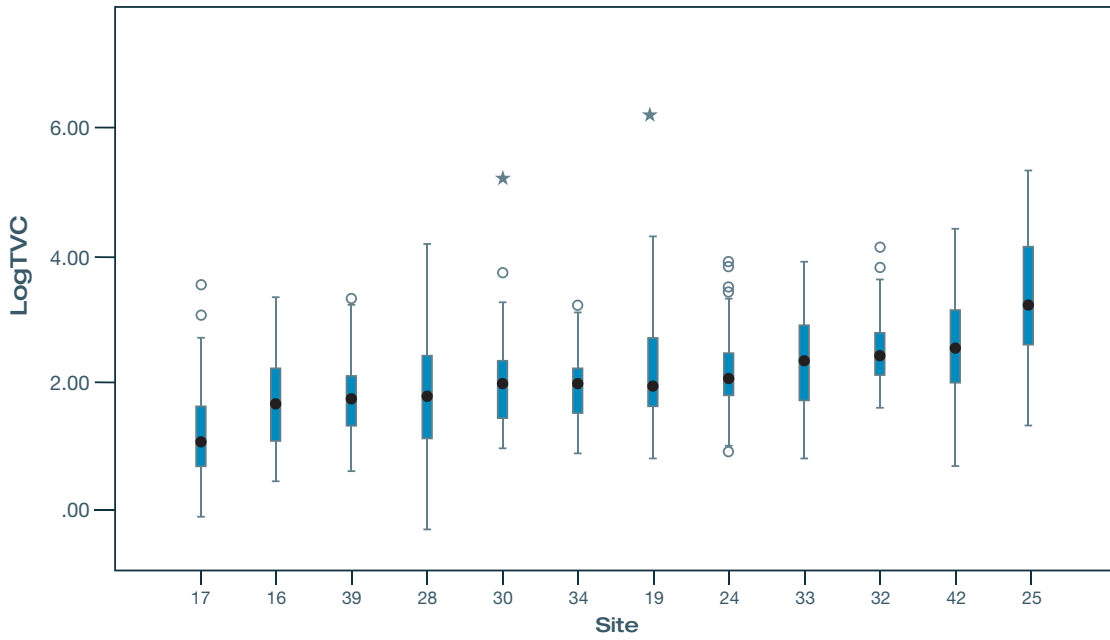


Figure 4: Variation in Total Viable Count (\log_{10} TVC/cm²) of sheep primals within and between establishments (arranged from the lowest to the highest median)

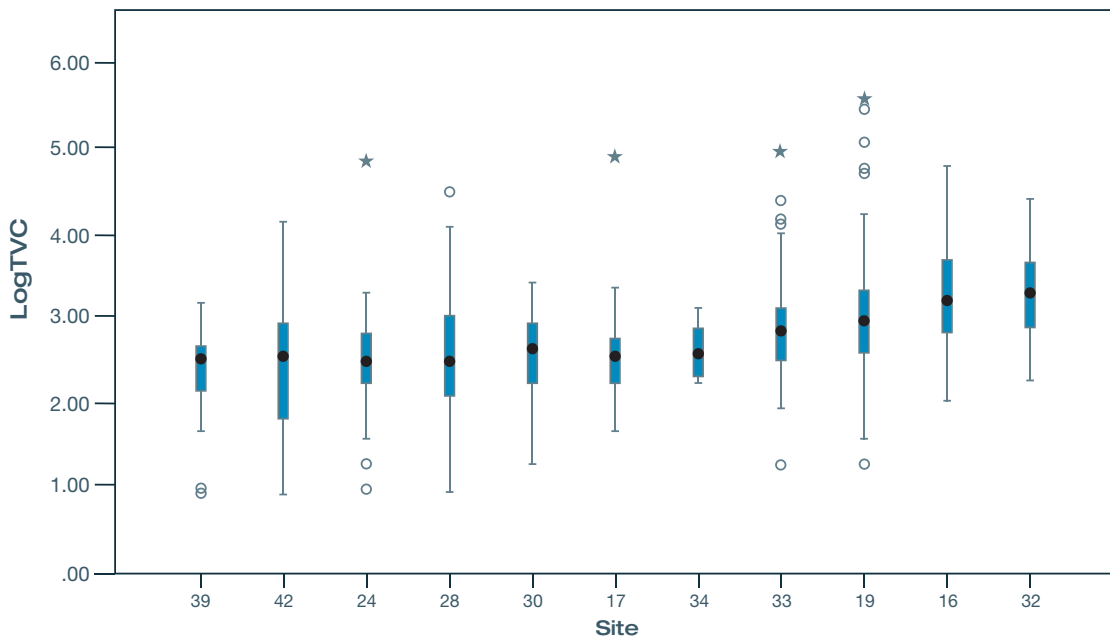


Figure 5: Variation in Total Viable Count (\log_{10} TVC/g) of boneless sheep meat within and between establishments (arranged from the lowest to the highest median)

Comparison with previous baseline surveys

There were differences in counts for indicator organisms (TVC, Coliforms and *E. coli*) between the Summer and Winter samplings but the differences were not great. For beef, mean counts and prevalence were generally slightly higher in Summer than Winter, while for sheep samples the opposite applied.

Because there were similarities in methodology of all four surveys it is possible to compare the microbiological status of boneless product over time.

Comparison between the mean \log_{10} TVC between surveys is shown in Figures 6 and 7 for beef and sheep meat frozen boneless trim, respectively.

Comparison between the prevalence of *E. coli* between surveys is shown in Figures 8 and 9 for beef and sheep meat frozen boneless trim, respectively.

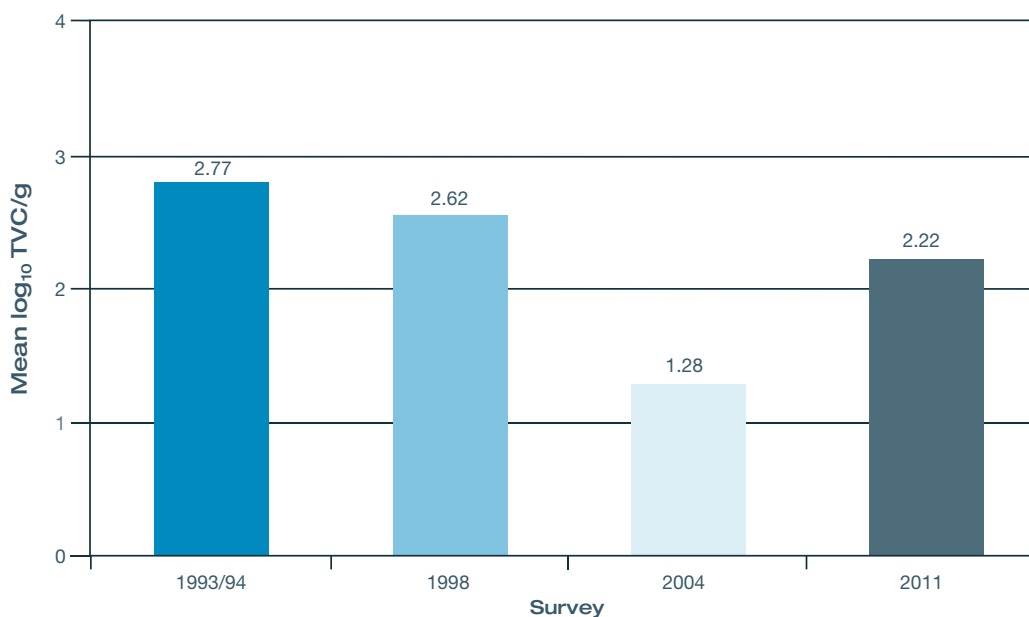


Figure 6: Historical comparison of the mean \log_{10} TVC/g of frozen boneless beef

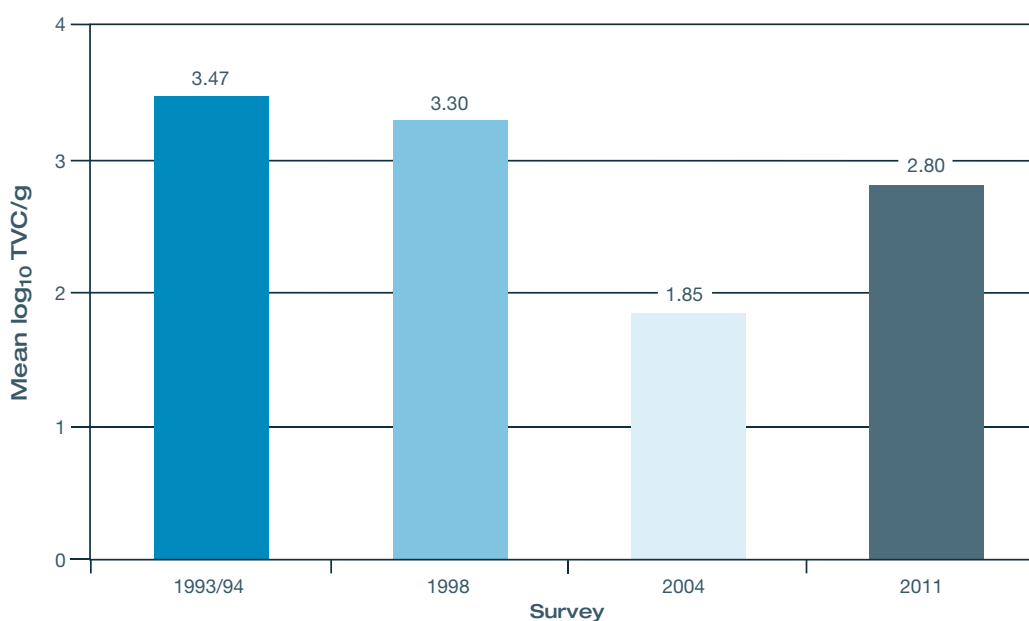


Figure 7: Historical comparison of the mean \log_{10} TVC/g of frozen boneless sheepmeat

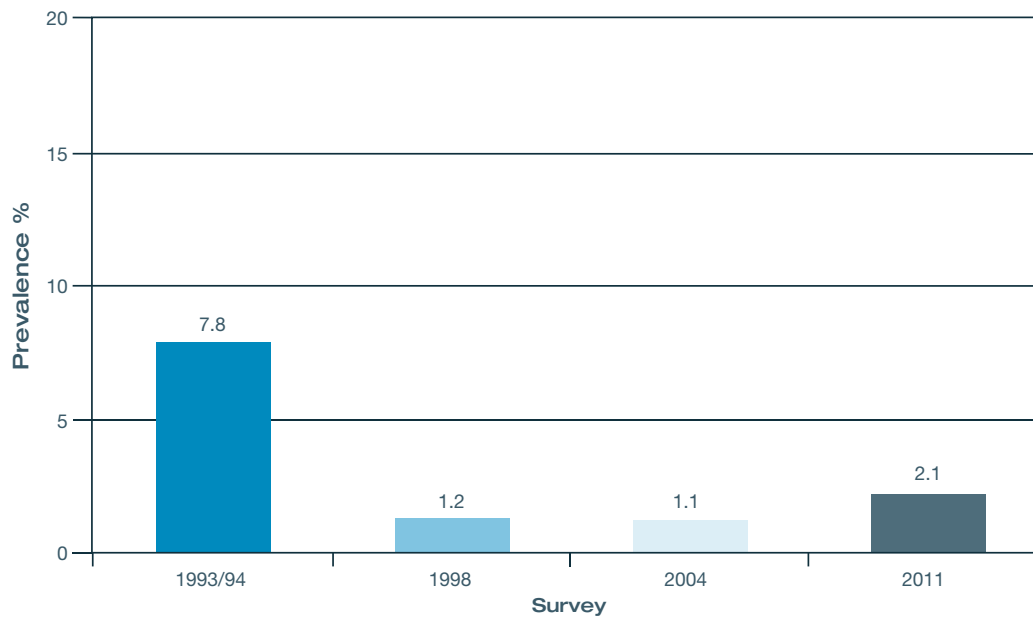


Figure 8: Historical comparison of the prevalence of E. coli detections on frozen boneless beef

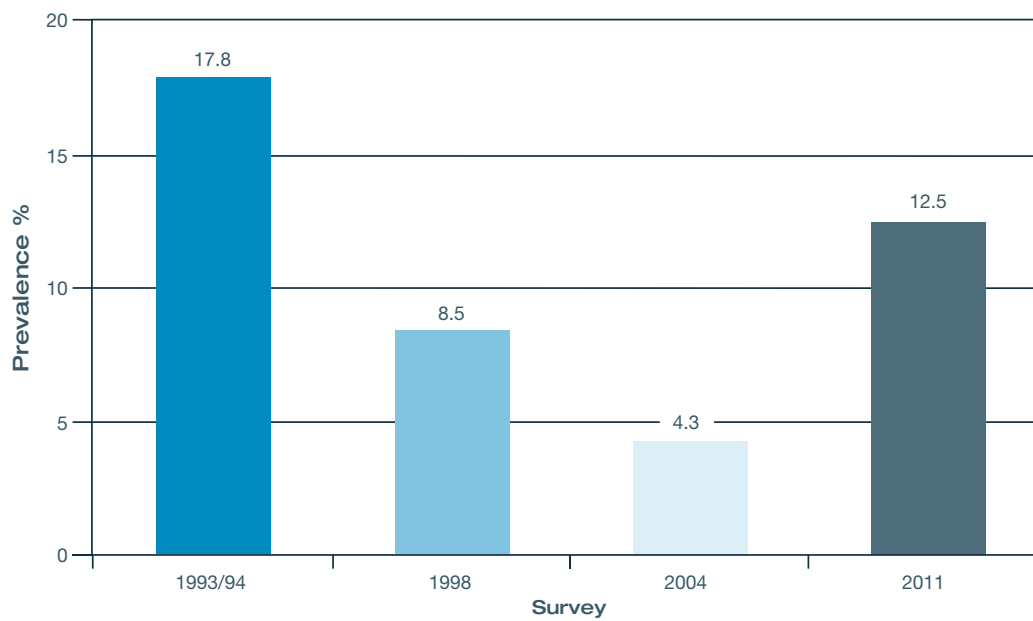


Figure 9: Historical comparison of the prevalence of E. coli detections on frozen sheepmeat

Higher levels in 2010-11 survey

The Summer sampling took place in January-March 2011 and, as seen in Figures 6-9, resulted in counts of indicator organisms (TVC, *E. coli*) being higher than in the last baseline study (2004) for beef and sheep frozen meat.

However, levels of *S. aureus* were lower in this baseline than in previous baselines studies, probably reflecting the almost universal use of gloves by operators on slaughter floors and boning rooms.

The baseline study represents a series of snapshots of product at establishments and contrasts with data generated under the *E. coli* *Salmonella* Monitoring (ESAM) program, which provides a continuous microbiological record from all export plants.

When the ESAM database was interrogated it was found that, while the national microbiological profile had been stable over the previous two years, in early-2011 counts on beef and sheep carcasses began to rise and, as shown in Figures 10 and 11, this was so for both TVC and *E. coli*.

As can be seen from Figure 12, in 2009-10 much of Australia had either "normal" rainfall, or was in drought, while in Figure 13 the early part of 2011 was characterised by unusual rain patterns in much of the eastern states of Australia; by contrast, in Western Australia drought conditions continued.

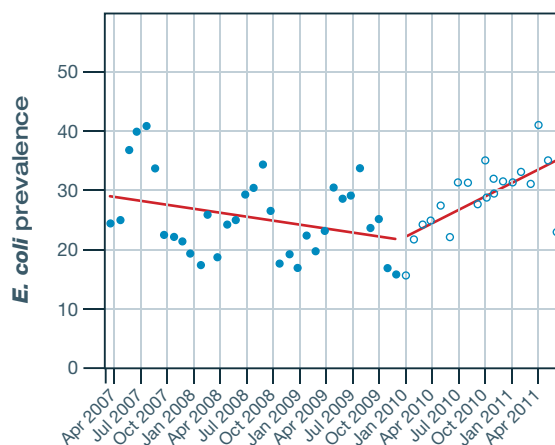
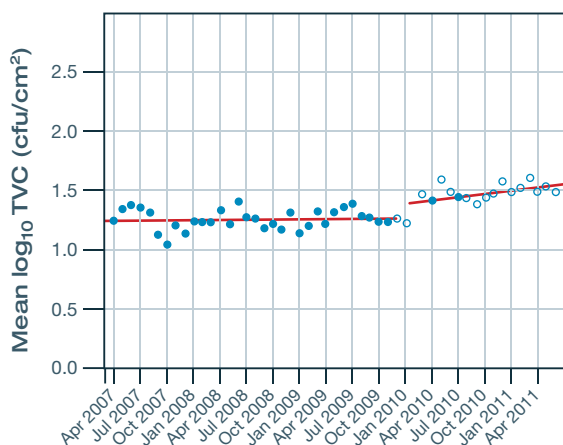


Figure 10: ESAM national data for TVC (left) and *E. coli* (right) on sheep carcasses

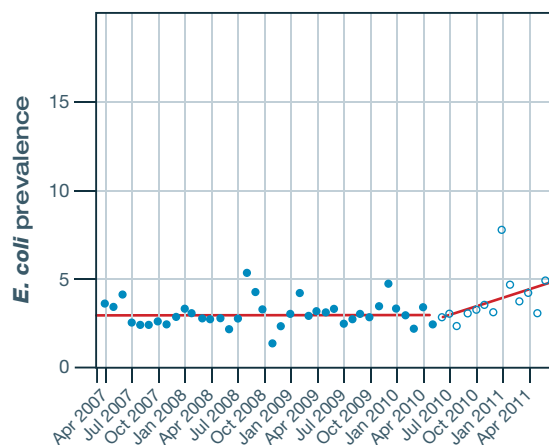
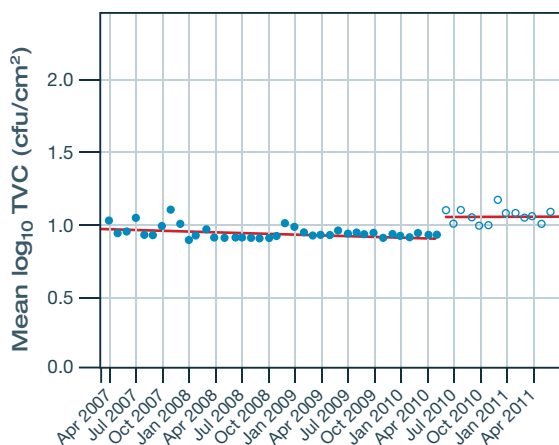


Figure 11: ESAM national data for TVC (left) and *E. coli* (right) on beef carcasses

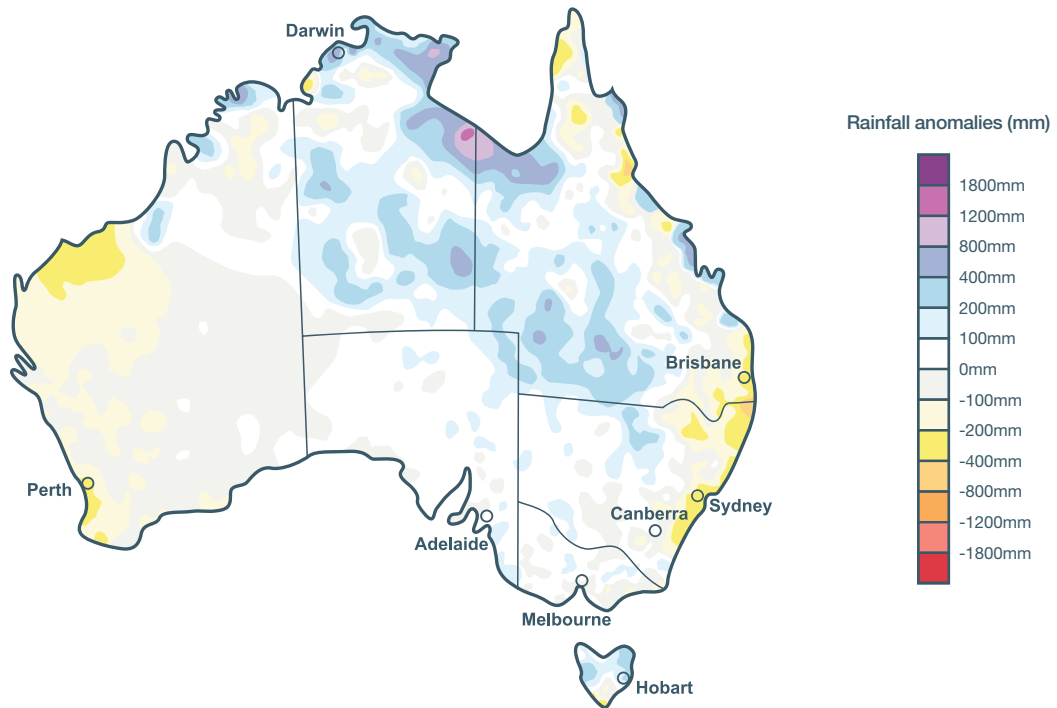


Figure 12: Rainfall anomalies (mm) for the period 1 July 2009 – 30 June 2010
(Bureau of Meteorology, available at <http://www.bom.gov.au/jsp/awap/rain/index.jsp>)

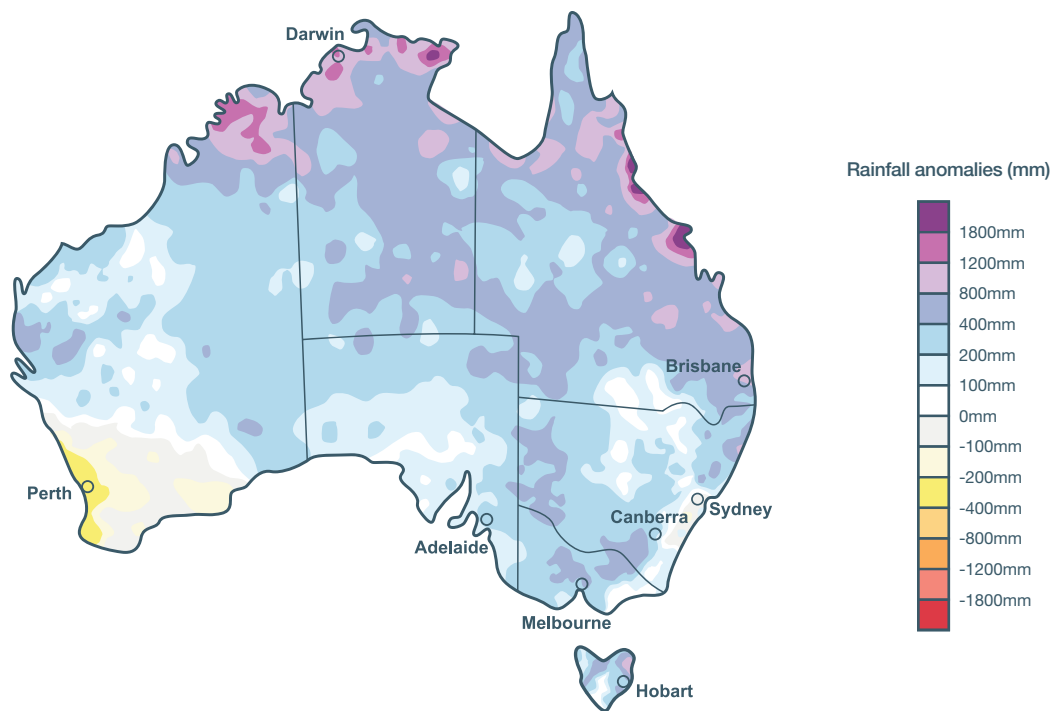


Figure 13: Rainfall anomalies (mm) for the period 1 July 2010 – 30 June 2011
(Bureau of Meteorology, available at <http://www.bom.gov.au/jsp/awap/rain/index.jsp>)

The South Australian Research and Development Institute (SARDI) compared trend counts on sheep and on steer/heifer carcasses in WA against those in the rest of Australia. There were clear differences between trend lines. For carcasses in WA the trend line was either flat or declined slightly over the period, while in the rest of Australia there was an abrupt upward trend beginning in mid-2010, as seen in the graphs above (Figures 10 and 11).

The SARDI analysis provides evidence of an alignment between microbiological counts on carcasses (ovine and bovine) and extreme rainfall events which occurred in eastern Australia during 2010-2011. Studies in Australia have shown that the condition of fleece and hide of animals presented for slaughter can present an “incoming problem” which exceeds the capacity of slaughter and dressing operations to maintain the usual hygienic status of the carcass (Kiermeier et al. 2006, 2009).

The SARDI work provides circumstantial evidence that the unusually high rainfalls in eastern Australia were the source of higher than usual counts of indicator organisms on carcasses. At slaughter establishments the negative effects of rainfall may have been due to a combination of:

- Stock being received with unusual levels of mud/faeces on wet pelts and hides, and this continuing over a long period of time
- More lush pasture resulting in loose stools and greater spread over the fleece and hide
- Stock being denied feed for protracted periods by floods
- Increased livestock transport times
- Processing schedules being disrupted

How does the present survey compare with others?

In the present survey primal cuts were chosen mainly because there is little known about their microbiological status. Not surprisingly then, there are not many studies published either nationally or internationally with which to compare the present one. This was the case specifically with sheepmeat and sheep primals; hence comparisons were only made with beef.

Beef Primals

There have been four international studies where methods used were similar to those used in the fourth baseline study, and which may be compared.

In New Zealand two studies were undertaken as part of a larger study on how hot boned primals performed at the retail level. Bell et al. (1996) found that hot boned striploins had a mean TVC of \log_{10} 2.5 cfu/cm² as did Penney et al. (1998), who also found that conventionally boned striploins were \log_{10} 3.0 cfu/cm².

In the USA, Ware et al. (2001) sponged fat and lean surfaces of two primals: Clod (shoulder) and Top butt (Striploin) for mean TVCs of \log_{10} 2.25 cfu/cm² and 2.27 cfu/cm², respectively.

Also in the USA Stopforth et al. (2006) carried out a six-month study on beef primals from two establishments in which 1022 samples were taken from 10 primal cuts. TVCs ranged from \log_{10} 4.0 cfu/g for butts and rib eye rolls to \log_{10} 6.2 cfu/g on club ends; on striploins the mean \log_{10} TVC was 5.9 cfu/g. The researchers also isolated *E. coli* O157 from 3/1022 (0.3%) and *Salmonella* from 22/1022 (2.2%) of samples; *E. coli* O157 was not isolated from striploins but 5/52 (9.6%) of striploins yielded *Salmonella*.

In the present baseline study the mean TVC for striploins was \log_{10} 1.25 cfu/cm² and for outsides \log_{10} 1.51 cfu/cm², significantly lower than levels estimated in other studies; as well, *E. coli* and *Salmonella* were not isolated in any of the 1,144 samples tested.

Boneless Beef

Comparisons are difficult to make because most countries do not publish the profile of boneless beef, or because of differences in methodology. However, a comparison using identical methodology was carried out by researchers at the US Department of Agriculture, who compared the microbiology of beef destined for grinding from Australia, New Zealand and Uruguay with that of domestic product (Bosilevac et al. 2007) The researchers analysed indicator organisms such as Total count, *Enterobacteriaceae*, coliforms/*E. coli*, *Staphylococcus aureus* and pathogens: *Campylobacter*, *Listeria*, *Salmonella* and non-O157 STEC.

Summary data of the survey are presented in Tables 1 and 2 and show levels of indicator organisms and pathogens were invariably lower in Australian beef trimmings compared with other countries. This was especially so when isolations of Shiga toxin-producing *E. coli*, in general, and HUS serotypes, in particular, are compared.

The USDA researchers commented: “Overall the results provide objective evidence that standards of hygiene during the slaughter and processing of beef in Australia continue to be very high.”

Table 1: Microbiological profile of indicator organisms in beef trimmings destined for ground beef

	Mean log cfu/g	Prevalence (%)		
	<i>TVC</i>	<i>Enterobacteriaceae</i>	<i>E. coli</i>	<i>S. aureus</i>
Australia	1.6	8.2	1.0	4.0
New Zealand	2.2	9.0	0.5	8.2
Uruguay	2.8	31.3	9.5	29.5
USA	2.5	37.8	7.2	4.2

Table 2: Prevalence of pathogens in beef trimmings destined for ground beef

	Prevalence (%)			Number of isolations	
	<i>Salmonella</i>	<i>Campylobacter</i>	<i>L. monocytogenes</i>	<i>STEC</i>	<i>HUS serotypes</i>
Australia	0	0	2.0	9	0
New Zealand	0.4	0.5	2.3	4	2
Uruguay	0.4	0.4	24	40	6
USA	0.8	1.3	5.0	28	5

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Appendix 1: Testing Methods

Study design

Samples were collected during January-March and June-August, 2011 from meat processing establishments (MPEs) selected on the basis that they collectively accounted for at least 80% of either beef or sheep meat processing volume in Australia. In all, samples were taken from 29 MPEs processing beef and 12 processing sheep meat. All establishments in the survey were under the jurisdiction of the Australian Quarantine and Inspection Service (AQIS) (now DAFF Biosecurity). The number of samples taken at each MPE reflected its volume of processing with an upper limit of 30 samples of any one type (eg frozen trim, primal cuts) taken on any one day. Where samples were taken from a single MPE on more than one day, the duration between visits was at least 5 weeks.

Sampling of beef and sheep primals

Samples were collected by a team of trained technicians. A polyurethane sponge (Whirlpak, USA) moistened with buffered peptone water (10ml) was used to sample primal cuts by sponging a 300cm² area. To provide for all testing required, three sponges were used for each sample. Where the total area of 900cm² was unavailable on a single primal, a second primal was used. The primal cuts selected for sampling were for beef, striploins and outsides, and for sheep, bone-in legs and shoulders. At some sheep MPEs only bone-out primals were available and these were therefore sampled. At each establishment every attempt was made to sample the respective primals as close as possible to final packaging. Samples were taken during the course of normal production at each establishment typically over a period of approximately one hour.

Sampling of frozen boneless beef and sheep meat trim

Samples of boneless meat trim were collected from frozen cartons which had been in the freezer store usually no longer than one month but typically within one week. Approximately 300g of meat drilled from 8-9 different locations in each carton using a sterile drill bit were transferred into sterile plastic bags by a technician wearing sterile gloves. Wherever possible samples were taken from cartons that covered a range of production dates and/or chemical lean (CL) contents. The range of product presented for sampling was constrained by the degree of access to cartons within a freezer store at each participating establishment.

Transport of samples to the laboratory

All samples were packed in insulated containers with chiller packs for transportation to an ISO 17025-accredited laboratory for testing. Upon arrival at the laboratory, samples were held at 2-4°C until examination. To standardise the time between sample collection and analysis, samples were analysed at least 18 hours after collection. In most cases this was on the day of arrival at the laboratory. All samples were less than 10°C upon receipt at the laboratory.

Summary of microbiological tests conducted

Test	Method reference	Limit of detection	
		Primals	Frozen trim
Total count (TVC)	Petrifilm AS5013.1-2004 (25°C, 4d)	0.08 cfu/cm ²	10 cfu/g
Coliforms/ <i>E. coli</i>	Petrifilm AOAC method 991.14 (35°C, 48hr)	0.08 cfu/cm ²	10 cfu/g
<i>Salmonella</i>	AS5013.10-2009 = ISO 6579:2002	present/300cm ²	present/25g
Coagulase positive Staphylococci	AS5013.12.1-2004 = ISO 6888-1:1999/Amd 1:2003	0.08 cfu/cm ²	10 cfu/g
<i>Listeria sp</i>	AS 5013.24.2 = ISO 11290-2:1998	0.08 cfu/cm ²	10 cfu/g
<i>Campylobacter</i>	AS 5013.6-2004	present/250cm ²	not tested
<i>E. coli</i> O157:H7	Bax - AOAC Certificate No. 050501	present/300cm ²	not tested

Microbiological analysis of sponge samples

Three sponge samples were collected from each primal, one of which was used for detection of *E. coli* O157:H7, one for *Salmonella* and *Listeria* detection with the remainder for all other analyses (TVC, *E. coli*, coliforms, coagulase positive staphylococci and *Campylobacter*).

Detection of *E. coli* O157:H7

A 225 ml volume of *E. coli* O157:H7 MP enrichment broth (Dupont Qualicon Bax®, Sydney, Australia) was added to one of the sponge bags and squeezed by hand 10 times prior to incubation at 42°C overnight and tested according to AOAC Certificate No. 050501. The following day, presumptive positives were tested using the Dynalbeads anti O157 method of immunomagnetic separation (Dynal Australia, Melbourne, Australia) as per the manufacturer's instructions using Rainbow Agar. Positive samples were subcultured onto blood agar and isolates selected for the detection of genes encoding for Shiga toxins. *E. coli* strains positive for the O157 antigen and containing a gene for shiga toxin (Stx1 or Stx2) were reported as *E. coli* O157:H7 detected in 300 cm².

Detection of *Salmonella* and *Listeria*

Buffered peptone water (225 ml) was added to the second sponge bag and squeezed by hand 10 times prior to incubation for 1 h at room temperature. For *Listeria* detection, the resuscitated culture was inoculated onto Palcam agar plates (Oxoid, Adelaide, Australia) for incubation at 37°C for 48 hours. Typical *Listeria* species colonies on the Palcam agar plates were transferred to TSYE agar (Oxoid, Adelaide, Australia) and confirmed by the catalase test and Gram stain. *Listeria monocytogenes* confirmation was by haemolysis on blood agar plates, xylose and rhamnose fermentation tests and the CAMP test (AS 5013.24.2). For *Salmonella* detection, the BPW culture was further incubated for 16-20 h at 37°C to allow resuscitation of damaged cells. Aliquots of resuscitated cultures were inoculated into the selective enrichment broths Muller-Kauffmann Tetrathionate Novobiocin broth (Oxoid, Adelaide, Australia) for incubation at 37°C for 24 hours and Rappaport-Vassiliadis medium (Oxoid, Adelaide, Australia) for incubation at 42°C for 24 hours according to Australian Standard method AS5013.10-2009. Each enriched culture was inoculated onto brilliant green agar and xylose lysine desoxycholate agar (Oxoid, Adelaide, Australia) and incubated at 37°C for 24 h.

Biochemical confirmation of typical colonies was by the use of the oxidase test and Microbact 12A strips (Oxoid, Adelaide, Australia) and agglutination tests with *Salmonella* O and H antisera. Positive samples were subcultured onto nutrient agar slopes and sent to the Queensland Public Health laboratory, Brisbane, Australia for serotyping. Results were reported as detected/not detected per 300 cm².

Determination of Total Viable Count (TVC), Coliforms, *E. coli* biotype I, coagulase positive staphylococci and *Campylobacter*

Buffered peptone water (40 ml) was added to the third sponge bag, which was squeezed by hand 10 times. Serial dilutions were prepared in 0.1% peptone water using 1 ml aliquots. For TVC, Petrifilm™ plates (3MTM, Sydney, Australia) were prepared and incubated at 25°C for 4 days at which time the colonies were counted and the TVC result in cfu/cm² calculated. The limit of detection was 0.08 cfu/cm². *E. coli* were estimated by placing 1ml aliquots of both the initial solution and appropriate dilutions onto *E. coli* Petrifilm™ (3MTM, Sydney, Australia) and incubating at 35°C for 48 h. Colonies were counted as per the manufacturer's instructions and AOAC method 991.14. The limit of detection was 0.08 cfu/cm². Coagulase positive staphylococci were determined using Australian Standard method AS5013.12.1-2004 where 0.33 ml aliquots were spread onto six dried plates of Baird Parker agar (Merck, Melbourne, Australia) and incubated at 37°C for 48 h. Colonies with typical morphology (grey-black, shiny, convex colony with a narrow entire margin surrounded by a zone of clearing) were picked off the plate for coagulase testing using rabbit blood plasma. The limit of detection for coagulase-positive staphylococci was 0.08 cfu/cm². *Campylobacter* was determined according to Australian Standard method AS 5013.6-2004 in which 100 ml Preston medium (Oxoid, Adelaide, Australia) was added to approximately 21 ml of the initial carcass sponge solution and incubated at 42°C for 48 h. Sixteen streak plates were prepared on Preston agar and on Skirrow agar (Oxoid, Adelaide, Australia). Plates were incubated at 42°C for 48 h. Typical colonies were streaked onto blood agar plates and incubated under micro-aerobic conditions at 42°C for 24 hours. Suspect colonies were confirmed using a classical oxidase test, gram stain, motility test, sensitivity to nalidixic acid and cephalothin and growth under aerobic and micro-aerobic conditions. The result was expressed as detected/not detected in 250cm².

Microbiological analysis of frozen boneless meat trimmings

From each sample of approximately 300g a subsample of 25g were examined for *Salmonella* spp. and *Listeria monocytogenes* as described above and reported as detected/not detected in 25g. A 10g subsample of the boneless meat sample was added to peptone salt solution (90mL) and macerated in a Seward Stomacher BA 7021 (Seward, United Kingdom). Aliquots (1 ml) were plated for TVC and Coliforms/*E. coli* and 0.33 ml aliquots for coagulase positive staphylococci as described above. The limit of detection was 10 cfu/g.

Appendix 2: Microbiological data of beef primals

Table 2.1: Mean Total Viable Count (\log_{10} TVC at 25°C) of Australian beef primals

	Overall		Summer		Winter	
	Striploin	Outside	Striploin	Outside	Striploin	Outside
Samples	572	572	272	272	300	300
Mean ^(a)	1.25	1.51	1.39	1.38	1.12	1.62
Median \log_{10}	1.11	1.32	1.26	1.31	1.03	1.36
Standard deviation	1.00	0.98	1.15	1.07	0.81	0.88
90th percentile	2.59	2.88	2.93	2.81	2.11	2.92
95th percentile	3.11	3.24	3.52	3.22	2.77	3.26
99th percentile	4.30	4.01	4.30	4.02	3.63	4.03
Maximum	5.26	4.22	5.15	4.22	5.26	4.22

^a Limit of detection 0.08 cfu/cm²

Table 2.2: Prevalence of generic E. coli on sponges from Australian beef primals and descriptive statistics for \log_{10} counts from positive sponges.

	Overall		Summer		Winter	
	Striploin	Outside	Striploin	Outside	Striploin	Outside
Samples	572	572	272	272	300	300
Prevalence (% detection)	10.7	25.2	12.9	27.2	8.7	23.3
Mean ^(a, b)	-0.49	-0.26	-0.36	-0.18	-0.65	-0.34
Median	-0.60	-0.54	-0.48	-0.30	-0.69	-0.77
Standard deviation	0.69	0.88	0.81	0.77	0.44	0.98
90th percentile	0.09	1.08	0.92	0.90	-0.01	1.31
95th percentile	1.19	1.50	1.22	1.40	0.10	2.33
99th percentile	1.88	2.38	1.88	1.52	0.12	2.36
Maximum	2.30	2.40	2.30	2.11	0.12	2.40

^a Limit of detection 0.08 cfu/cm²

^b \log_{10} counts of positive samples

Table 2.3: Prevalence (%) of *Salmonella*, *E. coli* O157:H7, *Listeria* and *Campylobacter* in sponges from Australian beef primals

	Overall		Summer		Winter	
	Striploin	Outside	Striploin	Outside	Striploin	Outside
Samples	572	572	272	272	300	300
<i>Salmonella</i>	nd	nd	nd	nd	nd	nd
<i>E.coli</i> O157:H7	nd	nd	nd	nd	nd	nd
<i>Listeria</i> spp.	0.2%	nd	nd	nd	0.3%	nd
<i>Campylobacter</i>	nd	nd	nd	nd	nd	nd

nd – not detected in 300 cm² (*E. coli* O157, *Salmonella* and *Listeria* spp) and in 250cm² (*Campylobacter*)

Table 2.4: Prevalence of coagulase positive staphylococci on sponges from Australian beef primals and descriptive statistics for log₁₀ counts from positive samples

	Overall		Summer		Winter	
	Striploin	Outside	Striploin	Outside	Striploin	Outside
Samples	572	572	272	272	300	300
Prevalence (% detection)	7.7	8.4	12.5	12.5	3.3	4.7
Mean ^(a, b)	0.19	0.18	0.32	0.24	-0.28	0.03
Median	0.23	0.29	0.29	0.40	-0.21	0.03
Standard deviation	0.65	0.68	0.57	0.73	0.75	0.55
90th percentile	1.12	0.95	1.16	1.27	0.93	0.78
95th percentile	1.21	1.41	1.18	1.51	0.94	0.83
99th percentile	1.22	1.48	1.22	1.48	0.94	0.83
Maximum	1.40	1.62	1.40	1.62	0.94	0.83

^a Limit of detection 0.08 cfu/g

^b log₁₀ counts of positive samples

Table 2.5: Prevalence of coliforms on sponges from Australian beef primals and descriptive statistics for log₁₀ counts from positive samples

	Overall		Summer		Winter	
	Striploin	Outside	Striploin	Outside	Striploin	Outside
Samples	572	572	272	272	300	300
Prevalence (% detection)	33.0	43.5	40.4	50.7	26.3	37.0
Mean ^(a, b)	-0.46	-0.28	-0.47	-0.30	-0.43	-0.26
Median	-0.60	-0.48	-0.69	-0.48	-0.60	-0.48
Standard deviation	0.70	0.80	0.71	0.74	0.68	0.86
90th percentile	0.60	1.03	0.54	1.00	0.77	1.09
95th percentile	0.94	1.40	1.16	1.19	0.92	1.84
99th percentile	1.92	2.36	2.25	1.93	0.98	2.39
Maximum	2.30	2.40	2.30	2.11	1.44	2.40

^a Limit of detection 0.08 cfu/g

^b log₁₀ counts of positive samples

Appendix 3: Microbiological profile of boneless frozen beef

Table 3.1: Total Viable Count (\log_{10} TVC at 25°C) of Australian frozen boneless beef

	Overall	Summer	Winter
Samples	1165	565	600
Mean ^(a)	2.22	2.31	2.13
Median	2.11	2.23	2.00
Standard deviation	0.81	0.78	0.84
90th percentile	3.28	3.35	3.19
95th percentile	3.85	3.85	3.90
99th percentile	4.61	4.60	4.66
Maximum	5.53	5.18	5.53

^a Limit of detection 10 cfu/g

Table 3.2: Prevalence of generic *E. coli* in core samples of Australian frozen boneless beef and descriptive statistics for \log_{10} counts from positive samples

	Overall	Summer	Winter
Samples	1165	565	600
Prevalence (% detection)	2.1	4.4	0
Mean ^(a,b)	1.32	1.32	-
Median	1.30	1.30	-
Standard deviation	0.39	0.39	-
90th percentile	1.99	1.99	-
95th percentile	2.41	2.41	-
99th percentile	2.51	2.51	-
Maximum	2.51	2.51	-

^a Limit of detection 10cfu/g

^b \log_{10} counts of positive samples only

Table 3.3: Prevalence of coagulase positive staphylococci in core samples of Australian frozen boneless beef and descriptive statistics for log counts from positive samples

	Overall	Summer	Winter
Samples	1165	565	600
Prevalence (% detection)	3.4	6.0	1.0
Mean ^(a,b)	1.93	2.09	1.00
Median	1.62	1.78	1.00
Standard deviation	1.04	1.05	0
90th percentile	3.50	3.52	1.00
95th percentile	4.26	4.26	1.00
99th percentile	4.30	4.30	1.00
Maximum	4.76	4.76	1.00

^a Limit of detection 10 cfu/g

^b log₁₀ counts of positive samples only

Table 3.4: Prevalence of coliforms in core samples of Australian frozen boneless beef and descriptive statistics for log₁₀ counts from positive samples

	Overall	Summer	Winter
Samples	1165	565	600
Prevalence (% detection)	7.8	13.3	2.7
Mean ^(a,b)	1.42	1.46	1.23
Median	1.30	1.30	1.00
Standard deviation	0.53	0.53	0.47
90th percentile	2.26	2.30	1.48
95th percentile	2.78	2.79	1.60
99th percentile	2.88	2.88	2.79
Maximum ^b	2.92	2.92	2.79

^a Limit of detection 10 cfu/g

^b log₁₀ counts of positive samples only

Appendix 4: Microbiological data for sheep

Table 4.1: Total Viable Count (\log_{10} at 25°C) from sponges of Australian chilled sheep primals

	Overall		Summer		Winter	
	Legs	Shoulders	Legs	Shoulders	Legs	Shoulders
Samples	613	613	310	310	303	303
Mean ^(a)	2.02	2.29	1.95	2.23	2.09	2.35
Median	2.00	2.15	1.92	2.10	2.03	2.22
Standard deviation	0.81	0.96	0.78	0.87	0.84	1.05
90th percentile	3.09	3.54	3.06	3.33	3.19	3.82
95th percentile	3.46	4.23	3.22	3.92	3.82	4.72
99th percentile	4.15	4.97	3.69	4.40	4.42	5.28
Maximum	4.64	6.21	3.96	6.21	4.64	5.33

^a limit of detection 0.08/cm²

Table 4.2: Prevalence of generic E. coli in sponges from Australian sheep primals descriptive statistics for \log_{10} counts from positive sponges.

	Overall		Summer		Winter	
	Legs	Shoulders	Legs	Shoulders	Legs	Shoulders
Samples	613	613	310	310	303	303
Prevalence (%)	42.9	34.6	27.7	33.5	58.4	35.6
Mean ^(a,b)	-0.44	-0.63	-0.68	-0.51	-0.33	-0.74
Median	-0.60	-0.77	-0.78	-0.69	-0.38	-0.77
Standard deviation	0.69	0.56	0.61	0.67	0.71	0.40
90th percentile	0.44	0	0.33	0.31	0.55	-0.08
95th percentile	0.98	0.33	0.66	1.15	1.23	-0.04
99th percentile	1.77	1.78	1.23	1.82	1.88	0.29
Maximum	2.36	1.82	1.83	1.82	2.36	0.30

^a Limit of detection 0.08 cfu/cm²

^b \log_{10} counts of positive samples

Table 4.3: Prevalence of *Salmonella*, *E. coli* O157:H7, *Listeria* and *Campylobacter*, in sponges from Australian chilled sheep primals (2011)

	Overall		Summer		Winter	
	Legs	Shoulders	Legs	Shoulders	Legs	Shoulders
Samples	613	613	310	310	303	303
<i>Salmonella</i>	2.8%	0.8%	1.6%	1.3%	4.0%	0.3%
<i>E.coli</i> O157:H7	0.3%	0.2%	nd	0.3%	0.7%	nd
<i>Listeria</i> spp.	0.2%	nd	0.3%	nd	nd	nd
<i>Campylobacter</i>	nd	0.2%	nd	nd	nd	0.3%

nd – not detected in 300 cm² (*E. coli* O157, *Salmonella* and *Listeria* spp.) and in 250cm² (*Campylobacter*)

Table 4.4: Prevalence of coagulase positive staphylococci on sponges from Australian sheep primals and descriptive statistics for log₁₀ counts from positive samples

	Overall		Summer		Winter	
	Legs	Shoulders	Legs	Shoulders	Legs	Shoulders
Samples	613	613	310	310	303	303
Prevalence (%)	4.2	5.2	2.6	3.5	5.9	6.9
Mean ^(a,b)	-0.21	0.34	0.03	0.90	-0.32	0.04
Median	-0.09	0.21	-0.09	0.92	-0.28	0
Standard deviation	0.60	0.93	0.70	1.10	0.54	0.68
90th percentile	0.50	1.49	0.62	2.78	0.26	0.58
95th percentile	0.97	1.52	1.15	2.96	0.28	1.23
99th percentile	1.15	2.07	1.15	2.96	0.45	1.52
Maximum	1.15	2.96	1.15	2.96	0.45	1.52

^a Limit of detection 0.08 cfu/cm²

^b log₁₀ counts of positive samples

Table 4.5: Prevalence of coliforms on sponges from Australian sheep primals and descriptive statistics for log counts from positive samples

	Overall		Summer		Winter	
	Legs	Shoulders	Legs	Shoulders	Legs	Shoulders
Samples	613	613	310	310	303	303
Prevalence (%)	52.0	46.2	39.7	43.5	64.7	48.8
Mean ^(a,b)	-0.36	-0.54	-0.50	-0.39	-0.27	0.68
Median	-0.48	-0.77	-0.77	-0.48	-0.34	0.77
Standard deviation	0.72	0.63	0.69	0.76	0.71	0.44
90th percentile	0.57	0.12	0.41	0.39	0.73	0.08
95th percentile	1.00	0.55	1.00	1.54	1.03	0.18
99th percentile	2.02	2.02	2.03	2.74	2.08	0.81
Maximum	2.36	3.10	2.10	3.10	2.36	1.52

^a Limit of detection 0.08 cfu/cm²

^b log₁₀ counts of positive samples only

Appendix 5: Microbiological profile of frozen boneless sheepmeat

Table 5.1: Total Viable Count (\log_{10} TVC at 25°C) of frozen, boneless sheepmeat

	Overall	Summer	Winter
Samples	551	251	300
Mean ^(a)	2.80	2.97	2.65
Median	2.76	2.90	2.63
Standard deviation	0.70	0.64	0.72
90th percentile	3.61	3.85	3.38
95th percentile	4.02	4.11	4.00
99th percentile	4.94	4.94	5.09
Maximum	5.51	5.09	5.51

^a Limit of detection 10 cfu/g

Table 5.2: Prevalence of generic *E. coli* in core samples of Australian boneless sheepmeat and descriptive statistics for \log_{10} counts from positive samples.

	Overall	Summer	Winter
Samples	551	251	300
Prevalence (%) ^a	12.5	14.7	10.7
Mean ^(b)	1.51	1.43	1.61
Median	1.30	1.30	1.30
Standard deviation	0.67	0.57	0.77
90th percentile	2.81	2.00	2.95
95th percentile	2.95	2.46	3.00
99th percentile	3.00	3.00	3.00
Maximum	3.30	3.30	3.00

^a Limit of detection 10 cfu/g

^b \log_{10} counts of positive samples

Table 5.3: Prevalence of *Salmonella* and *Listeria* in samples of frozen, boneless sheepmeat (2011)

	Overall	Summer	Winter
Samples	551	251	300
<i>Salmonella</i>	3.1%	1.2%	4.7%
<i>Listeria</i>	nd	nd	nd

nd – not detected in 25g sample

Table 5.4: Prevalence of coagulase positive staphylococci in core samples of Australian frozen boneless sheepmeat and descriptive statistics for \log_{10} counts from positive samples

	Overall	Summer	Winter
Samples	551	251	300
Prevalence (% detection)	1.8	0.4	3.0
Mean ^(a,b)	1.66	1.85	1.64
Median	1.70	1.85	1.70
Standard deviation	0.40	-	0.42
90th percentile	2.20	1.85	2.20
95th percentile	2.32	1.85	2.32
99th percentile	2.32	1.85	2.32
Maximum	2.32	1.85	2.32

^a Limit of detection 10 cfu/g

^b \log_{10} counts of positive samples

Table 5.5: Prevalence coliforms in core samples of Australian frozen boneless sheep meat and descriptive statistics for \log_{10} counts from positive samples

	Overall	Summer	Winter
Samples	551	251	300
Prevalence (% detection)	19.4	25.5	14.3
Mean ^(a,b)	1.54	1.52	1.59
Median	1.30	1.30	1.30
Standard deviation	0.68	0.63	0.76
90th percentile	2.78	2.48	3.23
95th percentile	3.23	2.79	3.26
99th percentile	3.85	3.00	3.30
Maximum	3.90	3.90	3.30

^a Limit of detection 10 cfu/g

^b \log_{10} counts of positive samples

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