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The prevalence and spread of *Escherichia coli* O157:H7 at a commercial beef abattoir

J.M. McEvoy[1], A.M. Doherty[1], J.J. Sheridan[1], F.M. Thomson-Carter[2], P. Garvey[1], L. McGuire[1], I.S. Blair[3] and D.A. McDowell[3]

[1]Teagasc, The National Food Centre, Ashtown, Dublin, [2]Department of Medical Microbiology, University of Aberdeen, Aberdeen, and [3]School of Applied Medical Science, University of Ulster at Jordanstown, Newtownabbey, UK

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ABSTRACT

J.M. MCEVOY, A.M. DOHERTY, J.J. SHERIDAN, F.M. THOMSON-CARTER, P. GARVEY, L. MCGUIRE, I.S. BLAIR AND D.A. MCDOWELL. 2003.

Aims: To investigate the prevalence and virulence characteristics of *Escherichia coli* O157:H7 after a number of beef process operations at a commercial Irish abattoir.

Methods and Results: Two 12-month studies were carried out. The first study (study 1) examined the prevalence of *E. coli* O157:H7 at up to six sites on carcasses at eight stages of the dressing, washing, chilling and boning process. The second study (study 2) examined the prevalence of *E. coli* O157:H7 in bovine faeces and rumen contents post-slaughter and on dressed, washed carcasses. Isolates from both studies were phage-typed and the presence of genes encoding verocytotoxin, enterohaemolysin and intimin production was determined. *E. coli* O157:H7 was isolated from four of 36 carcasses in study 1. *E. coli* O157:H7 was detected during hide removal and was detected at multiple carcass sites and multiple process stages, including boning. On two carcasses, contamination was first detected at the bung following its freeing and tying. All isolates from study 1 were phage type (PT) 2, *eaeA*O157 and *ehlyA* positive, but were verocytotoxin 1 (VT1) and verocytotoxin 2 (VT2) negative. In study 2, *E. coli* O157:H7 was isolated from 2·4% of faecal, 0·8% of rumen and 3·2% of carcass samples. In some cases, isolates recovered from the faeces of a particular animal, the resulting carcass and adjacent carcasses on the line had the same phage typing and virulence characteristic profile patterns. All isolates from study 2 were *eaeA*O157 and *ehlyA* positive and only one isolate was VT1 and VT2 negative. Most isolates were PT 32. A higher frequency of positive isolations was noted from samples taken during spring and late summer.

Conclusion: These studies show that in a typical Irish beef abattoir, carcass contamination with *E. coli* O157:H7 can occur during hide removal and bung tying and this contamination can remain on the carcass during subsequent processing.

Significance and Impact of the Study: This study provides data that is necessary for the understanding of how *E. coli* O157:H7 contamination of beef occurs.

Keywords: beef, carcass, *E. coli* O157:H7, faeces, rumen.

INTRODUCTION

Cattle have been identified as a major reservoir of *Escherichia coli* O157:H7 (Faith *et al.* 1996; Chapman *et al.* 1997; Hancock *et al.* 1997; Rice *et al.* 1997) and consumption of foods of bovine origin have been associated with some of the largest food poisoning outbreaks in which this organism was identified as the aetiologic agent (Anon. 1997; Meng and Doyle 1998).

E. coli O157:H7 has been reported in faeces (Chapman *et al.* 1993; Heuvelink *et al.* 1998a; Bonardi *et al.* 1999; Van

Correspondence to: J.M. McEvoy, Department of Veterinary and Microbiological Sciences, Van Es Hall, North Dakota State University, Fargo, ND 58105, USA (e-mail: john.mcevoy@ndsu.nodak.edu).

Donkersgoed *et al.* 1999; Elder *et al.* 2000), rumen contents (Van Donkersgoed *et al.* 1999), and on the hide (Elder *et al.* 2000) of cattle at slaughter. Possible contamination of edible carcass tissue is the most significant challenge to food safety, and the extent and nature of such contamination are related to the *E. coli* O157:H7 status of the preslaughter animal, and any processes which distribute the organism within or between carcasses during dressing operations. Thus a UK study isolated *E. coli* O157:H7 from 30% of carcasses derived from rectal swab-positive animals (Chapman *et al.* 1993), while a study in the US found a positive correlation between the total animal prevalence (prevalence in faeces and on the hide) and the prevalence of this pathogen on derived carcasses (Elder *et al.* 2000).

In many studies of the prevalence of *E. coli* O157:H7 on beef carcasses, samples for microbiological analysis have been collected from chilled carcasses (Chapman *et al.* 1993; Richards *et al.* 1998; Vanderlinde *et al.* 1998). While such studies provide information on the overall effects of processing operations in modulating the occurrence of the pathogen in finished carcasses, they are unable to identify the process stage(s) at which the carcass became contaminated, or the result of such contamination during subsequent processing. However, as it is clear that significant changes in the frequency of contamination can occur, a study reported a decrease in the proportions of *E. coli* O157:H7 carcass prevalence from 43% pre-evisceration to 2% after <2 h in the chill (Elder *et al.* 2000).

This paper describes two studies, each of 1 year duration, carried out at a typical Irish beef abattoir. Study 1 examined the occurrence, distribution and persistence of *E. coli* O157:H7 on beef carcasses. Study 2 surveyed the prevalence of *E. coli* O157:H7 in faecal and rumen samples of animals postslaughter and on resulting carcasses at the end of the dressing processes.

MATERIALS AND METHODS

Experimental design

Two separate 12 month studies were carried out to examine the prevalence of *E. coli* O157:H7 at a commercial beef abattoir. Study 1 examined the prevalence of *E. coli* O157:H7 at a number of carcass sites, at process stages between legging and boning. Study 2 examined the prevalence of *E. coli* O157:H7 in faeces, rumen contents and on entire carcass surfaces following washing.

Process description

The commercial abattoir used in both studies slaughtered cattle (predominantly steers and heifers) at a line speed of 40 to 80 animals per hour. The process used an upward pulling automatic hide puller. Dressed and graded carcasses were washed with potable water at a temperature of 35–40°C, before chilling.

Study 1

Sample collection. Thirty-six beef carcasses were sampled over a 12 month period between April 1997 and March 1998. Up to six carcass sites on the left side (i.e. the leading side) of each carcass were sampled at eight process stages (Table 1). An undelimited area of approx. 50 cm^2 was swabbed with cotton-tipped swabs using the wet and dry technique (Anon. 1993). Swabs were suspended in 50 ml of maximum recovery diluent (Oxoid Ltd, Basingstoke, UK), and transported to the laboratory within 1 h of collection.

Microbiological analysis. A 10 ml aliquot of swab suspension was added to 10 ml of double strength enterobacteriaceae enrichment (EE) broth (Oxoid) containing 0·04 g l^{-1}

Table 1 Carcass sites sampled at each sample point during processing

	Carcass sites					
Sample point	Hock	Brisket	Cranial back	Bung	Inside round	Outside round
Legging	✔					
Hide removal	✔	✔	✔	✔		
Bung tying*				✔		
Evisceration	✔	✔	✔	✔		
Splitting		✔	✔	✔	✔	
Washing	✔	✔	✔	✔	✔	
Chilling (24 h)	✔	✔	✔	✔	✔	
Boning†		✔	✔		✔	✔

✔ = Sample collected for microbiological analyses.

*After freeing and tying of the bung.

†Samples were taken from meat cuts corresponding to carcass sites sampled at previous stages of dressing.

novobiocin (Sigma Chemical Co., St Louis, MO, USA) and incubated at 37°C for 24 h. Using a sterile inoculation loop, an aliquot of the EE broth was transferred in duplicate to sorbitol MacConkey agar plates (Oxoid) containing 0·05 mg l^{-1} cefiximine (Cyanamid, Gosport Hampshire, UK) and 2·5 mg l^{-1} potassium tellurite (Sigma) and incubated at 37°C for 24 h. A maximum of eight non-sorbitol-fermenting colonies per plate were further screened by plating simultaneously onto eosin methylene blue agar (EMB) (Oxoid) and phenol red sorbitol agar containing 4-methylumbelliferyl-β-D-glucuronide (PRS-MUG) and incubating at 37°C for 24 h. Colonies exhibiting a green metallic sheen on EMB and failing to fluoresce under u.v. illumination on PRS-MUG plates were tested for the presence of the O157 antigen using a latex agglutination test (Wellcolex* *E. coli* O157:H7; Murex Biotech Ltd. Dartford, UK). Colonies positive for the O157 antigen by the latex agglutination assay were confirmed biochemically as described previously (Anon. 1994).

Study 2

Sample collection. A faecal, rumen and carcass sample was collected from each of five consecutive animals on a weekly basis from June 1998 until May 1999, excluding days between 21 and 28 December 1998 (total of 50 weeks). The total sample of 250 animals included 132 steers, 75 heifers and 42 cows. The factory identification number of each animal/carcass was recorded and used to trace its farm of origin.

Faecal samples were taken immediately after slaughter i.e. during de-legging. A sterile stomacher bag (Model 400 Bags 6041; Seward Ltd, London, UK) was inverted over the hand of the sampler, ensuring that the inside of the bag was not contaminated. The bag was inserted inside and around the rectum to remove faecal material. After sample collection, the bag was returned to its original shape containing the faeces samples, chilled to 4°C in an insulated box, transported to the laboratory, and examined within 2 h.

For rumen sample collection, an aseptic incision was made in the rumen wall immediately after evisceration. Sterile stomacher bag samples were recovered from inside the rumen, chilled and transported as described above. The rumen material was aseptically compressed and the resultant liquor decanted in, and stored in, a sterile screw-capped container.

Carcass samples were collected after entry into the chill using a previously described method (Lasta *et al.* 1992). Briefly, exterior surfaces of both carcass sides of each single animal were uniformly swabbed (from the hindquarter to the forequarter), using a sterile sponge (*ca* 90 cm^2; Spontex Ltd, Swansea, UK). Latex examination gloves were worn and changed between animals. The loaded sponge was placed in a sterile stomacher bag, chilled and transported to the laboratory as previously described.

Microbiological analysis. Faecal samples were stored overnight at 0°C and enriched by the following procedure. A sterile cotton-tipped swab (Copan, Brescia, Italy) was charged with faeces from the stomacher bag. The tip of the swab, loaded with the faecal sample, was broken off into 5 ml of buffered peptone water (Oxoid) containing 8 mg l^{-1} vancomycin (Sigma), 10 mg l^{-1} cefsulodin (Sigma) and 0·05 mg l^{-1} cefiximine (Cyanamid of Great Britain Ltd, Hampshire, UK) (BPW-VCC) and incubated at 37°C for 6 h.

Rumen samples were stored overnight at 0°C prior to enrichment. A 1 ml aliquot of rumen liquid was added to 5 ml of BPW-VCC and incubated at 37°C for 6 h.

Carcass samples were processed immediately upon return to the laboratory. Two hundred millilitres of BPW was added to the stomacher bag containing the charged sponge from each carcass sample. The sponge was rinsed by compression with 200 ml of BPW for 1 min and then compressed to recover the maximum volume of BPW. The sponge was removed and the remaining liquid was poured into a sterile screw-capped container and incubated at 37°C overnight.

E. coli O157:H7 was isolated from enriched faecal, rumen and carcass samples using immunomagnetic separation (Wright *et al.* 1994). Following the enrichment step, a 1 ml aliquot of the faecal, rumen or carcass enrichment broth was added to 20 μl of magnetic beads coated with an anti-*E. coli* O157 antibody (Dynabeads® anti-*E. coli* O157; Dynal A.S., Oslo, Norway). According to the manufacturer's instructions (Dynal A.S.), the beads were mixed (Dynal MX3) with the enrichment broth, separated in a magnetic particle concentrator (Dynal MPC®-M) and washed using phosphate-buffered saline (Oxoid) containing 0·05% Tween-20 (Sigma) (PBST). After the final wash and separation, the bacteria-bead complex was re-suspended in 100 μl of PBST. Duplicate 50 μl aliquots of the re-suspended bacteria-beads complex were dispensed onto sorbitol MacConkey agar (Oxoid) containing 0·05 mg l^{-1} cefiximine and 2·5 mg l^{-1} potassium tellurite (Sigma). The bacteria-bead complex was spread over one half of the plate using a sterile swab (Copan) and streaked to obtain isolated colonies over the second half of the plate using a sterile inoculating loop. The plates were incubated at 37°C for 24 h. Suspect colonies were screened as described in study 1 and subjected to biochemical confirmation as described previously (Anon. 1994).

Serotype confirmation and virulence factor determination of *E. coli* O157 isolates

All isolates from both studies, biochemically confirmed as *E. coli* O157, were subjected to serotype confirmation and virulence factor determination as follows: one bead containing a biochemically confirmed *E. coli* O157 isolate was aseptically transferred to 9 ml of tryptone soya broth

Table 2 Primers used during serotype confirmation and virulence characteristic determination of *Escherichia coli* O157:H7 isolates

Primer	Sequence (5′ → 3′)	Product size	Reference
rfb_{O157}			
O157-F	CGGACATCCATGTGATATGG	259 bp	(Paton and Paton 1998)
O157-R	TTGCCTATGTACAGCTAATCC		
$fliC_{H7}$			
FLICH7-F	GCGCTGTCGAGTTCTATCGAGC	650 bp	(Gannon *et al.* 1997)
FLICH7-R	CAACGGTGACTTTATCGCCATTCC		
VT1			
vt1-a	GAAGAGTCCGTGGGATAACG	130 bp	(Pollard *et al.* 1990)
vt1-b	AGCGATGCAGCTATTAATAA		
VT2			
vt2a	CTTCGGTATCCTATTCCCGG	478 bp	(Olsvik and Strockbine 1993)
vt2b	GGATGCATCTCTGGTCATTG		
*eaeA*O157			
ae19	ACGTTGCAGCATGGGTAACTC	1089 bp	(Gannon *et al.* 1993)
ae20	GATCGGCAACAGTTTCACCTG		
ehlyA			
MFS1F	ACGATGTGGTTTATTCTGGA	166 bp	(Fratamico *et al.* 1996)
MFS1R	CTTCACGTCACCATACATAT		

(Oxoid) and incubated at 37°C for 24 h. Cells from the broth culture were lysed in buffer containing 2% Triton-X-100 (Sigma), 1% sodium dodecyl sulphate (BDH Chemicals), 100 mM sodium chloride (BDH Chemicals), 10 mM tris base (Sigma) and 1 mM EDTA (Sigma). DNA was extracted from phenol : chloroform : isoamyl alcohol (25 : 24 : 1) and precipitated in one tenth volume of sodium acetate and two volumes of absolute ethanol. Following a wash in 70% ethanol, DNA was resuspended in 75 μl sterile distilled water.

The primers used in this study are detailed in Table 2. DNA amplification was performed in a total volume of 50 μl containing the following reagents: 5 μl PCR reaction buffer (Bioline, London, UK), 2 μl $MgCl_2$ (Bioline), 1 μl dNTPs, 0·25 μl of each primer, 0·25 μl of *Taq* polymerase (Bioline) and 40·25 μl of sterile distilled water. A negative control was included which consisted of all the reagents except DNA, which was substituted with 1 μl of sterile distilled water. Amplification was performed in a thermal cycler (PTC-200; MJ Research, DNA Engine, USA) with an initial denaturation step of 95°C × 5 min followed by 30 amplification cycles of denaturation at 94°C × 1 min, annealing at 55°C × 1 min (when using primers targeting rfb_{O157} and $fliC_{H7}$) or 50°C × 1 min (when using primers targeting all other sequences) and extension at 72°C × 1 min. A final extension step of 72°C × 5 min was included after the 30 cycles.

The PCR products were detected by electrophoresis of 15 μl of the amplification mixture on a 1% agarose gel containing 200 ng ml^{-1} of ethidium bromide. A 100 bp DNA ladder molecular weight marker was included in each electrophoretic run to allow identification of the size of the amplified product. PCR product bands were visualized under u.v. illumination and catalogued with a gel documentation system (Stratagene EE 2, Amsterdam, the Netherlands). A characteristic PCR product of expected size indicated a positive result (Table 2).

Phage typing of *E. coli* O157:H7 isolates

All biochemically confirmed isolates from both studies were phage-typed according to a previously described method (Khakria *et al.* 1990).

RESULTS

Study 1

Data showing the date of isolation and distribution of *E. coli* O157:H7 on positive carcasses are shown in Table 3. The two positive carcasses recovered on 12 August 1998 were separated on the slaughter line by at least 15 carcasses, but were placed side by side in the chill.

On two of four carcasses that tested positive for *E. coli* O157:H7, the organism was detected at the hock, cranial back and brisket after hide removal, while on a third positive carcass, the organism was detected at the hock following legging and at all carcass sites following hide removal.

On two carcasses, the bung site was negative before the bung tying operation but tested positive after the operation.

Carcass sites that were negative for *E. coli* O157:H7 immediately before evisceration did not test positive for the organism immediately after the operation. Conversely, on

Table 3 Study 1: Date of isolation and location of positive samples from four *Escherichia coli* O157:H7 positive carcasses

	Positive carcasses and date of occurrence			
Sample points and carcass sites	1 15 July	2 22 July	3 12 August	4 12 August
Legging				
Hock	–*	–	–	+
Hide removal				
Hock	–	+†	+	+
Bung	–	–	–	+
Cranial back	–	+	+	+
Brisket	–	+	+	+
Bung tying				
Bung	–	+	+	+
Evisceration				
Hock	–	+	+	+
Bung	–	+	+	+
Cranial back	–	+	+	+
Brisket	–	–	+	–
Splitting				
Inside round	–	+	–	–
Bung	–	+	+	–
Cranial back	–	+	+	–
Brisket	–	–	+	–
Washing				
Hock	–	+	+	–
Inside round	–	+	+	–
Bung	–	+	+	–
Cranial back	+	+	+	–
Brisket	–	+	–	–
Chilling (24 h)				
Hock	–	–	+	+
Inside round	–	–	+	+
Bung	–	–	+	+
Cranial back	–	–	+	+
Brisket	–	–	+	+
Boning				
Inside round	–	–	+	–
Outside round	–	–	+	+
Cranial back	–	–	+	–
Brisket	–	–	+	+

*Sites which were negative for *E. coli* O157:H7.
†Sites which were positive for *E. coli* O157:H7.

two occasions, *E. coli* O157:H7 was detected at the brisket immediately before, but not after evisceration. Also, *E. coli* O157:H7 was detected at the cranial back and bung sites before, but not after splitting.

Following washing, the cranial back, brisket and inside round each tested positive for *E. coli* O157:H7 having tested negative for the organism before washing. A brisket site that had tested positive for the *E. coli* O157:H7 before washing, tested negative immediately after.

There was a change from positive before chilling to negative after chilling at all sites tested on one carcass (22 July 1998) and at the cranial back on another carcass (15 July 1998). On the two remaining positive carcasses (12 August 1998), there was an increase in the prevalence of *E. coli* O157:H7 after chilling, with a change from negative to positive at all sites on one carcass and at the brisket on the other carcass.

Carcass sites that tested negative for *E. coli* O157:H7 immediately before boning did not test positive after boning.

The serotype of all *E. coli* isolates was confirmed as O157:H7 by PCR amplification of the rfb_{O157} and $fliC_{H7}$ sequences. All isolates carried genes encoding *eaeA*O157 and *ehlyA* but were VT negative.

Study 2

During the 12-month period of study 2, *E. coli* O157:H7 was isolated from 2·4% (6/250) of faecal, 0·8% (2/250) of rumen and 3·2% (8/250) of carcass samples (Fig. 1). All positive samples were from steers or heifers.

Data showing the isolation date and characteristics of strains from faecal, rumen and carcass samples are presented in Table 4.

The serotype of all *E. coli* isolates was confirmed as O157:H7 by PCR. All isolates carried the genes encoding *eaeA*O157 and *ehlyA*. Ninety-five per cent (19/20) of strains carried one or both the genes encoding verocytotoxin production. The overall prevalence of verocytotoxin genotypes was 44·5, 44·5, 5·5 and 5·5% for VT1/VT2, VT2, VT1 and VT negative, respectively.

All strains isolated during study 2 were PT 32, except one from a faecal sample, which did react with the phage panel, but did not conform to any known pattern (rdnc) and a PT 8 strain from a rumen sample.

Phage typing and VT genotyping revealed six sub-types. PT 32 VT2 (7/18) and PT 32 VT1/VT2 (7/18) were the most common sub-types, followed by PT 32 VT1 (1/18), PT 32 VT negative (1/18), PT 8 VT1/VT2 (1/18) and rdnc VT 2 (1/18) following VT2.

In samples withdrawn on 23 March 1999 and on 9 September 1998, two strains were isolated from a single faecal sample. A third strain was also isolated from the rumen of another animal on 9 September 1998. All animals tested on 9 September 1998 were from the same farm.

On 13 April 1999, *E. coli* O157:H7 was isolated from the faeces of two animals, the carcasses of these animals following washing and adjacent animals on the line. All isolates from faecal and carcass samples on this date were of the same phage type and virulence characteristic profile.

Data showing the frequency of isolation of *E. coli* O157:H7 from faecal, rumen and carcass samples each

Table 4 Study 2: Isolation date, phage type, serotype and virulence characteristic profiles of *Escherichia coli* isolates from faecal, rumen and carcass samples

Sample type and date	Animal no.*	Serotype		Virulence characteristic profile				Phage type
		O157	H7	*eaeA*O157	*ehlyA*	VT1	VT2	
Faeces								
23 March	4	+	+	+	+	+	–	32
	4†	+	+	+	+	–	–	32
13 April	3	+	+	+	+	+	+	32
	4	+	+	+	+	+	+	32
21 July	4	+	+	+	+	–	+	32
4 August	1	+	+	+	+	–	+	32
9 September	1	+	+	+	+	–	+	32
	1†	+	+	+	+	–	+	rdnc‡
Rumen								
21 July	3	+	+	+	+	–	+	32
9 September	4	+	+	+	+	+	+	8
Carcass								
13 April	2	+	+	+	+	+	+	32
	3	+	+	+	+	+	+	32
	4	+	+	+	+	+	+	32
	5	+	+	+	+	+	+	32
27 April	1	+	+	+	+	+	+	32
18 August	2	+	+	+	+	–	+	32
	3	+	+	+	+	–	+	32
3 November	5	+	+	+	+	–	+	32

*Animal no., where '1' is the first and '5' is the last of the five consecutive animals sampled.
†rdnc = reacts with phages but does not conform to any known pattern.
‡Second strain from a sample, as determined by virulence characteristic profile or phage type.

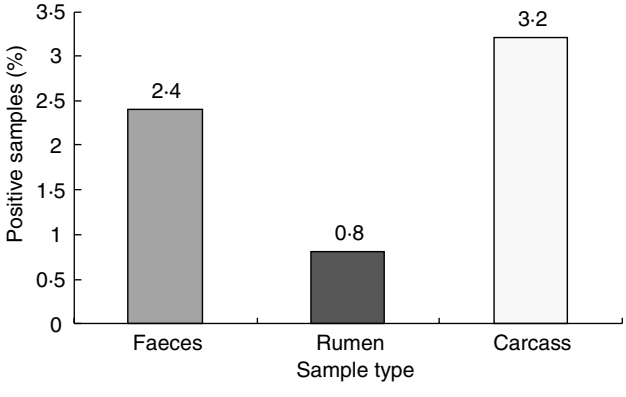

Fig. 1 Study 2: The percentage of each sample type positive for *Escherichia coli* O157:H7

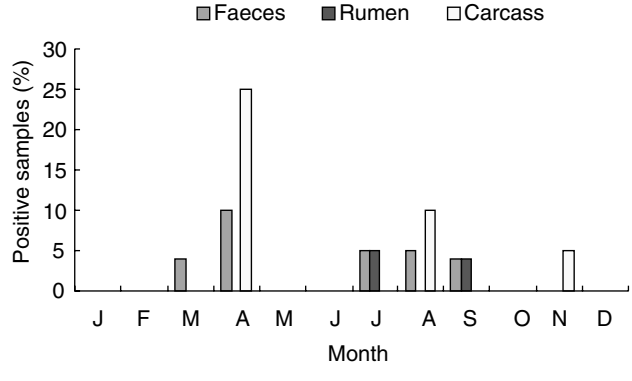

Fig. 2 Study 2: *Escherichia coli* O157:H7 isolations as a percentage of the total number of samples each month

month, are presented in Figure 2. Apart from one positive carcass sample in November, all isolations of this organism occurred during spring and late summer.

DISCUSSION

Data from study 1 showed that *E. coli* O157:H7 was widespread on three carcasses following hide removal and on one of those three carcasses the organism was recovered immediately after skinning the hock at legging. These results show that *E. coli* O157:H7 can be transferred to the carcass during hide removal operations. This finding is in agreement with a previous study that reported *E. coli* O157:H7 contamination of carcasses pre-evisceration, and also noted the presence of the organism on the hide of animals following stunning (Elder *et al.* 2000).

The bung tying operation, investigated during study 1, was found to contribute to *E. coli* O157:H7 contamination at the bung site, indicating that this operation may not prevent the transfer of contamination from the anus to the carcass. Similarly, a previous study showed that bung tying reduced, but did not eliminate the spread of an *E. coli* K12 strain to beef carcass tissue (Hudson *et al.* 1998). Carcass contamination during bung tying may result from contact with the workers hands or tools. Such worker-related cross-contamination may be reduced using an automated bung tying system. Automated bung tying has been reported to result in lower total, *E. coli* and coliform counts in the anal area than manual systems (Sheridan 1998).

During study 1, some carcass sites which were initially *E. coli* O157:H7 positive became negative for this organism at later stages of the dressing process. This was most evident on carcass 4 (12 August 1998), where all sites tested reverted from positive to negative between hide removal and splitting. A more general reduction (aerobic plate count) between hide removal and evisceration, in the absence of any decontamination treatment, has previously been reported (Nortjé and Naudé 1981). Such observations may be because of irreversible binding of some or most of the bacteria deposited on the carcass during hide removal, to carcass tissue. Bacterial attachment to meat surfaces has been described as a two-stage process whereby an initial reversible adsorption becomes an irreversible attachment over time, reducing the numbers which can be removed by surface swabbing (Firstenberg-Eden 1981). The significance of such attachment has been demonstrated in a study using both excision and swabbing in the recovery of *E. coli* O157:H7 from beef carcass surfaces, in which six of nine positive isolations were recovered by excision but only one of nine isolations were recovered by swabbing (Chapman *et al.* 1993).

Data from study 1 showed that a potable water wash at a temperature of 35–40°C can cause an increase or decrease in the prevalence of pathogens at particular carcass sites. These findings may be because of a redistribution of contamination on the carcass during the washing process (Prasai *et al.* 1995; Bell 1997). Such redistribution of contamination is reported to occur in a posterior to anterior direction as the wash water runs down the carcass surface (Bell 1997).

Data from study 1 showed that chilling could reduce the prevalence of *E. coli* O157:H7 on the carcass. This may be due to factors such as the irreversible attachment noted above, the effect of chilling and failure of the sampling method to recover the organism from the meat surface. Chilling may stress bacterial cells because of the synergistic effect of low a_w and temperature and subsequent growth of such stressed cells may be inhibited by the presence of bile salts and antibiotics in the enrichment media (Stephens and Joynson 1998; Hara-Kudo *et al.* 2000). Alternatively, the sampling method employed (i.e. surface swab) may be less effective in removing micro-organisms from chilled, as opposed to freshly slaughtered, carcasses. Surface swabbing (sponge swabbing) prechilling was shown to be as effective as excision at recovering total aerobic bacteria; however, it resulted in a significantly lower recovery than excision from carcasses after 24 h chilling (Ware *et al.* 1999).

The results from carcasses sampled on 12 August 1998 suggest that carcass sites may become contaminated with *E. coli* O157:H7 during the chilling process. This may be the result of cross-contamination during chilling, as carcass 3, which was positive at all sites except the brisket post-washing, was placed beside carcass 4 in the chill. However, it should be noted that, except for the inside round, all sites on carcass 4 had tested positive following hide removal.

The prevalence of *E. coli* O157:H7 at carcass sites did not increase following boning operations. This result was observed despite (i) 1–2$\log_{10}$ CFU cm^{-2} increase in the total viable count at the same carcass sites described in the present study, between chilling and boning (Doherty *et al.* 1999) and (ii) the identification of conveyor belts and cutting equipment used during beef carcass boning as sources of *E. coli* and coliforms (Gill *et al.* 1999).

Given that all isolates during study 1 could not be distinguished by the analyses used, it is possible that they originated from a common source, which may have persisted in the slaughter environment during the 1 month period when positive samples were recovered. Previous studies have reported isolating identical strains from different carcasses processed in the same place at the same time (Chapman *et al.* 1993; Richards *et al.* 1998). Alternatively, phage typing and virulence characteristic profiling may have been insufficient to differentiate among isolates. Some phage and VT genotypes have been reported to predominate in geographical areas, limiting the usefulness of these attributes as sub-typing tools. Phage type 2 has been reported to predominate among isolations from humans in England and Wales (Thomas *et al.* 1993; Thomas *et al.* 1996; Wilshaw *et al.* 1997) and in cattle from these countries (Mechie *et al.* 1997; Richards *et al.* 1998). The VT negative genotype isolated, in the present study, is generally less frequently noted in the literature than VT1/VT2 or VT2 genotypes, although there have been some reports (Chapman *et al.* 1993; Heuvelink *et al.* 1998b; Bonardi *et al.* 1999). VT encoding phages also have been lost from isolates during sub-culturing (Karch *et al.* 1992), masking the original virulence profile of the strain.

In study 2, the prevalence of *E. coli* O157:H7 in faecal samples was similar or higher than that reported in previous studies. In Northern Italy, 3% of preslaughter faecal samples from 419 feedlot cattle and 65 culled cows tested positive for *E. coli* O157:H7 (Conedera *et al.* 1997). Presenting results which are similar to the present study,

Conedera *et al.* (1997) did not find *E. coli* O157:H7 in faecal samples from cows, although in both their study, and the current study, the numbers of cows tested was a disproportionately small component (<17%) of the overall sample population. A 1 year study in Canada reported an average *E. coli* O157:H7 prevalence in preslaughter bovine faecal samples of 7·5% (Van Donkersgoed *et al.* 1999), while a similar study in the UK reported a prevalence of 15·7% (Chapman *et al.* 1997).

In this study, the prevalence of *E. coli* O157:H7 in the rumen was low relative to the prevalence in faeces. This observation is in line with the study by Van Donkersgoed *et al.* (1999), in which the authors found a 0·8% prevalence of the organism in rumen samples compared with 7·5% in faecal samples. These results support the hypothesis that *E. coli* O157:H7 does not generally proliferate in the rumen of adult cattle, and that multiplication occurs in the hindgut. In a previous study, *E. coli* O157:H7 was not found to persist in the rumen of artificially inoculated cattle fed various diets, although the organism was isolated from faecal samples for up to 67 days (Buchko *et al.* 2000). A study examining the distribution of *E. coli* O157:H7 in necropsied adult cattle, found the highest concentration in the large bowel and suggested this may be because of multiplication of the organism as it passed through the large bowel and the concentration of digesta as it moved towards the anus (Cray and Moon 1995).

The prevalence of *E. coli* O157:H7 on carcasses in study 2 was higher than the prevalence rates of 0·45% (Vanderlinde *et al.* 1998), 0·47% (Richards *et al.* 1998) and 2% (Elder *et al.* 2000) reported in previous studies. The higher prevalence may be because of a number of factors including different sampling regimes and techniques used in the various studies, geographical variation in the incidence of *E. coli* O157:H7 and different approaches to pathogen control in the abattoirs studied.

Unlike previous studies, where samples were taken from a reduced area of the carcass (Richards *et al.* 1998; Vanderlinde *et al.* 1998; Elder *et al.* 2000), in the present study, the entire outer surface of each carcass was swabbed. Given that a 10-fold increase in the area sampled was found to approximately double the number of *E. coli* and coliform-positive samples (Gill and Jones 2000), it is reasonable to assume that swabbing the entire carcass would result in a greater number of positive samples than swabbing a reduced area. Also, in previous studies (Richards *et al.* 1998; Vanderlinde *et al.* 1998) samples were taken following a period of chilling, as opposed to immediately after dressing, in the present study. Results from study 1 suggest that chilling may affect the recovery of *E. coli* O157:H7 from the carcass.

The use of anti microbial interventions in some of the abattoirs studied may have contributed to a lower prevalence of *E. coli* O157:H7 on carcasses relative to the current study, where no anti microbial interventions were used. Study 1 showed that *E. coli* O157:H7 occurring on the carcass during hide removal is unlikely to have been removed or inactivated during subsequent processing operations. This is in contrast to the study by Elder *et al.* (2000), which was carried out in a US abattoir where anti microbial interventions were applied to reduce carcass pathogen numbers. Those authors demonstrated a reduction in the prevalence of *E. coli* O157:H7 on carcasses from 18% before antimicrobial intervention to 2% after the intervention (Elder *et al.* 2000).

The most common phage type during study 2 (PT 32) was also the most common phage type from Irish human clinical infections at that time. In 1999, PT 32 accounted for 67% of human clinical isolations (Anon. 2000).

Data from study 2 shows that a single animal, or more than one animal from a single farm can be colonized with multiple *E. coli* O157:H7 strains. This is not surprising, as previous studies have shown that a large number of different *E. coli* biotypes may transiently colonize both calves and adult cattle on farms (Hinton *et al.* 1985; Hancock *et al.* 1998). In addition to farms, animals may also be exposed to different strains during transport and lairage. Organisms ingested during transport or lairage are more likely to be present in the rumen than faeces at slaughter, because the time required for the organism to pass through the animal's intestine relative to the short transport and lairage times in Irish abattoirs. Data from 9 September 1998 suggests that such transport or lairage-related contamination may have occurred as a strain present in the rumen of one animal differed from the strains present in the faeces of another animal from the same farm.

Strains present on six of eight positive carcasses could not be found in faecal or rumen samples of the respective animal. These results indicate that, on many occasions, carcass contamination with *E. coli* O157:H7 may result from a source other than the rumen and rectum. Data from study 1 showed *E. coli* O157:H7 can become widespread on the carcass during hide removal, suggesting the hide is an important source. *Escherichia coli* O157:H7 contaminated faeces, adhering to the hide during faecal shedding, could remain even after shedding has ceased, providing a reservoir for transfer to the carcass during hide removal. Previous studies have shown that *E. coli* O157:H7 can survive in faeces for prolonged periods (Wang *et al.* 1996; Bolton *et al.* 1999) even in a desiccated state (Delazari *et al.* 1998). Elder *et al.* (2000) found that 38% of cattle lots (a group of animals from a common source) at slaughter carried *E. coli* O157:H7 on their hide and all positive lots resulted in at least one positive carcass pre-evisceration.

On two occasions, strains indistinguishable by the typing method employed were isolated from the faeces of two

animals and the resulting carcasses. The contaminated faeces may have been transferred from the rectum during bung tying as noted in study 1. Alternatively, the source of contamination may have been contaminated faeces on the hide as discussed above. Adjacent carcasses on the line were also contaminated with the same strain. These findings are similar to those of a UK study which reported indistinguishable isolates from the carcasses of faecal positive and adjacent faecal negative animals (Chapman *et al.* 1993).

The seasonal prevalence of *E. coli* O157:H7 noted in the present study is in agreement with prevalence studies in cattle on farms in the UK (Chapman *et al.* 1997; Mechie *et al.* 1997). In a pattern similar to that previously noted (Mechie *et al.* 1997), the positive animals from 23 March to 13 April 1999 almost certainly originated from housed conditions, hence exposure to slurry during grazing (Hancock *et al.* 1994) was unlikely to be a factor in their carriage of *E. coli* O157:H7.

In conclusion, *E. coli* O157:H7 can be present in the faeces and rumen of Irish cattle at slaughter and can cross-contaminate carcasses during dressing operations. Carcass contamination can occur during hide removal and bung tying and remain during subsequent washing, chilling and boning operations. Most *E. coli* O157:H7 strains present in the faeces, rumen and on carcasses would be considered fully pathogenic as they contain the full complement of virulence factors.

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