

## Microbiological Baseline Survey of New Zealand Bovine Carcasses: A Preliminary Report

R. L. Cook,<sup>a</sup> S. C. Hathaway,<sup>a</sup> J. C. L. Harrison,<sup>b</sup> & N. H. Armitage<sup>a</sup><sup>a</sup>MAF Regulatory Authority (Meat & Seafood), PO Box 2526, Wellington, New Zealand<sup>b</sup>The Meat Industry Research Institute of New Zealand Inc. (MIRINZ), PO Box 617, Hamilton, New Zealand

## INTRODUCTION

The New Zealand meat industry in conjunction with the New Zealand MAF Regulatory Authority have recently completed a microbiological baseline survey of bovine carcasses intended to provide statistically significant data that: Establishes prevalences and levels of "indicator organisms and selected pathogens of food-borne significance on a fully representative national basis; evaluates relationships between the presence of specific pathogens and numbers/prevalence of indicator organisms; the latter providing a more practical means of on-going microbiological monitoring for process control; provides information on the epidemiological risk factors contributing to those microbial prevalences on particular classes of slaughtered animals; services the design and development of HACCP systems are validated in terms of achieving food safety objectives; provides baseline information for comparison with recent and future survey results from other countries.

This preliminary paper reports findings to date, and draws an initial comparison with the published findings of recent baseline surveys from the United States and Australia.

## METHOD

Carcass samples were collected over a 14 month period (December 1995 to January 1997) from 23 export bovine slaughter premises that contribute to 80% of the annual national throughput for bovine species, and for each class of slaughtered livestock. The number of each class of livestock to be sampled was determined as a proportion of the national annual kill and monthly kill for that class. As a result, carcasses of 320 heifers, 560 steers, 560 cows and 560 bulls (2000 total) were sampled.

Carcasses were sampled immediately after post-mortem inspection but prior to final trimming and carcass washing. Carcasses were sampled at three sites (outside leg, flank and brisket), and analysed as individual samples. Samples were collected using a multiple wet/dry swab technique, each from a 100 cm<sup>2</sup> carcass surface area. All samples were collected by one operator so as to achieve maximum consistency, and couriered under refrigeration within 24 h to MIRINZ for analysis. The swab samples were suspended in 15 ml of buffered peptone diluent, and analysed for aerobic plate count, and *Escherichia coli* by Petrifilm™ *E. coli* according to standard procedures (Cook, 1991). The limits of detection for the APC<sub>30</sub> and *E. coli* analyses were log<sub>10</sub> -0.12 cfu/cm<sup>2</sup> (0.76 cfu/cm<sup>2</sup>) and log<sub>10</sub> -1.12 cfu/cm<sup>2</sup> (0.08 cfu/cm<sup>2</sup>), respectively. In addition, samples were analysed for the presence of *Salmonella* by RVS enrichment (Cook, 1991) and for *E. coli* O157:H7 by EC broth / novobiocin enrichment and VIP<sup>®</sup> EHEC (*E. coli* O157:H7) screen (BioControl, United States).

## RESULTS AND DISCUSSION

In total, 996 carcasses were tested for the presence of *Salmonella*. All samples were negative, translating to a prevalence of not more than 0.1%. This result compares very favourably with the published prevalences for *Salmonella* in United States heifers/steers of 1% (USDA, 1994), in US cows/bulls of 2.7% (USDA, 1996), and Australian cattle of 0.4% (Vanderlinde & Murray, 1995). Failure to detect a single *Salmonella* in 996 carcasses provides statistical confidence at the 95% level that the prevalence of contaminated carcasses given a sampling regime of this kind is less than 0.1%.

*E. coli* O157:H7 was not detected from any of the 2000 bovine carcasses sampled. Failure to detect a single *E. coli* O157:H7 in 2000 carcasses provides statistical confidence at the 95% level that the prevalence of contaminated carcasses given a sampling regime of this kind is less than 0.05%. *E. coli* O157:H7 was not detected in a separate microbiological survey of 600 carcasses randomly selected from meat export slaughter houses sourcing cattle from within New Zealand's primary dairy farming region (unpublished data). Failure to detect a single *E. coli* O157:H7 in 2600 carcasses provides statistical confidence at the 95% level that the prevalence of contaminated carcasses is less than 0.04%. This compares very favourably with the published prevalences for *E. coli* O157:H7 in US heifers/steers of 0.2% (USDA, 1994), in US cows/bulls of <0.05% (USDA, 1996), and Australian cattle of 0.4% (Vanderlinde, 1995).

Preliminary results for the aerobic plate count and *E. coli* from the New Zealand survey, and published results from the US and Australian surveys, are presented in Tables 1 and 2, respectively. Despite differences in the size and presentation of the different classes of stock that could affect procedures that result in contamination of the carcass surface, differences in aerobic plate count between classes of animals appear to not differ.

As expected the counts on the flank site were on average higher than those on the outside leg and brisket sites, reflecting the greater level of handling on the flank site during evisceration. Similarly, the prevalence of *E. coli* at the flank site is higher than that of the other sites. The ranking of a site may relate to the degree of faecal contamination at that site (Nottingham *et al*, 1973). When detected, however, the mean log count of *E. coli* did not appear to differ between sites and classes, except for bulls where the leg and brisket mean counts were log<sub>10</sub> 0.3 to 0.5 less but the maximum count obtained was higher. The reason for this observation has yet to be determined. Nevertheless, the observation does support the process control and HACCP development utility envisaged in the design of survey protocols that analyse separate sites. The microbiological profile for New Zealand beef slaughter and dressing appears better than those of the US and Australia. Mean levels of APC and *E. coli* are considerably lower, and the prevalence of *E. coli* is similar, although higher than the prevalence on US heifers/steers. However, although seemingly reflective of better hygienic performance for most comparisons, differences need to be examined relative to the methodology used in different surveys: (1) Analysis of separate sites versus composite; (2) sampling immediately after post-mortem inspection vs after 12 h of refrigeration; (3) incubation of the APC at 30°C vs 35°C (US) and 25°C (Australian); (4) swab vs excision sampling.

A recently completed sampling method calibration study (unpublished data) suggests a 1.5 log count difference between swab sampling and excision results for the aerobic plate. Prevalence did not differ between the two methods. Adjustment of the New Zealand results to reflect this sampling method variation would result in similar mean counts to the US baseline surveys but still a lower maximum count than the US survey.

*E. coli* were detected only to a maximum of  $\log_{10}$  2.11 on New Zealand carcasses but up to  $\log_{10}$  6.0 on US carcasses. Similarly, *E. coli* were detected above the M value of  $\log_{10}$  2.0 described for the USDA Pathogen Reduction Rule (USDA, 1996b) on only one of the 2000 New Zealand carcasses. Adjusting individual carcass counts for perceived sampling method recovery differences would not have deemed any more than 5% of the NZ carcasses unacceptable. In contrast, the USDA described M-value was set at the 80<sup>th</sup> percentile for carcasses sampled in their baseline surveys. Consequently, the degree of faecal contamination on New Zealand carcasses, as indicated by the maximum numbers of *E. coli* detected, appears lower than that of the US. Consequently, the possibility of contamination with mesophilic pathogens during New Zealand slaughter and dressing appears more remote.

Comparing prevalences and means for *E. coli* is also difficult since the limit of detection for swab and excision sampling methods differ considerably. The limit of detection for the New Zealand swab sampling method is  $\log_{10}$  -1.12 cfu/cm<sup>2</sup> (0.08 cfu/cm<sup>2</sup>). While, it is difficult to calculate the limit of detection for the US and Australian methods in the absence of full method details being published, if one assumes that the total 300 cm<sup>2</sup> composite sample is used for the sample suspension, and that diluent is added at a volume ratio of 1:10, then the limit of detection would be 5 cfu/cm<sup>2</sup>. This limit is ~60x greater than that of the New Zealand method. Therefore, the observed differences in prevalence of *E. coli* between the New Zealand and US surveys (~24% versus ~8%) may be the result of differences in methods rather than a true difference. Similarly, because the New Zealand method can detect to a lower level, the mean counts cannot be compared as the New Zealand mean includes individual counts well below the limit of detection of the US method. The same argument cannot, however, be applied to the Australian survey data where the prevalence of *E. coli* is the same as that of the New Zealand survey and mean counts two (2) logs higher. The microbiological profile of New Zealand carcasses in terms of *E. coli* is, therefore, apparently better than that of Australian carcasses.

The international implication of these results requires careful consideration. Irrespective of what apparent differences signify, such differences would have a significant impact on the ability of New Zealand to achieve fixed microbiological criteria proposed in the US Pathogen Reduction/HACCP rule (USDA, 1996b). Nevertheless, the US and New Zealand share a common objective in seeking to minimise the potential for contamination of fresh meat by food-borne pathogens. Since the USDA has formulated performance criteria based on the results of their baseline survey, the criteria do not necessarily reflect the New Zealand processing standard. Therefore, it is imperative that New Zealand performance criteria are based on its own baseline survey, and additional ongoing microbiological monitoring programmes, and that the means of monitoring achievement of the above-stated objective be deemed equivalent to those of other countries.

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Table 1

Microbiological Baseline Survey of Bovine Carcasses: Aerobic Plate Count				
Class	Site	Prevalence %	Mean $\log_{10}$ cfu/cm <sup>2</sup>	Maximum $\log_{10}$ cfu/cm <sup>2</sup>
<b>New Zealand (30°C)</b>				
Heifers	Outside leg	92	1.21	4.67
	Flank	99	1.87	3.84
	Brisket	82	1.00	2.97
Steers	Outside leg	90	1.12	3.93
	Flank	98	1.70	4.24
	Brisket	83	1.20	3.69
Cows	Outside leg	87	1.10	4.15
	Flank	97	1.61	4.10
	Brisket	82	1.05	3.61
Bulls	Outside leg	89	1.21	4.38
	Flank	98	1.79	3.82
	Brisket	80	1.18	4.40
<b>United States (35°C)</b>				
Heifers/Steers	Composite*	99	2.68	6.00 - 7.00
Cows/Bulls	Composite*	100	3.05	>7.00
<b>Australia (25°C)</b>				
Cattle	Composite*	100	3.02	n/a

\* Composite of samples from rump, flank and brisket

Table 2

Microbiological Baseline Survey of Bovine Carcasses: <i>Escherichia coli</i>				
Class	Site	Prevalence %	Mean $\log_{10}$ cfu/cm <sup>2</sup>	Maximum $\log_{10}$ cfu/cm <sup>2</sup>
<b>New Zealand</b>				
Heifers	Outside leg	8	-0.78	0.24
	Flank	23	-0.64	1.39
	Brisket	6	-0.72	-0.05
Steers	Outside leg	11	-0.69	1.32
	Flank	24	-0.54	1.61
	Brisket	6	-0.81	1.62
Cows	Outside leg	10	-0.76	0.80
	Flank	21	-0.67	1.71
	Brisket	6	-0.77	1.31
Bulls	Outside leg	9	-0.34	2.04
	Flank	17	-0.57	2.11
	Brisket	6	-0.33	1.68
<b>United States</b>				
Heifers/Steers	Composite*	8	1.54	5.0-6.0
Cows/Bulls	Composite*	16	1.52	5.0-6.0
<b>Australia</b>				
Cattle	Composite*	22	1.05	n/a

\* Composite of samples from rump, flank and brisket