The incidence of *Escherichia coli* O157:H7 in bovine faecal, rumen and carcass samples.

J.M. McEvoy[1], A.M. Doherty[1], J.J. Sheridan[1], F.M. Thomson-Carter[2], L. McGuire[1], I.S. Blair[3] and D.A. McDowell[3]
[1]Teagasc, The National Food Centre, Castleknock, Dublin 15, Ireland.
[2]Department of Medical Microbiology, University of Aberdeen, Foresterhill, Aberdeen AB25 2ZD, Scotland.
[3]University of Ulster, Jordanstown, N. Ireland

Key words: *E. coli* O157:H7, cattle, beef, faeces, rumen, carcass

Background

Cattle have been identified as a major reservoir of *E. coli* O157:H7 (Chapman *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 etiologic agent (Meng and Doyle 1998). *E. coli* O157:H7 has been reported in faeces, rumen contents and on the hide of cattle at slaughter (Van Donkersgoed *et al.* 1999;Elder *et al.* 2000). Possible contamination of edible carcass tissue is the most significant challenge to food safety, and the extent and nature of such contamination may be related to the *E. coli* O157:H7 status of the pre-slaughter animal (Elder *et al.* 2000). To date, there has been no study of the incidence of *E. coli* O157:H7 in pre-slaughter cattle or on carcasses in an Irish abattoir.

Objective

The objective of this study was to assess the incidence and characteristics of *E. coli* O157:H7 in faecal and rumen samples post-slaughter and on resulting carcasses at an Irish beef abattoir.

Methods

At a commercial Irish beef abattoir, a faecal, rumen and carcass sample was collected from each of 5 consecutive animals on a weekly basis between June 1998 to May 1999. Faecal samples were collected in a sterile stomacher bag (Model 400 Bags 6041, Seward Ltd., London, England) during the legging operation. Rumen samples were collected in a sterile stomacher bag immediately after evisceration. Carcass samples were collected after entry into the chill using a method described by Lasta *et al.* (Lasta *et al.* 1992). All samples were placed in an insulated box with an ice pack and transported to the laboratory within 2 h of collection. Faecal and rumen samples were stored overnight at 0°C. An aliquot (*ca.* 0.5 g) of faeces was placed into 5 ml of buffered peptone water (Oxoid) containing 8 mg/l vancomycin (Sigma Chemical Co), 10 mg/l cefsulodin (Sigma Chemical Co.) and 0.05 mg/l cefiximine (Cyanamid of Great Britain Ltd., Hampshire, England) (BPW-VCC) and incubated at 37°C for 6h. A 1 ml aliquot of rumen liquid was enriched in a similar manner. Carcass samples were processed immediately upon return to the laboratory. The sponge was rinsed in 200 ml of BPW, removed and the remaining liquid was incubated at 37°C overnight. *E. coli* O157:H7 was isolated from enriched faecal, rumen and carcass samples using immunomagnetic separation (IMS) (Wright *et al.* 1994), followed by culture onto sorbitol MacConkey agar (Oxoid) containing 0.05 mg/l cefiximine and 2.5 mg/l potassium tellurite (Sigma Chemical Co.). Suspect colonies were subjected to biochemical confirmation. All biochemically confirmed isolates were subjected to serotype confirmation and virulence factor determination using PCR with primers targeting rfb_{O157} (Paton and Paton 1998), $fliC_{H7}$ (Gannon *et al.* 1997), *eaeA*O157 (Gannon *et al.* 1993) and *ehlyA* (Fratamico *et al.* 1995) gene sequences and sequences from genes encoding verotoxin 1 (VT1) (Pollard *et al.* 1990) and verotoxin 2 (VT2) (Olsvik and Strockbine 1993) production. Biochemically confirmed isolates were also phage typed according to the method of Khakria *et al.* (Khakria *et al.* 1990).

Results and discussion

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. The rumen prevalence was low relative to the prevalence in faeces. This observation is in line with the report of Van Donkersgoed (Van Donkersgoed *et al.* 1999) who noted a 0.8 % prevalence of the organism in rumen samples compared to 7.5 % in faecal samples. These results support the hypothesis that *E. coli* O157:H7 does not proliferate in the rumen of adult cattle, and that multiplication occurs in the hind-gut.

The serotype of all *E. coli* isolates was confirmed as O157:H7 by PCR (Table 1). All isolates carried the genes encoding *eaeA*O157 and *ehylA*. Ninety five percent (19/20) of strains carried one or both of the genes encoding verotoxin production. The overall prevalence of verotoxin genotypes was 44.5, 44.5 and 5.5 and 5.5 % for VT1/VT2, VT2, VT1 and VT negative, respectively. A similar low prevalence of the VT 1 genotype was found in previous studies in cattle (Chapman *et al.* 1997), and humans (Thomas *et al.* 1996).

All strains 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 (Table 1). PT 32 was the most common phage type associated with human infection in Ireland in 1999, accounting for 67 % of isolations (Anonymous 2000).

Results from samples taken on 13-Apr suggest a relationship between the pre-slaughter status of the animal and the status of the resulting carcass, as indistinguishable strains were isolated from the faeces of 2 animals and the resulting carcasses (Table 1). These findings are similar to those of Chapman *et al.* (Chapman *et al.* 1993)

Data showing the frequency of isolation of *E. coli* O157:H7 from faecal, rumen and carcass samples each month, are presented in Figure 1. Apart from one positive carcass sample in November, all isolations of this organism occurred during spring and late summer. This observation is in agreement with a prevalence study in cattle on farms in the UK (Chapman *et al.* 1997).

Conclusions

E. coli O157:H7 was shown to be present in faeces, rumen contents and on carcasses. The organism was most prevalent during spring and late summer. Most isolates would be considered fully pathogenic as they contain the full complement of virulence factors. The predominant phage type (PT 32) was also the predominant phage type recovered from human cases of *E. coli* O157:H7 infection in Ireland during the period of the study (1998-1999).

References

1. Anonymous (2000) Epidemiology of *E. coli* O157:H7 in Ireland in 1999. *National Disease Surveillance Centre, Sir Patrick Dunns Hospital, Dublin 2.*

2. Chapman,P.A., Siddons,C.A., Cerdan Malo,A.T. and Harkin,M.A. (1997) A 1-year study of *Escherichia coli* O157 in cattle, sheep, pigs and poultry. *Epidemiology and Infection* **119**, 245-250.

3. Chapman,P.A., Siddons,C.A., Wright,D.J., Norman,P., Fox,J. and Crick,E. (1993) Cattle as a possible source of verocytotoxin-producing *Escherichia coli* O157 infections in man. *Epidemiology and Infection* **111**, 439-447.

4. Elder,R.O., Keen,J.E., Siragusa,G.R., Barkocy-Gallagher,G.A., Koohmaraie,M. and Laegreid,W.W. (2000) Correlation of enterohemorrhagic *Escherichia coli* O157 prevalence in feces, hides and carcasses of beef cattle during processing. *Proceedings of the National Academy of Sciences* **97**, 2999-3003.

5. Fratamico,P.M., Sackitey,S.K., Wiedmann,M. and Deng,M.Y. (1995) Detection of Escherichia coli O157:H7 by multiplex PCR. *Journal of Clinical Microbiology* **33**, 2188-2191.

6. Gannon,V.P., Rashed,M., King,R.K. and Thomas,E.J. (1993) Detection and characterization of the eae gene of Shiga-like toxin-producing Escherichia coli using polymerase chain reaction. *Journal of Clinical Microbiology.* **31**, 1268-1274.

7. Gannon,V.P.G., D'Souza,S., Graham,T., King,R.K., Rahn,K. and Read,S. (1997) Use of the flagellar H7 gene as a target in multiplex PCR assays and improved specificity in identification of enterohaemorrhagic *Escherichia coli* strains. *Journal of Clinical Microbiology* **35**, 656-662.

8. Khakria,R., Duck,D. and Lior,H. (1990) Extended phage typing scheme for *Escherichia coli* O157:H7. *Epidemiology and Infection* **105**, 511-520.

9. Lasta,J.A., Rodriguez,R., Zanelli,M. and Margaria,C.A. (1992) Bacterial count from bovine carcasses as an indicator of hygiene at slaughtering places: A proposal for sampling. *Journal of Food Protection* **54**, 271-278.

10. Meng, J. and Doyle, M. P. (1998) Microbiology of shiga toxin producing *Escherichia coli* in foods. In *Escherichia coli O157:H7 and other shiga-toxin producing E. coli strains* ed. Kaper,J.B. and O'Brien,A.D. pp. 92-108. Washington D.C.: American Society for Microbiology.

11. Olsvik, Ø. and Strockbine, N. A. (1993) Polymerase chain reaction for detection of heat-stable, heat-labile and Shiga-like toxin genes in *Escherichia coli*. In *Diagnostic Molecular Microbiology* ed. Persing,D.H., Tenover,F.C., Smith,T.F. and White,T.J. American Society for Microbiology, Washington DC.

12. Paton,A.W. and Paton,J.C. (1998) Detection and characterization of shiga toxigenic *Escherichia coli* by using multiplex PCR assays for stx_1, stx_2, *eae*A, enterohemorrhagic *E. coli hly*A, rfb_{O111}, and rfb_{O157}. *Journal of Clinical Microbiology* **36**, 598-602.

13. Pollard,D.R., Johnson,W.M., Lior,H., Tyler,D. and Rozee,K.R. (1990) Rapid and specific detection of verotoxin genes in *Escherichia coli* by the polymerase chain reaction. *Journal of Clinical Microbiology* **28**, 540-545.

14. Thomas,A., Cheasty,T., Frost,J.A., Chart,H., Smith,H.R. and Rowe,B. (1996) Vero cytotoxin-producing Escherichia coli, particularly serogroup O157, associated with human infections in England and Wales: 1992-4. *Epidemiology and Infection* **117**, 1-10.

15. Van Donkersgoed,J., Graham,T. and Gannon,V. (1999) The prevalence of verotoxins, *Escherichia coli* O157:H7 and *Salmonella* in the faeces and rumen of cattle at processing. *Canadian Veterinary Journal* **40**, 332-338.

16. Wright,D.J., Chapman,P.A. and Siddons,C.A. (1994) Immunomagnetic separation as a sensitive method for isolating *Escherichia coli* O157 from food samples. *Epidemiology and Infection* **113**, 31-39.

Table 1 Isolation date, phage type, serotype and virulence characteristic profiles of *E. coli* isolates from faecal, rumen and carcass samples.

Sample type & date	Animal no.[a]	Serotype O157	Serotype H7	Virulence characteristic profile *eae*AO157	Virulence characteristic profile *ehyl*A	Virulence characteristic profile vt1	Virulence characteristic profile vt2	Phage type
Faeces								
23-Mar	4	+	+	+	+	+	-	32
	4*	+	+	+	+	-	-	32
13-Apr	3	+	+	+	+	+	+	32
	4	+	+	+	+	+	+	32
21-Jul	4	+	+	+	+	-	+	32
04-Aug	1	+	+	+	+	-	+	32
09-Sep	1	+	+	+	+	-	+	32
	1*	+	+	+	+	-	+	rdnc[b]
Rumen								
21-Jul	3	+	+	+	+	-	+	32
09-Sep	4	+	+	+	+	+	+	8
Carcass								
13-Apr	2	+	+	+	+	+	+	32
	3	+	+	+	+	+	+	32
	4	+	+	+	+	+	+	32
	5	+	+	+	+	+	+	32
27Apr	1	+	+	+	+	+	+	32
18-Aug	2	+	+	+	+	-	+	32
	3	+	+	+	+	-	+	32
03-Nov	5	+	+	+	+	-	+	32

[a] Animal number, where '1' is the first and '5' is the last of the five consecutive animals sampled.

[b] 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.

Figure 1 **Study 2: Number of faecal, rumen and carcass samples testing positive for *E. coli* O157:H7 each month.**