Journal of Applied Microbiology ISSN 1364-5072

ORIGINAL ARTICLE

The potential use of chilling to control the growth of *Enterobacteriaceae* on porcine carcasses and the incidence of *E. coli* O157:H7 in pigs

M. Lenahan[1,2], H. Crowley[1], S.B. O'Brien[1], C. Byrne[1], T. Sweeney[2] and J.J. Sheridan[1]

1 Teagasc, Ashtown Food Research Centre, Ashtown, Dublin, Ireland
2 School of Agriculture, Food Science and Veterinary Medicine, University College Dublin, Belfield, Dublin, Ireland

Keywords
carcasses, chilling, *E. coli* O157:H7, *Enterobacteriaceae,* faeces, pigs.

Correspondence
Mary Lenahan, Ashtown Food Research Centre, Ashtown, Dublin 15, Ireland.
E-mail: mary.lenahan@teagasc.ie

2008/1346: received 1 August 2008, revised 7 October 2008 and accepted 9 October 2008

doi:10.1111/j.1365-2672.2008.04112.x

Abstract

Aims: To (i) monitor the presence of *Enterobacteriaceae* as indicators of faecal contamination on pig carcasses, (ii) examine the potential use of chilling as a critical control point (CCP) and establish its influence on pig carcass categorization by Decision 471/EC and (iii) determine the incidence of *E. coli* O157:H7 in pigs.

Methods and Results: Porcine faecal samples and carcass swabs were collected before and after chilling at four Irish pig abattoirs and examined for *Enterobacteriaceae* and *E. coli* O157:H7. Chilling generally reduced *Enterobacteriaceae* counts on carcasses, but increases were also observed, particularly in one abattoir. *E. coli* O157:H7 was absent from carcasses before chilling, present on 0·21% after chilling and was recovered from 0·63% of faecal samples. All of the isolates were found to contain virulence genes associated with clinical illness in humans.

Conclusions: The data show that overall chilling had the capacity to reduce the numbers of carcasses positive for the presence of *Enterobacteriaceae*.

Significance and Impact of Study: The influence of chilling on the categorization of pig carcasses suggests that it has the potential to improve the numbers of acceptable carcasses and the process could be used as a CCP within a HACCP plan.

Introduction

In EC Commission Decision 2001/471/EC and Commission Regulation 2073/2005, the *Enterobacteriaceae* are used as indicators of faecal contamination of fresh meat carcasses (Anonymous 2001; Anonymous 2005). The performance criteria for the mean *Enterobacteriaceae* counts ($\log_{10}$CFU cm^{-2}) on pigs are: acceptable <2·0, marginal 2·0–3·0 and unacceptable >3·0 (Anonymous 2001, 2005). These criteria are applied to carcasses at the end of the slaughter line and before chilling, as it is intended that they be used to demonstrate the efficacy of the slaughter process only. There may however be an advantage in assessing the presence of these indicators after chilling, as this process has the potential to reduce their presence on pig carcasses and is therefore a better measure of the actual numbers of these organisms on carcasses prior to retail sale.

Chilling is used successfully as a critical control point (CCP) in slaughter plants in Australia based on the efficacy of the chilling process to reduce the numbers of *E. coli* on carcass surfaces (Anonymous 2004). Meat companies are required to validate their carcass-chill procedures to comply with the Australian Meat Standard AS 4696 : 2007 (Anonymous 2007). However, this is not the case in the European Union (EU) and in practice, the capabilities of chillers and chill-carcass cycles vary from plant to plant (Sheridan 2004). The basis of HACCP (Hazard Analysis and Critical Control Point) in relation to fresh meat is that the numbers of pathogenic organisms

on carcass surfaces are reduced or their growth is prevented as elimination is not possible (Sheridan 2000). Within this definition, chilling has the potential to accomplish these two objectives, but the refrigeration conditions must be shown to be capable of achieving them. As pathogenic bacteria are infrequently present on carcass surfaces, generic *E. coli* and other *Enterobacteriaceae* are used to indicate the potential presence of enteric pathogens. As already outlined above, the indicator used in the EU is the *Enterobacteriaceae* and in this study, the behaviour of these organisms during chilling was monitored.

E. coli O157:H7 causes a wide range of clinical symptoms in humans including haemorrhagic colitis (HC) and haemolytic uraemic syndrome (HUS) (Griffin and Tauxe 1991). Ruminants are regarded as the main reservoir of *E. coli* O157:H7 and the pathogens have been recovered from both Irish cattle (McEvoy *et al.* 2003) and sheep (Lenahan *et al.* 2007). Research has indicated that while pigs are potential carriers of *E. coli* O157:H7, the reported incidence is generally much lower than in ruminants (Wasteson 2001).

This study investigated the levels of *Enterobacteriaceae* on carcasses as indicators of faecal contamination and examined the potential use of chilling as a means of reducing their numbers or controlling their growth. The incidence of *E. coli* O157:H7 on carcasses before and after chilling and in faeces was also determined, as this is an important human pathogen and no previous work on its prevalence in Irish pigs has been carried out.

Materials and methods

Study design and slaughter process

Four pig abattoirs (A, B, C, and D) were each visited four times over a eleven-month period from January to November 2004. Abattoirs were visited once during each of the four seasons – visit 1 was from January to March, visit 2 from April to July, visit 3 from August to September and visit 4 from October to November. Three of the abattoirs (A, B, C) slaughtered 250 pigs per hour and the fourth (D) 75 pigs per hour. In general, pigs were slaughtered by the following process in the four abattoirs. Animals were held in lairage, stunned and immediately exsanguinated by sticking before dressing. They were scalded at 61°C (+ 2°C) for approx. 8 min, dehaired, ejected onto a gambrelling table and hoisted onto an overhead conveyor rail. The carcasses were passed through a singer, operating at approx. 1200°C for 15 s, polished and transferred into a separate evisceration area. Carcasses were debunged by detaching the rectum with a knife, which was then enclosed in a plastic bag to limit faecal leakage during subsequent processing. Carcasses were eviscerated, split from hind to fore using a manual splitting saw, and the head and spinal cord removed. Finally, carcasses were trimmed, weighed, graded, spray washed and chilled overnight.

At each visit, carcass hygiene levels were assessed by collecting 30 carcass swabs before chilling and 30 swabs after chilling, from different sides of the same animal, to determine *Enterobacteriaceae* numbers. The presence of *E. coli* O157:H7 was investigated by taking 30 whole animal body swabs before chilling and whole body swabs from 30 different animals after chilling at each visit. In addition, 30 faecal samples were collected and examined for the presence of the pathogen. In total, the *Enterobacteriaceae* were enumerated on 960 carcasses and the presence of *E. coli* O157:H7 was determined from 1440 carcass and faecal samples.

Sample collection

Carcass samples

Enterobacteriaceae numbers were enumerated by swabbing the lead side or carcass of an animal at the end of the slaughter line before chilling and the opposite trailing side of the same animal 24 h after chilling. Carcass sides (area 6270 cm^2) were swabbed using a 150 cm^2 polyurethane sponge (Sydney Heath & Son, Stoke-on-Trent, UK) starting from the hindquarter downward to the forequarter (O'Brien *et al.* 2007).

To determine the presence of *E. coli* O157:H7, whole body swabs (area 12 540 cm^2) were taken from animals before chilling and a different set of animals after chilling. Separate polyurethane sponges (75 cm^2) were used to swab the two carcass sides from each animal and these were pooled to give a composite whole body sample (Lenahan *et al.* 2007).

Faecal samples

Faecal samples were obtained during evisceration by aseptically slitting the distal colon with a sterile scalpel and removing faecal material from the colon using a sterile wooden tongue depressor (Medical Supply Co., Dublin, Ireland) (Lenahan *et al.* 2007). The faecal samples were taken from a different set of animals from those tested for *E. coli* O157:H7 before and after chilling. All carcass swabs and faecal samples were transported to the laboratory in a cool box containing ice packs and stored overnight in a laboratory chill (1°C) for examination within 24 h.

Microbiological analysis

Enumeration of *Enterobacteriaceae*

Carcass swabs were examined for the presence of *Enterobacteriaceae* using the procedure described in ISO 4833. In brief, swabs (10 g) in 90 ml MRD (Oxoid) were

stomached in a model 400 stomacher (A.J. Seward, UK) for 30 s at 260 rev min^{-1} and 1 ml aliquots of the resultant suspension used to make (1 : 10 v/v) serial dilutions in MRD. A 1 ml aliquot of the swab/MRD homogenate and the serial dilutions were pour plated with 15 ml of Violet Red Bile Glucose agar (VRBGA) (Oxoid), allowed to solidify and overlaid with 10 ml VRBGA. Plates were incubated at 37°C for 24 h.

Isolation of E. coli *O157:H7*

A 90 ml volume of Brilliant Green Bile Broth (BGBB) (Oxoid) was added to each whole body composite sample (10 g) and stomached for 30 s at 260 rev min^{-1}. Sterile cotton-tipped swabs (Nuova Aptaca, Canelli, Italy) used to sample 0·5 g faeces were broken off into 5 ml volumes of BGBB and vortexed for 10 s. The carcass swabs and faeces were selectively enriched in BGBB by incubating at 37°C for 6 h (Feder *et al.* 2003). One millilitre aliquots of the carcass or faecal enrichment broth were added to 20 μl of immunomagnetic beads coated with anti-*E. coli* O157 antibody (Dynabeads® anti-*E. coli* O157; Dynal A.S., Oslo, Norway) and processed according to the manufacturer's instructions. The bead/sample suspensions were plated in duplicate onto Sorbitol MacConkey agar (SMAC) (Oxoid) supplemented with cefixime-tellurite (CT) (Oxoid) and incubated at 37°C for 24 h. Where present, up to five nonsorbitol fermenting colonies were selected from each duplicate plate and subjected to biochemical confirmation. Colonies from CT-SMAC were streaked onto Nutrient Agar (Oxoid) to obtain a purified culture and incubated for 24 h at 37°C. Colonies from the Nutrient agar were biochemically confirmed by streaking onto Levines Eosin Methylene Blue agar (EMB) (Oxoid), Phenol Red Sorbitol agar (Oxoid) supplemented with 4-methylumbelliferyl-B-D-Glucuronide (Oxoid) (PRS-MUG), inoculated into Tryptone Tryptophane medium (TT) (Oxoid) and all were incubated at 37°C for 24 h. Colonies displaying a green metallic sheen on EMB, no fluorescence on PRS-MUG when illuminated with UV light, and which were indole positive in TT medium on addition of Kovacs reagent (Oxoid), were examined using a *E. coli* O157:H7 latex agglutination kit (Wellcolex, Basingstoke, UK) according to the manufacturer's instructions to check for the presence of the O157 antigen. All presumptive *E. coli* O157 isolates were stored at −20°C on Protect™ Stock Culture Beads (Technical Service Consultants Ltd, Heywood, UK) to enable PCR analysis to be carried out at a later date.

Detection of virulence genes

All presumptive *E. coli* O157 isolates were subjected to PCR analysis to confirm the presence of the O157 antigen and H7 flagellum and to determine presence or absence of 11 other genes that encode proteins considered to have a role in clinical illness. Porcine isolates stored at −20°C on Protect™ Stock Culture Beads were resuscitated by incubating one bead in 10 ml Brain Heart Infusion broth (BHI) (Oxoid) at 37°C for 18 h. After incubation, genomic DNA was extracted from a 1 ml portion of bacterial cells in BHI using a DNeasy blood & tissue kit (Qiagen™, Crawley, UK) as per the manufacturer's instructions for gram-negative bacteria. For each assay, 5 μl of genomic DNA was used in each 50 μl PCR reaction. PCR primer sequences and reaction conditions were obtained from previously published work on the genes for *O157, vt*1, *vt*2, *eaeA, hlyA* (Paton and Paton 1998), *H7* (Fratamico *et al.* 2000), *tir* (Higgins *et al.* 2003), *espA*, *espB*, *espP* (McNally *et al.* 2001), *espF* (Crane *et al.* 2001), *etpD* (Schmidt *et al.* 1999) and *katP* (Brunder *et al.* 1996). An *E. coli* O157:H7 isolate, ATCC 43895, obtained from the Central Meat Control Laboratory, Dept. of Agriculture, Abbotstown, Dublin, Ireland was included as a positive control in each PCR assay while sterile-filtered, DNase & RNase free water (Sigma-Aldrich, Munich, Germany) was used as a negative control. Isolates confirmed as *E. coli* O157:H7 by PCR were phage typed at the Laboratory of Enteric Pathogens, Centre of Infections, 61 Colindale Avenue, London NW9 5HT, UK.

Statistical analysis of *Enterobacteriaceae* counts

The experiment was designed as a split-split-plot with abattoirs (A, B, C, D), visits (1, 2, 3, 4) and stages (before and after chilling) on the main plots, sub-plots and sub--sub-plots respectively. The limit of detection for *Enterobacteriaceae* counts was 0·002 CFU cm^{-2} (−2·70 $\log_{10}$CFU cm^{-2}). Zero counts were recorded for carcasses when *Enterobacteriaceae* numbers were below the limit of detection. To cater for zero values, all bacterial counts were increased by 0·001 prior to applying a log transformation to the data and analysis of variance was carried out using Genstat (VSN International, Hemel Hemprtead, UK). When the results were tested for normality by the Anderson-Darling, Cramer-von Mises and Watson tests, the analysis indicated that the data were not log normally distributed (Genstat) at the 1% level.

In consequence, the data were analysed using the non-parametric Mann–Whitney and Kruskal–Wallis tests. The former test was used to compare before and after chilling at each visit for individual abattoirs. The latter test was used to compare the four abattoirs at each visit before and after chilling. The analyses were performed using Genstat. Subsequent to the Kruskal–Wallis test, comparisons between individual abattoirs were made using a multiple comparison test (Conover 1980).

Results

Enterobacteriaceae

Using the Mann–Whitney test, it was established that before and after chilling there were significant differences ($P < 0{\cdot}05$) in carcass counts at each visit for individual abattoirs (Table 1). Exceptions were noted in relation to carcass counts at abattoir C during visits two and four and at abattoir A during visit three, where carcass counts were not statistically different. When carcass counts were compared using the Kruskal–Wallis test, significant differences were observed before chilling between abattoirs during all visits (Table 1). Counts on carcasses in abattoir A were similar to those in abattoirs B, C and D during visits one, two and four, while abattoirs B and C were similar during visits two and four. When carcass counts were compared after chilling, significant differences were observed between abattoirs during all visits. It was noted that at visit two, there were no differences between carcass counts at abattoir C and the remaining three abattoirs.

Table 1 *Enterobacteriaceae* counts (mean $\log_{10}$CFU cm^{-2}) on pig carcasses. Individual abattoirs at each visit were compared before and after chilling using the Mann–Whitney test. The four abattoirs at each visit were compared (i) before and (ii) after chilling using the Kruskal–Wallis test

		Mean $\log_{10}$CFU cm^{-2}			Kruskal–Wallis†	
Visit	Abattoir	Before chill	After chill	Mann–Whitney*	Before chill	After chill
1	A	1·58	0·68	*	f	w
	B	1·38	0·65	*	f	w
	C	1·05	2·21	*	g	x
	D	2·56	2·18	*	h	x
2	A	0·93	0·47	*	f, g	w
	B	0·60	−0·05	*	g, h	x
	C	0·29	0·42	ns	h	w, x
	D	1·24	0·44	*	f	w, x
3	A	−0·05	−0·06	ns	f	w, y
	B	1·06	0·02	*	g	w, x
	C	−0·003	nd‡	*	h	x
	D	1·56	0·10	*	i	y
4	A	−0·07	0·27	*	f	w
	B	0·12	0·06	*	g	x
	C	0·03	0·86	ns	f, g	w
	D	1·65	1·05	*	h	w

*Significant at least at the 5% level.
†At each visit, significant differences between abattoirs were determined using mean rank values. The rank values are not shown and differences are indicated using letters. Abattoirs with no letter in common are significantly different at the 5% level.
‡nd, below the limit of detection.
ns, not significant.

To determine the initial levels of contamination on carcasses and the exact influence of chilling on the *Enterobacteriaceae* counts on carcasses, the data from each of the 30 carcasses swabbed before and after chilling during each visit in the four abattoirs were examined. The numbers of carcasses showing reductions, increases or unchanged counts after chilling are recorded in Table 2. These data are not intended to show statistical differences between *Enterobacteriaceae* counts on carcasses before and after chilling, but demonstrate changes in counts resulting from the chilling process on a carcass-by-carcass basis.

The highest mean initial counts were almost exclusively (7/16) on carcasses in the four abattoirs during visit 1 and abattoir D during all visits, indicating a much higher level of contamination on these carcasses. Reductions in counts as a result of chilling were recorded in all abattoirs and from all visits. When considered across abattoirs, the highest reductions were in abattoir D and the lowest in abattoir C. The data in Table 2 also clearly demonstrate that in abattoir C, while there were reductions in counts, increases also occurred after chilling during three of the four visits.

The *Enterobacteriaceae* are used to assess the hygienic status of fresh meat carcasses, using the performance criteria set down in EC Commission Decision 2001/471/EC and Commission Regulation 2073/2005, which assigns carcasses to one of three categories of contamination based on mean counts ($\log_{10}$CFU cm^{-2}) of acceptable <2·0, marginal 2·0–3·0 and unacceptable >3·0 (Anonymous 2001, 2005). Using these criteria, the numbers of acceptable, marginal and unacceptable carcasses before and after chilling are shown in Table 3. Before chilling, a majority of carcasses at all abattoirs and visits were found to be acceptable, except for abattoir D. In abattoir D, at each visit, there were fewer acceptable and more marginal and unacceptable carcasses compared with the other abattoirs indicating that carcass hygiene was poorer at this plant. This result is related to the data in Table 2, which show a higher level of contamination on carcasses in this abattoir, before chilling and after chilling.

Chilling increased the numbers of acceptable carcasses at abattoirs A, B and D, but not in abattoir C during visits 1, 2 and 4. The numbers of marginal and unacceptable carcasses were also reduced by chilling, in particular, in abattoir D. After chilling, in abattoir C, the numbers of acceptable carcasses were reduced during visits 1, 2 and 4, as were the numbers of marginal carcasses during these visits. In addition, the numbers of unacceptable carcasses increased during visit 1. These data suggest that carcass contamination levels increased during chilling in abattoir C, a result noted previously in Table 2.

Table 2 The influence of chilling in relation to reductions (R), increases (I), or no changes (NC) in *Enterobacteriaceae* counts (mean $\log_{10}$CFU cm^{-2}) on pig carcasses in four abattoirs during four visits

		Abattoir A			*Abattoir B*			*Abattoir C*			*Abattoir D*		
Visit	Effect of chilling	No. of carcasses	Before chill	After chill	No. of carcasses	Before chill	After chill	No. of carcasses	Before chill	After chill	No. of carcasses	Before chill	After chill
1	R	26	1·71	0·65	19	1·64	0·17	5	1·68	1·02	20	2·81	1·98
	I	2	0·75	1·05	7	1·33	2·19	24	0·93	2·51	10	2·07	2·58
	NC	2	0·75	0·75	4	0·23	0·23	1	0·90	0·90	0	–	–
2	R	16	1·48	0·45	22	0·78	−0·11	16	0·54	0·04	21	1·69	0·31
	I	7	0·60	0·99	5	0·12	0·37	10	0·03	1·23	6	0·30	1·10
	NC	7	nd*	nd	3	nd	nd	4	−0·08	−0·08	3	nd	nd
3	R	8	−0·15	nd	28	1·14	0·02	18	−0·01	nd	27	1·70	0·01
	I	8	−1·05	−0·30	0	–	–	0	–	–	2	0·45	1·35
	NC	14	nd	nd	2	nd	nd	12	nd	nd	1	nd	nd
4	R	4	0·38	−0·15	19	0·22	0·13	7	0·17	nd	21	1·97	0·73
	I	17	−0·18	0·55	5	−0·04	0·21	16	nd	1·63	8	1·01	2·03
	NC	9	−0·07	−0·07	6	−0·1	−0·1	7	−0·04	−0·04	1	nd	nd

*nd, below the limit of detection.

Table 3 Categorization of pig carcasses as a result of chilling

			Category											
			Acceptable				Marginal				Unacceptable			
		Total number of samples analysed	Abattoir											
Visit	Chilling		A	B	C	D	A	B	C	D	A	B	C	D
1	Before	120	20	23	29	6	9	7	1	15	1	-	-	9
	After	120	30	30	12	11	-	-	15	16	-	-	3	3
2	Before	120	28	29	30	24	2	1	-	6	-	-	-	-
	After	120	30	30	27	30	-	-	3	-	-	-	-	-
3	Before	120	30	26	30	23	-	4	-	7	-	-	-	-
	After	120	30	30	30	29	-	-	-	1	-	-	-	-
4	Before	120	30	30	30	20	-	-	-	10	-	-	-	-
	After	120	29	30	22	24	1	-	8	6	-	-	-	-

E. coli O157:H7

The survey detected *E. coli* O157:H7 in 0·63% (3/480) of faecal samples and only 0·21% (1/480) on carcasses after chilling. All samples found to contain *E. coli* O157:H7 were taken during the third quarter of the sampling period from August to September 2004. Of the four isolates recovered, two were found in faecal samples taken during the same visit to abattoir A. The other two isolates recovered from a faecal sample and from an after chill carcass swab were isolated during the same visit to abattoir C (Table 4). DNA from the *E. coli* O157:H7 isolates was extracted and characterized using PCR analysis. Although genetic profiles were established for a maximum of ten nonsorbitol fermenting isolates from CT-SMAC per sample (i.e. up to five per duplicate plate when colonies present), a single genetic profile was found to represent all isolates from each positive sample. Analysis by PCR established that the faecal *E. coli* O157:H7 isolates ($n = 3$) carried all the genes investigated in this study, while the carcass isolate ($n = 1$) contained less than half the genes detected in the faecal samples (Table 4). All faecal isolates possessed the genes for both verotoxins, while the carcass isolate carried only the gene for verotoxin 1. Both *E. coli* O157:H7 isolates from abattoir A were phage type PT 8, while the faecal isolate from abattoir C was phage type PT 32. The carcass isolate found after chilling at abattoir C had an unidentifiable phage type.

Discussion

Examination of the data using analysis of variance revealed that the *Enterobacteriaceae* were not log normally distributed over the surface of carcasses and in

Table 4 Phage types and presence (+) or absence (–) of virulence genes in *E. coli* O157:H7 isolated from porcine faeces and after chill carcass

Isolation date	Sample source	Abattoir	Phage type	Virulence genes												
				O157	*H7*	*vt1*	*vt2*	*eaeA*	*hlyA*	*tir*	*katP*	*espA*	*espB*	*espF*	*espP*	*etpD*
17 Aug 2004	Faeces	A	PT 8	+	+	+	+	+	+	+	+	+	+	+	+	+
17 Aug 2004	Faeces	A	PT 8	+	+	+	+	+	+	+	+	+	+	+	+	+
02 Sept 2004	Faeces	C	PT 32	+	+	+	+	+	+	+	+	+	+	+	+	+
02 Sept 2004	After chill carcass	C	Unidentifiable	+	+	+	–	+	+	–	–	–	–	–	–	–

consequence, it was not possible to compare mean log values statistically. Lack of a normal distribution of these organisms on fresh meat carcasses has been encountered previously (Gill 2000; Gill and Jones 2000). However, nonparametric analysis revealed that there were significant differences ($P < 0{\cdot}05$) in carcass counts before and after chilling and between abattoirs. Chilling generally decreased the numbers of *Enterobacteriaceae* positive carcasses and when the abattoirs were considered over the four visits, these varied from 93% to 59% before chilling, to 68–30% after chilling. These data show that overall chilling had the capacity to reduce the numbers of carcasses positive for the presence of *Enterobacteriaceae*. The implications of this for the categorization of carcasses in Decision 471/EC will be discussed below.

In terms of *Enterobacteriaceae* counts, this study recorded reductions during all visits in all abattoirs. Previous studies have noted reductions (Chang *et al.* 2003; Nesbakken *et al.* 2008), increases (Bolton *et al.* 2002) or no change (Gill and Jones 1997) in the numbers of coliforms and *E. coli* on pig carcasses due to the chilling process and these variations are attributed to differences in chilling parameters between plants (Sheridan 2000). The carcasses in this study were cooled in conventional chillers at all four abattoirs, but the chilling parameters of temperature, carcass spacing, air velocity and relative humidity were not measured. For the successful implementation of chilling as a CCP to control the growth of pathogens on carcasses, critical limits need to be established and the effectiveness of the chilling procedure validated at each plant (Gill and Jones 1997; Sheridan 2000; Anonymous 2004, 2007). According to Gill (2005), the proper control of a carcass cooling process requires the definition and subsequent maintenance of operating parameters that ensure at least the containment and preferably the reduction in the numbers of *E. coli* on carcasses.

It was observed that there were increases in counts after chilling in abattoir C, which occurred during three of the four visits. The counts at this abattoir increased in such a consistent manner that it suggests growth of these organisms occurred or that contamination during processing was responsible for the observed effect. The possibility of growth was most likely during visit 1 and 4, as the increases were sufficiently high for such an event to have occurred. Under normal chilling conditions (5–6°C), it has been shown that *Enterobacteriaceae* were not significantly different before and after chilling, which is related to the very long lag and generation times at these temperatures (Newton *et al.* 1978; Smith 1985). It is assumed therefore to facilitate this level of growth, the temperature in the chill would have been about 12–15°C, indicating a failure in the refrigeration system on that day (Smith 1985). Where smaller increases occurred in this abattoir, it is more difficult to determine whether the observed effect is due to growth or contamination during or after chilling. It is not possible to determine the source of this contamination accurately because the use of a whole carcass swab technique precluded the collection of information from specific sites and whether it occurred before or during chilling. In this experiment, the lead side (carcass) of an animal was swabbed before chilling and the opposite trailing side swabbed separately after chilling. The most plausible explanation, however, is that the carcasses were contaminated just before or during chilling, based on the relatively low numbers of positive carcasses and the low counts before chilling. This could have arisen from aerial sources, handling by personnel during chill loading or from carcasses touching other carcasses during chilling.

The increased counts on carcasses in all abattoirs during visit 1 compared to other visits suggest that there may have been a seasonal effect in the contamination of carcasses. This is not a true seasonal effect, however, because the visits to the abattoirs during this period were carried out on different dates during January, February and March. The higher counts observed during this time may be a reflection of the cleanliness of the animal housing or transport of animals to the abattoir, resulting in the presentation of animals that are dirtier than at other times of the year. As the presence of *Enterobacteriaceae* on carcasses is synonymous with faecal contamination, it seems reasonable to suggest that the higher levels of contamination with these organisms is associated with

increased faeces on the live animal (Heuvelink *et al.* 2001). A relationship between increased levels of soil on live animals and increased bacterial counts on beef carcasses has been established in the past (McEvoy *et al.* 2000). The highest levels of carcass contamination were observed in abattoir D during each of the four visits. The increased levels of contamination could not be explained on the basis of geographical, husbandry or lairage issues. The most likely explanation for the increased faecal contamination was from inadequate attention to detail during evisceration, which has been observed previously in another Irish abattoir (Pearce *et al.* 2004). It has been shown previously that line speed facilitates good manufacturing practices especially during evisceration, which is an important source of faecal contamination (Heuvelink *et al.* 2001). In this study, however, abattoir D had the slowest line speed (75 pigs per hour) and this factor was therefore unlikely to be responsible for the higher levels of contamination observed.

One of the objectives of this study was to determine the possible influence of chilling on pig carcass categorization in relation to Decision 471/EC. The data showed that in general, chilling improved the numbers of acceptable carcasses in all abattoirs at each visit, with the exception of abattoir C, where the effect of increased counts was clearly evident. This was also the case in relation to the marginal carcasses in abattoirs A and B and unacceptable carcasses in abattoir D. Although the reductions in counts were generally small as a result of chilling, it was apparent that this process could have a significant influence on the categorization of carcasses. An efficient chilling regime, designed to deliver consistent reductions in *Enterobacteriaceae* counts would seem a worthwhile endeavour, as it could improve carcass safety. This is particularly important because, as discussed elsewhere, the system of corrective action presently described in Decision 471/EC to deal with problems leading to contamination of carcasses during slaughter is unworkable (Hutchison *et al.* 2005; O'Brien *et al.* 2007). While not an ideal solution, reducing the numbers of marginal and unacceptable carcasses using chilling could help improve this problem by reducing the need for corrective action.

This study investigated the prevalence of *E. coli* O157:H7 on carcasses before and after chilling and in addition from faecal samples. The pathogen was recovered from 0·21% (1/480) of carcasses after chilling and 0·63% (3/480) of faecal samples. As the pathogen was detected at such low levels, it was not possible to determine if chilling had any influence on its isolation from carcasses. *E. coli* O157:H7 has been detected in pig faeces at levels comparable to this study, in America (2%; Feder *et al.* 2003) and Norway (0·1%; Johnsen *et al.* 2001). *E. coli* O157 has also been recovered from pig faeces in the Netherlands (0·1%; Heuvelink *et al.* 1999) and Great Britain (0·3%; Milnes *et al.* 2008). *E. coli* O157:H7 has been recovered in Irish cattle (McEvoy *et al.* 2003) and sheep (Lenahan *et al.* 2007), but this is the first record of its presence in pigs. The only report to date of *E. coli* O157:H7 from a pork product causing clinical illness appears to be from the consumption of a dry fermented traditional salami manufactured from pork meat (Conedera *et al.* 2007).

PCR analysis detected the *vt1, vt2*, *eaeA* and *hlyA* genes associated with human pathogenesis in all of the faecal *E. coli* O157:H7 isolates recovered by this study, while the one isolate recovered on an after chill carcass carried the genes for *vt1*, *eaeA* and *hlyA*. The genetic profile of *E. coli* O157:H7 isolated in this study were typical of those isolated from Irish patients suffering clinical illness and were the same phage types. In 2006, 87% of *E. coli* O157:H7 strains isolated from Irish clinical patients carried the *vt2* gene only, while 13% carried genes for both *vt1* and *vt2* (Garvey *et al.* 2008). One of the faecal isolates recovered in our study was PT 32 which was the predominant phage type (47%) in clinical patients in the Republic of Ireland in 2006 and the two remaining faecal isolates recovered were PT 8, which accounted for 11% of the pathogen isolated from Irish clinical patients in that year (Garvey *et al.* 2008). Molecular characterization of the *E. coli* O157:H7 isolated from pigs by our study suggests that they may be potentially pathogenic to humans, as their genetic profiles and phage types were similar to those outlined above in relation to clinical infections. However, the incidence of *E. coli* O157:H7 in pigs was extremely low and they are not considered a primary reservoir for clinical infection in Ireland.

In conclusion, it was important to establish if chilling had the ability to reduce *Enterobacteriaceae* counts consistently on pig carcasses which would be necessary if it were to be used as a CCP in a pig HACCP plan. The data revealed that reductions occurred on 57% of the carcasses examined, while 17% showed no change. Of considerable importance was the observation that 26% of carcasses showed increased counts. While this observation was related largely to contamination problems suggested in abattoir C, small increases also occurred in the other abattoirs.

Finally, in the EU the *Enterobacteriaceae* are used as indicators of faecal contamination on carcasses (Anonymous 2001, 2005), but it has been suggested that the use of *E. coli* as an indicator organism would be more appropriate for this purpose (McEvoy *et al.* 2004; Stuijk and Mossel 2005; Jordan *et al.* 2007). The *Enterobacteriaceae* family comprise a large number of organisms while *E. coli* are a smaller group more specifically associated with faeces and are therefore better indicators of faecal contamination on carcasses. *E. coli* are the preferred indicator

organism in Australia, the United States and elsewhere (Gill *et al.* 1996; Sofos *et al.* 1999; Jordan *et al.* 2007).

Acknowledgements

The authors would like to thank Dr Dermot Harrington for performing the statistical analysis on the *Enterobacteriaceae* results and the Laboratory of Enteric Pathogens, London, UK, for carrying out phage typing of isolates. We acknowledge with gratitude funding from the Department of Agriculture and Food under the Food Institutional Research Measure (FIRM), Project no. 01/R&D/D/170.

References

Anonymous (2001) Commission decision of 8 June 2001 (2001/471/EC). *OJEU* **L165**, 48–53.

Anonymous (2004) Validation of critical limits during chilling. *Meat Tech Update* **4/04**, 1–4.

Anonymous (2005) Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. *OJEU* **L338**, 1–26.

Anonymous (2007) Beef carcase chilling – opportunities for customising programs. *Meat Tech Update* **2/07**, 1–4.

Bolton, D.J., Pearce, R.A., Sheridan, J.J., Blair, I.S., McDowell, D.A. and Harrington, D. (2002) Washing and chilling as critical control points in pork slaughter hazard analysis and critical control point (HACCP) systems. *J Appl Microbiol* **92**, 893–902.

Brunder, W., Schmidt, H. and Karch, H. (1996) KatP, a novel catalase-peroxidase encoded by the large plasmid of enterohaemorrhagic *Escherichia coli* O157:H7. *Microbiology* **142**, 3305–3315.

Chang, V.P., Mills, E.W. and Cutter, C.N. (2003) Reduction of bacteria on pork carcasses associated with chilling method. *J Food Prot* **66**, 1019–1024.

Conedera, G., Mattiazzi, E., Russo, F., Chiesa, E., Scorzato, I., Grandesso, S., Bessegato, A., Fioavanti, A. *et al.* (2007) A family outbreak of *Escherichia coli* O157 haemorrhagic colitis caused by pork meat salami. *Epidemiol Infect* **135**, 311–314.

Conover, W.J. (1980) *Practical Nonparametric Statistics*, 2nd edn. New York: John Wiley and Sons.

Crane, J.K., McNamara, B.P. and Donnenberg, M.S. (2001) Role of EspF in host cell death induced by enteropathogenic *Escherichia coli*. *Cell Microbiol* **3**, 197–211.

Feder, I., Wallace, F.M., Gray, J.T., Fratamico, P., Fedorka-Cray, P.J., Pearce, R.A., Call, J.E., Perrine, R. *et al.* (2003) Isolation of *Escherichia coli* O157:H7 from intact colon fecal samples of swine. *Emerg Infect Dis* **9**, 380–383.

Fratamico, P.M., Bagi, L.K. and Pepe, T. (2000) A multiplex polymerase chain reaction assay for rapid detection and identification on *Escherichia coli* O157:H7 in foods and bovine faeces. *J Food Prot* **63**, 1032–1037.

Garvey, P., McKeown, P., Carroll, A. and McNamara, E. (2008) Epidemiology of verotoxigenic *E. coli* in Ireland, 2006. *Epi-Insight* **9**, 2–3.

Gill, C.O. (2000) HACCP in primary processing: red meat. In *HACCP in the Meat Industry* ed. Brown, M. pp. 81–122. Cambridge: Woodhead Publishing.

Gill, C.O. (2005) HACCP in the processing of fresh meat. In *Improving the Safety of Fresh Meat* ed. Sofos, J.N. pp. 630–672. Cambridge: Woodhead Publishing.

Gill, C.O. and Jones, T. (1997) Assessment of the hygienic performances of an air-cooling process for lamb carcasses and a spray-cooling process for pig carcasses. *Int J Food Microbiol* **38**, 85–93.

Gill, C.O. and Jones, T. (2000) Microbiological sampling of carcasses by excision or swabbing. *J Food Prot* **63**, 167–173.

Gill, C.O., McGinnis, J.C. and Badoni, M. (1996) Use of total or *Escherichia coli* counts to assess the hygienic characteristics of a beef carcass dressing process. *Int J Food Microbiol* **31**, 181–196.

Griffin, P.M. and Tauxe, R.V. (1991) The epidemology of infections caused by *Escherichia coli* O157:H7, other Enterohemorrhagic *E. coli*, and the associated hemolytic uremic syndrome. *Epidemiol Rev* **13**, 60–98.

Heuvelink, A.E., Zwartkruis-Nahuis, J.T.M., van de Biggelaar, F.L.A.M., van Leeuwen, W.J. and de Boer, E. (1999) Isolation and characterization of verocytotoxin-producing *Escherichia coli* O157 from slaughter pigs and poultry. *Int J Food Microbiol* **52**, 67–75.

Heuvelink, A.E., Roessink, G.L., Bosboom, K. and de Boer, E. (2001) Zero-tolerance for faecal contamination of carcasses as a tool in the control of O157 VTEC infections. *Int J Food Microbiol* **66**, 13–20.

Higgins, J.A., Nasarabadi, S., Karns, J.S., Shelton, D.R., Cooper, M., Gbakima, A. and Koopman, R.P. (2003) A handheld real time thermal cycler for bacterial pathogen detection. *Biosens Bioelectron* **18**, 1115–1123.

Hutchison, M.L., Walters, L.D., Avery, S.M., Reid, C., Wilson, D., Howell, M., Johnston, A.M. and Buncic, S. (2005) A comparison of wet-dry swabbing and excision sampling methods for microbiological testing of bovine, porcine, and ovine carcasses at red meat slaughterhouses. *J Food Prot* **68**, 2155–2162.

Johnsen, G., Wasteson, Y., Heir, E., Berget, O.I. and Herikstad, H. (2001) *Escherichia coli* O157:H7 in faeces from cattle, sheep and pigs in the southwest part of Norway during 1998 and 1999. *Int J Food Microbiol* **65**, 193–200.

Jordan, D., Philips, D., Sumner, J., Morris, S. and Jenson, I. (2007) Relationships between the density of different indicator organisms on sheep and beef carcasses and in frozen beef and sheep meat. *J Appl Microbiol* **102**, 57–64.

Lenahan, M., O'Brien, S., Kinsella, K., Sweeney, T. and Sheridan, J.J. (2007) Prevalence and molecular characterisation

of *Escherichia coli* O157:H7 on Irish lamb carcasses, fleece and in faeces samples. *J Appl Microbiol* **103**, 2401–2409.

McEvoy, J.M., Doherty, A.M., Finnerty, M., Sheridan, J.J., McGuire, L., Blair, I.S., McDowell, D.A. and Harrington, D. (2000) The relationship between hide cleanliness and bacterial numbers on beef carcasses at a commercial abattoir. *Lett Appl Microbiol* **30**, 390–395.

McEvoy, J.M., Doherty, A.M., Sheridan, J.J., Thomson-Carter, F.M., Garvey, P., McGuire, L., Blair, I.S. and McDowell, D.A. (2003) The prevalence and spread of *Escherichia coli* O157:H7 at a commercial beef abattoir. *J Appl Microbiol* **95**, 256–266.

McEvoy, J.M., Sheridan, J.J., Blair, I.S. and McDowell, D.A. (2004) Microbial contamination on beef in relation to hygiene assessment based on criteria used in EU Decision 2001/471/EC. *Int J Food Microbiol* **92**, 217–225.

McNally, A., Roe, A.J., Simpson, S., Thomson-Carter, F.M., Hoey, D.E., Currie, C., Chakraborty, T., Smith, D.G. *et al.* (2001) Differences in levels of secreted locus of enterocyte effacement proteins between human disease-associated and bovine *Escherichia coli* O157. *Infect Immun* **69**, 5107–5114.

Milnes, A.S., Stewart, I., Clifton-Hadley, F.A., Davies, R.H., Newell, D.G., Sayers, A.R., Cheasty, T., Cassar, C. *et al.* (2008) Intestinal carriage of verocytotoxigenic *Escherichia coli* O157, *Salmonella*, thermophilic *Campylobacter* and *Yersinia enterocolitica*, in cattle, sheep and pigs at slaughter in Great Britain during 2003. *Epidemiol Infect* **136**, 739–751.

Nesbakken, T., Eckner, K. and Røtterud, O. (2008) The effect of blast chilling on occurrence of human pathogenic *Yersinia enterocolitica* compared to *Campylobacter* spp. and numbers of hygienic indicators on pig carcasses. *Int J Food Microbiol* **123**, 130–133.

Newton, K.G., Harrison, J.C.L. and Wauters, A.M. (1978) Sources of psychrotrophic bacteria on meat at the abattoir. *J Appl Bacteriol* **45**, 75–82.

O'Brien, S.B., Lenahan, M., Sweeney, T. and Sheridan, J.J. (2007) Assessing the hygiene of pig carcasses using whole-body carcass swabs compared with the four-site method in EC Decision 471. *J Food Prot* **70**, 432–439.

Paton, A.W. and Paton, J.C. (1998) Detection and characterization of shiga toxigenic *Escherichia coli* by using multiplex PCR assays for stx1, stx2, *eae*A, enterohemorrhagic *E. coli hlyA*, *rfb* (O111), and *rfb* (O157). *J Clin Microbiol* **36**, 598–602.

Pearce, R.A., Bolton, D.J., Sheridan, J.J., McDowell, D.A., Blair, I.S. and Harrington, D. (2004) Studies to determine the critical control points in pork slaughter hazard analysis and critical control point systems. *Int J Food Microbiol* **90**, 331–339.

Schmidt, H., Geitz, C., Tarr, P.I., Frosch, M. and Karch, H. (1999) Non-O157:H7 pathogenic Shiga toxin-producing *Escherichia coli*: phenotypic and genetic profiling of virulence traits and evidence for clonality. *J Infect Dis* **179**, 115–123.

Sheridan, J.J. (2000) Monitoring CCPs in HACCP systems. In *HACCP in the Meat Industry* ed. Brown, M. pp. 203–230. Cambridge: Woodhead Publishing.

Sheridan, J.J. (2004) Decontamination. In *Encyclopedia of Meat Sciences* ed. Jensen, W.K., Devine, C. and Dikeman, M. pp. 389–396. Oxford: Elsevier Science.

Smith, M.G. (1985) The generation time, lag time, and minimum temperature of growth of coliform organisms on meat, and the implications for codes of practice in abattoirs. *J Hyg* **94**, 289–300.

Sofos, J.N., Kochevar, S.L., Reagan, J.O. and Smith, G.C. (1999) Extent of beef carcass contamination with *Escherichia coli* and probabilities of passing U.S. regulatory criteria. *J Food Prot* **62**, 234–238.

Stuijk, C.B. and Mossel, D.A.A. (2005) Letter to the editor. *Int J Food Microbiol* **99**, 113–114.

Wasteson, Y. (2001) Zoonotic *Escherichia coli*. *Acta Vet Scand Suppl* **95**, 79–84.

