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Cattle as a possible source of verocytotoxin-producing *Escherichia coli* O157 infections in man

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SUMMARY

In May–June 1992 cases of infection with verocytotoxin-producing (VT^+) *Escherichia coli* O157 in South Yorkshire could have been associated with prior consumption of beef from a local abattoir. During investigation of the abattoir, bovine rectal swabs and samples of meat and surface swabs from beef carcasses were examined for *E. coli* O157, isolates of which were tested for toxigenicity, plasmid content and phage type. *E. coli* O157 was isolated from 84 (4%) of 2103 bovine rectal swabs; of these 84, 78 (93%) were VT^+, the most common phage types being 2 and 8, the types implicated in the cluster of human cases. Positive cattle were from diverse sources within England. *E. coli* O157 was isolated from 7 (30%) of 23 carcasses of rectal swab-positive cattle and from 2 (8%) of 25 carcasses of rectal swab-negative cattle. The study has shown that cattle may be a reservoir of VT^+ *E. coli* O157, and that contamination of carcasses during slaughter and processing may be how beef and beef products become contaminated and thereby transmit the organism to man.

INTRODUCTION

Strains of *Escherichia coli* that produce a powerful cytotoxin active against cultured Vero cells are now recognized as important human pathogens [1]. These verocytotoxin-producing *E. coli* (VTEC) have been associated with both outbreaks and sporadic cases of haemorrhagic colitis (HC) in N. America [2, 3] and in England and Wales [4, 5], and with sporadic cases of haemolytic-uraemic syndrome (HUS) in Canada [6, 7] and in England and Wales [5, 8]. HUS, which is more common in young children, is now the single commonest cause of renal failure in children in N. America and in England and Wales [1, 9].

Beef, beef products and untreated milk have been suggested as possible sources of VTEC infection for man [2, 10]. Verocytotoxin-producing (VT^+) *E. coli* O157, the most common serogroup associated with illness in man, has been isolated previously from cattle at a Sheffield abattoir [5], but although these were of the same phage type and plasmid content as strains isolated from human infections in Sheffield, a route of transmission from cattle to man was not found [11].

In the Sheffield area, there was a cluster of cases of human infection with VT^+ *E. coli* O157 during May–June 1992, and epidemiological evidence, although not

conclusive, suggested that infection might have been associated with prior consumption of beef from a South Yorkshire abattoir. The aims of this study were, therefore, to investigate cattle after arrival at the abattoir, to determine whether they were carrying VT$^+$ *E. coli* O157 in their intestines, and to attempt to elucidate further how this organism may be transmitted to man.

MATERIALS AND METHODS

Human cases

Faecal samples from patients with bloody diarrhoea or clinical evidence of HUS, or both, were examined for VT$^+$ *E. coli* O157 at Sheffield Public Health Laboratory. The samples were selected from those submitted to the laboratory by general practitioners and the Communicable Diseases Unit of Lodge Moor Hospital, on the basis of information on the request form, or on the basis of the presence of blood in the sample.

Samples were inoculated onto sorbitol MacConkey (SMAC) medium (Oxoid - CM813). After overnight incubation at 37 °C, apparently sorbitol non-fermenting colonies were tested for agglutination with a latex test kit (Oxoid - DR622) for detecting *E. coli* O157. Isolates that gave positive results with this test, and isolates referred to Sheffield PHL following detection by similar methods in other laboratories in our area, were further characterized as described below.

Abattoir study period

The study was conducted from 8 July to 25 September 1992. From 8 July to 18 August rectal swabs only were examined, but for the remaining period of the study meat trimmings and surface swabs from carcasses were also examined.

Collection and examination of abattoir samples

Bovine rectal faeces

Swabs (Transwabs, Medical Wire Co.) were taken from cattle immediately after slaughter, placed in transport medium supplied by the swab manufacturer, and were stored at 4 °C prior to being transported to the laboratory within 24 h. Swabs were used to inoculate cefixime rhamnose sorbitol MacConkey (CR-SMAC) agar [12] which was then incubated for 18–20 h at 37 °C. Colonies that did not apparently ferment sorbitol and rhamnose were tested by latex agglutination and characterized as above.

Source of cattle

When available, the ear tag number and market lot number of each animal were recorded at the time of taking the rectal swab. The numbers were used to trace, whenever possible, the location of the farm of origin of the animal.

Carcass samples

These were collected from carcasses, when still available, of cattle shown presumptively (positive latex test) to be carrying *E. coli* O157 in their intestines, and if possible, from carcasses of adjacent rectal swab-negative animals. In each case about 100 g of meat trimmings were taken from the neck end of the carcass.

and a sterile absorbent gauze swab, with a flattened surface diameter of *c.* 5 cm, was used to sample an adjacent area of the carcass of about 0·5 m^2. Meat samples and surface swabs were stored at 4 °C prior to transport to the laboratory within 24 h. Samples were placed in 250 ml buffered peptone water (Oxoid - CM509) supplemented with vancomycin 8 mg/l, cefixime 0·05 mg/l, and cefsulodin 10 mg/l, respectively to inhibit the growth of Gram-positive organisms, aeromonads and *Proteus* spp.; this medium used for enrichment was derived from the results of studies of animal faeces and food samples (Sheffield PHL, unpublished data). After brief manual agitation broths were incubated at 37 °C for 6 h, subcultured onto CR-SMAC medium and processed as above.

Characterization of isolates

Identification

Isolates were confirmed as *E. coli* using biochemical tests and confirmed as serogroup O157 by agglutination to titre with antiserum (Laboratory for Microbiological Reagents, Central Public Health Laboratory, Colindale, London) to *E. coli* O157 [13].

Verocytotoxin production

Toxigenicity was determined by Vero cell culture assay [5]. Toxin type was determined by specific hybridization with DNA probes for the VT_1 and VT_2 genes. Using published sequence data [14], two primers 5′-CTTATCTGGATT-TAATGTGGC and 5′-AAGGAAACTCATCAGATGCC, corresponding to bases 810-830 and 1172-1153 respectively of the A cistron of the VT_1 gene, and two primers 5′-GAAGCCTTACGCTTCACGC and 5′-GTGACTTTCTGTTCAGAAACG, corresponding to bases 800-818 and 1169-1149 respectively of the A cistron of the VT_2 gene were constructed using phosphoramidite chemistry on an Applied Biosystems oligonucleotide synthesiser. Polymerase chain reaction was carried out in 50 μl volumes consisting of template DNA 10 ng, 10 pmol each primer, 10 mM-Tris-HCl pH 8·8, 2 mM-$MgCl_2$, 50 mM-KCl, nonidet P-40 (Sigma Chemical Co.) 0·05%, 200 μM each dNTP (Pharmacia Biotechnology) and 2·5 units of *Taq* DNA polymerase (Promega). Mixtures were processed for 35 cycles each of 94 °C for 1 min, 53 °C for 1 min and 72 °C for 2 min. Specific PCR products of 362 bp (VT_1) and 369 bp (VT_2) were purified by agarose gel electrophoresis, eluted from the gel, random-prime labelled with digoxigenin-11-dUTP, and used in colony hybridization reactions [15]. Specificity of the probes for the VT_1 and VT_2 genes had been confirmed previously by comparison with probes derived from cloned fragments of the VT_1 and VT_2 genes and known VT_1^+, VT_2^+ and VT^- strains were included as controls in each batch of tests.

Plasmid analysis

Plasmids were extracted by an alkaline detergent method [10] and were separated by submerged gel electrophoresis in Tris-acetate-EDTA buffer with agarose 1%, stained by ethidium bromide and visualized on an ultraviolet transilluminator. A control *E. coli* K-12 strain (NCTC 50192-39R861) harbouring plasmids of 148, 63·4, 36, and 6·9 kb was included with each batch of tests. For this control strain, log of plasmid size was plotted against distance migrated through

the agarose gel and approximate sizes of plasmids from strains of *E. coli* O157 were estimated from this graph.

Phage typing

All *E. coli* O157 were phage typed by the Laboratory for Enteric Pathogens, Central Public Health Laboratory, Colindale, London.

RESULTS

Human cases

During 1992 VT$^+$ *E. coli* O157 was isolated from 31 patients; 17 were female and 14 male. With the exception of two small clusters of cases in early April and in May–June, cases were evenly distributed throughout the period March–September.

Abattoir samples

Bovine rectal swabs

Organisms that did not apparently ferment sorbitol and rhamnose were isolated from 303 (14%) of 2103 bovine rectal swabs and included 84 (4%) *E. coli* O157 (Table 1).

Source of cattle

The farm of origin was traced for 34 cattle that had *E. coli* O157 in their rectal contents and comprised 17 farms in the vicinity of 13 towns or cities. Locations, with number of cattle in parentheses were: Barnsley, S. Yorks (3); Boroughbridge, N. Yorks (1); Budely, Worcs (1); Chesterfield, Derbs (12); Leominster, Worcs (2); Ludlow, Shrops (1); Newark, Notts (1); Retford, Notts (1); Ripon, N. Yorks (2); Rotherham, S. Yorks (4); Sheffield, S. Yorks (3); Thirsk, N. Yorks (2); Worcester, Worcs (1).

Carcass samples

E. coli O157 was isolated from 7 (30%) of 23 carcasses of cattle where the organism had been previously isolated from the rectal contents; in each case the carcass and rectal swab isolates were of the same phage type and plasmid profile. *E. coli* O157 was also isolated from 2 (8%) of 25 carcasses of cattle where the organism had not been previously isolated from the rectal contents; in each case the carcass isolate was of the same phage type and had the same plasmid profile as that isolated from the rectal contents of an animal adjacent on the production line. Of the 9 *E. coli* O157 isolated from carcasses, 6 were isolated from meat only, 2 from both meat and surface swabs, and 1 from surface swab only.

Characterization of isolates of E. coli *O157*

Human isolates

All isolates from human cases produced VT. Details of the phage types, toxin production and plasmid content are shown in Table 2. Sixteen (52%) of 31 VT$^+$ *E. coli* O157 were phage type 2; these included five strains isolated in early April from an outbreak of infection in a residential home for the elderly (Dr L. Jewes, personal communication) and three strains, which together with two strains of phage type 8, were isolated in May–June and may have been associated with

Table 1. *Sorbitol and rhamnose non-fermenting organisms isolated from the rectal faeces of 2103 cattle. Numbers in parentheses are percentages*

Organism	Number isolated
E. coli O157	84 (4)
Other *E. coli*	73 (3·5)
Hafnia alvei	29 (1·4)
Aeromonas hydrophila	19 (0·9)
Pseudomonas spp.	6 (0·3)
Other organisms*	92 (4·4)
Total	303 (14·5)

* This group includes organisms where genus was not identified.

Table 2. *Characteristics of 31 strains of* E. coli *O157 isolated from human patients during 1992*

Phage type	Vero cell assay for VT	DNA probe for VT_1	DNA probe for VT_2	Plasmids (kb)	Number of isolates
1	+	+	+	92, 6·6	3
				92, 63, 6·6	1
2	+	−	+	92	12
				92, 60	4
4	+	+	+	92, 63, 7	1
8	+	+	+	92, 6·2	3
14	+	+	+	92	1
				92, 44	1
49	+	−	+	92	5

consumption of beef originating from the abattoir. Variation in plasmid profile was observed within strains of phage types 1, 2 and 14. All isolates harboured the large *c.* 92 kb plasmid.

Bovine isolates

Seventy-eight (93%) of 84 isolates of *E. coli* O157 produced VT. The phage types, toxin production and plasmid profiles are summarized in Table 3. Twenty-four (31%) of 78 VT^+ *E. coli* O157 isolates were phage type 2, and 7 (9%) phage type 8. Considerable variation in plasmid profile was observed within strains of several phage types. All strains, other than non-toxigenic *E. coli* O157 of phage type RDNC, harboured the large *c.* 92 kb plasmid. One non-toxigenic *E. coli* O157 strain was phage type 32.

DISCUSSION

VT^+ *E. coli* O157 have been isolated from healthy cattle while attempting to find a source of human infections in Canada [16] and the USA [10, 17, 18]. Studies of sporadic cases, or less well delineated outbreaks in N. America have often suspected food of bovine origin as a vehicle of infection, but have been unable to confirm this epidemiologically or microbiologically [3, 19–21]. However, the seasonal incidence of infection, and epidemiological evidence from N. America, support the hypothesis that VT^+ *E. coli* O157 infection is a foodborne zoonosis.

The reservoir of VT^+ *E. coli* O157 and mode of transmission to man are less clear

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Table 3. *Characteristics of 84 strains of* E. coli *O157 isolated from bovine rectal faeces during 1992*

Phage types	Vero cell assay for VT	DNA probes for VT_1	DNA probes for VT_2	Plasmids (kb)	Number of isolates
1	+	+	+	92, 6·6	6
				92, 48, 6·6	1
				92, 33, 6·6	1
2	+	−	+	92	17
				92, 60	3
				92, 8·3	2
				92, 34, 8·3	1
				92, 34	1
4	+	+	+	92, 63, 7	3
				92	1
				92, 63, 40, 6·3	1
	+	−	+	92, 40	2
				92, 40, 11	1
8	+	+	+	92, 6·3	4
				92, 40	1
				92	1
	+	−	+	92	1
14	+	+	+	92	3
	+	−	+	92	1
	+	+	−	92	1
24	+	+	+	92, 83, 63, 7·4	1
31	+	−	+	92, 2	1
32	+	−	+	92, 2	3
				92, 7·4, 2	1
	−	−	−	92, 2	1
34	+	−	+	92	2
				92, 11	1
				92, 69, 11	1
				92, 52	1
				92, 69	1
43	+	+	+	92	1
49	+	−	+	92	3
				92, 7	2
				92, 69	1
				92, 4	1
RDNC*	+	−	+	92	4
				92, 4	2
	−	−	−	155, 148, 69, 52, 4, 2·5	5

* RDNC, reacted with the phage set but did not conform to a recognized phage type.

in the UK. Two outbreaks of infection have been reported: possible sources of infection were the handling, though not consumption of unwashed potatoes [22], and sliced turkey roll [23]; neither source was confirmed microbiologically.

In May–June 1992 there was a suspicion, though not epidemiologically proven, that 5 cases of VT^+ *E. coli* O157 infection in the Sheffield area, 3 of phage type 2 and 2 of phage type 8, may have been associated with consumption of beef originating from a South Yorkshire abattoir. *E. coli* O157 was subsequently isolated from the rectal contents of 84 (4%) of 2103 cattle at the abattoir, and thus

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confirmed that they may be a source of this organism. Seventy-eight (93%) of the 84 isolates were VT$^+$ and were of the same phage type, toxin type and plasmid profile as strains implicated in human disease in the UK; 24 (31%) of 78 VT$^+$ *E. coli* O157 were phage type 2, the most common type implicated in human infections in the UK, and 7 (9%) were phage type 8. Cattle from which *E. coli* O157 were isolated were from a diverse geographical origin, suggesting that carriage of the organism by cattle is widespread.

For the next stage of the study we tested the hypothesis that VT$^+$ *E. coli* O157 may be transmitted from cattle to man by contamination of carcass meat during the slaughter process. Carcasses were sampled by meat excision and surface swabbing with absorbent gauze, techniques that have previously given good recovery of other organisms from carcasses [24]. According to Ingram and Roberts [24], although the process may be applicable to only small areas of the carcass, excising thin sections of surface meat from the carcass is the best method for monitoring the microbiological quality of red meat carcasses. *E. coli* O157 was isolated from 30% of carcasses of rectal swab-positive cattle and from 8% of carcasses of rectal swab-negative cattle; the latter may be due to cross-contamination of carcasses within the abattoir, but it is also possible that the animals were carrying *E. coli* O157 in numbers sufficiently low to be undetectable in the faeces on the rectal swab. A strain of VT$^+$ *E. coli* O157 phage type 1, harbouring plasmids of 92 and 6·6 kb, was isolated from a bovine rectal swab and carcass during early August and was followed within the next 10 days by the occurrence of three human infections by strains with the same characteristics; though speculative, this may provide further evidence of transmission of the organism from cattle to man.

Of the 9 *E. coli* O157 isolated from beef carcasses in our study, 8 were isolated from meat and 3 from surface swabs. This confirms that excised meat is a better means of sampling the carcass than surface swabbing. This could be explained by penetration of the tissue by bacteria during the slaughter process: Anderson and colleagues [25] used a dye model to show that bacteria may not be removed from the carcass during washing, and may be driven deeper into the beef tissue by the washing process. Microbiological support for this theory is provided by Davidson and colleagues [26] who showed that bacterial contamination can occur to a depth of 6 mm in the tissue of a carcass.

This is the first description of isolation of *E. coli* O157 from bovine carcass meat, and provides further evidence that beef and beef products may be a vehicle of transmission of this organism from cattle to man. Although the study has shown that cattle might be a source of VT$^+$ *E. coli* O157, other possible sources of infection have been little studied, and cannot be overlooked. Doyle and Schoeni [27] isolated VT$^+$ *E. coli* O157, apparently typical of those causing human infections, from a variety of meats, but this has not been reported by others, and meats other than beef have not often been implicated as vehicles of infection for man. Non-meat food may also be a source of VT$^+$ *E. coli* since illness associated with the pathogen has been recorded in strict vegetarians [11].

VTEC causing human infections most commonly belong to serogroup O157 and further subdivision of this group is necessary to elucidate the epidemiology of VT$^+$ *E. coli* infections. Both phage typing [28] and plasmid analysis [11, 29] of strains

of VT$^+$ *E. coli* O157 have proved useful. In the present study we found variation in plasmid profile and toxin type within strains from several different phage types. phage type 4 for example containing strains of two different toxin types and five different plasmid profiles. This indicates that more useful epidemiological information may be obtained from a combination of phage type, plasmid profile and toxin type, than may be obtained from any characteristic alone, and further development of typing methods for VT$^+$ *E. coli* O157 is required.

Our study was not conducted for a sufficient length of time to determine if carriage of VT$^+$ *E. coli* O157 by cattle is seasonal, and whether or not that season may correspond to that observed in human infections. A long-term abattoir survey, and a study of various associated foodstuffs may help to elucidate the epidemiology of VT$^+$ *E. coli* O157 infection.

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REFERENCES

1. Karmali MA. Infection by verocytotoxin-producing *Escherichia coli*. Clin Microbiol Rev 1989; **2**: 15–38.
2. Riley LW, Remis RS, Helgerson SD et al. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. N Engl J Med 1983; **308**: 681–5.
3. Pai CH, Gordon R, Sims HV, Bryan LE. Sporadic cases of hemorrhagic colitis associated with *Escherichia coli* O157:H7. Ann Intern Med 1984; **101**: 738–42.
4. Smith HR, Rowe B, Gross RJ, Fry NK, Scotland SM. Haemorrhagic colitis and verocytotoxin-producing *Escherichia coli* in England and Wales. Lancet 1987; **i**: 1062–5.
5. Chapman PA, Wright DJ, Norman P. Verotoxin-producing *Escherichia coli* infections in Sheffield: cattle as a possible source. Epidemiol Infect 1989; **102**: 439–45.
6. Karmali MA, Steele BT, Petric M, Lim C. Sporadic cases of haemolytic-uraemic syndrome associated with faecal cytotoxin and cytotoxin-producing *Escherichia coli* in stools. Lancet 1983; **i**: 619–20.
7. Karmali MA, Petric M, Lim C, Fleming PC, Arbus GS, Lior H. The association between idiopathic hemolytic-uremic syndrome and infection by verotoxin-producing *Escherichia coli*. J Infect Dis 1985; **151**: 775–82.
8. Scotland SM, Rowe B, Smith HR, Willshaw GA, Gross RJ. Vero cytotoxin-producing strains of *Escherichia coli* from children with haemolytic-uraemic syndrome and their detection by specific DNA probes. J Med Microbiol 1988; **25**: 237–43.
9. Report. Third Annual Report (1988–1989) of the British Paediatric Surveillance Unit. 5 St Andrews Place, London. BPSU 1989.
10. Martin ML, Shipman LD, Wells JG, et al. Isolation of *Escherichia coli* O157:H7 from dairy cattle associated with two cases of haemolytic-uraemic syndrome. Lancet 1986; **ii**: 1043.
11. Chapman PA, Jewes L, Siddons CA, Norman P, George SL. Verotoxin-producing *Escherichia coli* infections in Sheffield during 1989. PHLS Microbiol Digest 1990; **7**: 163–6.
12. Chapman PA, Siddons CA, Zadik PM, Jewes L. An improved selective medium for the isolation of *Escherichia coli* O157. J Med Microbiol 1991; **35**: 107–10.
13. Chapman PA. Evaluation of a commercial latex slide test for the identification of *Escherichia coli* O157. J Clin Pathol 1989; **42**: 1109–10.
14. Jackson MP, Neill RJ, O'Brien AD, Holmes RK, Newland JW. Nucleotide sequence analysis and comparison of the structural genes for Shiga-like toxin I and Shiga-like toxin II encoded by bacteriophages from *Escherichia coli* 933. FEMS Microbiol Lett 1987; **44**: 109–14.

15. Chapman PA, Daly CM. An evaluation of a non-radioactive trivalent DA probe (LTh, ST1a, ST1b) for detecting enterotoxigenic *Escherichia coli*. J Clin Pathol 1993; **46**: 309–12.
16. Borczyk AA, Karmali MA, Lior H, Duncan LM. Bovine reservoir for verotoxin-producing *Escherichia coli* O157:H7. Lancet 1987; **i**: 98.
17. Ostroff SM, Griffin PM, Tauxe RV, et al. A statewide outbreak of *Escherichia coli* O157 infections in Washington state. Am J Epidemiol 1990; **132**: 239–47.
18. Wells JG, Shipman LD, Greene KD, et al. Isolation of *Escherichia coli* serotype O157:H7 and other shiga-like toxin-producing *E. coli* from dairy cattle. J Clin Microbiol 1991; **29**: 985–9.
19. Ryan CA, Tauxe RV, Hosek GW, et al. *Escherichia coli* O157:H7 diarrhea in a nursing home: clinical, epidemiological, and pathological findings. J Infect Dis 1986; **154**: 631–8.
20. MacDonald KL, O'Leary MJ, Cohen ML, et al. *Escherichia coli* O157:H7, an emerging gastrointestinal pathogen – results of a one-year, prospective, population-based study. JAMA 1988; **259**: 3567–70.
21. Bryant HE, Athar MA, Pai CH. Risk factors for *Escherichia coli* O157:H7 infection in an urban community. J Infect Dis 1989; **160**: 858–64.
22. Morgan CM, Newman C, Palmer SR, et al. First recognised community outbreak of haemorrhagic colitis due to verotoxin-producing *Escherichia coli* O157:H7 in the UK. Epidemiol Infect 1988; **101**: 83–91.
23. Salmon RL, Farrell ID, Hutchison JGP, et al. A christening party outbreak of haemorrhagic colitis and haemolytic-uraemic syndrome associated with *Escherichia coli* O157:H7. Epidemiol Infect 1989; **103**: 249–54.
24. Ingram PM, Roberts TA. The microbiology of the red meat carcass and the slaughterhouse. Roy Soc Health J 1976; **96**: 270–6.
25. Anderson ME, Marshall RT, Dickson JS. Estimating depths of bacterial penetration into post-rigor carcass tissue during washing. J Food Safety 1992; **12**: 191–8.
26. Davidson CM, Taylor M, Zellerman GG. Method for sampling beef carcasses. Appl Environm Microbiol 1978; **35**: 811–2.
27. Doyle MP, Schoeni JL. Isolation of *Escherichia coli* O157:H7 from retail fresh meats and poultry. Appl Environ Microbiol 1987; **53**: 2394–6.
28. Ahmed R, Bopp C, Borczyk A, Kasatiya S. Phage typing scheme for *Escherichia coli* O157:H7. J Infect Dis 1987; **155**: 806–9.
29. Ostroff SM, Tarr PI, Neill MA, Lewis JH, Hargrett-Bean N, Kobayashi JM. Toxin genotypes and plasmid profiles as determinants of systemic sequelae in *Escherichia coli* O157:H7 infections. J Infect Dis 1989; **160**: 994–8.