Microbiological Quality of Australian Sheep Meat

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ABSTRACT

Microbiological quality of sheep carcasses and boneless sheep meat produced in Australia was surveyed during the period June to November 1998. Sponge samples were collected from 917 carcasses, and meat samples were drilled from 467 cartons of frozen boneless meat. Carcass and boneless meat samples were respectively collected from 7 and 10 establishments that concentrated on export, and from 36 and 5 establishments supplying the Australian domestic market of which 31 were very small plants slaughtering cattle and sheep but no more than 1,200 sheep equivalents per week. The mean log total viable counts were 3.55/cm² and 3.30/g for carcasses and boneless meat, respectively. *Escherichia coli* was detected on 29.2% of carcasses and 24.5% of boneless meat samples and coagulase-positive staphylococci on 24.1% of carcasses, and 38.6% of boneless meat samples. *Salmonella* was detected on 0.1% of carcasses and 1.3% of boneless meat samples, *E. coli* O157:H7 was recovered from 0.7% of carcasses and 1.3% of boneless sheep meat. There were statistically significant differences between establishment types for some microbiological criteria, although there were no significant differences in prevalence of *Salmonella* or *E. coli* O157:H7 between establishment types. While there were differences in sampling and microbiological techniques between this study and another conducted in 1993 to 1994 that require detailed consideration, there were small but significant improvements in several microbiological criteria for boneless meat. While data that would allow for comparison of carcass data were not gathered, it is unlikely that improvements in the microbiological quality of boneless sheep meat could accrue without improvements to carcasses.

The hygienic quality of red meats continues to attract attention globally with regulatory bodies considering it a major cause of food poisoning incidents. By far the most attention is focused on beef, particularly in fractions that are intended for grinding into hamburger patties. By contrast, there is little information on the hygienic quality of sheep meats, even though they may be components in a range of processed meat products.

In 1993 to 1994 the Australian meat industry commissioned a baseline study of the microbiological quality of Australian red meat, including sheep meat (4). In the ensuing period the Australian meat industry has undergone dramatic change, with all slaughter and boning facilities implementing comprehensive quality assurance plans in which the hazard analysis critical control point concept is an integral part. Commensurate with implementing quality assurance systems, there have been significant inputs in operator training, improved refrigeration, and provision of laboratory facilities.

It was against this background that, in 1998, the industry commissioned a second baseline study of the microbiological quality of Australian meat based predominantly on sponge samples of carcasses that had been chilled for at least 12 h and on samples drilled from frozen meat. This study also incorporated carcass samples from very small plants (VSPs) that are also known as slaughterhouses.

The aims of the present study were to monitor the microbiological quality of meat produced by current manufacturing practices and compare that quality with the first baseline study (4).

MATERIALS AND METHODS

Study design. The survey was undertaken between June and November 1998. Samples were taken at each of 15 large and medium-sized slaughter and 15 boning establishments located across all states, selected to represent sheep meat production statistically in Australia. Slaughter volume ranged from around 1,000 head/day to 5,000/day, with most plants slaughtering 2,000 to 3,000 head/day. In addition, 31 VSPs were sampled in Queensland, South Australia, and Tasmania. VSPs slaughter both cattle and sheep but less than 1,200 sheep equivalents per week (eight sheep are equivalent to one cattle beast). They are located in remote areas of Australia and undertake a service kill for local farmers and retail beef, lamb, mutton, and pork to local, small communities. Irrespective of its throughput, each establishment was sampled on one occasion, although each state was visited on two to four separate occasions (contingent on the total number of samples taken) for 3 days at a time to minimize any environmental effect of time of year. For establishments other than VSPs 34 to 39 samples were taken either from carcasses that had been chilled for at least 12 h or from frozen cartons of boneless meat. For VSPs, 1 to 30 samples were taken from carcasses that had been chilled for at least 12 h. Samples were collected on Tuesdays, Wednesdays, and Thursdays by trained technicians.

Carcass sampling. A polyurethane sponge (Micro Diagnostics, Brisbane, Australia) moistened with buffered peptone water (10 ml) was used to sample carcasses, a composite sample being taken by sponging a 25-cm² area at each of the rump, flank, and

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brisket regions of the carcass. Two sponge samples were collected from the right and left sides of each carcass.

Frozen boneless sheep meat sampling. Samples of boneless meat were collected from frozen cartons that had been in the freezer no longer than 6 months. Approximately 100 g in total of meat was drilled from five to six different locations in each carton using a sterile drill bit and transferred into a sterile sticker bag using a sterile pair of kitchen tongs.

Transport of samples to the laboratory. All samples were packed in insulated containers with chiller packs and transported to a National Association of Testing Authorities accredited laboratory for testing. Upon arrival at the laboratory, samples were placed in a refrigerator until analyzed. To standardize the time between sample collection and analysis, samples were analyzed at least 18 h after collection. In most cases this was on the day of arrival at the laboratory. 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 with regard to any abnormally high results. Temperature histories obtained from data loggers enclosed with the samples during transit were used to assist this decision in order to determine whether temperature abuse had occurred during transit.

Microbiological analysis of sponge samples. One sponge sample was used for detection of *Escherichia coli* O157:H7 and the other for all other analyses (total viable count [TVC], *E. coli*, coagulase-positive staphylococci, and *Salmonella*). To eliminate bias between the right and left sides of the carcass, sponges were directed at random to either test.

**Determination of *E. coli* O157:H7.** Modified EC broth (Amyl Media, Melbourne, Australia) plus 0.002% novobiocin (240 ml) was added to one of the sponge bags and the sample sponge squeezed by hand 10 times prior to incubation at 37°C overnight. The following day, the Dynalbeads anti O157 method (Dynal Australia, Melbourne, Australia) was followed as per the manufacturer’s instructions and the Association of Official Analytical Chemist’s method 991.4 (3). The limit of detection was 0.33 CFU/cm². Coagulase-positive staphylococci were determined using the Australian Standard method AS 1766.2.4 (3). E. coli and coagulase-positive staphylococci testing, buffered peptone water (200 ml) was added to the sponge bag, and the bag was incubated for 24 h at 37°C to allow resuscitation of damaged cells. Aliquots of resuscitated cultures were inoculated into mannitol selenite cystine broth (Oxoid, Melbourne, Australia) for incubation at 37°C for 2 days. Colonies were counted as per the manufacturer’s instructions and the Association of Official Analytical Chemist’s method 991.4 (3). The limit of detection was 3.3 CFU/cm².

**Determination of TVC, *E. coli* biotype 1, coagulase-positive staphylococci, and *Salmonella*.** Buffered peptone (Oxoid, Melbourne, Australia) water (40 ml) was added to the second sponge bag that was squeezed by hand 10 times. Serial dilutions were prepared in 0.1% peptone water using 1-ml aliquots. Duplicate pour plates were prepared according to the Australian Standard method AS 1766.1.3 (1) and incubated at 25°C for 4 days at which time the colonies were counted and the count/cm² recorded.

**TABLE 2.** *E. coli* counts of Australian sheep meat (1998)

<table>
<thead>
<tr>
<th>No. samples</th>
<th>Carcasses</th>
<th>Boneless meat</th>
</tr>
</thead>
<tbody>
<tr>
<td>921</td>
<td>469</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>29.2</td>
<td>24.5</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>1.07</td>
<td>0.94</td>
</tr>
<tr>
<td>Median</td>
<td>0.17</td>
<td>0.94</td>
</tr>
<tr>
<td>80th percentile</td>
<td>0.60</td>
<td>0.65</td>
</tr>
<tr>
<td>95th percentile</td>
<td>Not detected</td>
<td>Not detected</td>
</tr>
<tr>
<td>Maximum</td>
<td>2.17</td>
<td>2.98</td>
</tr>
</tbody>
</table>

* a Sponge samples (log count/cm²).
* b Drill samples (log count/g).

The limit of detection was 3.3 CFU/cm². *E. coli* were estimated by placing 1-ml aliquots of both the initial solution and appropriate dilutions onto duplicate *E. coli* Petrifilm (3M, Sydney, Australia) and incubating at 37°C for 2 days. Colonies were counted as per the manufacturer’s instructions and the Association of Official Analytical Chemist’s method 991.4 (3). The limit of detection was 0.33 CFU/cm². Coagulase-positive staphylococci were determined using the Australian Standard method AS 1766.2.4 (3). E. coli and coagulase-positive staphylococci testing, buffered peptone water (200 ml) was added to the sponge bag, and the bag was incubated for 20 h at 37°C to allow resuscitation of damaged cells. Aliquots of resuscitated cultures were inoculated into mannitol selenite cystine broth (Oxoid, Melbourne, Australia) for incubation at 37°C for 24 h and into Rappaport-Vassiliadis medium (Oxoid) for incubation at 42°C for 24 h. Each enriched culture was inoculated onto brilliant green agar (gray-black, shiny, convex colony with a narrow entire margin surrounded by a zone of clearing) were picked off for coagulase testing using rabbit blood plasma (Micro Diagnostics, Brisbane, Australia). The limit of detection for coagulase-positive staphylococci was 3.3 CFU/cm². After aliquots had been taken from the sponge bag for TVC, *E. coli* and coagulase-positive staphylococci testing, buffered peptone water (200 ml) was added to the sponge bag, and the bag was incubated for 20 h at 37°C to allow resuscitation of damaged cells. Aliquots of resuscitated cultures were inoculated into mannitol selenite cystine broth (Oxoid, Melbourne, Australia) for incubation at 37°C for 24 h and into Rappaport-Vassiliadis medium (Oxoid) for incubation at 42°C for 24 h. Each enriched culture was inoculated onto brilliant green agar (Oxoid) and lysine mannitol glycerol agar (Amyl Media) and incubated at 37°C for 48 h. Biochemical confirmation of typical colonies was by the use of Medvet Microbact 24E strips (Medvet Science, Australia) and serological confirmation was done using Serobact *Salmonella* latex agglutination kit (Medvet Science). Positive samples were subcultured onto nutrient agar slopes and sent to the Queensland Public Health laboratory, Brisbane, Australia for serotyping. Results were reported as present or absent in 70 cm².

**TABLE 3.** Prevalence of *Salmonella*, *E. coli* O157:H7, and coagulase-positive staphylococci on Australian sheep meat (1998)

<table>
<thead>
<tr>
<th></th>
<th>Carcasses</th>
<th>Boneless meat</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella</em></td>
<td>1/921 (0.1%)</td>
<td>6/469 (1.3%)</td>
</tr>
<tr>
<td><em>E. coli</em> O157:H7</td>
<td>6/921 (0.7%)</td>
<td>6/470 (1.3%)</td>
</tr>
<tr>
<td>Coagulase-positive staphylococci</td>
<td>222/921 (24.1%)</td>
<td>181/469 (38.6%)</td>
</tr>
</tbody>
</table>

* a Sponge samples.
* b Drill samples.

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(a) Sponge samples (log count/cm²).
(b) Drill samples (log count/g).

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TABLE 1. TVC at 25°C of Australian sheep meat (1998)

<table>
<thead>
<tr>
<th>No. samples</th>
<th>Carcasses</th>
<th>Boneless meat</th>
</tr>
</thead>
<tbody>
<tr>
<td>917</td>
<td>467</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>3.55</td>
<td>3.30</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0.88</td>
<td>1.04</td>
</tr>
<tr>
<td>Median</td>
<td>3.55</td>
<td>3.13</td>
</tr>
<tr>
<td>80th percentile</td>
<td>4.21</td>
<td>3.91</td>
</tr>
<tr>
<td>95th percentile</td>
<td>5.03</td>
<td>5.44</td>
</tr>
<tr>
<td>Maximum</td>
<td>6.68</td>
<td>7.86</td>
</tr>
</tbody>
</table>

* a Sponge samples (log count/cm²).
* b Drill samples (log count/g).
Microbiological analysis of boneless sheep meat. Testing was carried out as described above although for *E. coli O157:H7* a 25-g sample of meat was added to 225 ml of modified EC broth (Amyl Media) plus 0.002% novobiocin and agitated for 2 min using a Seward Stomacher BA 7021 (Seward, London, UK). For TVC, *E. coli*, coagulase-positive staphylococci, and *Salmonella* a 25-g sample of meat was added to 75 ml of buffered peptone (Oxoid) water and agitated for 2 min using a Seward Stomacher BA 7021 (Seward). After aliquots had been taken from the sample bag for TVC, *E. coli* and coagulase-positive staphylococci testing, buffered peptone water (150 ml) was added to the sample bag, and the bag was incubated for 20 h at 37°C to allow resuscitation of damaged cells for *Salmonella* testing.

*E. coli O157:H7* and *Salmonella* were reported as present or absent in 25 g. For TVC, the limit of detection was 20 CFU/g, and for *E. coli* and coagulase-positive staphylococci the limit of detection was 2 CFU/g.

Statistical analysis. All counts were converted to log values to enable statistical analysis based on the assumption of normal distributions. For TVC, nondetections were assigned a value of 1 (log = 0). For all other microorganisms, nondetections were assigned a missing value prior to calculation of the log values. Where microbial counts are reported as CFU, they were calculated as the antilog of the respective mean log values. All data were analyzed using the statistical package SPSS Base 8.0 for Windows.

**RESULTS**

Microbiological status of Australian sheep meat. The microbiological status of Australian sheep carcasses and frozen boneless sheep meat is summarized in Tables 1 through 3. Mean log TVC for carcasses was 3.55, and for boneless sheep meat 3.30 (Table 1). Maximum TVCs, log 6.68 for carcasses and log 7.86 for boneless meats, represented radical departures from both the 80th and 95th percentiles. This suggested occasional loss of process control rather than temperature abuse during transit of samples to the laboratory, because temperature data loggers were included with each shipment.

On carcasses and boneless meat, respectively, generic *E. coli* was detected at a prevalence of 29.2% and 24.5% (Table 2). For carcasses the mean log of the positives was 0.17 CFU/cm² and for boneless meat, 0.94 CFU/g. Coagulase-positive staphylococci were detected on 24.1% of sheep carcasses and on 24.6% of boneless meat samples (Table 3). On those samples on which coagulase-positive staphylococci were detected, the mean count was 10.4 CFU/cm² on sheep carcasses and 13.5 CFU/g on boneless sheep meat. The prevalence of *Salmonella* and *E. coli O157:H7* was 0.1 and 0.7%, respectively, on carcasses and 1.3% for boneless sheep meat.

**Comparison of establishment types.** Summary data for export and domestic establishments and for VSPs are presented in Table 4. For carcasses, the mean log TVC was significantly lower (*P* < 0.05) at export establishments (2.90) compared with domestic (3.75) and VSPs (3.91), as was the prevalence of coagulase-positive staphylococci (12.2, 34.0, and 24.6%, for export, domestic, and VSPs, respectively). By contrast, generic *E. coli* was less prevalent on sheep carcasses from VSPs (21.4%) compared with domestic (32.7%) and export plants (35.2%). There were no significant differences in prevalence of *Salmonella* and *E. coli O157:H7* between establishment types.

**Comparison with 1993 to 1994 survey.** Because the 1993 and 1998 surveys relied on drilled samples of frozen boneless sheep meat, it is possible to compare the microbiological quality of boneless sheep meat. However, there were differences in sampling and in some microbiological techniques between the two surveys. The first survey esti-
mated generic *E. coli* using the five-tube most probable number, which has a lower detection limit than the duplicate Petrifilm (3M) used in the present survey. Isolation of *E. coli* O157 in the first survey was by enrichment followed by plating on Petrifilm (3M) plates and confirmation using sorbitol MacConkey agar (1). In the present survey *E. coli* O157:H7 was estimated after enrichment, then attachment on Dynal beads anti O157, a method considered more sensitive than that used in the 1993 to 1994 survey. Thus, any interpretation of the present comparison must take into account differences in methodology between the two surveys.

Because they were not part of the 1993 to 1994 survey, data from VSPs are excluded from this comparison, which is confined to data from export and domestic establishments. Comparisons of the hygienic quality of sheep meats processed at export and domestic establishments are presented in Table 5. There are a number of small, but statistically significant, improvements in microbiological quality for generic *E. coli* and for the prevalence of *Salmonella* in boneless sheep meat. None of the other criteria differed significantly between the surveys.

**Microbiological profile of export sheep meat and comparison with 1993 to 1994 survey.** A microbiological profile of boneless sheep meat produced by export establishments in 1993 to 1994 and in 1998 is presented in Table 6. Differences in methodology notwithstanding, the data indicate small but significant improvements in prevalence of generic *E. coli* and *Salmonella*.

**DISCUSSION**

Comparison of establishment types. The data represent product from systems where standards of construction and inspection are considered to be radically different. It is agreed that standards of construction are generally superior at export versus domestic establishments, which in turn are superior to VSPs. The latter are usually situated in remote locations and provide a service kill for local communities, slaughtering less than 150 cattle equivalents per week.

Government oversight is more intense at export than at domestic establishments where an inspection team with veterinary oversight is employed to provide the command-and-control surveillance required by Australia’s trading partners. Domestic establishments, by contrast, since the mid-1990s have operated comprehensive quality systems that are subject to periodic audit by the Controlling Authority or its agents.

There were differences in some mean data between the different sectors (export, domestic, and VSPs) with export establishments generally having lower mean log TVCs and lower prevalence of coagulase-positive staphylococci while VSPs recorded a lower prevalence of *E. coli*. These findings require some qualification to take account of operational and sampling differences between export and domestic establishments. Firstly, sampling was done during the lamb season when domestic plants typically operate at high line speeds. Secondly, in export establishments carcasses are sealed in chillers overnight before being sampled next morning when the sampling team has access to a full day’s production that has received uninterrupted chilling. In the domestic sector, by difference, chillers are opened and bodies moved to allow assemblage of orders for overnight delivery to the retail system. Domestic samples may therefore have been temporarily moved out of refrigeration during the picking operation, when they will also have received additional handling.

If mean log TVCs of sheep carcasses of each establishment are distributed according to sector, it becomes clear that there are considerable overlaps between each sector with the microbiological quality of product being related to establishment performance rather than sectoral performance.

**ACKNOWLEDGMENTS**

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**REFERENCES**