Food Protection Trends, Vol. 31, No. 8, Pages 514–520

6200 Aurora Ave., Suite 200W, Des Moines, IA 50322-2864

Verification of Hygiene in Australian Manufacturing Beef Processing — Focus on *Escherichia coli* O157

JOHN SUMNER,[1*] ANDREAS KIERMEIER[2] and IAN JENSON[1]
[1]Meat and Livestock Australia, Locked Bag 991, North Sydney, Australia 2059;
[2]South Australian Research and Development Institute, P.O. Box 397, Adelaide, Australia 5001

ABSTRACT

In 1996, authorities in the United States implemented a series of important regulations surrounding the hygiene of meat destined for grinding and in ground beef. These included the Pathogen Reduction Final Rule (known as "the MegaReg") and the declaration of *Escherichia coli* O157 as an adulterant. As a major supplier to the U.S. market, the Australian beef industry responded by implementing the *E. coli Salmonella* Monitoring (ESAM) program and a program for testing manufacturing beef for *E. coli* O157; these programs are augmented by regular national baseline surveys. In line with increased sensitivity of test methods used for detecting *E. coli* O157 in meat, the prevalence of detections has increased. Currently, *E. coli* O157 is detected in around 0.1% of lots. Intensive sampling of five lots in which *E. coli* O157 had been detected (and removed from the export stream) failed to detect the pathogen in three lots, and the highest concentration observed in the other two lots was 0.093 MPN/cm^2, equivalent to 790 organisms per carton (per 27.2 kg). By compiling historical and contemporary monitoring data and by citing recently completed studies to gauge the effectiveness of these responses, the present paper documents how the Australian meat industry has responded to regulatory changes in the United States.

A peer-reviewed article

[*]Author for correspondence: Phone and Fax: +1 61.3.6394.7640
E-mail: john_sumner2@biopond.com

TABLE 1. Testing of manufacturing beef for *E. coli* O157 at Australian processing establishments

Year	Number of tests	Number (%) of lots with *E. coli* O157 confirmed
1998–2002	184,843	32 (0.017)
2005	24,029	4 (0.016)
2006 and 2007	45,000	8 (0.017)
2008[a]	30, 647	36 (0.117)
2009[a]	34,433	35 (0.106)[b]
2010[a]	31,615	21 (0.066)

[a]N-60 testing implemented.

[b]Note that there were 20 additional isolations from bobby veal (calves less than one week old). However, since this product is not destined for grinding, the data have not been included.

INTRODUCTION

For more than four decades, Australia has been a major exporter to the United States of manufacturing beef for grinding into hamburger patties. Typically, each year around 250,000 tons are supplied frozen in 60 lb (27.2 kg) cartons, equivalent to 2,500,000,000 quarter-pound patties, or around 10% of hamburgers consumed in the United States. As a commodity, manufacturing and ground beef have come under close focus from the food safety viewpoint because of contamination with enteric pathogens, particularly *E. coli* O157. The first documented outbreak involving hamburgers occurred in Oregon and Michigan in 1982, with the report citing "a rare *E. coli* serotype O157:H7" *(28)*. In 1992–93, outbreaks involving more than 500 people in the western United States revealed the risk of *E. coli* O157 illness from consumption of undercooked hamburgers *(10)*. In the ensuing two decades there have been numerous outbreaks from consumption of hamburgers, some of which are summarized by Rangel et al. (*24)*.

Over the same period there have been a series of changes aimed at enhancing control of pathogenic *E. coli* in meat used for grinding and in ground beef, most notable of which are:

- Declaration of *E. coli* O157 as an adulterant *(15)*
- The requirement to test meat destined for grinding *(2)*
- N60 ("robust") testing *(2, 17)*
- Increasing attention being paid to non-O157 serotypes *(16)*

In the present paper, the purpose of which is to document how the Australian meat industry has responded to regulatory changes in the United States, we present a compilation of historical and contemporary data together with recently completed studies. To gauge the effectiveness of industry development we present trends in prevalence of indicator organisms on beef carcases and of *E. coli* O157:H7 on trim destined for grinding in the United States. We also cite data for indicators and pathogens on beef trim gathered in the third national baseline study, together with data on the prevalence and concentration of *E. coli* O157 in five lots of beef trim from which the pathogen was detected in surveillance sampling at abattoirs.

E. coli O157:H7 as an adulterant and testing programs

As a result of declaration of *E. coli* O157 as an adulterant in ground beef in 1994, and the consequent "zero tolerance" policy, testing of manufacturing beef for the presence of *E. coli* O157 has become a significant aspect of the control of this pathogen in the beef supply chain. In effect, the company's testing program has become a "disposition CCP" under which a lot of production is not released to the trade unless and until there is confirmation that the pathogen has not been detected in the sample *(8)*.

Concomitant with the adoption of a zero tolerance policy came the development of testing programs designed to support the concepts of "adulterant" and "zero tolerance." Early sampling plans involved a sample size of 25 g per lot of production, later increased to 325 g, comprising 5 × 65 g samples *(18)*. More recently, the collection of 60 surface slices from the external carcass surface has been introduced, the so-called N60 or "robust" testing. Improvement in analytical techniques has also increased the sensitivity of testing for *E. coli* O157 *(17)*.

In Australian boning (fabrication) rooms over the period 1998–2007, sampling involved accumulating small pieces of trim during the period when a lot was produced, from which a 25 g sample was taken. Since late 2007, N60 sampling has been used. All export establishments testing for the presence of *E. coli* O157 in beef destined for grinding in the United States do so under the supervision of AQIS, using laboratories accredited to ISO 17025 by the National Association of Testing Authorities (NATA). Samples are drawn from 12 cartons selected at random from the lot, and test results are provided to the Australian Quarantine and Inspection Service (AQIS) for entry into the National Microbiological Database *(2)*.

In Table 1 is summarized results of sampling for the period 1998–2010, from which it can be seen that the inception of N60 testing in 2008 has led to an increased detection rate. AQIS also performs verification testing on lots prior to the establishment release of the product and, during 2008–2010, in a total of 528 lots sampled, *E. coli* O157 was detected in 1 (0.19%).

From the outset, there have been criticisms that testing is not effective at detecting lots that contain the microorganism of concern when its prevalence is low. In 1999, thirty-five international

FIGURE 1. Quarterly average prevalence of chilled beef carcass samples with detactable (> 0.08 CFU/cm^2) *E. coli* (closed circles Cow/Bull, open circles Steer/Heifer)

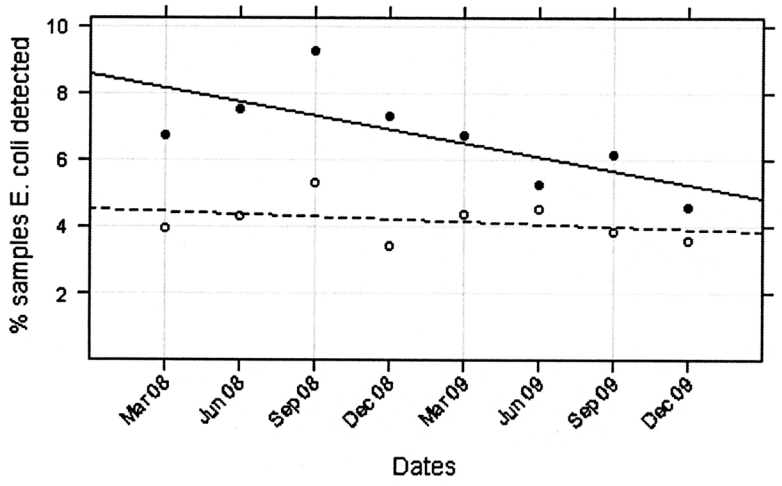
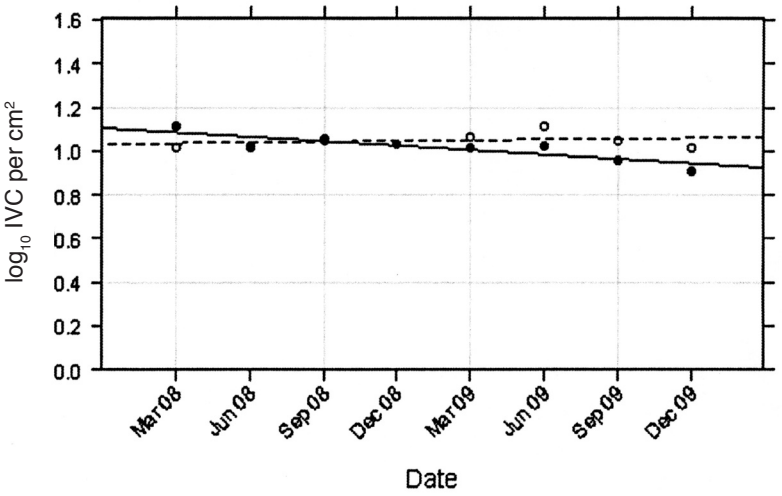


FIGURE 2. Quarterly average Aerobic Plate Counts (log_{10} CFU/cm^2) on chilled beef carcass samples (closed circles Cow/Bull, open circles Steer/Heifer)



experts assembled by the American Meat Science Association established that, if *E. coli* O157 were present at 0.1% prevalence, the numbers of samples from a contaminated lot needed to detect the pathogen with probabilities of 0.90, 0.95 and 0.99 were 2,303, 2,996 and 4,605, respectively *(1)*. Because of the low prevalence and non-random distribution, the same consensus also counselled against pathogen testing to assess process control. A subsequent gathering of meat safety experts, under the *aegis* of the International Livestock Congress, concurred with the AMSA findings and concluded that testing programs should focus on enumerating indicator organisms, particularly when pathogens are present at low concentration *(7)*.

Indicator organisms on beef carcasses

While testing of individual lots for *E. coli* O157 gives some degree of certainty about the safety of product, more confidence can be gained by also considering microbial indicators of good process hygiene. In Australia, the *E. coli* and *Salmonella* Monitoring (ESAM) program is an AQIS-supervised monitoring program for beef carcasses to which many establishments also submit Aerobic Plate Count (APC) data.

The ESAM program is performed by all export establishments, which are required to take action on results considered unacceptable, based on a three-class sampling plan and a moving window *(26)*. The results are stored in the National Microbiological Database which, after 13 years of operation, now contains > 500,000 data sets. The database is active, with each export establishment receiving monthly summaries of its own, and of the national, microbiological profile. Few samples have *E. coli* above the limit of detection (0.08 CFU/cm^2), so national benchmarks focus on the rate at which samples have detectable *E. coli* (Fig. 1 and 2). The proportion of samples in which *E. coli* is detected is low and trending downwards (Fig. 1), while APCs are low and are stable or trending downwards (Fig. 2).

Indicator organisms on beef for grinding

In 2004 a third baseline survey was conducted of Australian boneless beef destined for grinding in the United State*s (22)*. Meat was sampled by drilling cores through the cartons of frozen meat, which approximates the result that would be obtained from grinding the contents of the carton. In this national survey, the number of samples collected from establishments was proportional to their throughput; establishments representing about 75% of the national throughput were sampled.

The microbiological quality of manufacturing beef in this survey was excellent. In 17.5% of samples the APC was below the limit of detection (5 CFU/g), and, when detected, the mean concentration was 1.3 log_{10} CFU/g; *Enterobacteriaceae*, coliforms and *E. coli* were rarely detected (limit of detection 5 CFU/g), and then usually at low concentrations (Table 2).

Contamination levels in lots containing *E. coli* O157

To date there has been no study of the distribution of *E. coli* O157 in

TABLE 2. Microbiological profile of Australian frozen boneless beef (n = 1082)

	APC	Coliforms[a]	*E. coli*[a]	*Enterobacteriaceae*[a]
Prevalence (%)	82.5	5.5	1.8	7.1
Mean $\log_{10}$ CFU/g	1.3	1.3	1.5	1.3
Standard deviation ($\log_{10}$ CFU/g)	0.8	0.6	0.8	0.6

[a]Counts are $\log_{10}$ CFU/g of positive samples only.

contaminated lots of beef destined for grinding, nor of its concentration. To redress this data gap, lots of manufacturing beef that failed to meet Australian requirements for export to the United States were examined. Accordingly, when the presence of *E. coli* O157 was confirmed in a lot, the cartons of frozen manufacturing beef from which samples had been taken were shipped to the laboratory for analysis. In total, cartons from which five lots had yielded *E. coli* O157 were obtained and submitted to intensive sampling.

Cartons were maintained at -18°C until thawing at 0 ± 1°C for 48 hours, after which each carton was opened and the number of pieces of meat, the weight of each piece and the external (i.e., carcass) surface area were determined. Samples were collected for analysis using a surface slice method *(2)*. From each carton, 75 × 5 g samples were collected from pieces with external carcass surfaces, resulting in a total of 900 samples per lot.

Surface slices, weighing 5 g and representing 10 cm^2, were enriched and tested for *E. coli* O157:H7 by use of the *E. coli* O157:H7 MP BAX kit and the Q7 BAX System; isolation was performed using immunomagnetic separation.

A total of 5 lots (A to E) were subjected to intensive sampling; *E. coli* O157 was not detected in three lots (A, B, and C), while two lots (D and E) resulted in 2 and 74 further *E. coli* O157 detections, respectively. The two detections in lot D originated from a single large piece of meat, while those for lot E originated from 27 beef trim pieces that had been sampled from two of the twelve cartons.

The median concentration (MPN/cm^2) for all cartons in which *E. coli* O157 was not further detected was estimated to be less than 0.0014 MPN/cm^2, which is equivalent to less than 9 organisms per 27.2 kg carton. The highest concentration observed was 0.093 MPN/cm^2, equivalent to 790 organisms per carton *(21)*.

From the investigation of lots where *E. coli* had been detected, it is concluded that only a small fraction of lots of Australian beef is contaminated with *E. coli* O157 and that when contamination does occur, it is restricted to only a small part of the lot, possibly just a few cartons.

This contention that contamination is restricted is supported by two Australian studies. First, Fegan et al. *(14)* found a low prevalence of *E. coli* O157 in the feces of Australian cattle, 10% for grass-fed and 15% for lot-fed cattle, and almost always at low concentration. Second, Fegan et al. *(13)* studied transfer of *E. coli* from feces and hides to carcasses. Most groups of cattle contained very few shedders, though an occasional "super shedder" led to more widespread hide contamination. Nonetheless, when these highly contaminated animals were processed, only sporadic carcasses were contaminated with *E. coli* O157, and then only at very low levels. These data are consistent with other studies, reviewed by Arthur et al. *(4)*, which suggest that only *E. coli* O157 from "super shedders" survive current processes, resulting in a low prevalence of contaminated carcasses.

It is possible that the low prevalence and concentrations of *E. coli* O157 detected in this study were the result of the meat having been frozen, a process that may result in a reduction of *E. coli* O157:H7 *(3, 11, 12, 25)*. It should be noted that cartons destined for the USA grinding market are in the frozen transport chain for several weeks or months.

It appears from this analysis that the prevalence and concentration of *E. coli* O157 in Australian boxed beef are very low.

Non-O157 shiga toxin-producing *E. coli* (STEC)

In early 1995, mettwurst contaminated with *E. coli* O111 caused an outbreak of food poisoning in South Australia involving > 150 victims, one of whom died *(9)*. There was speculation that the fermentation had been inadequate, and a processing standard has been developed under which each manufacturer must validate that the fermentation process is capable of inactivating *E. coli* Biotype 1 from ingoing raw materials.

There is increasing global interest in *E. coli* strains, other than O157, that occur in food and that cause gastrointestinal disease in humans. The USA lists six serotypes (O26, O111, O145, O103, O121 and O45), five of which are also of interest to the European Union (O121 is the exception), that cause serious infections. All of these serotypes produce Shiga Toxin plus other virulence factors, and methods for detecting them are in development.

Australian researchers *(5)* have examined cattle feces at the time of slaughter, searching for non-O157 STEC of concern in Europe and the United States. Three hundred fecal samples were tested, of which 78 (15%) contained genes of at least one of the serotypes of interest in the United States and the EU, together with a Shiga Toxin gene and the Intimin gene (the most significant virulence factor gene). However, while genes were present in 78 fecal samples, only 21 (7%) yielded an isolate, and none of these contained both the Shiga Toxin and Intimin

TABLE 3. Beef carcass contamination in Australia 1937–2004

	Log TVC/cm²	*E. coli*	Reference
		Prevalence (%) >10/cm²	
1937	3.88	-	19
1964	3.90	22.5	19
1978	2.79	15.6	19
1994	3.02	9.2	27
1998	2.43	2.4	23
2004	1.33	0.2	22

TABLE 4. Microbiological profile of indicator organisms in beef trimmings destined for ground beef (after Bosilevac et al. *(6)*)

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

genes. These results indicate that while non-O157 STEC can be detected in the feces of Australian cattle, the likelihood of these strains causing serious disease in humans is not high.

CONCLUDING REMARKS

Apart from the mettwurst incident already described, there have been no reports of meat-borne outbreaks involving either *E. coli* O157 or non-O157 serotypes. While this may in part reflect the fact that Australian consumers do not undercook meat, particularly ground meat, unique aspects of the Australian meat industry may also contribute to the low incidence of meat-associated disease in Australia. Based on the protocol of Jordan et al. *(20)*, cattle have been shown to enter the slaughter facility with a low tag score. Australian line speeds are slow (mean 75 head/hour) and the Australian workforce is generally stable and well trained, with all export establishments having a training facility and many employing full-time trainers. The industry invests heavily in routine microbiological monitoring and in national baseline surveys that are used to drive industry improvement. The prevalence and concentration of indicator bacteria (Figs. 1 and 2) demonstrate the effectiveness of the processing systems.

On the basis of work carried out by CSIRO on the microbiology of beef carcasses, it is possible to construct a 70-year comparative profile by utilizing data from Grau *(19)* and three national baseline studies (Table 3). It should be emphasized that the data quoted by Grau *(19)* were gathered at a single abattoir whereas baseline data were industry-wide. Nonetheless, the progressive reduction in bacterial loading in general, and in *E. coli* in particular, appear to be associated with the radical changes that the industry underwent beginning with the introduction of HACCP-based QA systems in the mid-1990s.

In 2005, researchers at the US Department of Agriculture compared the microbiology of beef destined for grinding from Australia, New Zealand and Uruguay with that of domestic product *(6)*. Researchers analyzed indicator organisms such as Total count, *Enterobacteriaceae*, Coliforms/*E. coli*, *Staphylococcus aureus*, and pathogens: *Campylobacter*, *Listeria*, *Salmonella* and non-O157 STEC. Summary data of the survey are presented in Tables 4 and 5. The researchers counsel against making inter-country comparisons, because imported meat is frozen while domestic is chilled, and because imported meat was sourced during the high-prevalence season for pathogens while that for domestic meat was from the low-prevalence season. Nonetheless, the researchers concluded that Australian and New Zealand beef trim had lower levels of contamination than did U.S. and Uruguayan trim, and that differences were more likely to be associated with the processing environment and processes in use, rather than seasonal differences.

After considering trends in prevalence of indicator organisms on beef carcasses and of *E. coli* O157:H7 on trim, together with data generated by three national baseline studies undertaken since 1995, we conclude that the hygienic status of beef trim destined for grinding in the United States continues to improve.

REFERENCES

1. Anonymous. 1999. The role of microbiological testing in beef food safety programs: the scientific perspective. Consensus of the 1999 symposium under the aegis of the American Meat Science Association, Kansas City, Missouri, January 1999.
2. Anonymous. 2010. *Escherichia coli* O157:H7 testing of raw ground beef components destined for export to the US and US territories. AQIS [Australian Quarantine Inspection Service] Meat Notice 2010/03. http://www.daffa.gov.au/aqis/export/meat/elmer-3/notices accessed 15 March, 2010.
3. Ansay, S., K. Darling, and C. Kaspar. 1999. Survival of *Escherichia coli* O157:H7 in ground beef patties during storage at 2, -2, 15 and

TABLE 5. Prevalence of pathogens in beef trimmings destined for ground beef (after Bosilevac et al. *(6)*)

	Prevalence (%)				
	Salmonella	***Campylobacter***	***L. monocytogenes***	**STEC**	**HUS serotypes**
Australia	0	0	2.0	4.09	0
New Zealand	0.4	0.5	2.3	1.79	0.89
Uruguay	0.4	0.4	2.4	15.62	2.34
USA	0.8	1.3	5.0	5.74	1.02

then -2°C, and -20°C. *J. Food Prot.* 62:1243–1247.

4. Arthur, T., D. Brichta-Harhay, J. Bosilevac, N. Kalchayanand, S. Shackelford, T. Wheeler, and M. Koohmaraie. 2010. Super shedding of *Escherichia coli* O.157:H7 by cattle and the impact on beef carcass contamination. *Meat Sci.* 86:32–37
5. Barlow, R., and G. Mellor. 2010. Prevalence of Enterohemorrhagic *Escherichia coli* serotypes in Australian beef cattle. *Foodborne Path.Dis.* 7(10):1239–1245.
6. Bosilevac, J., M. Guerini, D. Brichta-Harhay, T. Arthur, and M. Koohmarie. 2007. Microbiological characterization of imported and domestic boneless beef trim used for ground beef. *J. Food Prot.* 70:440–449.
7. Brown, M., C. Gill, J. Hollingsworth, R. Nickelson, S. Seward, J. Sheridan, T. Stevenson, J. Sumner, D. Theno, W. Usborne, and D. Zink. 2000. Review: The role of microbiological testing in systems for assuring the safety of beef. *Int. J. Food Microbiol.* 62:7–16.
8. Butler, F., G. Duffy, D. Engeljohn, A. Lammerding, and B. Tompkin. 2006. Case study: *Escherichia coli* O157:H7 in fresh raw ground beef. Background paper for the Joint FAO/WHO Expert Consultation on Development of Practical Risk Management Strategies based on Microbiological Risk Assessment Outputs. Kiel, Germany, 3-7 April 2006. http://www.fao.org/ag/agn/agns/jemra_riskmanagement_en.asp. Accessed 10 June 2010.
9. Cameron, S., C. Walker, M. Beers N. Rose, and E. Anear. 1995. Enterohaemorrhagic *Escherichia coli* outbreak in South Australia associated with the consumption of mettwurst. *Communic. Dis. Intell.* 19:70–71.
10. CDC. 1993. Update: multistate outbreak of *Escherichia coli* O157:H7 infections from hamburgers – western United States 1992–1993. Centers for Disease Control and Prevention, Atlanta, Ga. *Morbid. Mortal. Weekly Rep.* 42:258–263.
11. Dykes, G. 2000. The effect of freezing on the survival of *Escherichia coli* O157:H7 on beef trimmings. *Food Res. Int.* 33: 387–392.
12. Dykes, G. 2006. Laboratory-based simulation of freezing profiles of beef trim for *Escherichia coli* O157 survival determinations. *J. Microbiol. Methods* 64:266–274.
13. Fegan, N., G. Higgs, P. Vanderlinde, and P. Desmarchelier. 2005. An investigation of *Escherichia coli* O157 contamination of cattle during slaughter at an abattoir. *J. Food Prot.* 68:451–457.
14. Fegan, N., P. Vanderlinde, G. Higgs, and P. Desmarchelier. 2004. The prevalence and concentration of *Escherichia coli* O157 in faeces of cattle from different production systems at slaughter. *J. Appl. Microbiol.* 97:362–370.
15. Food Safety Inspection Service (2007a) Timeline of events related to *E. coli* O157:H7. http://www.fsis.usda.gov/Science/Ecoli_O157_Timeline/index.asp. Accessed 15 March 2011.
16. Food Safety Inspection Service 92007b) Agenda, The public health significance of non-O157 shiga toxin-producing *Escherichia coli* (STEC) Public Meeting. http://www.fsis.usda.gov/News_&_Events/Agenda_Ecoli_101707/index.asp Accessed 15 March 2011.
17. Food Safety Inspection Service (2010a) Verification activities for *Escherichia coli* O157:H7 in raw beef products. FSIS Directive 10,010.1 revision 3. Accessed 15 March 2011.
18. Food Safety Inspection Service (2010b) Microbiological results of raw ground beef products analyzed for *Escherichia coli* O157:H7, summarized by Calendar Year. http://www.fsis.usda.gov/Science/Ecoli_O157_Summary_Tables/index.asp Accessed 15 March 2011.
19. Grau, F. 1979. Fresh meats: bacterial association. *Archiv für Lebensmittel.* 30:81–116.
20. Jordan, D., S. McEwen, J. Wilson, W. McNab, and A. Lammerding. 1999. Reliability of an ordinal rating system for assessing the amount of mud and feces (tag) on cattle hides at slaughter. *J. Food Prot.* 62:520–525.
21. Kiermeier, A., G. Mellor, R. Barlow, and I. Jenson. Assumptions of acceptance sampling and the implications for lot contamination: *E. coli* O157 in lots of Australian manufacturing beef. *J. Food Prot.* (in press).
22. Phillips, D., D. Jordan, S. Morris, I. Jenson, and J. Sumner. 2006. A national survey of the microbiological quality of beef carcasses and frozen boneless beef in Australia. *J. Food Prot.* 69:1113–1117.
23. Phillips D., J. Sumner, J. Alexander and K. Dutton. 2001. Microbiological quality of Australian beef. *J. Food Prot.* 64:692–696.

24. Rangel, I., P. Sparling, C. Crowe, P. Griffin, and D. Swerdlow. 2005. Epidemiology of *Escherichia coli* O157:H7 outbreaks, United States, 1982–2002. *Emerg. Infect. Dis.* 11:603.
25. Sage, J., and S. Ingham. 1998. Survival of *Escherichia coli* O157:H7 after freezing and thawing in ground beef patties. *J. Food Prot.* 61: 1181–1183.
26. Vanderlinde, P., I. Jenson, and J. Sumner. 2005. Using national microbiological data to set meaningful performance criteria for slaughter and dressing of animals at Australian export abattoirs. *Int. J. Food Microbiol.* 104:155–159.
27. Vanderlinde, P., B. Shay, and J. Murray. 1998. Microbiological quality of Australian beef carcass meat and frozen bulk packed beef. *J. Food Prot.* 61:437–443.
28. Wells, J., B. Davis, K. Wachsmuth, L. Riley, R. Remis, R. Sokolow, and G. Morris. 1983. Laboratory investigation of hemorrhagic colitis outbreaks associated with a rare *Escherichia coli* serotype. *J. Clin. Microbiol.* 18:512–520.