The Incidence of *Listeria* spp. and *Escherichia coli* O157:H7 on Beef Carcasses

McEvoy, J.M., Doherty, A.M., Sheridan, J.J. and McGuire, L.

Teagasc, The National Food Centre, Dunsinea, Castleknock, Dublin 15, Ireland.

Background

The aim of the Hazard Analysis Critical Control Point (HACCP) system in slaughter operations is to prevent, eliminate or reduce both the incidence and levels of microorganisms pathogenic for humans (USDA-NACMCF, 1993). Before the initiation of a HACCP system, a survey of the types and levels of microorganisms present on the carcass should be carried out and used as a baseline by which future hygiene modifications can be measured. *Listeria* spp., including *L. monocytogenes*, have been shown to be ubiquitous in the environment of beef slaughterhouses (Skovgaard and Morgen, 1988, Gobat and Jemmi, 1990). Given this widespread distribution it is inevitable that some transfer to the carcass will occur during dressing operations.. *Escherichia coli* O157:H7 is an intestinal pathogen which can be introduced into the slaughter house environment on the hide or in the intestine of animals. It has been involved in a number of outbreaks of food poisoning resulting from the consumption of undercooked beef (Buchanan and Doyle, 1997)

Objectives

The aim of this study was to establish a baseline level of the occurrence of *Listeria* spp. and *E.coli* O157:H7 on beef carcasses at different stages of dressing. This information can be used to identify sources of contamination and the effect of control measures such as carcass washing in reducing contamination. Baseline data is essential for measuring the effect of the HACCP system on process hygiene.

Methods

Thirty six beef carcasses were sampled over a twelve month period at a local abattoir for the presence of *Listeria* spp. and *E. coli* O157:H7. Samples were taken from 5 carcass sites at different processing stages and from 4 meat cuts after boning (Table 1). An undelimited area of approximately 50 cm^2 was swabbed wet and dry. Swabs were suspended in 50 ml of maximum recovery diluent (Oxoid), transported to the laboratory within 1 h of collection and processed immediately. Listeria spp. were isolated by enriching 10 ml of the original sample in 10 ml of double strength buffered peptone water (Oxoid) at 30°C for 24 h before plating onto Palcam agar (Oxoid) and incubating at 30°C for 48 h. Up to 5 colonies per plate showing characteristic blackening and dimpled centres were re-streaked onto tryptone soya agar (Oxoid) and incubated at 30°C for 24 h. Isolates were confirmed and speciated using the API *Listeria* system (Biomerieux) or according to Sheridan *et al.* (1994). *E. coli* O157:H7 was isolated by enriching 10 ml of the original sample in 10 ml of double strength EE broth (Oxoid) containing 0.02 g/l novobiocin, at 37°C for 24 h before plating onto sorbitol MacConkey agar (Oxoid) containing 0.05 mg/l cefiximine and 2.5 mg/l potassium tellurite and incubating at 37°C for 24 h. Up to 8 sorbitol non fermenting colonies per plate were screened by plating onto eosin methylene blue agar (Oxoid) and phenol red sorbitol agar containing 4-methylumbelliferyl-ß-D-glucuronide. Colonies showing typical *E. coli* O157:H7 reactions were tested using the O157:H7 latex agglutination kit (Wellcolex) and confirmed biochemically (USDA-FSIS, 1994).

Results and Discussion

Of a total of 156 positive *Listeria* samples, 20.5 % were found to be *L. monocytogenes* with the other 79.5 % made up of *L. innocua* (54.5 %) and *L. welshimeri* (25.0 %) (Table 1). Samples were sometimes contaminated with more than one species of *Listeria* (data not shown). Looking at the total occurrence of *Listeria* certain trends can be identified. The overall incidence of *Listeria* in this study was 16 % (156 out of 952 (34 carcasses x 28 samples/carcass)). The number of *Listeria* isolated from the hock at each process stage as a percentage of the total number of hock samples taken at that stage, showed an increase between legging (3 %) and hide removal (20 %) and a decrease at evisceration (14 %) and carcass washing (6 %). Considering that the hock is not touched after legging, this trend indicates possible airborne contamination of the hock especially at hide removal. *Listeria* was not detected at the cranial back until after carcass washing where 12 % of the samples were positive, indicating possible re-distribution of the organism during this step. Grouping the sites at each process stage highlighted 2 problem areas, namely carcass washing and boning. Eighteen percent of samples taken after carcass washing and 50 % of samples after boning were positive for *Listeria* as opposed to 3 % at legging and 9-10 % at the other process stages. The frequency of detection of *Listeria* shown here provides a baseline against which improvements in process hygiene can be measured.

E. coli O157:H7 was isolated from 11 % (4 out of 36) of the carcasses sampled (Table 2). The positive samples were collected in July and August with 2 of the positive carcasses occurring on the same day. These carcasses were separated on the slaughter line by at least 15 carcasses, however, they were placed side by side in the chill. The detection of *E. coli* O157:H7 during these months is consistent with the reported seasonal incidence of this pathogen (Mechie *et al.*, 1997). It must be noted that the sample size in this study was small and this must be taken into account when interpreting the results. A more detailed survey of the presence of the organism will be carried out over the coming year at the same abattoir. On 1 of the carcasses, *E. coli* O157:H7 was only detected on the cranial back after carcass washing However, on the other 3 carcasses, widespread distribution of *E. coli* O157:H7 was evident. These findings suggest that there may be many opportunities for the organism to transfer to the carcass during dressing and deboning. Contamination of the cranial back as early as hide removal is unexpected as this area is untouched by the operatives or hide at this stage. On carcass 4, *E. coli* O157:H7 was generally detected on all sites as far as evisceration. The pathogen was not detected at

carcass splitting or carcass washing. However, it was detected again on all the carcass sites sampled after chilling and on the outside round and brisket after deboning. This may reflect a lack of sensitivity in the detection method It might also be due to cross contamination from operatives placing the carcasses in the chill or from the close proximity of carcasses in the chill (noting that *E. coli* O157:H7 was detected on carcass 3 before entry to the chill and placed beside carcass 4 in the chill).

Conclusion

This study demonstrates that, when present, *E. coli* O157:H7 can become widely distributed on the carcass and in the factory environment in much the same way as *Listeria*. While it must be recognised that *Listeria* is not an enteric organism and could not be used as an indicator for enteric pathogens such as *E. coli* O157:H7, it might be useful as an indicator of global factory hygiene (Gobat and Jemmi, 1990). Improvements in Good Manufacturing Practice resulting in a decrease in the baseline occurrence of *Listeria* could also have a positive effect in reducing the spread of *E. coli* O157:H7 when present in the factory environment.

References

- Buchanan, R.L., and Doyle, M.P. 1997. Foodborne disease significance of *Escherichia coli* O157:H7 and other enterohemorrhagic *E. coli*. Food Tech. **51**, 10: 69-76
- Gobat, P.F. and Jemmi, T. 1990. Epidemiological studies on *Listeria* spp. in slaughterhouses. Fleischwirtsch. **70**, 12: 1448-1450
- Mechie, S.C., Chapman, P.A. and Siddons, C.A. 1997. A fifteen month study of *Escherichia coli* O157:H7 in a dairy heard. Epidemiol. Infect. **118**: 17-25
- Sheridan, J.J., Duffy, G., McDowell, D.A. and Blair, I.S. 1994. The occurrence and initial numbers of *Listeria* in Irish meat and fish products and the recovery of injured cells from frozen products. Int. J. Food Micro. **22**: 105-113
- Skovgaard, N. and Morgen, C.A. 1988. Detection of Listeria spp. in faeces from animals, in feeds and in raw foods of animal origin. Int. J. Food Micro. **6**:229-242
- United States Department of Agriculture. 1994 Microbiological testing programme for *Escherichia coli* O157:H7 in raw grouned beef. FSIS notice
- United States Department of Agriculture, NACMCF. 1993. Generic HACCP for raw beef. Food Micro. **10**: 449-488

Table 1: Frequency of isolation of Listeria *spp.* from beef carcasses (n=34) during dressing and boning

	Legging	Hide Removal					Evisceration				Carcass Splitting				Carcass Washing					After 24 h Chilling					Boning			
	A[a]	A	B	C	D	E	A	C	D	E	F	C	D	E	A	F	C	D	E	A	F	C	D	E	G	H	I	J
L. monocytogenes	0	3	1	0	0	1	0	2	0	0	1	2	0	0	0	2	2	1	1	1	1	0	0	0	2	8	1	2
L. innocua	1	3	1	1	0	5	5	4	0	1	7	2	0	1	0	3	2	1	4	1	1	2	2	2	8	17	5	7
L. welshimeri	0	1	0	0	0	0	0	1	0	0	0	0	0	0	2	4	4	2	2	0	1	0	2	3	6	3	5	3
TOTAL	**1**	**7**	**2**	**1**	**0**	**6**	**5**	**7**	**0**	**1**	**8**	**4**	**0**	**1**	**2**	**9**	**8**	**4**	**7**	**2**	**3**	**2**	**4**	**5**	**16**	**28**	**11**	**12**

[a]A = Hock, B = Bung - before it is cut and freed,. C = Bung - after it is cut and freed, D = Cranial Back, E = Brisket, F = Inside Round, G = Inside Round - after boning, H = Outside Round - after boning, I = Cranial back - after boning, J = Brisket - after boning

Table 2: Isolation of *E. coli* O157:H7 from beef carcasses (n=36) during dressing and boning

Positive carcasses and sample dates	Legging	Hide Removal					Evisceration				Carcass Splitting				Carcass Washing					After 24 h Chilling					Boning			
	A[a]	A	B	C	D	E	A	C	D	E	F	C	D	E	A	F	C	D	E	A	F	C	D	E	G	H	I	J
Carcass 1 (15/7'97)	-[b]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	-	-	-	-	-	-	-	-	-	-
Carcass 2 (22/7'97)	-	*	-	*	*	*	*	*	*	-	*	*	*	-	*	*	*	*	*	-	-	-	-	-	-	-	-	-
Carcass 3 (12/8/'97)	-	*	-	*	*	*	*	*	*	*	-	*	*	*	*	*	*	*	-	*	*	*	*	*	*	*	*	*
Carcass 4 (12/8/'97)	*	*	*	*	*	*	*	*	*	-	-	-	-	-	-	-	-	-	-	*	*	*	*	*	-	*	-	*

[a]See Table 1 for detailed description of sites.

[b]- = Sites which tested negative for *E. coli* O157:H7; * = Sites which tested positive for *E. coli* O157:H7