A National Survey of the Microbiological Quality of Beef Carcasses and Frozen Boneless Beef in Australia

DAVID PHILLIPS,1,* DAVID JORDAN,2 STEPHEN MORRIS,2 IAN JENSON,2 AND JOHN SUMNER2

1Symbio Alliance, P.O. Box 4312, Eight Mile Plains, Queensland, Australia 4064; 2Wollongbar Agricultural Institute, Department of Primary Industries, 1243 Bruxner Highway, Wollongbar, New South Wales, Australia 2477; and 3Meat and Livestock Australia, Locked Bag 991, North Sydney, Australia 2059

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ABSTRACT

The third national baseline microbiological survey of Australian beef carcasses and frozen boneless beef was conducted in 2004. Carcasses (n = 1,155) sampled at 27 slaughter establishments had a mean aerobic plate count (at 25°C) of 1.3 log CFU/cm². Escherichia coli was isolated from 8.0% of the carcasses, with a mean count of ~0.8 log CFU/cm² for positive samples. On samples from 24 boning (fabrication) plants (n = 1,082), the mean aerobic plate count for frozen boneless beef was 1.3 log CFU/g, and the mean count for the 1.8% of samples with detectable E. coli was 1.5 log CFU/g. E. coli O157:H7 was isolated from 1 of 1,143 carcasses and from 0 of 1,082 boneless samples. Salmonella was isolated from 0 of 1,155 carcasses and from 1 of 1,082 samples of boneless product. No Campylobacter spp. were isolated from carcasses or boneless beef. Coagulase-positive staphylococci were isolated from 28.7% of beef carcasses and 20.3% of boneless beef samples, and positive samples had a mean count of 0.3 log CFU/cm² and 0.8 log CFU/g, respectively.

National microbiological surveys of meat products assist in the identification of opportunities for improving food safety. They provide data that can be used to validate regulatory systems and to define the performance standards that are incorporated into regulations. Individual processors can use the findings to refine process control and to judge progress in improving product hygiene relative to the remainder of their industry. The data from these surveys may also be useful for risk assessment and for defining future research priorities. When the surveys are repeated at regular intervals using comparable techniques, they provide valuable insight into medium- and long-term trends in product hygiene. In 1993 through 1994, the Australian meat industry commissioned its first baseline study of the microbiological quality of Australian meat (16). Data were collected from samples excised from chilled carcass surfaces and from core samples drilled from cartons of frozen boneless meat destined for manufacturing. In this study, mean aerobic plate counts (APCs) for beef carcasses and boneless beef were in the 2 to 3 log units; Escherichia coli was detected on 22% of beef carcasses and in 17% of boneless beef samples. Pathogens were present at a very low prevalence. The first baseline study established a benchmark for Australian meat against which subsequent performance of the industry could be compared.

In 1998, the industry commissioned a second baseline study in the wake of substantial changes in domestic regulation and reforms initiated in major export markets. For example, all slaughter and boning facilities had recently implemented quality assurance plans based on hazard analysis critical control point systems, with commensurate investment in operator training, improved refrigeration, and on-site laboratory facilities. The United States had implemented a pathogen reduction final rule (known as the MegaReg (14), which required many changes to Australian processing of meat for export to the United States. The industry’s second baseline, designed primarily to assess the initial impact of these changes, was based on data from sponge samples taken from chilled carcasses to be consistent with the MegaReg and on samples drilled from cartons of frozen meat. To provide a basis for comparison with the first baseline study, excision samples also were taken from beef carcasses.

The second national baseline established that mean log APCs for carcasses and frozen manufacturing meat as a whole remained in the range of 2 to 3 log units. E. coli was detected on 10.3% of carcass samples and on 5.3% of frozen meat samples, and pathogen prevalence was similar to that in the first baseline study (11).

Since these surveys, the factors that drive changes in meat hygiene both in Australia and abroad have again altered. All Australian processing establishments are now required to operate under a single Australian standard for the production of meat products for human consumption (6). Thus, unlike in earlier surveys, there is presently no difference in mandatory quality assurance practices between establishments that service the export market and those that serve the domestic market. Concerns about product safety in the international meat market and among consumers have not abated.

The third national survey of microbiological attributes of beef carcasses and frozen boneless beef in Australia was conducted to provide objective evidence of the combined
effectiveness of the Australian regulatory system and industry initiatives for improving the safety of these products. The aims of the present study were to (i) assess the change in microbiological quality of chilled beef carcasses since 1998 as measured by the sponge-swabbing method and (ii) assess the change in microbiological quality of frozen boneless beef since the 1993 through 1994 survey.

MATERIALS AND METHODS

Design of the study. Samples were collected from February through April (summer sampling) and from July through October (winter sampling) 2004. Samples were collected from abattoirs in each of the five mainland Australian states. In all, samples were collected from 27 abattoirs and 24 boning (fabricating) establishments whose combined production accounted for >75% of Australian beef production; participation in the survey was optional. Almost all of the establishments in the survey were under the jurisdiction of the Australian Quarantine and Inspection Service. The number of carcasses sampled at each abattoir was weighted according to the volume of processing, which varied from 300 to 3,800 head per day. At larger abattoirs, a limit of 30 carcasses per day was imposed to ensure the plant was visited on more than one occasion.

The livestock processed during this study reflected the full range of husbandry practices, age, breed, and climatic effects across Australia. Cattle were most typically from grass-fed or rangeland production systems (beef breeds) or were dairy types culled from predominantly grass-fed milking herds. Approximately 10% of all cattle processed in Australia have spent a minimum 70 days in a feedlot prior to slaughter.

Samples from beef carcasses. Selection of carcasses and subsequent sampling was performed by a team of trained technicians. Samples were collected after approximately 14 h of active chilling (range, 12 to 24 h). Sample collection days were Tuesday through Friday (kill days were Monday through Thursday) to allow for adequate carcass chilling. Individual carcasses were selected for sampling using a systematic random approach. From the total lot of carcasses accessible to the technicians at a plant at any time, samples were collected at regular intervals until the required number of carcasses had been sampled. Separate polyurethane sponges (Whirlpak speci-sponge, Nasco, Fort Atkinson, Wis.) moistened with buffered peptone water (25 ml) were used to obtain swab samples from each side of the selected carcasses; a composite sample was taken by sponging a 100-cm² area at each of the butt, flank, and brisket regions of each carcass side as detailed in the MegaReg methodology. A third sponge was used to swab areas adjacent to those swabbed with the second sponge on the same carcass side.

Although excision samples are considered more accurate than swabs (10), swabs are now the standard method for appraising carcass hygiene in many countries, including Australia, New Zealand, and the United States. For large-scale surveys in commercial settings, the swab technique is preferred.

Samples from frozen boneless beef. Samples of boneless beef were collected from randomly selected frozen cartons that had been in a freezer for usually no longer than 1 month. A systematic random selection process similar to that used for carcasses and constrained by the degree of access to cartons within a cold room was applied but with no intentional bias with regard to the day of processing. Approximately 150 g of meat was drilled from eight or nine different locations in each carton with a sterile drill bit and transferred to sterile plastic bags with a sterile pair of kitchen tongs.

Transport of samples to the laboratory. All samples were packed in insulated containers with chiller packs and a temperature logger for transportation to a laboratory accredited by the National Association of Testing Authorities. Upon arrival at the laboratory, samples were held at 2 to 4°C until examination. To standardize the times between sample collection and analysis, samples were analyzed between 18 and 24 h of collection. In most cases, analyses were conducted on the day of arrival at the laboratory. Internal air temperature histories of the sample transit container obtained during transit were used to determine whether temperature abuse had occurred. Samples whose temperatures had exceeded 10°C were not analyzed.

Microbiological analysis of sponge samples. Three sponge samples were collected from each carcass; one was used for detection of *E. coli* O157:H7, one was used for *Salmonella*, and the third was used for other analyses (APC, *E. coli*, *Enterobacteriaceae*, coagulase-positive staphylococci, and *Campylobacter*). To eliminate bias between the right and left sides of the carcass, sponges were randomly directed to each test.

Detection of *E. coli* O157:H7. A 225-ml volume of modified EC broth (Oxoid, Adelaide, Australia) with 0.02 g/liter novobiocin (Calbiochem, Bad Soden, Germany) was added to one of the sponge bags, which was then squeezed by hand 10 times and incubated at 37°C overnight according to AOAC method 991.14 (7). The following day, the Dynalbeads anti-O157 method of immunomagnetic separation (Dynal Australia, Melbourne, Australia) was followed as per the manufacturer’s instructions. Positive samples were subcultured, and isolates were sent to the laboratories of Queensland Health (Brisbane, Australia) or to Food Science Australia (Brisbane) for the detection of genes encoding Shiga toxins. *E. coli* strains positive for the O157 antigen and containing a gene for Shiga toxin were reported as number of *E. coli* O157:H7 cells detected in 300 cm².

Detection of *Salmonella*. Buffered peptone water (225 ml) was added to the sponge bag, which was then squeezed by hand 10 times and 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) and incubated at 37°C for 24 h or into Rappaport-Vassiliadis medium (Oxoid) and incubated at 42°C for 24 h according to Australian Standard method AS 1766.2.5 (2). Each enriched culture was inoculated onto brilliant green agar and xylose lysine desoxycholate agar (Oxoid) and incubated at 37°C for 24 h. The identity of typical colonies was confirmed biochemically with Microbact 24E or 12A strips (Oxoid). Positive samples were subcultured onto nutrient agar slopes and sent to the Queensland Public Health Laboratory (Brisbane, Australia) for serotyping. Results were reported as presence or absence of *Salmonella* cells per 300 cm².

Determination of APCs and concentrations of coliforms, *E. coli*, *Enterobacteriaceae*, coagulase-positive staphylococci, and *Campylobacter* on carcasses. Buffered peptone water (25 ml) was added to the sponge bag, which was then squeezed by hand 10 times. Serial dilutions were prepared in 0.1% peptone water with 1-ml aliquots. For APCs, duplicate pour plates were prepared according to the Australian Standard method AS 1766.1.3 (1) and incubated at 25°C for 96 h. Colonies were then counted, and the number of CFU per square centimeter was recorded. The limit of detection (LOD) was 0.4 CFU/cm². *E. coli* concentrations were estimated by placing 1-ml aliquots of both the initial solution and
TABLE 1. Microbiological profile of Australian chilled beef carcasses (n = 1,147)

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Prevalence (%)</th>
<th>Mean</th>
<th>Median</th>
<th>SD</th>
<th>90th percentile</th>
<th>95th percentile</th>
<th>99th percentile</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>APCs(^a,b)</td>
<td>96.0</td>
<td>1.3</td>
<td>1.2</td>
<td>0.8</td>
<td>2.3</td>
<td>2.8</td>
<td>3.5</td>
<td>5.8</td>
</tr>
<tr>
<td>Coliforms(^c,d)</td>
<td>15.2</td>
<td>-0.6</td>
<td>-0.8</td>
<td>0.8</td>
<td>0.6</td>
<td>1.0</td>
<td>2.1</td>
<td>2.2</td>
</tr>
<tr>
<td>E. coli(^c,d)</td>
<td>8.0</td>
<td>-0.8</td>
<td>-1.1</td>
<td>0.7</td>
<td>0.4</td>
<td>0.7</td>
<td>1.7</td>
<td>1.7</td>
</tr>
<tr>
<td>Enterobacteriaceae(^c,d)</td>
<td>20.5</td>
<td>-0.6</td>
<td>-0.8</td>
<td>0.8</td>
<td>0.6</td>
<td>1.2</td>
<td>2.2</td>
<td>2.3</td>
</tr>
<tr>
<td>Coagulase-positive staphylococci(^b,c)</td>
<td>28.7</td>
<td>0.3</td>
<td>0.3</td>
<td>0.7</td>
<td>1.4</td>
<td>1.6</td>
<td>2.4</td>
<td>3.0</td>
</tr>
</tbody>
</table>

\(^a\) All counts were incremented by 1 CFU/cm\(^2\) before log transformation.

\(^b\) Limit of detection is 0.4 CFU/cm\(^2\).

\(^c\) Counts are for positive samples only.

\(^d\) Limit of detection is 0.04 CFU/cm\(^2\).

appropriate dilutions onto duplicate E. coli Petriflms (3M, Sydney, Australia) that were then incubated at 37°C for 48 h. Colonies were counted as per the manufacturer’s instructions and AOAC method 991.14 (7). The LOD was 0.04 CFU/cm\(^2\). Enterobacteriaceae numbers were estimated by placing 1-ml aliquots of both the initial solution and appropriate dilutions onto duplicate Enterobacteriaceae Petriflms that were then incubated at 37°C for 24 h according to AFNOR method 3M 01/6-09/97 (5). Colonies were counted as per the manufacturer’s instructions. The LOD was 0.04 CFU/cm\(^2\). Coagulase-positive staphylococci concentrations were determined using Australian Standard method AS 1766.2.4 (4), where 0.1-ml aliquots were spread onto dried plates of Baird Parker agar (Merck, Melbourne, Australia) and incubated at 37°C for 48 h. Colonies with typical morphology (gray black, shiny, and convex with a narrow entire margin surrounded by a zone of clearing) were picked off the plate for coagulase testing using rabbit blood plasma. The LOD for coagulase-positive staphylococci was 0.4 CFU/cm\(^2\).

RESULTS AND DISCUSSION

Microbiological status of beef carcasses and frozen boneless beef. All samples received at the laboratory had been kept below 10°C and were analyzed. A microbiological profile of Australian beef carcasses produced at 27 establishments is presented in Table 1. The mean APC was 1.3 log CFU/cm\(^2\); counts at the 90th, 95th, and 99th percentiles were 2.3, 2.8, and 3.5 log CFU/cm\(^2\), with a maximum of 5.8 log CFU/cm\(^2\). Coliforms were detected on 15.2% of samples, for which the mean APC was -0.6 log CFU/cm\(^2\) and the maximum was 2.2 log CFU/cm\(^2\). E. coli was detected on 8.0% of samples, for which the mean APC was -0.8 log CFU/cm\(^2\) and the maximum was 1.7 log CFU/cm\(^2\). Enterobacteriaceae were detected on 20.5% of samples, for which the mean APC was -0.6 log CFU/cm\(^2\) and the maximum was 2.3 log CFU/cm\(^2\). Coagulase-positive staphylococci were detected on 28.7% of samples, for which the mean APC was 0.3 log CFU/cm\(^2\) and the maximum was 3.0 log CFU/cm\(^2\).

A microbiological profile of boneless beef produced at 24 establishments is presented in Table 2. The mean APC was 1.3 log CFU/g, and counts at the 90th, 95th, and 99th percentiles were 2.3, 2.7, and 3.5 log CFU/g, with a maximum of 5.5 log CFU/g. Coliforms were detected on 5.5% of samples, for which the mean APC was 1.3 log CFU/g and the maximum was 2.9 log CFU/g. E. coli was detected on 1.8% of samples, for which the mean APC was 1.5 log CFU/g and the maximum was 2.8 log CFU/g. Enterobacteriaceae were detected on 7.1% of samples, for which the mean APC was 1.3 log CFU/g and the maximum was 3.0 log CFU/g. Coagulase-positive staphylococci were detected on 20.3% of samples, for which the mean APC was 0.8 log CFU/g and the maximum was 2.3 log CFU/g.

On chilled beef carcasses, Salmonella and Campylobacter were not recovered from any of 1,155 samples, and...
TABLE 2. Microbiological profile of Australian frozen boneless beef (n = 1,082)

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Prevalence (%)</th>
<th>Mean</th>
<th>Median</th>
<th>SD</th>
<th>90th percentile</th>
<th>95th percentile</th>
<th>99th percentile</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>APC</td>
<td>82.5</td>
<td>1.3</td>
<td>1.3</td>
<td>0.8</td>
<td>2.3</td>
<td>2.7</td>
<td>3.5</td>
<td>5.5</td>
</tr>
<tr>
<td>Coliforms</td>
<td>5.5</td>
<td>1.3</td>
<td>1.2</td>
<td>0.6</td>
<td>2.4</td>
<td>2.6</td>
<td>2.9</td>
<td>2.9</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>1.8</td>
<td>1.5</td>
<td>1.3</td>
<td>0.8</td>
<td>2.7</td>
<td>2.8</td>
<td>2.8</td>
<td>2.8</td>
</tr>
<tr>
<td><em>Enterobacteriaceae</em></td>
<td>7.1</td>
<td>1.3</td>
<td>1.2</td>
<td>0.6</td>
<td>2.0</td>
<td>2.6</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Coagulase-positive staphylococci</td>
<td>20.3</td>
<td>0.8</td>
<td>0.7</td>
<td>0.3</td>
<td>1.0</td>
<td>1.7</td>
<td>2.2</td>
<td>2.3</td>
</tr>
</tbody>
</table>

*a* Limit of detection is 5 CFU/g.  
*b* All counts were incremented by 1 CFU/g before log transformation.  
*c* Counts are for positive samples only.

*E. coli* O157:H7 was recovered from only 1 sample (0.1%). *E. coli* O157:H7 and *Campylobacter* were not recovered from any of 1,082 drilled samples of frozen boneless beef, and *Salmonella* was recovered from 1 sample (0.1%).

**Comparison with previous baseline surveys.** Similarities in the methodology used in all three surveys allowed comparison of the microbiological status of beef carcasses and boneless beef. In the 1998 and 2004 surveys when carcass samples were obtained by sponge sampling, there was a reduction in mean APC from 2.4 to 1.3 log CFU/cm² (distribution of counts is shown in Fig. 1). Of 17 beef-processing establishments providing data for both the 1998 and 2004 surveys, 15 improved their mean APCs. For comparing the prevalence of *E. coli* between surveys, a common LOD of 5 log CFU/g was used. The prevalence was 11.6% in the 1993 through 1994 survey and 1.8% in both the 1998 and 2004 surveys.

A comparison of the results of this survey with those of previous surveys conducted in 1993 through 1994 and 1998 revealed an improvement in the microbiological quality of Australian beef. The most plausible explanation for the improvement is the investment made by the Australian industry in food safety systems during the past decade and the heightened awareness of safety issues among personnel at all levels of the cattle and beef industries. Investments have been made by both regulators and industry officials. In this coregulatory framework, new regulations have been enacted and enforced by the regulators, and the industry has implemented risk-based quality systems and staff training and has invested in buildings, equipment, and chilling systems.

The coregulatory approach has been adopted across all of the Australian meat industry. Most of the establishments in the industry are part of the present survey, i.e., they are medium and large plants with slaughter volumes of 300 to 3,800 head per day. These products are exported to global markets and used to supply the domestic market. Excluded from this survey were the very small plants (VSPs), which typically exist to service communities that are often remote from main population centers. Information on the hygienic performance of these VSPs in the coregulatory environment recently became available from a separate survey in South Australia of 3 VSPs slaughtering 60 to 250 head per day and 11 slaughtering 1 to 30 head per day (13).

**FIGURE 1.** Frequency distribution of APCs (log CFU per square centimeter) from Australian beef carcasses, 1998 to 2004.
The mean APCs at abattoirs and VSPs were 1.7 and 1.8 log CFU/cm², respectively, compared with 1.3 log CFU/cm² for the plants surveyed in the current national baseline study. In the current survey of large plants, *E. coli* was detected on 8.0% of carcasses compared with 8.4 and 28.4% carcasses at South Australian VSPs and abattoirs, respectively. At 9 of 11 VSPs, *E. coli* was not detected on any of 54 carcasses sampled; prevalences at the other 2 plants were 16 and 50%. At the three abattoirs processing beef, prevalence was 0, 4, and 80%.

**Comparison with international data.** Two types of information can be used to compare the quality of Australia’s meat with that of the meat produced in the rest of the world. The first type is the expectation of the rest of the world as expressed through government standards or guidelines and commercial purchasing specifications. The second type is the performance of companies in other countries as reported in studies on process change or as conformance with regulatory specifications set by an importing country. The lack of standard sampling, testing, and reporting makes direct comparisons difficult, especially for organisms that are detected only rarely, such as *E. coli* and *Salmonella*. When the Australian data were compared with European Union performance criteria for swab sampling (9), U.S. *Salmonella* detection in ground beef and on beef carcasses (15), and New Zealand data from their National Microbiological Database (8), we found that Australian beef is at least similar in microbiological quality to that produced by some of Australia’s major trading partners. In a recent study on imported and domestic beef trim conducted in the United States (12), the lowest levels of bacterial indicators were reported for Australian beef.

These data indicate the effectiveness of a coregulatory approach between industry and regulators in both the Australian domestic and export sectors. The results of this survey should be useful for public health risk assessments and provide objective evidence that standards of hygiene during the slaughter and processing of beef in Australia continue to be very high.

**ACKNOWLEDGMENTS**

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**REFERENCES**