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# A one year study of *Escherichia coli* O157 in raw beef and lamb products

P. A. CHAPMAN*, C. A. SIDDONS, A. T. CERDAN MALO AND M. A. HARKIN

*Public Health Laboratory, Herries Road, Sheffield S5 7BQ*

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## SUMMARY

Between April 1996 and March 1997 we examined 5093 samples of raw beef and lamb products for the presence of *E. coli* O157. Samples were purchased from 81 small butchers' shops in south Yorkshire. In March 1997 we also examined five samples of dried mint for the presence of *E. coli* O157.

Strains of *E. coli* O157 were isolated by enrichment culture in modified buffered peptone water followed by immunomagnetic separation and culture of magnetic beads onto cefixime tellurite sorbitol MacConkey agar. Strains were characterized by phage typing, toxin genotyping and plasmid analysis.

Strains of *E. coli* O157 were isolated from 72 (1·4%) of 5093 samples; it was isolated from 36 (1·1%) of 3216 samples of beef products and from 29 (2·9%) samples of lamb products. The highest prevalence was found in lamb sausages and lamb burgers where *E. coli* O157 was isolated from 3 (4·1%) of 73 and 18 (3·7%) of 484 samples respectively. Strains of *E. coli* O157 were isolated most frequently during early summer. Strains of *E. coli* O157 were also isolated from 2 of 5 samples of dried mint although we did not determine how the mint had become contaminated.

All isolates of *E. coli* O157 were Verocytotoxin-producing as determined by both Vero cell assay and DNA hybridization for the genes encoding Verocytotoxin and all were positive for the *eaeA* gene. A combination of phage typing, toxin genotyping and plasmid profile subdivided the 72 strains of *E. coli* isolated into 20 different subtypes, of which 18 were indistinguishable from strains isolated previously from cattle and sheep; of these 18 strains, 8 were indistinguishable from strains isolated from human cases of infection during the study period.

## INTRODUCTION

Verocytotoxin-producing ($VT^+$) *E. coli* (VTEC) cause haemorrhagic colitis (HC), the haemolytic-uraemic syndrome (HUS) and occasionally mild non-bloody diarrhoea in man, although some infections may be asymptomatic. In the United Kingdom, $VT^+$ *E. coli* O157, the most common serogroup associated with illness in man, has been isolated from cattle[1–4] and beef, beef products, milk and milk products have been identified as sources of human infection [2, 3, 5, 6]. Since the first isolation of *E. coli* O157 from food in England and Wales [3], there have been several reports of isolation of the organism from food, either associated with cases of infection, or sampled during small surveys. At present there have been no reported large surveys of the prevalence of *E. coli* O157 in raw meat products.

The aims of this study were (i) to examine samples of raw beef and lamb products, purchased from small butchers' shops in south Yorkshire, for the presence of *E. coli* O157, (ii) to compare any strains of the organism isolated with those previously isolated from

* Author for correspondence.

the rectal contents of cattle and sheep at slaughter [7] and with those isolated from human infection during the course of the survey, and (iii) to attempt to identify any differences in practices between butchers' shops from which samples containing *E. coli* O157 had been purchased and those where purchased products did not contain *E. coli* O157.

## MATERIALS AND METHODS

### Sample collection

Between 400 and 430 samples of raw processed meat products were purchased each month from April 1996 to March 1997 inclusive. All were purchased from small butchers' shops within South Yorkshire, all of which obtained meat habitually, although not exclusively, from an abattoir used for a previous study of the prevalence of *E. coli* O157 in cattle and sheep [7]. Meat samples were stored at 2–8 °C between collection and delivery to Sheffield Public Health Laboratory, which was within 24 h. During March 1997, 20 samples of mixed spices and 5 samples of dried mint were collected from 5 of the butchers' shops.

### Isolation of *E. coli* O157

Strains of *E. coli* O157 were isolated by an immunomagnetic separation technique [8, 9] and culture of magnetic beads on to cefixime tellurite sorbitol MacConkey agar (CT-SMAC) [10]. Twenty-five gram portions of meat samples were placed in 225 ml of buffered peptone water (Oxoid, CM509) supplemented with vancomycin 8 mg/l, cefixime 0·05 mg/l, and cefsulodin 10 mg/l (BPW-VCC) [2] and blended in a stomacher (Colworth 400) for 30 s at the medium speed setting. Samples of herb and spice mixture or dried mint were treated similarly but 10 g of sample were added to 1 l of medium. Any samples remaining were stored at −20 °C for future use. Suspensions were incubated at 37 °C for 6 h and 1 ml of broth was added to 20 $\mu$l of magnetic beads coated with an antibody prepared to the lipopolysaccharide of *E. coli* O157 (Dynabeads anti-*E. coli* O157, Dynal, Oslo) in a 1·5 ml microcentrifuge tube. The beads were suspended, mixed, separated in a magnetic particle concentrator (MPC-10, Dynal, Oslo) and washed as described previously [9]. After the final wash and separation the beads were resuspended in approx. 25 $\mu$l of nutrient broth, inoculated on to CT-SMAC medium and incubated overnight at 37 °C. Colonies not fermenting sorbitol from CT-SMAC were tested for agglutination with a latex test kit (Oxoid, DR622) for detecting *E. coli* O157. Isolates that gave positive results were confirmed as *E. coli* by biochemical tests (Crystal ID, Becton Dickinson) and a serogroup O157 by agglutination to titre with a rabbit antiserum prepared to *E. coli* O157 (Laboratory for Microbiological Reagents, Central Public Health Laboratory, Colindale, London) [1].

### Characterization of isolates

#### *Verocytotoxin production*

The ability of strains to express Verocytotoxin was determined by Vero cell culture assay [1] and toxin type by specific hybridization with DNA probes for the $VT_1$ and $VT_2$ genes. Presence of the *eaeA* gene was also determined by DNA hybridization. From published sequence data [11, 12] DNA probes specific for the A cistrons of the $VT_1$ and $VT_2$ genes, and for the *eaeA* gene, were prepared and labelled with digoxygenin-11-dUTP by the polymerase chain reaction and used in colony hybridization reactions [2, 13]. Known $VT_1^+$, $VT_2^+$, $VT^-$, $eaeA^+$ and $eaeA^-$ strains were included as controls in each batch of tests.

#### *Plasmid analysis*

Plasmids were extracted by an alkaline detergent method [14], separated by submerged gel electrophoresis in Tris-acetate-EDTA buffer with agarose 1%, stained by ethidium bromide and visualized on an ultraviolet transilluminator. A strain of *E. coli* K-12 (NCTC 50192-39R861) carrying plasmids of 148, 63·4, 36, and 6·9 kb was included with each batch of tests so the size (kb) of the plasmid could be estimated.

#### *Phage typing*

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

#### *Determination of meat species*

Where sufficient sample remained, the meat species was determined for beefburgers and lamb burgers from which *E. coli* O157 had previously been isolated and from at least an equal number where *E. coli* O157 had not been detected. This was determined by enzyme immunoassays for bovine and sheep serum proteins (Bio-Kit Meat Species Identification, Cortecs Diagnostics) and for bovine and sheep collagen (Bio-

Table 1. *Samples of beef and lamb products from 81 retail outlets examined for the presence of* E. coli *O157*

| Retail outlet no. | No. of samples purchased | No. of samples from which *E. coli* O157 was isolated (%) |
|---|---|---|
| T113 | 450 | 11 (2·5) |
| W121 | 439 | 13 (3·0) |
| J67 | 382 | 4 (1·0) |
| K78 | 336 | 1 (0·3) |
| P91 | 281 | 6 (2·1) |
| G38 | 272 | 7 (2·6) |
| B2 | 219 | 4 (1·8) |
| J62 | 192 | 4 (1·1) |
| A1 | 186 | 1 (0·5) |
| B10 | 178 | 4 (2·3) |
| P94 | 152 | 3 (2·0) |
| S106 | 146 | 1 (0·7) |
| J63 | 134 | 0 |
| H52 | 102 | 2 (2·0) |
| J64 | 101 | 0 |
| W125 | 95 | 0 |
| C15 | 94 | 0 |
| W114 | 93 | 0 |
| T112 | 86 | 0 |
| B11 | 79 | 0 |
| S108 | 58 | 0 |
| M89 | 57 | 1 (1·8) |
| S102 | 44 | 1 (2·3) |
| G44 | 34 | 1 (2·9) |
| G43 | 29 | 1 (3·4) |
| R99 | 27 | 0 |
| M85 | 24 | 0 |
| C17 | 22 | 0 |
| B4 | 20 | 2 (10·0) |
| All others | 761 | 7 (0·9) |
| Total | 5093 | 72 (1·4) |

Table 2. *Detection of* E. coli *O157 in 5093 meat samples purchased between 1 April 1996 and 31 March 1997*

| Food type | No. examined | No. positive for *E. coli* O157 | Percentage positive for *E. coli* O157 |
|---|---|---|---|
| Beef burger | 1120 | 13 | 1·2 |
| Beef mince | 2075 | 23 | 1·1 |
| Beef sausage | 21 | 0 | 0 |
| Beef, all products | 3216 | 36 | 1·1 |
| Lamb burger | 484 | 18 | 3·7 |
| Lamb mince | 463 | 8 | 1·7 |
| Lamb sausage | 73 | 3 | 4·1 |
| Lamb, all products | 1020 | 29 | 2·9 |
| Mixed meat burger | 80 | 1 | 1·3 |
| Mixed meat mince | 526 | 5 | 1·0 |
| Mixed meat sausage | 251 | 1 | 0·4 |
| Mixed meat, all products | 857 | 7 | 0·8 |
| Total | 5093 | 72 | 1·4 |

Kit Cooked Meat Species Identification, Cortecs Diagnostics). Saline extracts were prepared for determination of species serum proteins. For determination of species collagen, samples were blended in saline, heated to 95 °C for 15 min and filtered. Both were performed according to the manufacturer's instructions. For both assays, portions of the extract or filtrate were added to plastic microwells which had been pre-coated with species-specific anti-serum protein or anti-collagen antibodies. With washing steps between, wells were then sequentially treated with a biotinylated secondary antibody, a streptavidin/horseradish peroxidase (HRP) conjugate and a chromogenic substrate for HRP. Positive and negative control samples were provided by the manufacturer who indicated that an optical density reading of greater than 2·5 times that of the negative control was a positive result.

*Questionnaires*

Premises from which burgers containing *E. coli* O157 had been obtained and an equal number of premises that had supplied only burgers without *E. coli* O157 were asked by the sampling officer, during early March, to complete a questionnaire about burger production. The questionnaire aimed to determine: (i) the type of meat used to prepare burgers, (ii) whether mechanically reclaimed meat, or any other specially purchased meat, was used, (iii) details of cleaning regimes for equipment, especially between different species of meat, (iv) whether any non-meat additives were used in the burgers, e.g. cereals, herbs, spices, etc., (v) the storage temperature of the prepared burgers, and (vi) the maximum time the burgers were stored prior to sale.

## RESULTS

Over the 1-year period, 5093 meat samples were purchased from a total of 81 different butchers' shops. Fewer than 20 samples were purchased from 52 of the shops (761 samples in total) and 4332 (85%) of the samples were purchased from the remaining 29 shops (Table 1).

Strains of *E. coli* O157 were isolated from 72 (1·4%) of 5093 samples; it was isolated from 36 (1·1%) of 3216 samples of beef products and from 29 (2·9%) samples of lamb products (Table 2). The highest prevalence was found in lamb sausages and lamb burgers where *E. coli* O157 was isolated from 3 (4·1%) of 73 and 18 (3·7%) of 484 samples respectively

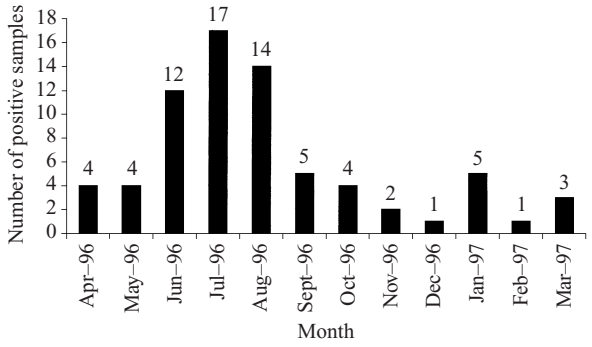


**Fig. 1.** Seasonal prevalence of *E. coli* $O_{15}$ in raw beef and lamb products.

(Table 2). *E. coli* O157 was isolated most frequently during early summer, the prevalence varying from 0·2% in December and February to 4% in July (Fig. 1). Strains of *E. coli* O157 were also isolated from 2 of 5 samples of dried mint but not from any of 20 samples of spice mixture. All isolates of *E. coli* O157 were $VT^+$ as determined by both Vero cell assay and DNA hybridization for VT genes and all carried the *eaeA* gene. A combination of phage type, plasmid profile and toxin genotype divided the isolates into 20 different subtypes (Table 3).

Sufficient sample was available to determine the meat species of 13 lamb burgers and 11 beef burgers that were positive for *E. coli* O157, and of 20 lamb burgers and 20 beef burgers that were negative for the organism. Results are shown in Table 4.

Questionnaires were completed by 14 premises from which *E. coli* O157 had been isolated from burgers and from 10 premises from which we had not isolated *E. coli* O157 from burgers. There were few apparent differences between the two groups: (i) all used meat trimmings from the fore, hind and neck regions of the carcases of both cattle and sheep for burger production, (ii) none purchased any meat specially for burger production, (iii) none cleaned preparation equipment between different species of meat and one butcher (from whom we had purchased only *E. coli* O157-negative burgers) cleaned the equipment once or twice weekly whereas the remainder cleaned the equipment at the end of every day, (iv) all added cereals and mixed spices to both beef and lamb burgers and 9 of 14 butchers from whom we had isolated *E. coli* O157 from burgers and 5 of 11 butchers from whom we had not isolated *E. coli* O157 from burgers added dried mint to lamb burgers (either incorporated within the lamb burger or as a surface coating) but not to beef burgers, (v) all stored burgers at 4–8 °C prior to sale and (vi) one

Table 3. *Characteristics of 72 strains of* E. coli *O157 isolated from 5093 meat samples*

| Phage type | Plasmid profile (sizes in kb) | Vero cell assay | Hybridization with DNA probe for | | | No. of Strains |
|---|---|---|---|---|---|---|
| | | | $VT_1$ | $VT_2$ | *eaeA* | |
| 1 | 92, 6·9 | + | + | + | + | 2 |
| 2 | 92 | + | − | + | + | 2 |
| | 92, 60 | + | − | + | + | 3 |
| | 92, 2 | + | − | + | + | 1 |
| 4 | 92 | + | − | + | + | 17 |
| | 92, 32 | + | − | + | + | 6 |
| | 92, 60 | + | − | + | + | 1 |
| | 92, 63, 32, 6·9 | + | + | + | + | 1 |
| 8 | 92 | + | + | + | + | 23 |
| | 92, 60 | + | + | + | + | 1 |
| 14 | 92 | + | − | + | + | 1 |
| | 92, 6·9 | + | − | + | + | 1 |
| 21 | 92, 2 | + | − | + | + | 1 |
| | 92, 63, 2 | + | − | + | + | 1 |
| 32 | 92, 2 | + | − | + | + | 2 |
| 54 | 92 | + | − | + | + | 1 |
| RDNC | 92 | + | − | + | + | 4 |
| | 92, 2 | + | − | + | + | 2 |
| | 92, 32, 2 | + | − | + | + | 1 |
| | 92, 60 | + | − | + | + | 1 |
| Total | | | | | | 72 |

Table 4. *Meat species present in beef burgers and lamb burgers with and without* E. coli *O157*

| Burger type | *E. coli* O157 isolated | No. | Enzyme immunoassay for | | | |
|---|---|---|---|---|---|---|
| | | | Sheep | | Bovine | |
| | | | Serum proteins | Collagen | Serum proteins | Collagen |
| Lamb | + | 13 | 13 | 13 | 1 | 1 |
| | − | 20 | 18 | 18 | 6 | 6 |
| Beef | + | 11 | 3 | 3 | 11 | 11 |
| | − | 20 | 0 | 0 | 20 | 20 |

butcher (from whom we had purchased only *E. coli* O157-negative burgers) stored burgers for up to 5 days prior to sale whereas the remainder stored them for a maximum of 1 full day after the day of preparation.

## DISCUSSION

Although beef products have been widely implicated as vehicles of *E. coli* O157 infection, we know very little about the prevalence of the organism in retail raw meat products in the United Kingdom. Results in surveys in Europe and N. America have generally shown a very low prevalence of the organism in beef samples. Tarr and colleagues [15] examined 1400 ground beef samples in Seattle but failed to find *E. coli* O157; however, samples were collected from only three different retail outlets, only 10 g of each sample was tested and an insensitive method of enrichment and subculture to plain sorbitol MacConkey agar was used. Lindqvist and colleagues [16] examined 543 samples of minced beef in Sweden but failed to find *E. coli* O157, despite using sensitive methods such as IMS and PCR; however, all their samples were purchased in the winter, a time of low prevalence of the organism in the animal population [7, 17]. Other small surveys of minced beef in Belgium [18], The Netherlands [19] and the United Kingdom [20] have found *E. coli* O157 in no more than 0·3% of samples tested.

In the present study we found *E. coli* O157 in 1·4% of all beef and lamb samples tested. This is higher than the prevalence reported in the above studies. Several factors may have influenced this. Firstly, over the period of a full year we examined a large number of samples (5093) collected from a wide range of retail outlets (81). Secondly, we used the IMS technique throughout the study; we have previously shown this to be 10- to 100-fold more sensitive than enrichment and subculture for the isolation of *E. coli* O157 from minced beef [9]. Thirdly, the samples were purchased from small butchers' shops within South Yorkshire, all of which obtained meat habitually, although not exclusively, from an abattoir where we had previously isolated *E. coli* O157 from the cattle and sheep.

Most of the *E. coli* O157 isolates were obtained from the samples collected in early summer, the prevalence varying from 0·2% in December and February to 4% in July (Fig. 1). This seasonal distribution is very similar to the seasonal prevalence observed in cattle [7, 17], sheep [7] and humans [1, 2] in the Sheffield area. All the isolates were biochemically typical, Verocytotoxigenic, harboured a 92 kb plasmid and were *eaeA* gene-positive and were therefore typical of strains causing infection in man. A combination of phage typing, toxin genotyping and plasmid profile subdivided the 72 strains of *E. coli* isolated into 20 different subtypes, of which 18 were indistinguishable from strains isolated previously from cattle and sheep [7]; of these 18 subtypes, 8 were indistinguishable from strains isolated from human cases of infection during the study period.

Although in an abbattoir study we isolated *E. coli* O157 from 15·7% of cattle and 2·2% of sheep, in the present study we isolated the organism more frequently from lamb products (2·9%) than from beef products (1·1%). The products most frequently contaminated were lamb sausages (4·1%) and lamb burgers (3·7%). On 16 occasions where beef mince, beef burger, lamb mince and lamb burger were purchased from the same retail outlet, *E. coli* O157 was isolated only from the lamb burger. This is difficult to explain. It is possible that the lamb burgers may have become contaminated with beef during their manufacture. However, immunoassays for species-specific serum proteins and collagen did not support this hypothesis. Indeed, lamb burgers which were positive for *E. coli* O157 contained beef less frequently than did lamb burgers which were negative for the organism. Also in contradiction to the hypothesis, beef burgers from which we had isolated *E. coli* O157 were more frequently contaminated with lamb than those beef burgers from which the organism was not isolated. The questionnaires, although showing very few differences between the ways in which the two types of burger were produced, did indicate that dried mint was frequently added to lamb burgers but was not added to beef burgers. We subsequently isolated *E. coli* O157 from 2 of 5 samples of dried mint. It is possible that the mint could have been grown in manured ground and contaminated in this way; vegetable crops contaminated with manure have been documented as vehicles of transmission of *E. coli* O157 infection [21–23] and other bacterial pathogens such as salmonella have been transmitted by contaminated herbs and spices [24]. However, it is also possible that the mint could have been contaminated with *E. coli* O157 in the retail premises. Both possibilities are being investigated further. If the mint was used only as a surface coating for the lamb burgers, then it is likely that any *E. coli* O157 contaminating it would be killed rapidly on cooking. This may explain why there have been no reported cases of *E. coli* O157 infection associated with consumption of lamb burgers, despite the relatively high prevalence of the organism in this product.

The study confirms that both beef and lamb products are potential sources of *E. coli* O157 infection and re-affirms the need for thorough cooking of these products. Although there have been no reported cases of the infection being acquired from lamb products, they should not be overlooked as possible vehicles of infection.

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