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The prevalence and concentration of *Escherichia coli* O157 in faeces of cattle from different production systems at slaughter

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ABSTRACT

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Aims: To determine the prevalence and concentration of *Escherichia coli* O157 shed in faeces at slaughter, by beef cattle from different production systems.

Methods and Results: Faecal samples were collected from grass-fed (pasture) and lot-fed (feedlot) cattle at slaughter and tested for the presence of *E. coli* O157 using automated immunomagnetic separation (AIMS). *Escherichia coli* O157 was enumerated in positive samples using the most probable number (MPN) technique and AIMS and total *E. coli* were enumerated using Petrifilm. A total of 310 faecal samples were tested (155 from each group). The geometric mean count of total *E. coli* was 5×10^5 and $2{\cdot}5 \times 10^5$ CFU g^{-1} for lot- and grass-fed cattle, respectively. *Escherichia coli* O157 was isolated from 13% of faeces with no significant difference between grass-fed (10%) and lot-fed cattle (15%). The numbers of *E. coli* O157 in cattle faeces varied from undetectable (<3 MPN g^{-1}) to $1{\cdot}1 \times 10^5$ MPN g^{-1}. Twenty-six (67%) of 39 O157 positive faeces had <10 MPN g^{-1} and three (8%) had counts between 10^3–10^5 MPN g^{-1}. There was no significant difference between concentrations of *E. coli* O157 in the faeces of grass-fed or lot-fed cattle.

Conclusion: The prevalence and numbers of *E. coli* O157 in the faeces of cattle at slaughter were not affected by the production systems evaluated in this study.

Significance and Impact of the Study: Information on the prevalence and numbers of *E. coli* O157 can be used for formulating intervention strategies and in quantitative risk assessments.

Keywords: cattle, *E. coli* O157, enumeration, grass-fed, lot-fed, prevalence, production systems, pulsed-field gel electrophoresis.

INTRODUCTION

Escherichia coli O157 is an important foodborne pathogen. A major reservoir of the bacterium is the gastrointestinal tract of cattle thus beef cattle and dairy cattle are an important point of entry of *E. coli* O157 into the human food chain (Hancock *et al.* 1998; Chapman *et al.* 2001; Renter *et al.* 2003). In developing strategies for the control of *E. coli* O157 in red meat production and in ensuring the safety of products such as beef patties qualitative and quantitative risk assessments have been conducted. From these through-chain assessments, the prevalence and concentration of *E. coli* O157 in cattle faeces have been identified as important factors that impact on the magnitude of risk associated with the consumption of a contaminated product (Cassin *et al.* 1998; Food Safety and Inspection Service. 2001; Centers for Disease Control and Prevention 2002). There is a lack of sufficient quantitative information on the presence of *E. coli* O157 at the various stages of the production pathway and this increases the uncertainty associated with the outputs of these risk assessments.

Several factors can influence the faecal shedding of cattle such as the animal's age, diet and husbandry. Australian beef

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cattle are produced on pasture or are finished on grain-supplemented diets in feedlots (lot-fed). The diet and the husbandry practices in these major production systems may influence the prevalence and numbers of total *E. coli* and *E. coli* O157 shed by the respective animals (Duncan *et al.* 2000; Stanton and Schutz 2000; Callaway *et al.* 2003). There is no published information of the prevalence or concentration of *E. coli* O157 shed by cattle at slaughter from the different local production systems.

Another significant factor in determining the prevalence and concentration of a specific serotype of *E. coli* among the total *E. coli* population is the specificity and the limit of detection of the methods used. Detection and isolation methods continue to improve with technological advances. Immunomagnetic separation (IMS) and immunocapture (IC) techniques are currently the preferred methods available for the isolation and detection of *E. coli* O157 (Cubbon *et al.* 1996; de Boer and Heuvelink 1998; Kerr *et al.* 2001), as the limit of detection is up to 100-fold greater than direct culture methods (Chapman *et al.* 1994). The use of IMS and the enrichment of larger amounts of faeces (e.g. 10 g instead of 1 g) increase the chance of detection of *E. coli* O157. As a result of using this combination, the prevalence of *E. coli* O157 shed by cattle has been shown to be higher at *ca* 23–28% (Elder *et al.* 2000; Smith *et al.* 2001) than 2% which was previously reported (Hancock *et al.* 1997).

The objective of this study was to determine the prevalence and concentration of *E. coli* O157 in cattle from grass and lot-fed production systems, and to gather quantitative data for inclusion in future risk assessment studies. This was achieved using specific methods with a low limit of detection.

MATERIALS AND METHODS

Sampling plan

A separate sampling plan was developed for grass-fed and lot-fed cattle so that the results could be interpreted independently, while still allowing comparisons between the two different production systems. The number of cattle to be sampled from each state in Australia was stratified based on the production of total beef or numbers of cattle on feed in that state as described below. Lot-fed animals were defined as those fed a grain-enriched diet for at least 60 days prior to slaughter. Specific data on the number of grass-fed cattle in Australia was unavailable, but as the majority of animals within Australia are on pasture, total beef production figures were used for the purpose of developing the sampling plan. The number of grass-fed cattle sampled from each state was determined based on that state's production as a percentage of total production and is listed in Table 1.

Table 1 Number of cattle faecal samples collected at slaughter per state

State	Number of samples from production system		Total
	Grass-fed*	Lot-fed†	
Queensland	75	75	150
New South Wales	30	60	90
Victoria	30	10	40
Western Australia	10	10	20
South Australia	5	0	5
Tasmania	5	0	5
Total	155	155	310

*Number of samples collected based on total beef production (tonnes of carcass weight) per state in 2000 (from Livestock Products Australia, Australian Bureau of Statistics, September quarterly, 2001).
†Number of samples collected based on number of animals on feed in each state for the December quarter 2000 (from Meat and Livestock Australia, Fast Facts: Australia's Beef Industry, July 2001).

The number of lot-fed cattle sampled in each state was determined from the number of Australian cattle on feed as shown in Table 1. Based on this data, 10 lot-fed cattle should have been sampled from South Australia (SA); however, during the course of the survey, it became apparent that these samples would not be available, as feedlots in SA had been emptied because of a decline in profitability. A further five samples from both Queensland (Qld) and New South Wales (NSW) were collected to make up for this shortfall.

Abattoir selection

Abattoirs from the AUS-MEAT abattoir accreditation list (http://www.ausmeat.com.au/standards/accreditation) were randomly selected, contacted and invited to participate in the study. One abattoir declined to participate in the survey and another was randomly selected from the same state to replace it. A maximum of five samples were collected from any abattoir on a given day. Abattoirs were randomly selected with replacement, such that an abattoir may have been required to provide multiple samples, collected on different processing days. This selection process was performed separately for grass-fed and lot-fed cattle; this required some abattoirs to provide five samples from a single production type, while others were to provide five samples each from both grass and lot-fed cattle. If an abattoir was unable to provide the specific type of samples required, another abattoir was randomly selected. The geographical origin and times of transport and holding prior to slaughter was not determined for any of the cattle.

Collection of faecal samples

Faecal samples were collected from cattle processed at randomly selected times during the day. This was achieved by breaking the processing day into 30-min sections and then randomly selecting the time period in which the sample was taken. Faecal samples were only identified based on the production type of the animals sampled and the abattoir from which they were collected. Faecal samples were collected postevisceration by cutting the intestine 15–30 cm from the bagged end and squeezing at least 30 g of material into a sterile jar. Samples were kept chilled and returned to the laboratory within 24 h (or 48 h for samples from Western Australia) by courier and processed on arrival at the laboratory.

Isolation of *E. coli* O157

Faecal slurries were prepared by transferring 30 g of faeces to a sterile container and diluting 10^{-1} with buffered peptone water (BPW; Oxoid, Basingstoke, UK). Faecal slurries were stored at 2°C overnight if they were not enriched immediately. *Escherichia coli* counts remained unchanged in faecal samples diluted in BPW and stored for up to 4–5 days at 2°C (data not shown). A 100 g portion of faecal slurry was incubated at 42°C for 6 h after which *E. coli* O157 were concentrated from 1 ml of this enrichment using automated immunomagnetic separation (AIMS) following the manufacturer's instructions (Dynal Pty Ltd, Oslo, Norway). Collected beads were plated onto Sorbitol MacConkey Agar containing 0·05 mg l^{-1} cefixime and 2·5 mg l^{-1} tellurite (CT-SMAC; Zadik *et al.* 1993) and CHROMagar O157 (CHROMagar, Paris, France) and incubated at 37°C for 18–20 h. Colonies showing the typical *E. coli* O157 phenotype were characterized as described below. The remaining unenriched slurry was stored at 2°C for enumeration of *E. coli* O157 if required.

Enumeration of generic *E. coli*

Escherichia coli numbers were estimated by plating 1 ml of serial dilutions of the 10^{-1} diluted faecal slurry onto *E. coli* Petrifilm (3 M Australia Pty Ltd, St Marys, Australia). The number of *E. coli* present was determined after incubation for 20 h at 37°C following the manufacturer's instructions.

Enumeration of *E. coli* O157

Enumeration was performed using a combination of a 5 × 3 tube (0·1–0·00001 g of faeces) most probable number (MPN) technique, followed by AIMS (Fegan *et al.* 2004). MPN tubes inoculated with faeces diluted in BPW were incubated for 6 h at 42°C and then tested for the presence of *E. coli* O157 using AIMS as described above. An MPN tube was considered positive by the presence of a colony on the selective and differential media, which showed the correct colony morphology and agglutinated with specific antisera. MPN values were calculated using the MPN Calculator Build 22 by Mike Curiale (http://members.ync.net/mcuriale/mpn/index.html). For reasons of economy, only the 0·1 and 0·01 g MPN tubes were tested directly after the 6 h incubation, the remainder were held at 2°C for 16 h until the results of the 0·1 and 0·01 g MPN tubes were determined. If any of the 0·01 g tubes were positive, the remaining MPN tubes were tested for *E. coli* O157 using AIMS.

Characterization of *E. coli* O157

Presumptive *E. coli* O157 colonies from CT-SMAC and CHROMagar O157 plates were serotyped using the *E. coli* O157 Test Kit (Oxoid) and tested for the presence of the O157 *rfb* gene following the method of Desmarchelier *et al.* (1998). Only one colony per sample was stored for further characterization. Isolates were tested for Shiga toxin genes (stx_1 and/or stx_2) and other virulence markers (*eae*A and *ehx*A) following the method of Paton and Paton (1998). The presence of the H7 antigen was determined by inoculating motility media (Speck 1976) and serotyping motile strains using the RIM® *E. coli* H7 test latex antiserum (Remel Lenexa, KS, USA). Pulsed-field gel electrophoresis (PFGE) was used to determine genetic relatedness among isolates. DNA was prepared following the method of Böhm and Karch (1992) while the digestion and running conditions used were those of Davis *et al.* (2003) except that the gels were made from Pulsed Field Certified Agarose (Bio-Rad, Hercules, CA, USA) and were run on a CHEF DRIII (Bio-Rad) for a period of 22 h. PFGE patterns were analysed using molecular analyst fingerprinting (MAF) software version 1·6 (Bio-Rad) using the DICE similarity coefficient and clustering by the unweighted pair group method using arithmetic averages (UPGMA).

Statistical analysis

Where appropriate, results were analysed using a statistical computer package (Minitab, Mintab Inc., PA, USA). A chi-squared test for independence was used to compare the prevalence of *E. coli* O157 between different production systems. A one-way analysis of variance (ANOVA) was performed on counts of total *E. coli* and *E. coli* O157 in grass-fed and lot-fed cattle faeces. For the purposes of statistical analysis, MPN counts of <3 MPN g^{-1} and generic *E. coli* counts <10 CFU g^{-1} were assigned an arbitrary value of 1 MPN g^{-1} and 1 CFU g^{-1} respectively.

RESULTS

Prevalence of *E. coli* O157

Escherichia coli O157 containing at least one Shiga toxin gene (stx_1 and/or stx_2), *eae*A and *ehx*A were isolated from 39 (13%) of 310 faecal samples, 23 (15%) from lot-fed cattle and 16 (10%) from grass-fed cattle. There was no significant difference in the observed prevalence of *E. coli* O157 between grass-fed and lot-fed cattle ($P = 0{\cdot}23$).

Enumeration of generic *E. coli*

The number of generic *E. coli* shed by lot-fed cattle varied from <10–$4{\cdot}4 \times 10^7$ CFU g^{-1}, with a geometric mean count (antilog of the mean of $\log_{10}$ transformed MPN values) of 5×10^5 CFU g^{-1} ($\log_{10}$ 5·7). The number of *E. coli* shed by grass-fed cattle varied from <10 and $3{\cdot}8 \times 10^8$ CFU g^{-1} with a geometric mean count of $2{\cdot}5 \times 10^5$ CFU g^{-1} ($\log_{10}$ 5·4). There was no significant difference between the number of *E. coli* shed by grass-fed and lot-fed cattle ($P = 0{\cdot}95$). A comparison of the range of counts found in cattle faeces is shown in Fig. 1.

Enumeration of *E. coli* O157

The *E. coli* O157 count was estimated for the 23 positive lot-fed and 16 positive grass-fed cattle faecal samples (Table 2). The counts ranged from undetectable (<3 MPN g^{-1}) to $1{\cdot}1 \times 10^5$ MPN g^{-1} using the MPN procedure. The majority of faecal samples (67%) contained <10 MPN g^{-1} of *E. coli* O157 while there was only one count at $1{\cdot}1 \times 10^5$ MPN g^{-1}. Counts of *E. coli* O157 shed by grass-fed cattle ranged from <3 MPN g^{-1} to $4{\cdot}3 \times 10^2$ MPN g^{-1}, while for lot-fed cattle the count ranged from <3 MPN g^{-1} to $1{\cdot}1 \times 10^5$ MPN g^{-1}. The geometric mean count of *E. coli* O157 in the faeces of lot-fed cattle was $\log_{10}$ 1·3 (20 MPN g^{-1}), standard deviation (S.D.) 1·3 while that for grass-fed cattle faeces was $\log_{10}$ 0·6 (4 MPN g^{-1}), S.D. 1·0. There was no significant difference between the *E. coli* O157 counts for grass-fed or lot-fed cattle ($P = 0{\cdot}06$). In two faeces from lot-fed cattle (samples 62 and 203) the *E. coli* O157 count was of the same magnitude as the generic *E. coli* count. For sample 62, the *E. coli* count determined using Petrifilm (detecting non-O157 *E. coli*) was 200 CFU g^{-1}, while the *E. coli* O157 count was 430 MPN g^{-1}, similarly for sample 203, the non-O157 *E. coli* and *E. coli* O157 counts were $1{\cdot}6 \times 10^5$ CFU g^{-1} and $1{\cdot}1 \times 10^5$ MPN g^{-1}, respectively (Table 2). The two counts (*E. coli* O157 and generic *E. coli*) were added together to provide the total generic *E. coli* count as *E. coli* O157 do not appear as *E. coli* on Petrifilm.

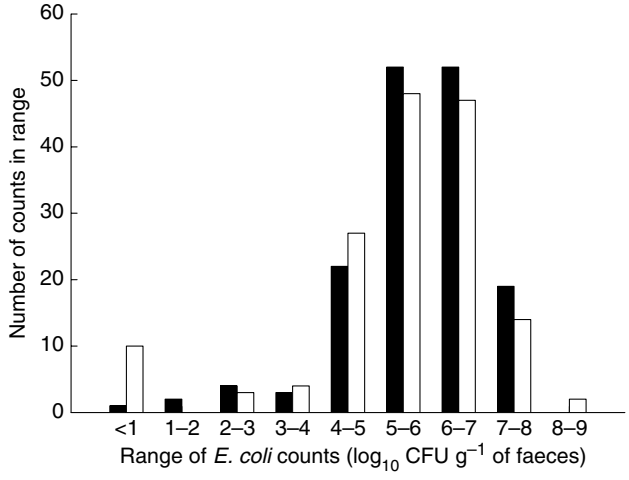

Fig. 1 The range of generic *Escherichia coli* counts in the faeces of cattle at slaughter [grass-fed (□) and lot-fed (■)]

Characterization of *E. coli* O157

The majority (59%) of the *E. coli* O157 isolates were O157:H- (Table 2). The proportion of *E. coli* O157 isolates that were *E. coli* O157:H- isolates for lot-fed cattle was 57% and for grass-fed cattle was 63%. All *E. coli* O157 isolates carried at least one of the genes encoding Shiga toxin and both the *eae*A and *ehx*A. The Shiga toxin genes found in *E. coli* O157 isolated from this study included $stx_{1\&2}$ (56% of isolates), stx_2 alone (38%) and stx_1 alone(5%). Equal numbers of *E. coli* O157 from grass-fed cattle carried $stx_{1\&2}$ and stx_2, with only two isolates carrying stx_1. *Escherichia coli* O157 isolates from lot-fed cattle were not found to carry stx_1 alone, with the majority of isolates (65%) carrying $stx_{1\&2}$ (Table 3).

There were 26 different restriction patterns as determined by PFGE. The phenogram generated from MAF showing the 26 different banding patterns and corresponding isolates is shown in Fig. 2. Two major clusters were present at a similarity of 80% with one cluster consisting entirely of *E. coli* O157:H- isolates while the other contained all of the *E. coli* O157:H7 isolates and two *E. coli* O157:H- isolates. Of the 26 different PFGE patterns, nine were common to multiple isolates and the other 17 patterns were distinct, but may have varied by only one or two bands from other patterns. PFGE patterns 1, 6, 10, 12, 18 and 22 each contained two isolates obtained from different samples collected from the same abattoir on the same day. PFGE patterns 8 and 9 each contained three isolates, two of which were isolated from different samples collected from the same abattoir on the same day, while the third isolate came from a sample collected at a different abattoir in another state on a different sampling day. Pattern 2 was present in four isolates, all isolated from different abattoirs in one state on different sampling days. Isolates with PFGE patterns which were indistinguishable from each other were isolated from cattle

Table 2 Concentrations of *Escherichia coli* O157 in the faeces of lot-fed and grass-fed cattle at slaughter

				Count	
Sample no.	Serotype	Production type	Date of collection	*E. coli* (CFU g^{-1})	O157 (MPN g^{-1})
176	O157:H7	Lot-fed	09/12/2002	$1{\cdot}3 \times 10^4$	<3
75	O157:H-		31/10/2002	$1{\cdot}4 \times 10^5$	<3
93	O157:H7		06/11/2002	$7{\cdot}2 \times 10^5$	<3
147	O157:H7		13/11/2002	$2{\cdot}8 \times 10^6$	<3
293	O157:H7		08/01/2003	5×10^5	3
273	O157:H-		19/12/2002	$1{\cdot}8 \times 10^7$	3·6
204	O157:H-		25/11/2002	$1{\cdot}7 \times 10^5$	3·6
295	O157:H7		08/01/2003	$1{\cdot}9 \times 10^5$	3·6
149	O157:H-		13/11/2002	$5{\cdot}1 \times 10^5$	3·6
287	O157:H-		27/12/2002	$2{\cdot}1 \times 0^6$	3·6
43	O157:H-		08/10/2002	$1{\cdot}6 \times 10^5$	7·4
202	O157:H-		25/11/2002	$3{\cdot}3 \times 10^5$	9·2
55	O157:H-		25/10/2002	$7{\cdot}6 \times 10^5$	9·2
275	O157:H-		19/12/2002	8×10^6	15
52	O157:H7		25/10/2002	$2{\cdot}2 \times 10^7$	15
274	O157:H-		19/12/2002	$1{\cdot}3 \times 10^6$	21
178	O157:H-		09/12/2002	$3{\cdot}4 \times 10^5$	93
271	O157:H7		19/12/2002	$8{\cdot}1 \times 10^4$	93
74	O157:H-		31/10/2002	$4{\cdot}6 \times 10^5$	93
62	O157:H7		29/10/2002	630*	430
272	O157:H7		19/12/2002	$4{\cdot}6 \times 10^5$	430
294	O157:H7		08/01/2003	$4{\cdot}1 \times 10^5$	$4{\cdot}3 \times 10^3$
203	O157:H-		25/11/2002	$2{\cdot}7 \times 10^5$ *	$1{\cdot}1 \times 10^5$
266	O157:H-	Grass-fed	17/12/2002	$4{\cdot}5 \times 10^5$	<3
122	O157:H-		11/11/2002	$1{\cdot}8 \times 10^7$	<3
116	O157:H7		12/11/2002	$1{\cdot}6 \times 10^4$	<3
10	O157:H-		25/09/2002	$2{\cdot}4 \times 10^5$	<3
9	O157:H-		25/09/2002	4×10^5	<3
17	O157:H-		01/10/2002	$1{\cdot}8 \times 10^6$	<3
194	O157:H7		20/11/2002	$3{\cdot}3 \times 10^6$	<3
217	O157:H-		27/11/2002	$3{\cdot}5 \times 10^7$	<3
168	O157:H7		15/11/2002	$1{\cdot}3 \times 10^6$	3
170	O157:H7		15/11/2002	$3{\cdot}2 \times 10^5$	3·6
100	O157:H7		06/11/2002	$1{\cdot}1 \times 10^6$	3·6
123	O157:H-		27/11/2002	$3{\cdot}2 \times 10^7$	3·6
191	O157:H7		20/11/2002	$1{\cdot}8 \times 10^7$	9·2
31	O157:H-		07/10/2002	$1{\cdot}2 \times 10^7$	15
220	O157:H-		27/11/2002	$5{\cdot}1 \times 10^5$	240
125	O157:H-		27/11/2002	$9{\cdot}9 \times 10^6$	$4{\cdot}3 \times 10^3$

**E. coli* counts were determined by adding the Petrifilm and *E. coli* O157 counts together.

Table 3 Type of Shiga toxin genes present in *Escherichia coli* O157 isolated from cattle from different production systems

Toxin type	Lot-fed	Grass-fed	Total
stx_1	0 (0)*	2 (13)	2 (5)
stx_2	8 (35)	7 (44)	15 (38)
$stx_{1\&2}$	15 (65)	7 (44)	22 (56)

*Values in paranthesis are given in percentages.

from the same production type, with the exception of isolates with PFGE pattern 2, which were isolated from both lot and grass-fed cattle. Different PFGE patterns were also observed in cattle slaughtered on the same day from one abattoir.

DISCUSSION

The Food Safety and Inspection Service has recently requested meat-processing establishments reassess their

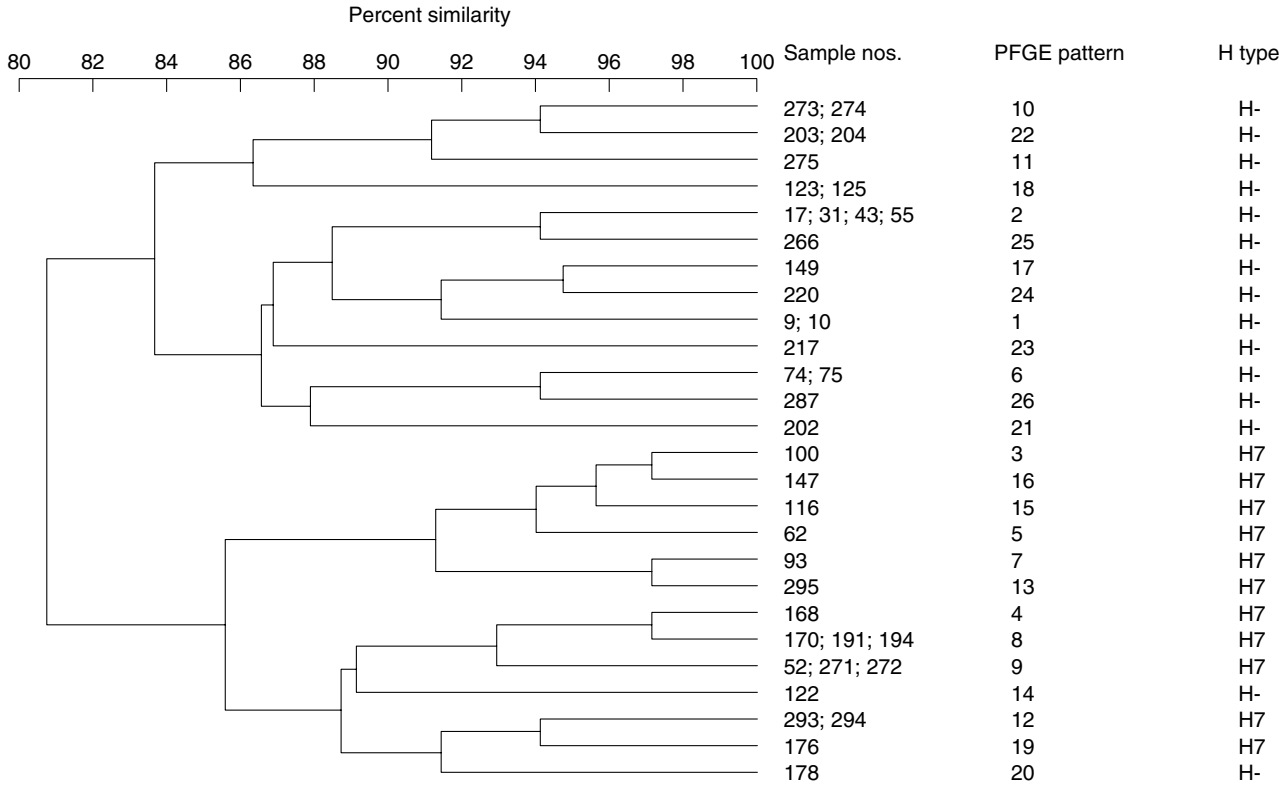

Fig. 2 Phenogram of pulsed-field gel electrophoresis patterns of *Escherichia coli* O157 isolates generated using Molecular Analyst Fingerprinting software

HACCP plans based on new scientific data, which indicates that *E. coli* O157 is more prevalent than previously thought (Food Safety and Inspection Service 2002). The two studies which led to this decision were those by Elder *et al.* (2000) where 28% of 327 cattle were found to carry *E. coli* O157 at slaughter, and the second study by Smith *et al.* (2001) found 23% of 3162 feedlot cattle carried this pathogen. These two studies used more sensitive methods (IMS) and larger sample sizes (10 g faeces) than earlier work and therefore it is not surprising that the observed prevalence was higher than previously thought. Few studies have been reported using 10 g faecal samples combined with IMS for the detection of *E. coli* O157. The observed prevalence of *E. coli* O157 in the current study of Australian cattle (13%) is somewhat lower than that found in US lot-fed cattle (23–28%; Elder *et al.* 2000; Smith *et al.* 2001) and in Italian cattle (17%; Bonardi *et al.* 2001). The results from the current study and those by Elder *et al.* (2000); Smith *et al.* (2001) and Bonardi *et al.* (2001) highlight the effect sample size and the method of analysis can have on estimations of prevalence.

There is conflicting evidence on the effect of high nutrient or high roughage diets on the prevalence of *E. coli* O157 in cattle, with some work suggesting *E. coli* O157 prevalence is lower in animals fed hay or roughage with other studies demonstrating no effect (Buchko *et al.* 2000; Tkalcic *et al.* 2000; Callaway *et al.* 2003). The results from our study support the latter conclusion, as there was no significant difference in the prevalence or counts of *E. coli* O157 between grass-fed and lot-fed cattle. The animals used in this study were naturally contaminated with *E. coli* O157 and were tested at the abattoir prior to slaughter. Cattle were tested after transport and it is not known if transport from farms and feedlots to the abattoirs and feed withdrawal before slaughter resulted in any change in the prevalence or numbers of *E. coli* O157 from that while still on feed. Little effect has been observed on the prevalence of *E. coli* O157 after transport in other studies (Barham *et al.* 2002; Minihan *et al.* 2003) but the effect on numbers of *E. coli* O157 is unknown. This study was designed using random selection of abattoirs and samples to reduce the effect of biases such as the time for transport and lairage of animals. It was observed that on some occasions, the faeces of grass-fed cattle contained remnants of grain. This could indicate supplemental feeding of such animals or changes in the husbandry of these animals during transport and/or holding before slaughter. However, such animals would not have fit the definition of lot-fed (60 days on grain) applied to animals

defined as lot-fed in the study. This survey was conducted during a period of widespread drought in Australia, and it is possible that some husbandry practices (e.g. supplemental feeding) may have changed in some areas and that this influenced the results of the survey.

In contrast to the *E. coli* O157 results described above, it has been suggested that cattle on grain diets shed higher numbers of *E. coli* than cattle fed high roughage or forage fed animals (Diez-Gonzalez *et al.* 1998; Jordan and McEwen 1998; Scott *et al.* 2000; Stanton and Schutz 2000). No significant differences in total *E. coli* between grain and grass fed cattle was found in this study, but as animals were tested only at slaughter, no conclusions can be drawn on the numbers of *E. coli* shed while they were still on full feed. The effects of transport, lairage and withholding of feed during this time may negate any effects that may have occurred on farm as discussed previously.

The counts of *E. coli* O157 in cattle faeces at slaughter were generally low, with the majority of *E. coli* O157 positive faecal samples containing <10 MPN g^{-1}. It is difficult to compare the numbers of *E. coli* O157 present in animal faeces with other studies as different enumeration methods have been used such as plating directly onto selective and differential media (Zhao *et al.* 1995; Omisakin *et al.* 2003), or by using a combination of direct plating and MPN/IMS (Strachan *et al.* 2001; Ogden *et al.* 2002) and these methods have different lower limits for counts. The range of counts of *E. coli* O157 in adult cattle faeces were similar to those found in other studies where the majority of counts were <100 CFU or MPN g^{-1} with few animals shedding >10^5 CFU or MPN g^{-1} (Lahti *et al.* 2003; Omisakin *et al.* 2003). The counts in adult cattle from these studies were lower than those found in calves (Zhao *et al.* 1995) and despite differences in methodology it is possible that young animals may not only have a higher prevalence of *E. coli* O157 (Blanco *et al.* 2001) but also may shed higher numbers.

Escherichia coli O157 isolates from this study were typical of those isolated from Australia in previous studies where the majority of *E. coli* O157 isolates carried $stx_{1\&2}$, followed by stx_2 and few isolates carried stx_1. In addition, when compared using PFGE, the isolates clustered based on H serotype (Fegan and Desmarchelier 2002). The presence of PFGE patterns which were indistinguishable among *E. coli* O157 from different herds of animals slaughtered at different times and from different production systems suggests there may be some clones of *E. coli* O157, which are widely distributed in Australian cattle. The presence of multiple patterns from animals slaughtered at the same plant on the same day, and from animals slaughtered at different plants on different days indicates that there is a wide variety of *E. coli* O157 isolates present in cattle at slaughter.

In at least two samples (62 and 203) the *E. coli* O157 count was similar to the generic *E. coli* count. Although the methods used to determine the concentration of *E. coli* (direct plate onto Petrifilm) and *E. coli* O157 (MPN) differed, this suggests that in these particular animals, *E. coli* O157 was a predominant *E. coli* type. One of these animals, 203, had the highest count of *E. coli* O157 ($1{\cdot}1 \times 10^5$ MPN g^{-1}) observed in this study. The effect of a few animals shedding high numbers of *E. coli* O157 vs more animals shedding lower numbers of *E. coli* O157 on the risk of carcase contamination has yet to be determined. Numbers of *E. coli* O157 shed by adult cattle at slaughter in this study were generally low, suggesting these animals pose a smaller risk to carcass contamination. It is possible that one high shedding animal, or supershedder as defined by Naylor *et al.* (2003), within a herd may be a higher risk for contaminating the hides and the abattoir environment than a group of animals where only low numbers of *E. coli* O157 are shed. The relationship between such supershedders and the risk of carcase contamination is a subject for further study.

Feeding history did not significantly affect the prevalence and numbers of both generic *E. coli* and *E. coli* O157 in cattle at slaughter. Further studies in this area could provide information that will help to identify the risks related to carcass contamination from infected animals. In addition, further studies are needed to determine if effects of feed, transport and lairage influence the prevalence and numbers of *E. coli* O157 in adult cattle to aid in the development of a whole-of-chain approach for managing the risk of exposure to *E. coli* O157.

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