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Microbiological quality of Australian beef and sheepmeat

Results of the industry's third national abattoir study

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Executive summary

The third national baseline microbiological survey of Australian beef and sheepmeat quality was conducted in 2004.

How the survey was conducted

Samples of chilled beef carcases and frozen boneless beef were collected from processors accounting for approximately 75% of Australia's throughput. Samples of chilled sheep carcases and frozen boneless sheepmeat were collected from processors accounting for approximately 78% of Australia's throughput. The number of samples collected from each processor was proportional to their estimated processing volume. Testing was conducted in an accredited laboratory following internationally accepted methods. Over 1000 samples of each type of beef product, over 1000 sheep carcases and over 500 frozen boneless sheepmeat products were tested.

Results of beef testing

Carcases were found to have an average total viable count (TVC, 25°C) of 21cfu/cm^2. *E. coli* was isolated from 4.9% carcases with an average count of 0.38 cfu/cm^2 on positive samples. The average TVC for boneless beef was 15 cfu/g and the average count for the 1.1% of samples with detectable *E. coli* was 79 cfu/g. *E. coli* O157:H7 was isolated from 1/1143 carcases and 0/1082 boneless samples. *Salmonella* was isolated from none of the carcases tested and from 1/1082 samples of boneless product. No *Campylobacter* sp. were isolated. Coagulase positive staphylococci were isolated from 20.1% and 2.6% of carcase and boneless beef samples with positive samples having an average count of 4 cfu/cm^2 and 34 cfu/g respectively.

Results of sheepmeat testing

Carcases were found to have an average total viable count (TVC, 25°C) of 191 cfu/cm^2. *E. coli* was isolated from 32.9% carcases with an average count of 2 cfu/cm^2 on positive samples. The average TVC for frozen boneless sheepmeat was 65 cfu/g. The average *E. coli* count for the 4.3% of positive samples was 105 cfu/g. *E. coli* O157:H7 was isolated from 0.6% carcases and 0.2% boneless samples. *Salmonella* was isolated from none of the carcases tested and from 0.5% samples of boneless product. *Campylobacter* sp. were isolated from 0.4% of carcases and 0.2% of boneless samples. Coagulase positive staphylococci were isolated from 15.9% and 14.1% of carcase and boneless samples with positive samples having an average count of 19 cfu/cm^2 and 55 cfu/g respectively.

What do these results tell us?

There has been a significant improvement in microbiological quality in the Australian industry. This is demonstrated by improved results in this survey over the previous surveys conducted in 1993-94 and 1998. Product hygiene is at a consistently high level. The differences in microbiological quality from processor to processor were mostly insignificant. Product characteristics, such as a long shelf-life can be attributed to the excellent microbiological quality of the product.

The data reflects the investment made by the Australian industry in food safety systems over the past decade which encompasses the farm-transport-slaughter/dressing and chilling continuum. Investments have been made by both regulators and industry. New regulations have been enacted and enforcement has developed in a co-regulatory environment. Industry has developed risk-based quality systems, trained staff and invested in buildings, equipment and chilling systems.

We expect that the results will be useful for the purpose of public health risk assessment. The results indicate that the public health risks due to the use and consumption of Australian red meat products are exceedingly low.

When compared to the microbiological standards and guidelines applied in other countries and by purchasers of Australian product, the quality of the product tested in this baseline survey exceeded requirements. When compared to data available on the performance of meat processors in other countries the results compared very favourably.

Introduction

Meat & Livestock Australia is pleased to present the results of the third national abattoir microbiological study prepared on behalf of the red meat sector.

This report will be of great interest to the industry, to the controlling authorities in both the Australian and state governments, to public health authorities and to the customers of Australian meat products.

The report provides data that contributes to the validation of Australian processing practices, the regulatory standards in place and the enforcement efforts of the controlling authorities. The results presented here will show that the hygienic quality of Australian red meat products are amongst the highest in the world and that international standards are met and exceeded.

For individual meat processing establishments these results will provide a benchmark against which performance may be judged.

Background
– the industry's first and second national baselines studies

In 1993-94 the Australian meat industry commissioned its first national baseline study of the microbiological quality of Australian meat (reported in Vanderlinde *et al*., 1998 and 1999). The study was based on samples excised from chilled carcase surfaces and from pieces of meat drilled from cartons of frozen manufacturing meat. The study was designed to define the performance of the industry using a recognised set of hygienic measures. At that time there was interest in comparing the performance of export and domestic plants and this became one of the main goals of the first study. Another goal was to quantify the benefits of quality assurance programs. Although Quality Assurance (QA) was in its infancy in 1993 it seemed destined to become the driving force for improvements in product safety and evidence of the benefits were needed by processors and regulators.

A summary of some of the results of the first baseline is found in *Table 1*.

Table 1: Results obtained from the first national baseline in 1993-94

	Beef		Sheep	
	Carcase (excised tissue per cm^2)	Frozen boneless (per gram)	Carcase (excised tissue per cm^2)	Frozen boneless (per gram)
TVC – mean (log_{10})	3.02	2.77	3.92	3.47
E. coli (% prevalence)	22	19	75	50
Salmonella (% prevalence)	0.4	6.5	5.7	6.6

Generally, product from export plants had lower microbiological counts than that from domestic plants. However, carcases at export plants measured after weekend chilling tended to have higher than average counts. The baseline study found that three domestic plants that had installed QA systems were manufacturing products of similar microbiological quality to that from plants which had not installed a QA system. In comparison with meat surveyed in countries with which Australia traded there was little difference in TVC but *E. coli* counts appeared lower. Overall, the first baseline study established a benchmark for Australian meat against which subsequent surveys could be compared.

In 1998 the industry commissioned a second baseline study which came after the Australian meat industry had undergone recent and significant change (reported in Phillips *et al*., 2001 a, b). For example, all slaughter and boning facilities (domestic and export) had implemented quality assurance plans based on HACCP and had invested heavily in operator training, improved refrigeration and provision of laboratory facilities. Importantly, the United States of America had implemented the Pathogen Reduction Final Rule (known as 'the MegaReg') which required many changes to Australian processing. The industry's second baseline, designed primarily to take into account the effect of these changes, was based on sponge samples of chilled carcases (to be consistent with the MegaReg) and on samples drilled from meat frozen in cartons. As well, to provide links with the first baseline study, excision samples were taken from beef carcases. The study also collected data from the hot boning sector, which was in an early stage of development and, for the first time, very small plants (VSPs, formerly known as slaughterhouses) were included in the national baseline.

The second national baseline established that TVCs, prevalence of *E. coli* and *Salmonella* were slightly lower than in the 1994 survey. Although product from export establishments had lower microbiological counts, the gap with domestic plants had narrowed and VSPs were found to produce carcases of similar microbiological quality to larger establishments. Frozen meat from hot boning establishments had a similar microbiological profile to that from plants using conventional boning methods.

The standardised methodology introduced in Australia and consistent with the MegaReg made it possible to compare the results of the 1998 baseline with similar data from other nations which either import Australian red meat products, or who are competitors in the international market for red meat, or both. Carcase testing in the first baseline was performed on excised tissue samples, whereas the second baseline was performed by sponging carcase surfaces.

A comparison of the two sampling methods was conducted on beef carcases. The average TVC was 0.14 $\log_{10}$ higher on excised samples. The baseline study revealed that Australian beef carcases and frozen boneless meats compared favourably on virtually all hygienic criteria.

In 2004 a third industry-wide baseline study was conducted. This time establishments were visited at least once during summer and at least once during winter to ensure results incorporated any seasonal variation in effects due to, for example, variation in pre-slaughter influences or processing. The 2004 baseline was conducted in an environment in which all establishments operated under the Australian Standard for the Production and Transportation of Meat and Meat Products for Human Consumption (AS 4696–2002). Unlike earlier baseline studies, in 2004 there was no clear difference between the mandatory quality assurance practices required in export and domestic establishments. Sampling and microbiological analysis in 2004 were almost identical to those undertaken for the 1998 baseline study and the results from both show the extent of improvement from consolidation of QA systems within the Australian red meat industry over the period 1998-2004. The results of the third baseline study are reported here.

The value of baseline surveys

Baseline studies have great value. Foremost is that they deliver objective evidence that the combination of hygienic measures mandated by regulation and those imposed at the discretion of company management result in a safe product likely to have good storage characteristics. Evidence of the amount of variation in performance between establishments is also very helpful for validating the regulatory system under which the industry operates. For example, if the study showed high microbiological counts across the industry, or showed some plants with acceptable levels and others with marginal or unacceptable levels, it could be seen as failure of the overall system under which the industry is regulated.

The standardised methods employed in a baseline study allow an individual company to compare its performance with other companies. If a company or an industry undergoes radical change to its process then baseline data will be able to quantify how that change has impacted on meat hygiene. The baseline results also provide a benchmark that individual companies can use to judge if improvement of their processes is required and for setting performance targets.

The standard testing methods used in baseline studies allows the results to be used to rate the hygienic quality of meat on an international basis. Increasingly this type of evidence is sought by clients and regulators in foreign markets as a way of ensuring their own requirements for product safety and shelf life are satisfied and that consumer's concerns about safety issues are addressed.

A fourth advantage is that the data generated by baseline studies are essential for risk assessments. These science-based appraisals of product safety are being increasingly relied upon at a national and international level for ensuring the management of food safety issues has a rational basis.

For the Australian meat industry, data generated in all three baselines have been invaluable in providing confidence in the state and federal regulatory systems. Globally, the microbiological status of Australia's meat products has been at least the equivalent of the best-performing industries. Finally, baseline data from the previous surveys played a key role in underpinning the industry's risk profile performed in 2003, the output from which guides the ongoing management of food safety in the Australian red meat sector.

How the study was done

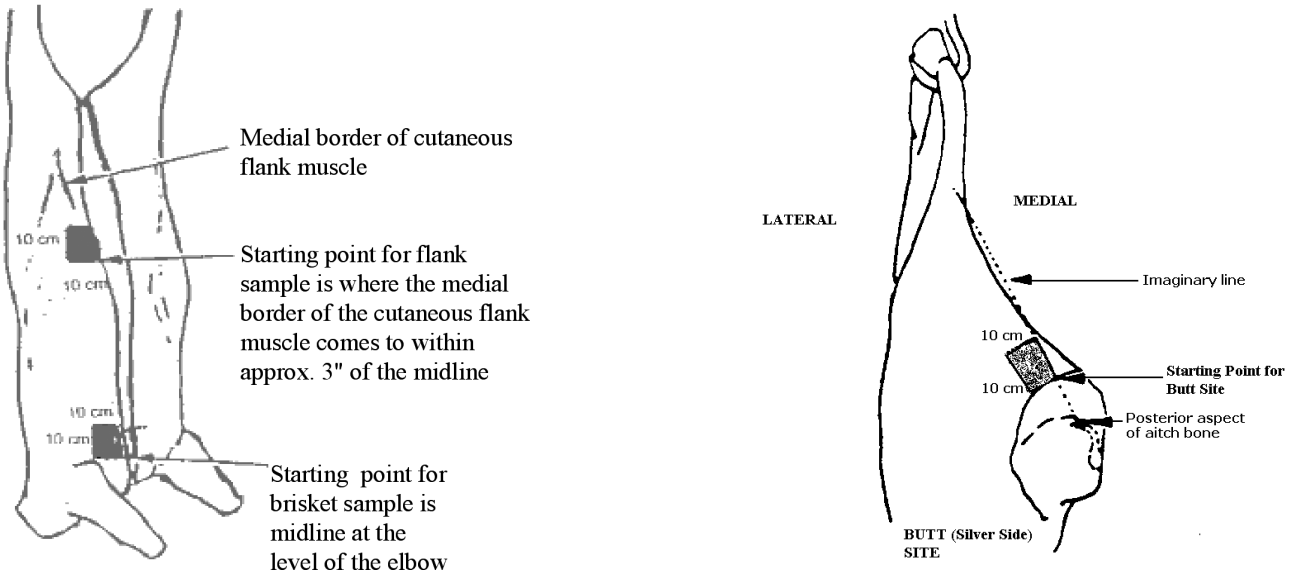
The overall aim of this study was to describe the microbiological quality of Australian red meat using internationally recognised systems of sampling and analysis.

Sampling occurred at two times of the year. The summer sampling was from February to April, 2004 and the winter sampling from July to October, 2004. Establishments were sampled at least once in each of the summer and winter periods and the establishments were distributed throughout each of the five mainland states of Australia. For beef, 27 abattoirs and 24 boning rooms were sampled with their combined production accounting for approximately 75% of Australian beef production. For sheep, 20 abattoirs and 10 boning rooms were sampled with their combined production accounting for approximately 78% of Australian sheepmeat production. The establishments were selected by their size with the target to sample establishments processing 80% of Australian throughput. Participation in the survey was optional. Almost all establishments in the study were under the jurisdiction of the Australian Quarantine and Inspection Service (AQIS). The number of carcases or boxes sampled at each abattoir or boning room was in proportion to its estimated processing volume. The production volumes of abattoirs ranged from 300 to 3800 carcases per day for cattle and from 2000 to 7000 small stock per day.

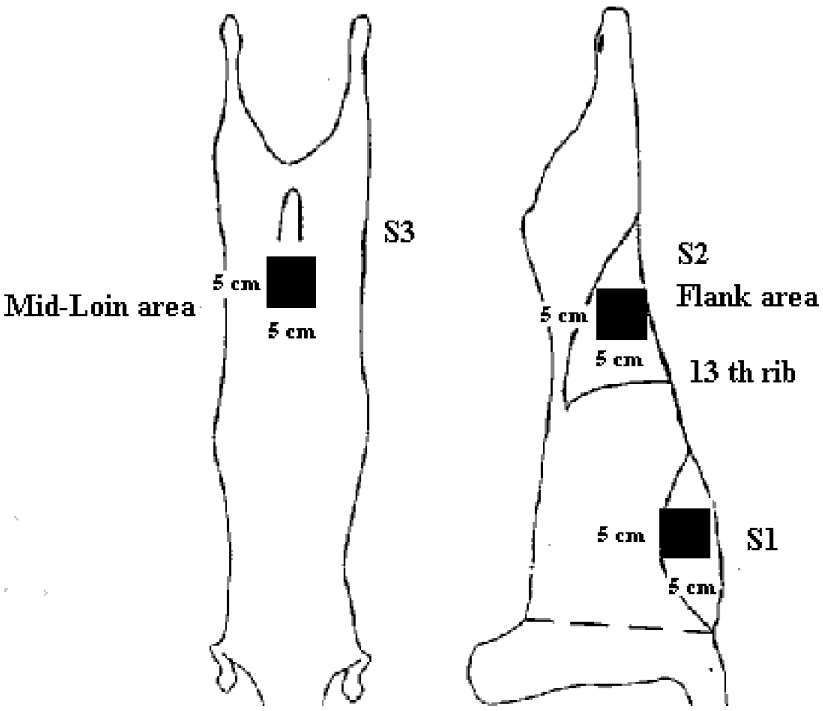
Samples were collected by a team of trained technicians who sponged carcases randomly at each of the ESAM sites (*Figure 1*) which are equivalent to the MegaReg requirements. For beef carcases, a composite sample was taken by sponging a 100cm^2 area at each of the butt, flank and brisket regions of each side of the carcase as detailed in AQIS Notice 2003/6. A third sponge sampled areas adjacent to those sampled with the second sponge (B) on the same carcase side. For sheep carcases a composite sample was taken by sponging a 25cm^2 area at each of the mid-loin, flank and brisket regions of each side of the carcase as detailed in AQIS Notice 2003/6. A third sponge sampled an area immediately cranial to that sampled with the second sponge on the same carcase side. In order to provide two mid-loin samples (A and B) on each carcase it was necessary to take them adjacent to the spinal column on each side of the carcase rather than across the spinal column as detailed in AQIS Notice 2003/6. Carcases were sampled after approximately 14h active chilling. Sample collection days were Tuesday to Friday (ie kill days Monday to Thursday) to minimise the confounding effect of sampling carcases held in chilled storage over a weekend.

Beef

Figure 1: ESAM sampling sites for beef and sheep (AQIS Meat Notice 2003/6)



Sheep



Samples of boneless beef were collected from frozen cartons which had been held in freezer store usually no longer than one month. Approximately 150g of meat drilled from 8–10 different locations in each carton using a sterile drill bit were transferred into sterile plastic bags using a sterile pair of kitchen tongs. Cartons were selected on a random basis from those accessible at the time of visit.

All samples were packed in insulated containers with chiller packs and transported to the laboratory for testing. Samples which arrived warmer than 10°C were discarded. If a consignment was delayed in transit, it was noted on the sample receipt form and the project manager notified so that a decision could be made in the event of any abnormally high results. Temperature histories obtained from data loggers enclosed with the samples during transit were used to assist a decision on whether temperature abuse had occurred during transit.

Three sponge samples were collected from each carcase one of which was used for detection of *E. coli* 0157:H7, one for *Salmonella* and the remainder for all other analyses (TVC, *E. coli*, *Enterobacteriaceae*, coagulase positive staphylococci and *Campylobacter*). To eliminate bias between the right or left side of the carcase, sponges were directed at random to either test.

Tests were conducted in a National Association of Testing Authorities (NATA) accredited laboratory operating to the ISO 17025 laboratory quality standard. 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 *Appendices 1* and *2*.

How we are reporting results

Some tests yielded qualitative (presence/absence) results and others yielded quantitative (numerical) results, which were treated in different ways.

Tests for *Salmonella*, *Campylobacter*, and *E. coli* O157:H7 yielded presence or absence findings for individual samples. These were analysed across plants and the entire survey to provide the proportion of samples yielding positive results. We refer to these results as prevalence of detection, or, proportion of detections.

Some tests involved estimating the number of a specific bacteria or group of bacteria present in the sample. This was so for generic *E. coli*, coliforms, *Enterobacteriaceae*, and coagulase positive staphylococci. In this case we may have reported the number of tests yielding any organism, particularly if a lot of specimens contained zero counts. Alternatively, or in addition, we have reported the actual count per unit of area of carcase sponged or the actual count per weight of meat sampled.

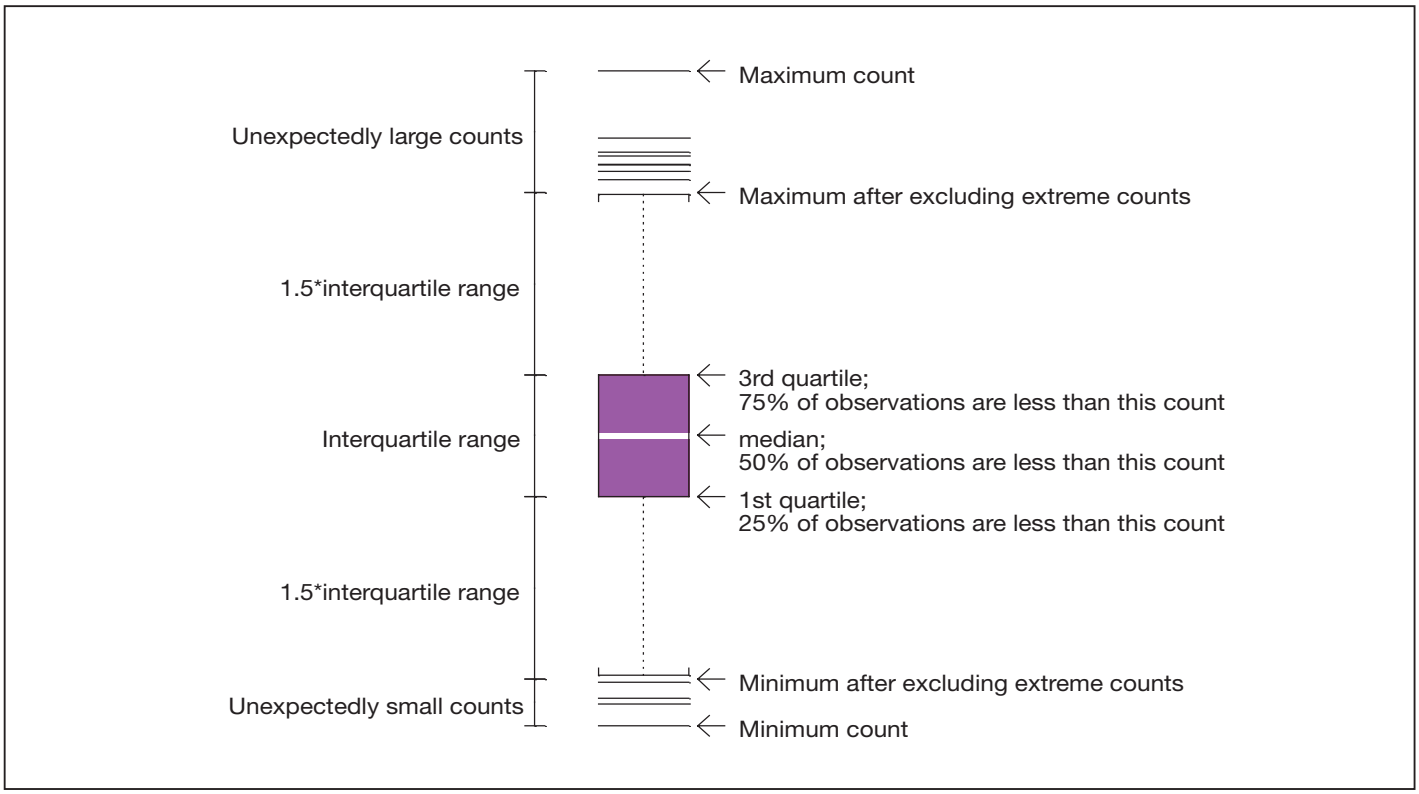
Laboratory tests for the detection and enumeration of bacteria have limitations when the number of bacteria present in the sample or on the carcase is very low. For many tests it is possible to calculate a 'limit of detection' (LOD) which represents the lowest density of organisms that is expected to yield a positive result. Thus all results that are reported as negative (or not detected) are either 'true negatives' (the organism was absent from sample) or 'false negative' (the organism was present but not detected, or because too few were present in the sample). LODs for the 2004 survey are listed in *Appendix 2*.

The data for total viable counts of aerobic (and facultative) bacteria (TVC) also provided an estimate of the density of bacteria per unit area or weight. However, in this case we rely on log-transformed data because they are easier to work with and interpret. We make this transformation by first adding the amount of 1 to each TVC observation then obtain the base 10 logarithm of each value. These transformed data are referred to as 'log TVC'. When we compare different baseline studies we apply this same transformation to the raw TVC data from each study (so the transformed data from each survey are directly comparable). The results for log TVC can be represented as a 'box-plot'.

Box-plots are a convenient graphical device for summarising data for each establishment or other subgroup. The box-plots used here have the features illustrated in *Figure 2*.

Log base 10 transformation of count data for *E. coli* was also applied. However, this was confined to those samples or sponges with a positive detection (zero counts were excluded). The log transformed generic *E. coli* data therefore summarises the concentration data for positive sponges only.

Figure 2: Explanation of the features of box-plots used for describing the bacterial counts in the baseline survey



Results – microbiology of beef carcases and frozen boneless beef

A total of 1155 carcases produced at 27 abattoirs were tested (see *Appendix 3.A* for detailed results). The mean log TVC was 1.33 $\log_{10}$cfu/cm^2 (equivalent to 21 cfu/cm2) with *E. coli* detected on 4.9% of samples with positive samples having an average count of -0.42 $\log_{10}$cfu/cm^2 (equivalent to 0.38 cfu/cm^2). Coagulase positive staphylococci were isolated from 20.1% samples with positive samples having an average count of 0.56 $\log_{10}$cfu/cm^2(equivalent to 4 cfu/cm^2).The mean TVC and the prevalence of *E. coli* and coagulase positive staphylococci in beef carcase samples were lower in the winter than in the summer survey.

For frozen boneless beef 1082 cartons were tested from 24 boning rooms (see *Appendix 3.B* for detailed results). The mean log TVC/g was 1.19 $\log_{10}$cfu/g (15 cfu/g) and *E. coli* was detected on 1.1% of samples with positive samples having an average count of 1.90 $\log_{10}$cfu/g (equivalent to 79 cfu/g). Coagulase positive staphylococci were isolated from 2.6% of samples with positive samples having an average count of 1.53 $\log_{10}$cfu/g (equivalent to 34 cfu/g). As for beef carcases, TVCs and prevalence of *E. coli* and coagulase positive staphylococci were lower in the winter survey.

On carcases, *Salmonella* and *Campylobacter* were not detected on any of the 1155 samples taken, while *E. coli* O157:H7 was recovered from one carcase (0.1%). On frozen, boneless beef *E. coli* O157:H7 and *Campylobacter* were not detected on any of 1082 drilled samples while *Salmonella* was detected in one sample (0.1%).

The hygienic quality of the meat tested in this survey, taken as a whole, was excellent. The prevalence of hygienic indicator organisms, such as *E. coli* or coagulase positive staphylococci was very low and the counts were also very low. The inability to isolate *Campylobacter* in any sample indicates that even though many animals carry this microbe in their gastrointestinal tract and shed the organism in their faeces, it does not appear to contaminate carcases after dressing or survive the chilling and freezing processes. The very low prevalence of contamination with *E. coli* O157:H7 and *Salmonella* also indicate a high standard of hygienic processing.

Data are presented here for individual establishments. Considerable care needs to be taken in interpreting these data. Each abattoir or boning room was visited 2–6 times during the survey (depending upon the number of samples to be collected). Therefore the results are an indication of the quality of production on those days and may not account for the variability in hygiene status of product at a particular establishment.

Figures 3 and *4* show the variation observed within and between plants sampled in this study for TVCs of beef carcases and boneless beef respectively. Although there are differences in the median count between some plants, the size of the differences are small and, for most comparisons, not meaningful. Some of the plants in this comparison utilise carcase decontamination technologies, whereas others rely on other aspects of their process to achieve a hygienically dressed carcase. The box plots help us to understand the process control at various plants. Shorter boxes indicate that there is very tight process control, whereas a longer box indicates less uniformity in process control. The number of outliers is small, which indicates consistency of processing.

The Meat Standards Committee has produced a guideline for interpreting microbiological results (*Table 2*) (Anon., 2002). Using the Meat Standards Committee guidelines, beef product results were overwhelmingly in the 'Excellent' category (*Table 3*)

Table 2: Meat Standards Committee classification of microbiological results

Category	**TVC/cm² or /g**	***E. coli*/cm² or /g**
Excellent	<1,000	Not detected
Good	1,000–10,000	1–10
Acceptable	10,000–100,000	10–100
Marginal(action required)	100,000–1,000,000	100–1,000

Table 3: Quality categorisation of results for TVC and E. coli *tests on beef carcases and frozen boneless beef according to the Meat Standards Committee classification*

	Carcase		**Frozen boneless**	
	TVC	*E. coli*	TVC	*E. coli*
Excellent	96.9%	94.5%	97.6%	98.9%
Good	2.6%	4.7%	2.1%	*
Acceptable	0.4%	0.2%	0.2%	0.6%
Marginal(action required)	0.1%	0%	0.1%	0.5%

* LOD <10 cfu/g

MLA has recently conducted research (MLA, 2004) to identify factors in processing that are most significantly correlated with good microbiological quality. The results suggest that improvements in the hygienic quality of carcases might be achieved by attention to two aspects of processing:

1. The contamination level on incoming livestock because it makes it more difficult for the process to cope with the contamination load. The important factors contributing to contamination level appear to be:
   - cleanliness of hides
   - time of transport
   - proportion of cows/bulls
2. Slaughter and dressing practices, particularly,
   - effective separation of hide-on and hide-off areas
   - evisceration straight onto evisceration tables
   - use of decontamination interventions

Figure 3: Variation within and between plants in log TVC/cm² of beef carcases. The box plot for all data is also shown.

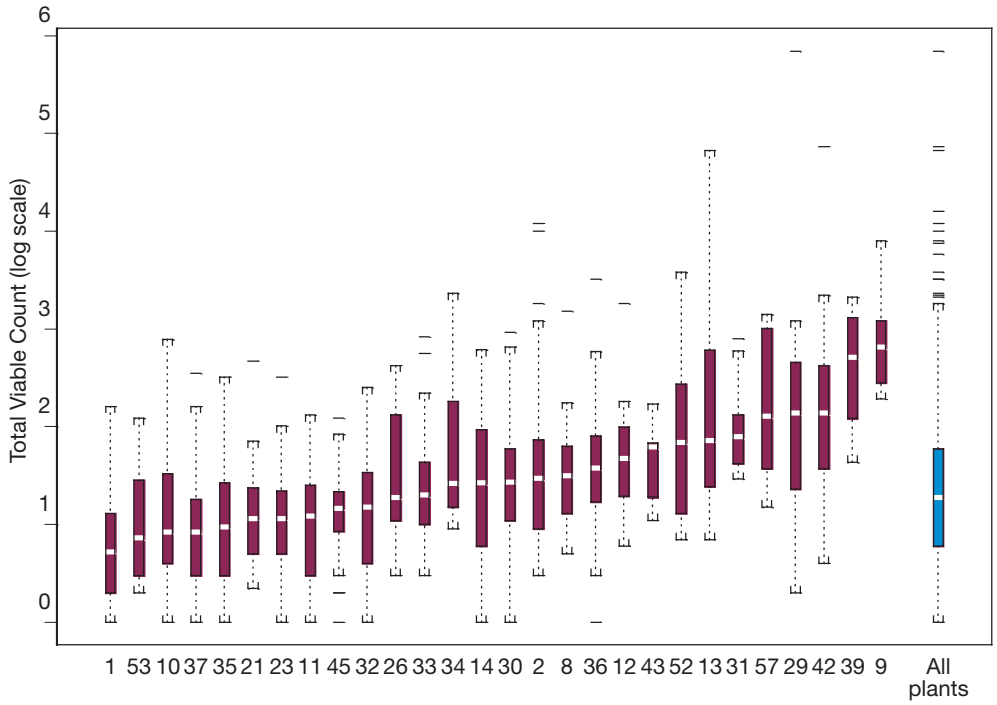
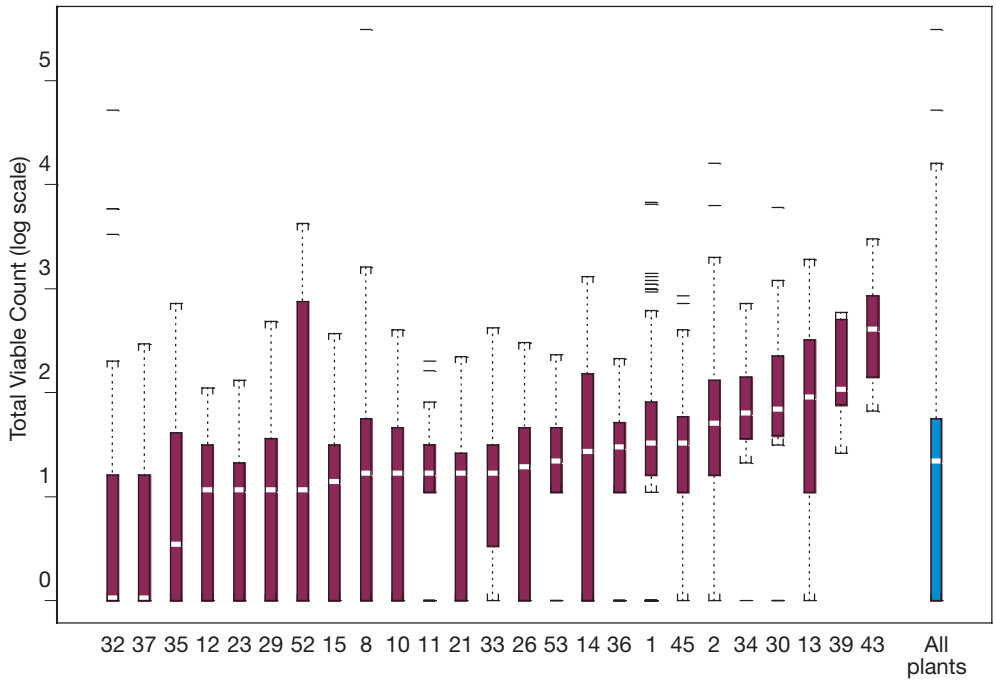


Figure 4: Variation within and between plants in log TVC/g of boneless beef. The box plot for all data is also shown.



Results – microbiology of sheep carcases and frozen boneless sheepmeat

A total of 1117 carcases processed at 20 abattoirs were tested (for detailed results see *Appendix 4.A*). The mean TVC was 2.28 $\log_{10}$cfu/cm^2 (equivalent to 191cfu/cm^2) with *E. coli* detected on 32.9% of samples with positive samples having an average count of 0.28 $\log_{10}$cfu/cm^2 (equivalent to 2 cfu/cm^2). Coagulase positive staphylococci were isolated from 15.9% carcase samples with positive samples having an average count of 1.27 $\log_{10}$cfu/cm^2 (equivalent to 19 cfu/cm^2). The mean TVC and the prevalence of *E. coli* and coagulase positive staphylococci on sheep carcase samples were lower in the winter than in the summer survey.

For frozen boneless sheepmeat 560 samples from 10 boning rooms were tested (for detailed results see *Appendix 4.B*). The mean TVC was 1.81 $\log_{10}$cfu/g (equivalent to 65 cfu/g) and *E. coli* was detected on 4.3% of samples with positive samples having an average count of 2.02 $\log_{10}$cfu/g (equivalent to 105 cfu/g). Coagulase positive staphylococci were isolated from 14.1% of samples with positive samples having an average count of 1.74 $\log_{10}$cfu/g (equivalent to 55 cfu/g). As for sheep carcases, TVCs and prevalence of *E. coli* and coagulase positive staphylococci were lower in the winter survey.

On carcases, *Salmonella* was not detected on any of the 1117 samples taken, while *E. coli* O157:H7 was recovered from 6/1117 samples (0.6%) and *Campylobacter* from 4/1117 samples (0.4%). On frozen, boneless sheepmeat *Salmonella* was detected on 3/557 (0.5%) of samples, *E. coli* O157:H7 on 1/557 (0.2%) and *Campylobacter* on 1/539 (0.2%) of samples.

The hygienic quality of the meat tested in this survey, taken as a whole, was excellent. The prevalence of hygienic indicator organisms, such as *E. coli* or coagulase positive staphylococci was very low and the counts were also very low. The low prevalence of *Campylobacter* in any sample indicates that even though many animals carry this microbe in their gastrointestinal tract and shed the organism in their faeces, it does not appear to frequently contaminate carcases after dressing or survive the chilling and freezing processes. The prevalence in some other meats after processing is much higher. The very low prevalence of *E. coli* O157:H7 and *Salmonella* also indicate a very high standard of hygienic processing. *E. coli* O157:H7 in sheepmeat is unlikely to be significant because people rarely eat non-intact (for example, ground), undercooked sheepmeats, which are established vehicles of infection in beef products.

Data are presented here for individual establishments. Considerable care needs to be taken in interpreting these data. Each abattoir or boning room was visited 2–6 times during the survey (depending upon the number of samples to be collected) and therefore the results are an indication of the quality of production on those days rather than their 'normal' production.

Figures 5 and *6* show the variation observed within and between plants sampled in this study for TVCs of sheep carcases and boneless sheepmeat respectively. Although there are differences in the median count between plants, the size of the differences is not considered important. All of the plants in this comparison rely on various aspects of their process to achieve a hygienically dressed carcase. The box plots help us to understand the process control at various plants. Shorter boxes indicate that there is very tight process control, whereas a longer box indicates less uniformity in process control, The number of outliers is small which indicates consistency of processing and a low likelihood of quality problems.

The Meat Standards Committee has produced a guideline for interpreting microbiological results (*Table 2*) (Anon., 2002). Using the Meat Standards Committee guidelines, sheep product results were overwhelmingly in the 'Excellent' and 'Good' categories (*Table 4*)

Table 4: Quality categorisation of results for TVC and E. coli *tests on sheep carcases and frozen boneless sheepmeat according to the Meat Standards Committee classification*

	Carcase		Frozen boneless	
	TVC	*E. coli*	TVC	E. coli
Excellent	80.7%	67.1%	91.4%	95.7%
Good	16.7%	27.8%	8.2%	*
Acceptable	1.9%	3.9%	0.4%	2.5%
Marginal(action required)	0.7%	1.1%	0%	1.3%

* LOD is <10 cfu/g

A previous study undertaken by MLA (MLA, 1998) evaluated a range of livestock and carcase slaughter and dressing procedures in relation to carcase visual and microbiological contamination levels. The study identified the contamination levels on livestock to be processed as major contributors to the final microbial loads of carcases. For sheep carcases the important livestock factors contributing to contamination level appeared to be:

- non-crutched or daggy pelts
- longer pelt
- proportion of older sheep processed
- transport distances to slaughter in excess of 200km
- proportion of saleyard purchases

A procedure scoring system was used to record defects throughout the slaughter and carcase dressing process. For sheep processors, procedure score was found to be associated with carcase coliform and *E. coli* microbial loads.

Figure 5: Variation within and between plants in log TVC/cm² of sheep carcases. The box plot for all data is also shown.

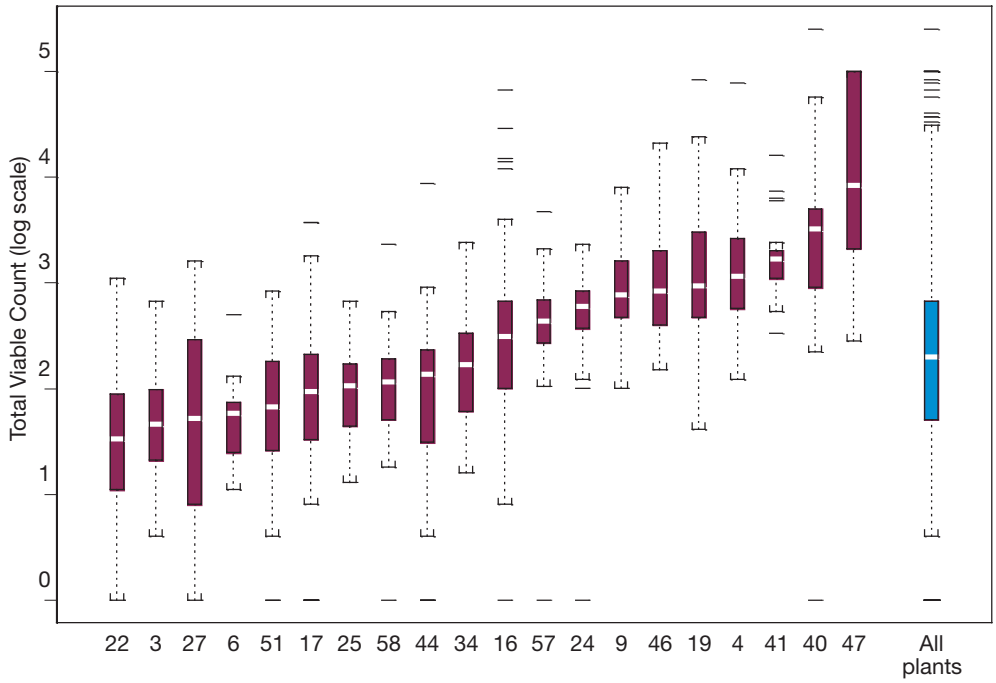
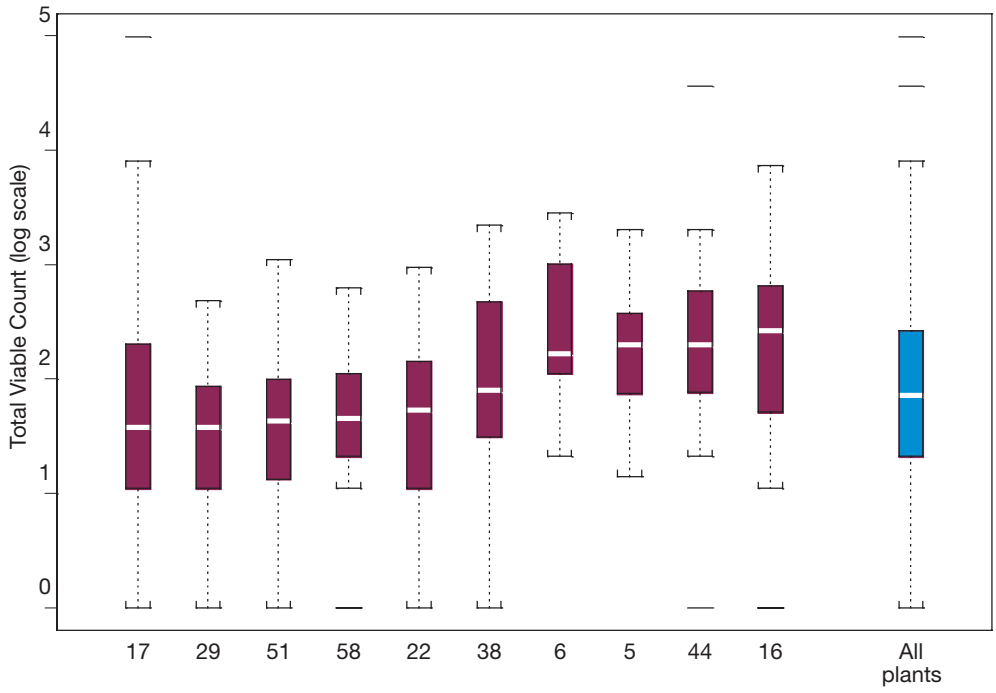


Figure 6: Variation within and between plants in log TVC/g of boneless sheepmeat. The box plot for all data is also shown.



Comparison with previous baseline surveys

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

Carcases

A comparison of the microbiological status of beef and sheep carcases in the 1998 and 2004 surveys, in which the microbiological analysis was identical, indicates significant improvement.

From 1998 to 2004 there was a 47.1% reduction in the mean log TVC of beef carcases sampled (distribution of counts is shown in *Figure 7*) and a 35.9% reduction for sheep carcases (distribution of counts is shown in *Figure 8*). Of 17 beef processing establishments providing data to both the 1998 and 2004 surveys, 15 improved their mean log TVC. For 7 sheep processing establishments that provided data to both surveys, 5 showed an improvement in their mean log TVC.

Figure 7: Comparison of the frequency distribution of beef carcase TVC/cm² 1998 to 2004

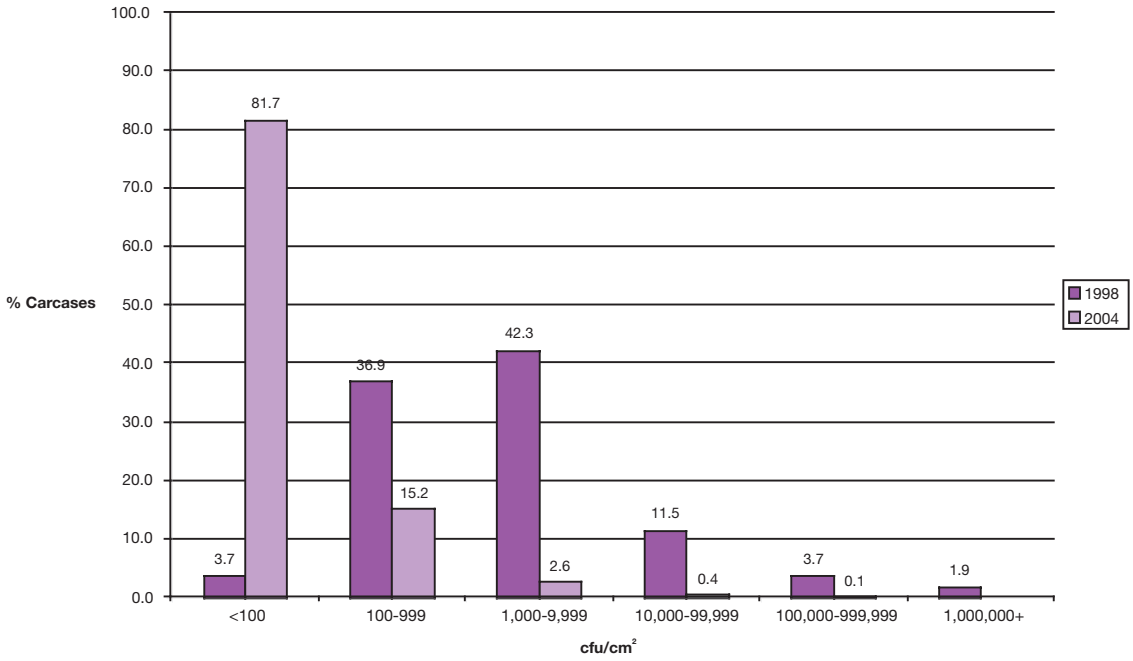
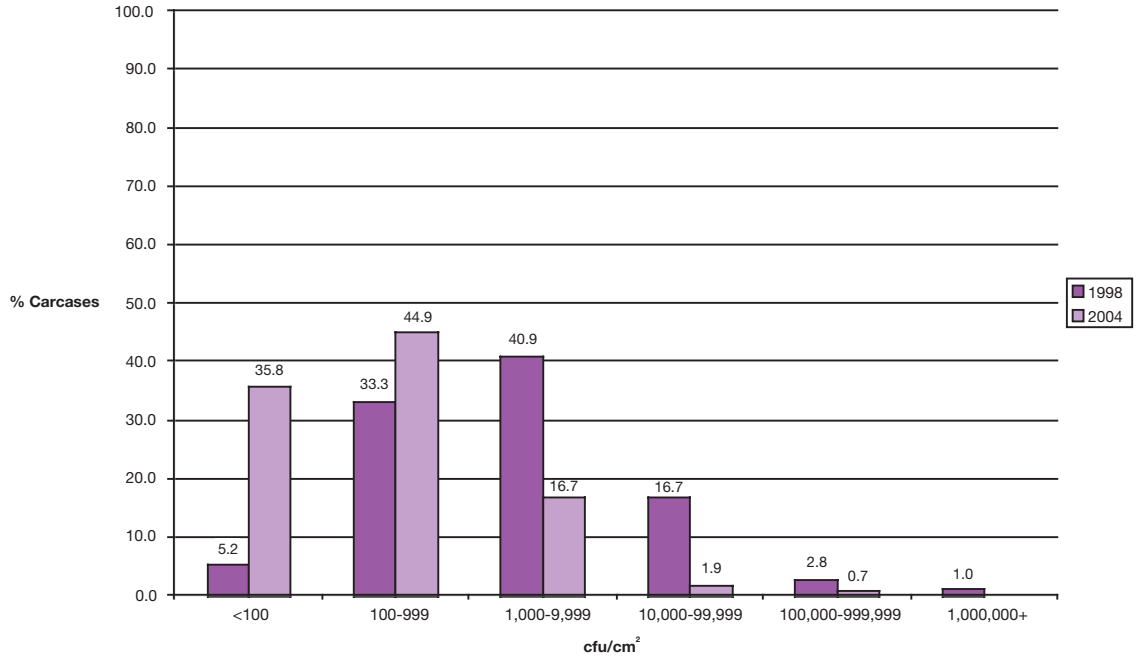


Figure 8: Frequency distribution of sheep carcase TVC/cm² 1998 versus 2004



From 1998 to 2004 there was a 48.6% reduction in the prevalence of *E. coli* on beef carcase samples (distribution of counts is shown in *Figure 9*) but only a 2.9% reduction for sheep carcases (distribution of counts is shown in *Figure 10*). As previously discussed, the result for sheep carcases place nearly 95% of samples in the Excellent or Good category defined by the Meat Standards Committee. Of 17 beef processing establishments providing data to both the 1998 and 2004 surveys, 10 recorded a lower prevalence of *E. coli* and 3 of 7 sheep processing establishments showed an improvement.

Figure 9: Comparison of the frequency distribution of E. coli *detections on beef carcases from 1998 to 2004*

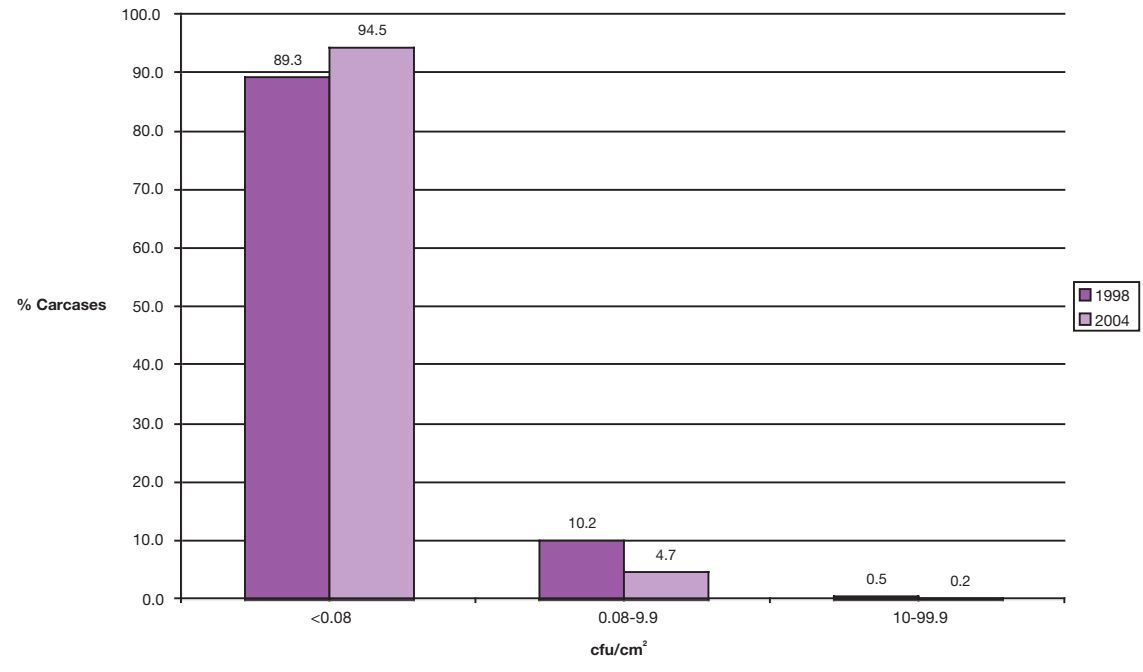
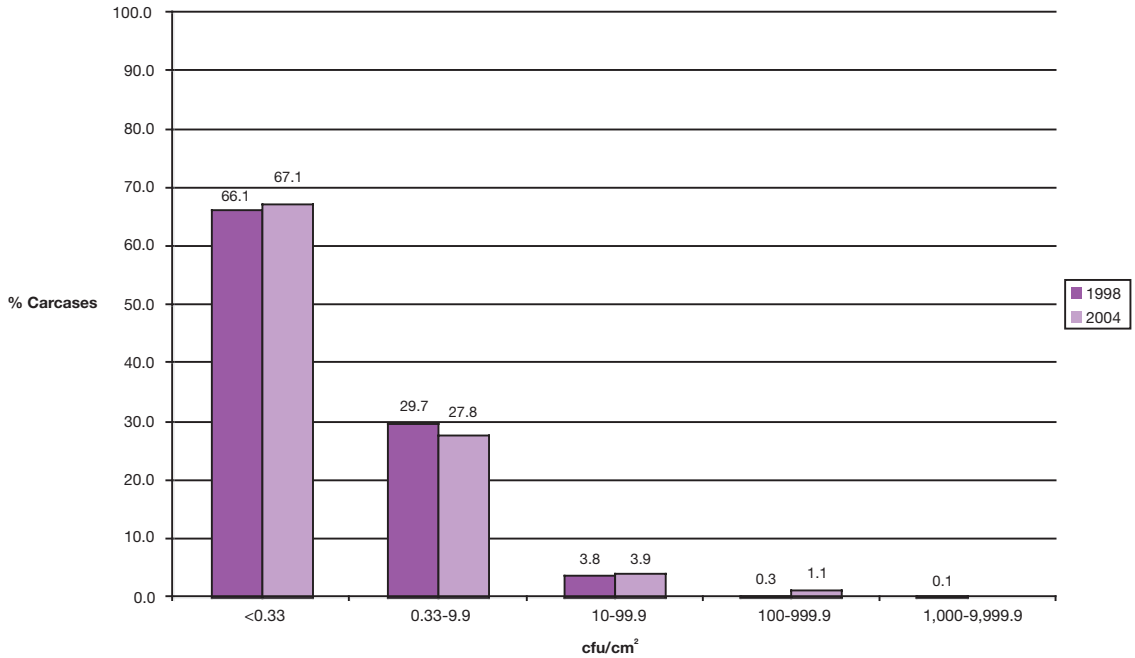


Figure 10: Comparison of the frequency distribution of E. coli *detections on sheep carcases from 1998 to 2004*



Frozen boneless meat

In all three baseline surveys samples of frozen, boneless meat were taken in exactly the same manner (drilling of cartons). However there were differences in some microbiological techniques between the 1993-94 and the 1998 and 2004 surveys. In the earliest survey TVC was determined on agar plates rather than on Petrifilm, which would probably have an insignificant impact on the results obtained. *E. coli* was determined by a most probable number (MPN) method in the earliest survey which might be expected to produce a higher count than the Petrifilm method used in the later surveys.

Since 1993-94 a 57.4% reduction in the mean log TVC of frozen boneless beef samples has been recorded with the largest reduction recorded from 1998 to 2004 (*Figure 11*). A similar picture has been recorded for frozen boneless sheepmeat samples with a 48.1% reduction in the mean log TVC from 1993-94 to 2004 (*Figure 12*). Of 11 beef processing establishments and 5 sheepmeat processing establishments providing data to both the 1998 and 2004 surveys, all improved their mean log TVC.

Figure 11: Comparison of the mean log TVC/g of frozen boneless beef 1993-94 to 2004

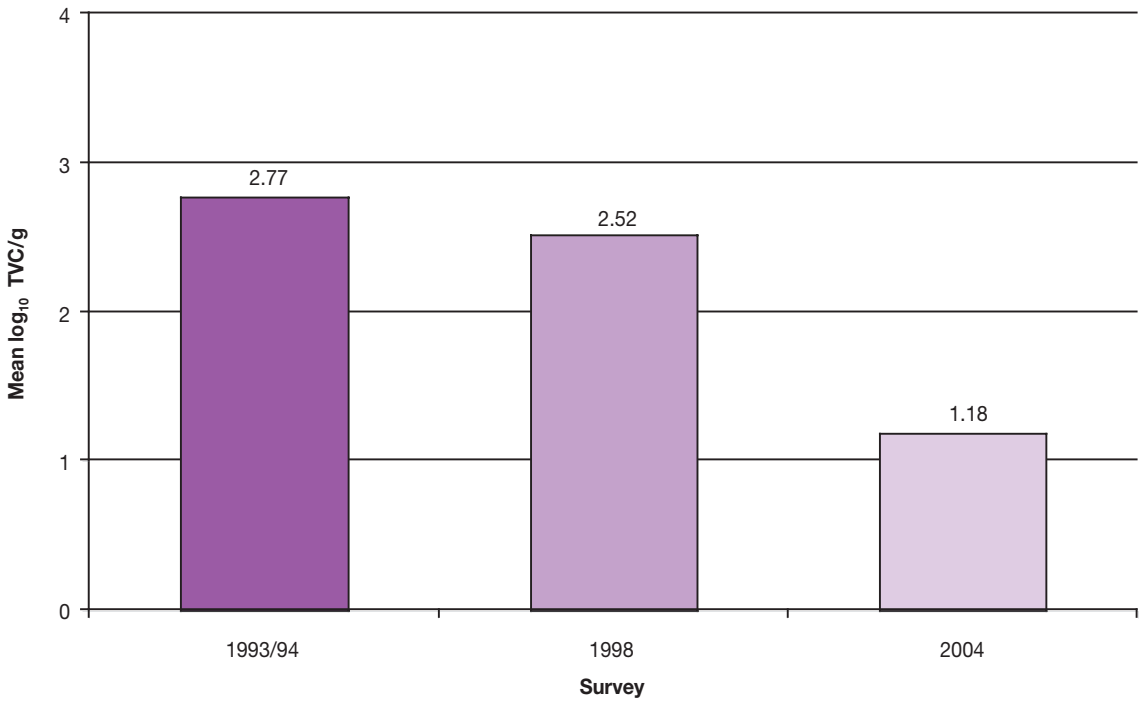
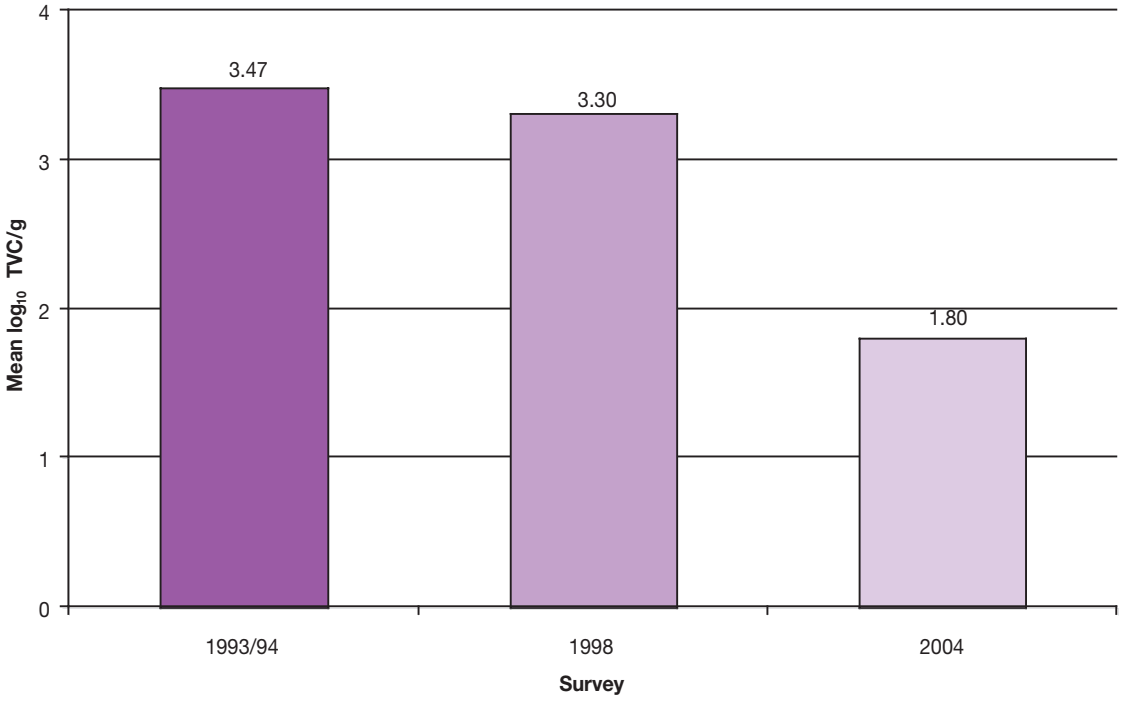


Figure 12: Comparison of the mean log TVC/g of frozen boneless sheepmeat 1993-94 to 2004



From 1993-94 to 2004 there was an 85.9% reduction in the prevalence of *E. coli* detection in frozen boneless beef samples (*Figure 13*) and a 75.8% reduction for frozen sheepmeat samples (*Figure 14*). All data presented in *Figures 13* and *14* have been corrected for a LOD of 10 cfu/g to enable comparisons to be made between the different surveys. All beef and sheep boning establishments providing data to both the 1998 and 2004 surveys recorded either a zero prevalence of *E. coli* detection in both surveys or a lower prevalence of *E. coli* detection in 2004.

Figure 13: Comparison of the prevalence of E. coli *detections on frozen boneless beef (LOD 10 cfu/g) from 1993-94 to 2004*

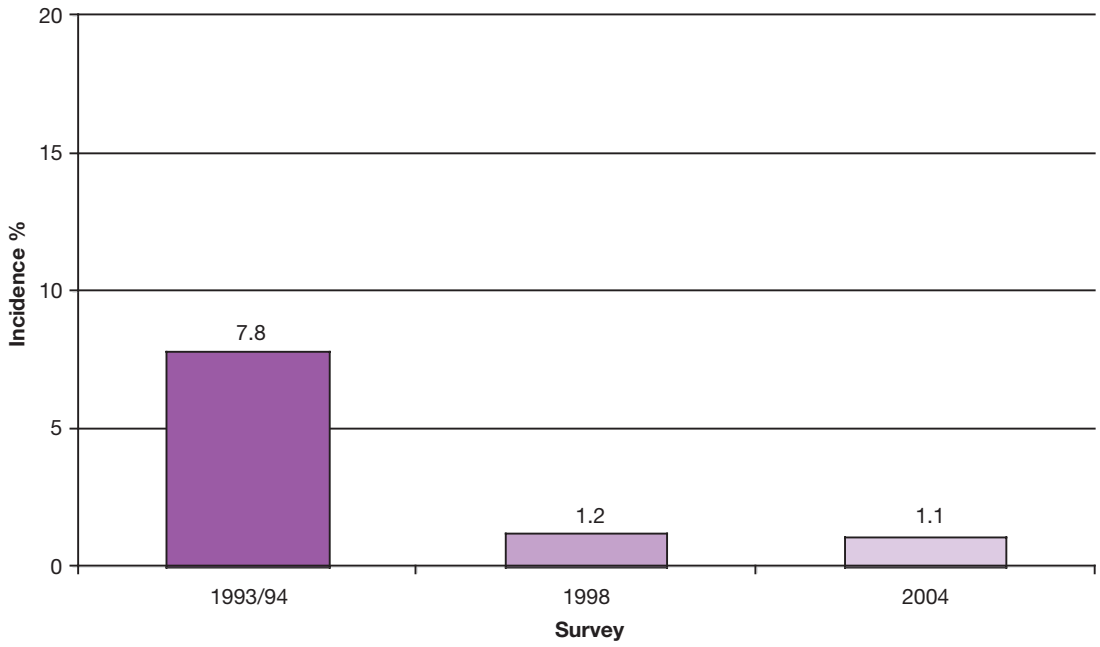
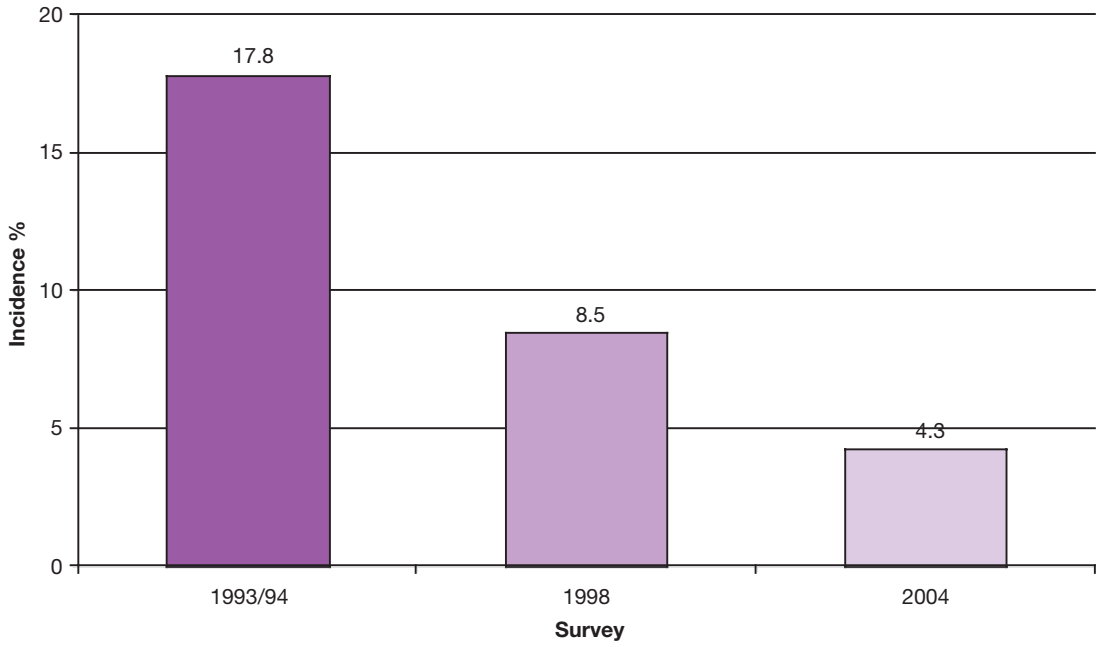


Figure 14: Comparison of the prevalence of E. coli *detections on frozen sheepmeat (LOD 10 cfu/g) from 1993-94 to 2004*



How Australia's results compare with international standards and data

Australian meat is of a very high microbiological quality, reflecting the care taken during animal production and processing. How do the results presented here compare to other international studies and to expectations stated in regulations, specifications and criteria?

Sources of information and how comparisons were made

There are two types of information that can assist us in assessing Australia's quality against the rest of the world. The first is the expectations of the rest of the world expressed through government standards or guidelines and commercial purchasing specifications. The second is on the performance of companies in other countries. These data can be found on government websites, and from publications in international scientific journals. From the latter source data reflect two main lines of investigation; firstly, studies on process changes and secondly, conformance with regulatory specifications set by an importing country.

There are problems in using all of these sources of data for purpose of comparison with the baseline results which can be summarised by saying that they have been collected for different purposes, in different ways and expressed in a different form.

Standards, guidelines and specifications are generally written in one of two forms. They either relate to a shipment (or a lot) of product or to day to day operations of an individual establishment. A certain number of samples are collected from the lot/shipment or from a proportion of production (such as 1 sample per 300 carcases). The baseline study lumps all of the results for Australia together into a single pool and it is not possible to separate them into discrete lots, shipments or days of production.

Samples may also be collected and tested in different ways. Swabbing of various kinds or excision sampling may be prescribed. Different amounts of product may be tested in presence/absence tests and the laboratory procedures (for example incubation temperatures, media used) may give different results. It is not possible to apply a correction factor for differing methods. We will assume that sampling and testing make no material difference to the decisions made, unless otherwise stated.

Since samples are collected in a different way and for a different purpose, results are often expressed in a different form. A decision is made on acceptability of the lot or the process control by dividing the product into three classes: acceptable, marginal and unacceptable (sometimes there is no marginal class). It is difficult to make this classification work for our results because we grouped them all into a single pool. This is especially true for marginally acceptable results which can usually only represent 25–30% of samples. Thus, the marginal results for Australia overall may be very small, but some plants will have none and others may have in excess of the allowed proportion which may require further regullatory action.

Despite all of these challenges, it is possible to make some comparison of the hygienic status of Australian product against the expectations and performance of the rest of the world.

Beef – international standards and specifications

There are a few government standards and commercial specifications for beef products with which Australian baseline data can be compared.

In Europe, EU Decision 2001/471/EC sets criteria based on monitoring of TVC and *Enterobacteriaceae* and, when sponge/swab sampling is used, the performance criteria are as set out in *Table 5*. From the processor's viewpoint, the test is used as an indication of how well faecal contamination is controlled. Microbial levels are expressed as log counts so, in brackets, arithmetic numbers are included.

Table 5: EU microbiological performance criteria for beef carcases (McEvoy, 2004)

	Log mean count/cm²		
	Acceptable	Marginal	Unacceptable
TVC	<2.8 (630)	2.8–4.3 (19,950)	>4.3 (>19,950)
Enterobacteriaceae	<0.8 (6)	0.8–1.8 (63)	>1.8 (>63)

When TVCs and *Enterobacteriaceae* counts for Australian beef carcases were arranged according to the EU performance criteria as set out in *Table 1* almost 95% of TVCs and 99% of *Enterobacteriaceae* counts were in the 'acceptable' category with 0.2% TVCs and 0.6% *Enterobacteriaceae* counts in the 'unacceptable' category (*Table 6*). It should be noted that *Enterobacteriaceae* were not detected on 964/1155 (83.5%) of beef carcase sample.

Table 6: Conformance of Australian beef carcases with the EU microbiological performance criteria (after McEvoy, 2004)

	Number/Total (%)		
Count/cm²	Acceptable	Marginal	Unacceptable
TVC	1095/1155 (94.8)	57/1155 (4.9)	3/1155 (0.2)
Enterobacteriaceae	1140/1155 (98.7)	8/1155 (0.7)	7/1155 (0.6)

In the USA, the MegaReg set performance standards for *E. coli* and *Salmonella*. These standards are based on the random sampling of a proportion of carcases processed within an establishment using on tissue excised from a carcase (*Table 7*).

Table 7: US microbiological performance standards

	Number of samples collected (n)	Number of samples in which the result is allowed to be >m but not >M (c)c	The level of contamination that separates acceptable from marginally acceptable product (m)	The level of contamination that separates marginally acceptable product from unacceptable product (M)
E. coli	13	3	Negative (LOD 5 cfu/cm²)	100 cfu/cm²
Salmonella (steers/heifers)	82	1	Detected	
Salmonella (cows/bulls)	58	2	Detected	

When the results of this baseline was compared to the US MegaReg requirements there was some difficulty because the MegaReg requirements are for individual sets of data from an individual establishment. Nevertheless, a comparison can be attempted. Almost any set of *E. coli* results would meet the US requirements because few establishments would have 3/13 samples positive for *E. coli* and the 95th percentile for positive samples was less than 10 cfu/g (by less sensitive methods than the US MegaReg).

No *Salmonella* was isolated from beef carcases, so it is likely that any product sampled would meet the US MegaReg requirements.

Large food companies also set specifications and, in *Table 8* is presented a commercial specification for frozen, boneless beef destined for grinding in the USA. Many of the criteria are covered by ESAM testing but *E. coli* O157:H7 testing is done via sampling programs which satisfy requirements of the purchasing company.

Table 8: Company microbiological specifications of frozen, boneless beef destined for grinding

Test	Target Maximum	Action Level
TVC (cfu/g)	50,000	200,000
Coliforms (cfu/g)	250	500
E. coli (cfu/g)	75	150
E. coli O157:H7	Negative	Confirmed positive
S. aureus (cfu/g)	50	100
L. monocytogenes	Negative	
Salmonella	Negative	

Baseline data can easily be compared with the commercial specification for boneless beef (*Table 9*).

A small number of samples of Australian product would fail to meet this commercial specification.

Table 9: Conformance of Australian frozen boneless beef with a commercial specification for grinding beef

Test	Target Maximum	Australian conformance
TVC (cfu/g)	50,000	>99% of product had counts <5,000
Coliforms (cfu/g)	250	>98% of product had counts <250
E. coli (cfu/g)	75	>99% of product had counts <10
E. coli O157:H7	Negative	100% of product complied
S. aureus (cfu/g)	50	>98% of product had counts <50
L. monocytogenes	Negative	Not tested in the baseline
Salmonella	Negative	99.9% of product complied

Beef – performance comparisons

A number of published sources allow comparison between this baseline (or other Australian data) and performance of other countries, or individual establishments in other countries.

The USA collates *Salmonella* monitoring data on an annual basis. In the baseline no *Salmonella* was found on beef carcases. However, it is possible to compare historic ESAM data with the US figures. *Table 10* shows prevalence over the period 2000–2003, during which time prevalence has varied from 0.26–0.43% in Australia. In the US there appears to have been a decline from 1.60 to 0.80%, perhaps reflecting the uptake and effectiveness of decontamination interventions such as thermal treatments on carcases.

Table 10: Comparison Australia/USA prevalence of Salmonella *on beef carcases (2000–2003)*

Positives/total (%)	2000	2001	2002	2003
Australia	12/2808 (0.43)	12/4583 (0.26)	12/4687 (0.26)	15/4222 (0.35)
USA	48/3087 (1.55)	62/3871 (1.60)	89/8986 (0.99)	57/7079 (0.80)

The New Zealand government maintains the National Microbiological Database (NMD) which is updated, on a quarterly basis, using data generated by on-plant laboratories under the MILAB system. Operation of the NMD has been described by Hathaway and Cook (1999) and the most recent version of the system is described in Anon. (2003). Unfortunately NZ data have not been reported since 1999. There is a great deal of similarity in the data between Australia and New Zealand. The differences in sampling and testing probably introduce more variation between the countries than might be due to microbiological quality. What comes through is that carcase hygiene, both beef and sheep is very similar in both countries, and at a level which is as low as that of any other country.

The advent of EU standards has led to a number of studies in which countries are keen to assess how well their EU-approved establishments will conform with the EC decision. These studies are useful comparisons for Australian establishments. In Northern Ireland, a baseline survey of seven plants (n=420) which export within the European Union was reported by Murray et al. (2001). A single 50cm x 20cm (1000cm^2) site was sponged on the brisket of chilled carcases and TVC and *Enterobacteriaceae* counts performed. More recently McEvoy et al. (2004) surveyed beef carcases at various stages of processing by swabbing with a cotton tipped stick 50cm^2 areas of chilled carcases (n=36) at five sites (hock, brisket, cranial back, bung and inside round); the mean log TVC/cm^2 of all sites is presented in *Table 11*.

Table 11: Comparison Australia/Northern Ireland TVC and Enterobacteriaceae *on chilled beef carcases*

		Mean log TVC/cm^2 (arithmetic count)	*Enterobacteriaceae* prevalence (mean log/cm^2 of positives)
Australia	Baseline study	1.28 (19)	16.5 (0.4)
Northern Ireland	Murray et al. 2001	2.80 (630)	21.4 (2.5)
Northern Ireland	McEvoy et al. 2004	2.72 (309)	28.4 (1.3)

The results (*Table 11*) indicate a much lower mean log TVC on Australian carcases, together with lower prevalence of *Enterobacteriaceae* and mean log of positives samples.

The prevalence of *Salmonella* was monitored by McEvoy et al. (2003) on beef carcases after entry to the chiller at an abattoir in Northern Ireland. The entire outer surface of a carcase was sponged and immunomagnetic separation used to recover *Salmonella* from enriched buffered peptone water samples. Salmonella was isolated in 19/250 (7.6%) of carcase swabs. While *Salmonella* was not isolated from the 1155 baseline study carcases it must be emphasised that the sample area (300cm^2) was much smaller than that of the Northern Ireland study and did not employ immunomagnetic separation, both of which make it much more likely that *Salmonella* would be found.

In Sweden, Hansson (2001) reported on the microbiological status of beef carcases (n=100) from four high capacity Swedish plants. Two 100cm^2 sites (loin and sternum) on pre-chill carcases were sampled using moistened cotton swabs and TVC and presumptive *E. coli* counts made. The results (*Table 12*) indicate lower mean log TVCs and prevalence of *E. coli* for Australian carcases, though differences in technique and stage of sampling should be considered.

Table 12: Comparison Australia/Sweden TVC and E. coli *on chilled carcases*

Mean log TVC/cm^2 (arithmetic count)		***E. coli* prevalence (%)**	
Australia	Sweden	Australia	Sweden
1.28 (19)	2.59 (390)	5.5	34

In Switzerland, Zweifel et al. (2005) monitored TVC (30°C) and *Enterobacteriaceae* of beef carcases (n=800) at five Swiss EU-approved abattoirs with combined annual production >50,000kg. Four sites (rump, flank, brisket and neck) were sampled prior to chilling using a wet and dry swab over an area of 100cm^2 (total 400cm2). Australian beef carcases had lower mean log TVCs and *Enterobacteriaceae* prevalence (*Table 13*), though there were differences in testing.

Table 13: Comparison Australia/Switzerland TVC and Enterobacteriaceae *on beef carcases (Zweifel, et al., 2005)*

Mean $\log_{10}$ TVC/cm^2 (arithmetic count)		**Enterobacteriaceae prevalence (mean $\log_{10}$/cm^2 of positives)**	
Australia	Switzerland	Australia	Switzerland
1.28 (19)	2.82 (660)	16.5 (0.4)	30.7 (0.88)

Sheep – performance comparison

There have been a number of studies on sheep carcase hygiene that may be used for comparisons.

In Canada, Gill and Baker (1998) found that unchilled sheep carcases had $\log_{10}$ TVC/cm^2 at the shoulder, loin and leg of 2.81, 2.80 and 2.56, respectively. These counts are about the same as found in Australia (given that unchilled carcases tend to have a higher count).

In USA, Duffy et al. (2001) surveyed 5,042 chilled lamb carcases at six USA plants finding mean log TVCs of 4.23/cm^2 (spring) and 4.61/cm^2 (winter) and overall prevalence of *E. coli* of 66.2%. Australian results are much better than these results.

In Switzerland Zweifel and Stephan (2003) used a wet/dry double swab to sample 10 sites (each 40cm^2) on carcases which had been chilled for less than 3 hours in three EU-approved abattoirs. Median log TVCs ranged between 2.5–3.8 cfu/cm^2 with the brisket and neck sites the most highly contaminated. *Enterobacteriaceae* were isolated from 68.1% of carcases. Australian results are a little better on TVCs but seem to be a lot better on *Enterobacteriaceae*, though sampling sites differed.

Global comparisons in summary

Two major findings come out of the summary. Firstly, the lack of standard sampling, testing and reporting makes direct comparisons difficult, even for experienced meat microbiologists. This is especially so when testing for organisms which are detected only rarely, such as *E. coli* and *Salmonella*. Then, sampling and testing methods can dramatically affect the chances of detection – and therefore comparisons become difficult.

The second major finding is that, whenever reliable comparisons are made, Australian product is, in most cases, of superior microbiological quality.

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Appendix 1: Key facts on bacteria

Total viable count (TVC) is a term which refers to all the bacteria that grow in agar under the conditions of the test (conducted at 25°C over 4 days). It is likely to be a similar number to tests for Aerobic Plate Count (APC) or standard plate count (SPC).

Enterobacteriaceae is a family of bacteria that includes several foodborne pathogens. They share some common characteristics such as the ability to ferment certain sugars and grow in the presence of bile. For this reason they can survive in the gut of man and animals. Some are considered a normal component of the bacterial community that inhabits the gut, and others may cause disease. Some *Enterobacteriaceae* are associated with plants rather than animals. Some countries, such as those in Europe, choose to use *Enterobacteriaceae* as an indicator of hygiene in the preparation of foods. In the case of meat and meat products, this is a reasonable choice because there are many Enterobacteriaceae found on the hide or fleece and in the gut of cattle and sheep.

Coliforms are a sub-group of the family *Enterobacteriaceae*. They ferment the sugar, lactose and grow in the presence of bile. They are also found in high numbers on the hide or fleece and in the gut of cattle and sheep, but are not as likely to be found in association with plants. Tests for coliforms have been popular for a long time because they are easy to perform and give a good indictor of hygiene. Coliform tests have been popular in English-speaking countries.

E. coli, short for *Escherichia coli*, is a species of coliform. It is generally found at high concentrations on the hide/fleece and in the gut of cattle and sheep. *E. coli* is considered to be more than an indicator of good hygiene because some strains of *E. coli* cause disease, particularly diarrhoea in man. The presence of *E. coli* is not unexpected occasionally on carcases because of the high numbers that may be found on or in animals, but high levels increase the chance that a disease-causing (pathogenic) strain of *E. coli* could be present.

***E. coli* O157:H7** is a particular strain (or serotype) of *E. coli* that has been known to cause epidemics of food-borne illness which, in some cases, may progress to serious kidney damage (Haemolytic Uraemic Syndrome) and sometimes, death. Outbreaks of illness due to this strain of *E. coli* are rare in Australia and have not been associated with meat. However, the presence of this serotype on meat represents a health risk if the meat is consumed without adequate cooking or fermentation.

Salmonella is a genus of *Enterobacteriaceae* that contains many distinct types of strains. Most of the *Salmonella* types (for example, *Salmonella typhimurium*) are pathogenic for man, causing gastroenteritis. Animals are considered to be the main source of *Salmonella* infections in man.

Campylobacter is a genus of bacteria that contains several species that cause gastroenteritis in man. In fact, there are more illnesses reported in Australia each year due to *Campylobacter* than Salmonella. It is an organism that is found in poultry and cattle quite frequently. Only low levels of *Campylobacter* are needed to cause an infection in man, so it is important to know whether this bacterium is found in meat that is reaching consumers.

Staphylococcus aureus is a bacterium that when present in large numbers in food can produce sufficient enterotoxin to cause illness in humans. Although an important pathogen, *S. aureus* is commonly carried on the body of healthy humans and is typically found in the nose, throat and skin. It may also be found on the skin of cattle, and these strains are considered less likely to produce enterotoxin. While it is unrealistic to expect that *S. aureus* could be eliminated, prevention of meat contamination relies on good hygiene and minimal handling practices. The test performed detects coagulase positive staphylococci which are probably all *S. aureus*, but may include a couple of other non-pathogenic species, which are found in animals.

Appendix 2: Testing methods

Sample handling, dilutions, inoculation and limit of detection

Beef and sheep carcases (sponge samples)

a) ***E. coli* 0157 H7:** Add 225mL modified EC broth +novobiocin broth to sponge bag. Squeeze the bag 10 times and incubate at 37ºC overnight. The following day, the Dynalbeads anti O157 method was followed as per the manufacturer's instructions. Report as present/absent in 300cm^2 for beef carcases and present/absent in 75cm^2 for sheep carcases.

b) ***Salmonella:*** AS1766.2.5-1991 – Add 225mL buffered peptone water and squeeze the bag 10 times. Incubate at 37ºC for 20 h. Aliquots of resuscitated cultures were inoculated into mannitol selenite cystine broth for incubation at 37°C for 24 hours and into Rappaport-Vassiliadis medium for incubation at 42°C for 24 hours. Each enriched culture was inoculated onto brilliant green agar and lysine mannitol glycerol agar and incubated at 37°C for 48 hours. Report as present/absent in 300cm^2 for beef carcases and present/absent in 75cm^2 for sheep carcases.

c) ***Campylobacter; E. coli* type 1; coliforms; Staphylococci; TVC, and *Enterobacteriaceae*:** Squeeze the bag 10 times and remove 2mL TVC, 2mL *E. coli*, 0.2mL *S. aureus*, 2mL *Enterobacteriaceae*. Remove an extra 5.3mL from bag and add 100mL Preston medium to sponge bag.

E. coli type 1/coliforms	AOAC 991.14 – Pipette 1mL onto duplicate 3M coliform/*E. coli* Petrifilms, incubate at 37°C for 48 h. Limit of detection: Beef: Average CFU (25mL/300cm^2) = 0.08cfu/cm^2 Sheep: Average CFU (25mL/75cm^2) = 0.33cfu/cm^2
Coagulase positive Staphylococci	AS1766.2.4-1995 – Pipette 0.1mL onto 2 Baird Parker agar plates. Incubate at 37°C for 48 h. confirmation by tube coagulase. Limit of detection: Beef: Average CFU(25mL/300cm^2) x 10 = 0.8cfu/cm^2 Sheep: Average CFU (25mL/75cm^2) x 10 = 3.3cfu/cm^2
TVC	AS1766.2.1-1991 – Prepare 10-fold serial dilutions (10^{-1}–10^{-4}). The actual dilutions required may be determined by experience with the type of sample. Proceed as per pour plate method. The TVC plates are to be incubated at 25ºC/4days. Use duplicate plates at each dilution. Limit of detection: Beef: Average CFU (25mL/300cm^2) x10 = 0.8cfu/cm^2 Sheep: Average CFU (25mL/75cm^2) x 10 = 3.3cfu/cm^2
Enterobacteriaceae	AFNOR 3MO/6-09/97 – Prepare 10-fold serial dilutions (10^{-1}–10-^{3}) Pipette 1mL onto duplicate 3M Enterobacteriaceae Petrifilms, incubate at 37°C for 24 h. Limit of detection: Beef: Average CFU (25mL/300cm^2) = 0.08cfu/cm^2 Sheep: Average CFU (25mL/75cm^2) = 0.33cfu/cm^2
Campylobacter	AS1766.2.13-1991 – Incubate in Preston Broth at 42°C for 48 h. Plate onto Preston Agar & Skirrow Agar and incubate at 42°C for 48 h. Limit of detection: Beef: Present/absent in 162cm^2 Sheep: Present/absent in 40.5cm^2

Beef and sheep frozen boneless

From 150g sample, subsample the following:

- 25g beef samples only for ***E. coli* 0157** H7: Add 225mL modified EC broth +novobiocin broth to sponge bag. Squeeze the bag 10 times and incubate at 37°C overnight. The following day, the Dynalbeads anti O157 method was followed as per the manufacturer's instructions. Report as present/absent in 25g.
- 25g for ***Salmonella***: AS1766.2.5-1991 – Add 225mL buffered peptone water and squeeze the bag 10 times. Incubate at 37°C for 20 h. Aliquots of resuscitated cultures were inoculated into mannitol selenite cystine broth for incubation at 37°C for 24 hours and into Rappaport-Vassiliadis medium for incubation at 42°C for 24 hours. Each enriched culture was inoculated onto brilliant green agar and lysine mannitol glycerol agar and incubated at 37°C for 48 hours.
- 25g for ***Campylobacter***: AS1766.2.13-1991 – Incubate in 100mL Preston Broth at 42°C for 48 h. Plate onto Preston Agar & Skirrow Agar and incubate at 42°C for 48 h. Report as present/absent in 25g
- 10g for ***E. coli* Type 1; coliforms; Staphylococci; TVC, *Enterobacteriaceae*:** Add 90mL 0.1% PW to make a 1:10 dilution and stomach for 2 minutes. (This is the '-1' dilution). From this solution, do the following:

E. coli type 1/coliforms	AOAC 991.14 – Pipette 1mL onto duplicate 3M coliform/*E. coli* Petrifilms, incubate at 37°C for 48 h. Limit of detection: Average CFU x 10 = 10cfu/g
Staphylococci	Pipette 0.5mL onto 4 Baird Parker agar plates. Incubate at 37°C for 48 h. Confirmation by tube coagulase. Limit of detection: Average CFU x10 = 10cfu/g
TVC	AS1766.2.1-1991 – Prepare 10-fold serial dilutions (10^{-1}–10^{-4}). The actual dilutions required may be determined by experience with the type of sample. Proceed as per pour plate method. The TVC plates are to be incubated at 25ºC/4days. Use duplicate plates at each dilution. Limit of detection: Average CFU x10 = 10cfu/g
Enterobacteriaceae	AFNOR 3MO/6-09/97 – Prepare 10-fold serial dilutions (10^{-1}–10^{-3}). Pipette 1mL onto duplicate 3M Enterobacteriaceae Petrifilms, incubate at 37°C for 24 h. Limit of detection: Average CFU x10 = 10cfu/g

Appendix 3: Microbiological data for beef

Appendix 3.A: Microbiological profile of beef carcases

Table A3A.1: Log transformed Total Viable Count (TVC at 25°C) of Australian beef carcases tested by sponging 300cm^2 of surface area.

	Overall	Summer	Winter
Number of samples	1147	550	597
Mean log	1.33	1.42	1.24
Median log	1.26	1.32	1.15
Standard deviation	0.79	0.80	0.78
90th percentile	2.34	2.49	2.21
95th percentile	2.81	2.89	2.72
99th percentile	3.51	3.76	3.34
Maximum	5.84	5.84	4.86

Table A3A.2: Prevalence of generic E. coli *on sponges from Australian beef carcases and descriptive statistics for log transformed counts from positive sponges*

	Overall	Summer	Winter
Number of samples	1155	558	597
Prevalence (% detection)[a]	4.9	7.3	2.7
Mean of $\log_{10}$ count [b]	-0.42	-0.38	-0.52
Median[b]	-0.77	-0.69	-0.77
Standard deviation[b]	0.70	0.72	0.64
90th percentile	0.68	0.68	0.72
95th percentile	0.89	1.05	0.89
99th percentile	1.72	1.72	0.89
Maximum	1.72	1.72	0.89

[a] Limit of detection 0.08 cfu/cm^2

[b] $\log_{10}$ transformed counts of positive samples only

Table A3A.3: Prevalence (%) of Salmonella*,* E. coli *O157:H7, Coagulase positive staphylococci, and* Campylobacter *in sponges from Australian beef carcases*

	Overall	Summer	Winter
Number of samples	1155	558	597
Salmonella	0	0	0
E. coli O157:H7	0.1	0.2	0
Coagulase positive staphylococci	20.1	28.4	12.4
Campylobacter	0	0	0

Table A3A.4: Prevalence of coagulase positive staphylococci on sponges from Australian beef carcases and descriptive statistics for log transformed counts from positive samples

	Overall	Summer	Winter
Number of samples	1147	550	597
Prevalence (% detection)[a]	20.1	28.4	12.4
Mean of $\log_{10}$ count [b]	0.56	0.76	0.43
Median[b]	0.59	0.70	0.35
Standard deviation[b]	0.61	0.64	0.49
90th percentile[b]	1.45	1.54	1.18
95th percentile[b]	1.83	2.1	1.38
99th percentile[b]	2.43	2.52	1.58
Maximum[b]	2.96	2.96	1.58

[a] Limit of detection 0.08 cfu/g

[b] Log_{10} transformed counts of positive samples only

Table A3A.5: Prevalence of coliforms on sponges from Australian beef carcases and descriptive statistics for log transformed counts from positive samples

	Overall	Summer	Winter
Number of samples	1147	550	597
Prevalence (% detection)[a]	10.4	15.1	6.0
Mean of log_{10} count[b]	-0.29	-0.21	-0.45
Median[b]	-0.48	-0.48	-0.77
Standard deviation[b]	0.80	0.83	0.71
90th percentile[b]	0.72	0.76	0.43
95th percentile[b]	1.58	1.60	0.96
99th percentile[b]	2.11	2.23	2.12
Maximum[b]	2.23	2.23	2.12

[a] Limit of detection 0.08 cfu/g

[b] Log_{10} transformed counts of positive samples only

Table A3A.6: Prevalence of Enterobacteriaceae *on sponges from Australian beef carcases and descriptive statistics for log transformed counts from positive samples*

	Overall	Summer	Winter
Number of samples	1147	550	597
Prevalence (% detection)[a]	16.1	23.1	9.7
Mean of log_{10} count[b]	-0.40	-0.31	-0.59
Median[b]	-0.77	-0.77	-0.77
Standard deviation[b]	0.80	0.84	0.66
90th percentile[b]	0.76	0.79	0.48
95th percentile[b]	1.26	1.66	0.88
99th percentile[b]	2.23	2.23	2.20
Maximum[b]	2.26	2.26	2.20

[a] Limit of detection 0.08 cfu/g

[b] Log_{10} transformed counts of positive samples only

Appendix 3.B: Microbiological profile of boneless frozen beef

Table A3B.1: Log transformed Total Viable Count (TVC at 25°C) of Australian frozen boneless beef (log cfu/g)

	Overall	Summer	Winter
Number of samples	1082	511	571
Mean log	1.19	1.54	0.88
Median log	1.32	1.56	1.04
Standard deviation	0.92	0.88	0.83
90th percentile	2.30	2.62	1.82
95th percentile	2.70	2.97	2.26
99th percentile	3.52	3.78	2.93
Maximum	5.49	4.72	5.49

Table A3B.2: Prevalence of generic E. coli *in core samples of Australian frozen boneless beef and descriptive statistics for log transformed counts from positive samples*

	Overall	Summer	Winter
Number of samples	1082	511	571
Prevalence (% detection)[a]	1.1	1.8	0.5
Mean of $\log_{10}$ count[b]	1.90	2.07	1.38
Median[b]	1.78	2.40	1.54
Standard deviation[b]	0.64	0.64	0.33
90th percentile[b]	2.67	2.79	1.60
95th percentile[b]	2.79	2.79	1.60
99th percentile[b]	2.79	2.79	1.60
Maximum[b]	2.79	2.79	1.60

[a] Limit of detection 10 cfu/g

[b] Log_{10} transformed counts of positive samples only

Table A3B.3: Prevalence (%) of Salmonella, E. coli *O157:H7, Coagulase positive staphylococci, and* Campylobacter *in samples of Australian frozen boneless beef*

	Overall	Summer	Winter
Number of samples	1082	511	571
Salmonella	0.1	0	0.2
E. coli O157:H7	0	0	0
Coagulase positive staphylococci	2.6	4.5	0.9
Campylobacter	0	0	0

Table A3B.4: Prevalence of coagulase positive staphylococci in core samples of Australian frozen boneless beef and descriptive statistics for log transformed counts from positive samples

	Overall	Summer	Winter
Number of samples	1082	511	571
Prevalence (% detection)[a]	2.6	4.5	0.9
Mean of $\log_{10}$ count[b]	1.53	1.59	1.28
Median[b]	1.63	1.67	1.00
Standard deviation[b]	0.45	0.42	0.52
90th percentile[b]	2.18	2.15	2.18
95th percentile[b]	2.26	2.26	2.18
99th percentile[b]	2.32	2.32	2.18
Maximum[b]	2.32	2.32	2.18

[a] Limit of detection 10 cfu/g

[b] Log_{10} transformed counts of positive samples only

Table A3B.5: Prevalence of coliforms in core samples of Australian frozen boneless beef and descriptive statistics for log transformed counts from positive samples

	Overall	Summer	Winter
Number of samples	1082	511	571
Prevalence (% detection)[a]	3.8	6.8	1.1
Mean of $\log_{10}$ count[b]	1.57	1.60	1.25
Median[b]	1.48	1.48	1.18
Standard deviation[b]	0.53	0.54	0.26
90th percentile[b]	2.48	2.49	1.60
95th percentile[b]	2.61	2.74	1.60
99th percentile[b]	2.93	2.93	1.60
Maximum[b]	2.93	2.93	1.60

[a] Limit of detection 10 cfu/g

[b] Log_{10} transformed counts of positive samples only

Table A3B.6: Prevalence of Enterobacteriaceae *in core samples of Australian frozen boneless beef and descriptive statistics for log transformed counts from positive samples*

	Overall	Summer	Winter
Number of samples	1082	511	571
Prevalence (% detection)[a]	5.0	8.8	1.6
Mean of $\log_{10}$ count[b]	1.56	1.58	1.44
Median[b]	1.54	1.54	1.30
Standard deviation[b]	0.52	0.53	0.42
90th percentile[b]	2.57	2.60	2.23
95th percentile[b]	2.76	2.70	2.23
99th percentile[b]	2.95	2.95	2.23
Maximum[b]	2.95	2.95	2.23

[a] Limit of detection 10 cfu/g

[b] Log_{10} transformed counts of positive samples only

Appendix 4: Microbiological data for sheep

Appendix 4.A: Microbiological profile of sheep carcases

Table A4A.1: Log transformed Total Viable Count (25° incubation) from sponges of Australian chilled sheep carcases tested by sponging 75cm² of surface area

	Overall	Summer	Winter
Samples	1117	557	560
Mean	2.28	2.43	2.13
Median log	2.28	2.32	2.23
Standard deviation	0.89	0.84	0.91
90th percentile	3.32	3.43	3.22
95th percentile	3.67	3.90	3.52
99th percentile	4.75	5.00	3.92
Maximum	5.40	5.40	4.83

Table A4A.2: Prevalence of generic E. coli *in sponges from Australian sheep carcases descriptive statistics for log transformed counts from positive sponges*

	Overall	Summer	Winter
Number of samples	1117	557	560
Prevalence (%)[a]	32.9	31.2	34.6
Mean of log_{10} count[b]	0.28	0.36	0.20
Median	0.11	0.11	0.11
Standard deviation	0.72	0.81	0.63
90th percentile	1.26	1.52	1.11
95th percentile	1.73	2.11	1.32
99th percentile	2.80	2.83	2.70
Maximum	3.04	2.83	3.04

[a] Limit of detection 3.3 cfu/cm²

[b] Log_{10} transformed counts of positive samples only

Table A4A.3: Prevalence of Salmonella*,* E. coli *O157:H7, Coagulase positive staphylococci, and* Campylobacter*, in sponges from Australian chilled sheep carcases (2004)*

	Overall	Summer	Winter
Salmonella	0/1117	0/557	0/560
E. coli O157:H7	6/1117 (0.6%)	3/557 (0.5%)	4/560 (0.7%)
Coagulase positive staphylococci	15.9%	18.9%	13.0%
Campylobacter	4/1117 (0.4%)	0/555	4/559 (0.7%)

Table A4A.4: Prevalence of coagulase positive staphylococci on sponges from Australian sheep carcases and descriptive statistics for log transformed counts from positive samples

	Overall	Summer	Winter
Number of samples	1117	557	560
Prevalence (% detection)[a]	15.9	18.9	13.0
Mean of $\log_{10}$ count[b]	1.27	1.27	1.27
Median[b]	1.23	1.23	1.23
Standard deviation[b]	0.51	0.55	0.46
90th percentile[b]	1.94	2.11	1.90
95th percentile[b]	2.32	2.32	2.04
99th percentile[b]	2.52	2.52	2.52
Maximum[b]	2.63	2.63	2.52

[a] Limit of detection 3.3 cfu/cm^2

[b] Log_{10} transformed counts of positive samples only

Table A4A.5: Prevalence of coliforms on sponges from Australian sheep carcases and descriptive statistics for log transformed counts from positive samples

	Overall	Summer	Winter
Number of samples	1117	557	560
Prevalence (% detection)[a]	38.6	39.3	37.9
Mean of $\log_{10}$ count[b]	0.31	0.39	0.22
Median[b]	0.11	0.11	0.11
Standard deviation[b]	0.76	0.84	0.67
90th percentile[b]	1.30	1.67	1.18
95th percentile[b]	1.92	2.32	1.43
99th percentile[b]	2.82	2.83	2.32
Maximum[b]	3.04	2.85	3.04

[a] Limit of detection 0.33 cfu/g

[b] Log_{10} transformed counts of positive samples only

Table A4A.6: Prevalence Enterobacteriaceae *on sponges from Australian sheep carcases and descriptive statistics for log transformed counts from positive samples*

	Overall	Summer	Winter
Number of samples	1117	557	560
Prevalence (% detection)[a]	47.2	47.8	46.6
Mean of $\log_{10}$ count[b]	0.39	0.48	0.30
Median[b]	0.11	0.30	0.11
Standard deviation[b]	0.78	0.84	0.71
90th percentile[b]	1.43	1.70	1.23
95th percentile[b]	2.04	2.23	1.63
99th percentile[b]	2.80	2.86	2.80
Maximum[b]	3.11	2.92	3.11

[a] Limit of detection 0.33 cfu/g

[b] Log_{10} transformed counts of positive samples only

Appendix 4.B: Microbiological profile of frozen boneless sheepmeat

Table A4B.1: Log transformed Total Viable Count (25° incubation) of frozen, boneless sheepmeat (log_{10}cfu/g)

	Overall	Summer	Winter
Samples	560	302	258
Mean	1.81	2.02	1.57
Median log	1.83	2.06	1.66
Standard deviation	0.94	0.87	0.96
90th percentile	2.96	3.00	2.79
95th percentile	3.18	3.30	3.04
99th percentile	3.78	3.82	3.69
Maximum	4.99	4.99	3.86

Table A4B.2: Prevalence of generic E. coli *in core samples of Australian boneless sheepmeat and descriptive statistics for log transformed counts from positive samples*

	Overall	Summer	Winter
Number of samples	560	302	258
Prevalence (%)[a]	4.3	5.6	2.7
Mean of log_{10} count[b]	2.02	2.28	1.41
Median	1.74	2.20	1.30
Standard deviation	0.94	0.97	0.48
90th percentile	3.48	3.62	2.26
95th percentile	3.62	4.48	2.26
99th percentile	4.48	4.48	2.26
Maximum	4.48	4.48	2.26

[a] Limit of detection 10 cfu/g

[b] Log_{10} transformed counts of positive samples only

Table A4B.3: Prevalence of Salmonella*,* E. coli *O157:H7, Coagulase positive staphylococci and* Campylobacter*, in samples of frozen, boneless sheepmeat (2004)*

	Overall	Summer	Winter
Salmonella	3/557 (0.5%)	1/299 (0.3%)	2/258 (0.8%)
E. coli O157:H7	1/557 (0.2%)	1/300 (0.3%)	0/257
Coagulase positive staphylococci	14.1%	22.5%	4.3%
Campylobacter	1/539 (0.2%)	0/295	1/244 (0.4%)

Table A4B.4: Prevalence of coagulase positive staphylococci in core samples of Australian frozen boneless sheepmeat and descriptive statistics for log transformed counts from positive samples

	Overall	Summer	Winter
Number of samples	560	302	258
Prevalence (% detection)[a]	14.1	22.5	4.3
Mean of $\log_{10}$ count[b]	1.74	1.78	1.45
Median[b]	1.72	1.78	1.30
Standard deviation[b]	0.61	0.61	0.49
90th percentile[b]	2.58	2.75	2.04
95th percentile[b]	2.94	2.94	2.43
99th percentile[b]	3.38	3.38	2.43
Maximum[b]	3.38	3.38	2.43

[a] Limit of detection 10 cfu/g

[b] Log_{10} transformed counts of positive samples only

Table A4B.5: Prevalence coliforms in core samples of Australian frozen boneless sheepmeat and descriptive statistics for log transformed counts from positive samples

	Overall	Summer	Winter
Number of samples	560	302	258
Prevalence (% detection)[a]	11.6	12.9	10.1
Mean of $\log_{10}$ count[b]	1.69	1.86	1.39
Median[b]	1.48	1.65	1.30
Standard deviation[b]	0.74	0.85	0.41
90th percentile[b]	2.65	2.92	1.96
95th percentile[b]	2.92	3.76	2.30
99th percentile[b]	4.70	4.70	2.65
Maximum[b]	4.70	4.70	2.65

[a] Limit of detection 10 cfu/g

[b] Log_{10} transformed counts of positive samples only

TABLE A4B.6: Prevalence Enterobacteriaceae *in core samples of Australian frozen boneless sheepmeat and descriptive statistics for log transformed counts from positive samples*

	Overall	Summer	Winter
Number of samples	560	302	258
Prevalence (% detection)[a]	14.1	15.6	12.4
Mean of $\log_{10}$ count[b]	1.68	1.87	1.40
Median[b]	1.48	1.70	1.30
Standard deviation[b]	0.74	0.84	0.45
90th percentile[b]	2.79	3.04	2.04
95th percentile[b]	3.04	3.63	2.38
99th percentile[b]	4.74	4.75	2.79
Maximum[b]	4.75	4.75	2.79

[a] Limit of detection 10 cfu/g

[b] Log_{10} transformed counts of positive samples only

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