A survey of the prevalence of *Escherichia coli* O157 in raw meats, raw cow’s milk and raw-milk cheeses in south-east Scotland

John E. Coia*, Yvonne Johnston, Nicholas J. Steers, Mary F. Hanson

*Department of Clinical Microbiology, Western General Hospitals NHS Trust, Crewe Road, Edinburgh EH4 2XU, UK*

**Abstract**

2429 samples of foodstuffs were examined for the presence of verocytotoxigenic *Escherichia coli* O157 (VTEC O157) by means of immunomagnetic separation (IMS) over a 2-year period commencing April 1997. Specimens comprised 1190 raw meats, 500 raw milks and 739 raw-milk cheeses. The meat and cheese samples were purchased from retail premises in south-east Scotland; raw milk samples were obtained directly from farms. In addition, total *E. coli* counts were performed on milk and cheese samples, and the pH of cheese specimens measured. The water activity (*A*<sub>w</sub>) was also measured for a representative sample of each cheese type, and for all of the samples with high levels of *E. coli*.

VTEC O157 was isolated from two samples of beef burger, both manufactured on the premises of the same butchers shop. Control studies with artificially inoculated foodstuffs demonstrated a sensitivity of detection of < 5 organisms 25 g<sup>-1</sup>. These findings, which contrast with the results of similar studies elsewhere in the UK, suggest that other sources of infection may be important in explaining the high rates of infection with this organism in south-east Scotland. © 2001 Elsevier Science B.V. All rights reserved.

**Keywords:** *Escherichia coli* O157; *Escherichia coli* infections; Epidemiology; Microbiology; Gastrointestinal infections; Food poisoning; Food surveillance; Immunomagnetic separation

**1. Introduction**

In the last decade, infections caused by verocytotoxigenic *E. coli* O157 (VTEC O157) and other VTEC have emerged as a major public health concern in North America and in Europe. This anxiety stems both from the increased numbers of infections which have been reported, and the wide spectrum of illness which may ensue, ranging from mild diarrhoea through to haemorrhagic colitis, haemolytic uraemic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) (Coia, 1998).

In Britain, the highest recorded rates of *E. coli* O157 infection in recent years have been in Scotland, where 506 cases (9.8/100,000) were recorded in 1996 (Reilly, 1997). Although meat and dairy products have been implicated in a number of studies, the relative paucity of isolations from food has meant that much remains unclear with respect to the
epidemiology of the infection (Advisory Committee on the Microbiological Safety of Food (ACMSF, 1995)).

A number of studies in Scotland have emphasised and developed the animal link, particularly with respect to bovines, but until recently the lack of suitable methods of sufficient sensitivity for the routine detection of the organism in foodstuffs have frustrated attempts to further define these associations (Sharp et al., 1995; Coia, 1998).

The technique of immunomagnetic separation (IMS) has revolutionised our ability to isolate the organism, with an increase in sensitivity of between 10- and 100-fold (Wright et al., 1994). In our laboratory, VTEC O157 was isolated from environmental samples and bottling apparatus at a dairy epidemiologically implicated in a large outbreak in West Lothian and from a bulk milk sample from a feeder dairy farm by means of this technique (Upton and Coia, 1994).

More recently, this technique permitted the isolation of VTEC O157 from a number of foodstuffs epidemiologically linked with the large, central Scotland outbreak (The Pennington Group, 1997). The technique has also been used to demonstrate the presence of the organism from carcass rectal swabs at slaughter (Chapman et al., 1997), and at least one survey has shown a small but significant level of contamination of raw retail meats (Chapman et al., 1996).

In the period 1990–1994, south-east Scotland had a rate of *E. coli* O157 infection of 5.6 per 100,000 (data supplied by the Scottish Centre for Infection and Environmental Health (SCIEH)) which was amongst the highest in the UK. The majority of these cases were not outbreak-associated (Cowden, 1997) and, with the exception of the West Lothian episode, the source of infection was not clearly identified.

Given these high rates of infection, the epidemiological data suggesting a link with bovine products (Coia, 1998), and the availability of the novel IMS methodology for the sensitive detection of the organism in foodstuffs, we believed it would be useful to test retail meats, unpasteurised milks and unpasteurised milk products from this area for the presence of VTEC O157 in order to establish if there were significant levels of contamination. This data would allow us to more accurately define the sources of this important infection, and subsequently to devise more effective intervention strategies.

2. Materials and methods

2.1. Sample collection

The duration of the study was 24 months, commencing April 1997. Retail samples of meats and meat products and raw-milk cheeses were purchased anonymously by local authority environmental health officers (EHOs) from butchers shops and other retail premises in south-east Scotland (City of Edinburgh, West Lothian, Midlothian, East Lothian and Scottish Borders local authority areas). Beef and lamb products were sampled in the ratio 80:20. Where samples of meat were taken from supermarket premises, care was taken to ensure that as far as possible, samples were of Scottish origin. Given the limited range of Scottish raw-milk cheeses available for retail sale, samples originating from other countries on sale within the area were also sampled. Raw milks were collected from farms in the area by local authority Milk Officers. As far as was possible, sampling was performed to reflect the relative output of individual farms, based on available data from the Scottish Office Agriculture Environment and Fisheries Department (SOAEFD).

After purchase, the samples in their original packaging were placed directly into cool boxes and transported to the laboratory within 2 h on the day of collection. Specimens were stored at 4°C in the laboratory prior to processing on the day following receipt.

2.2. Detection of *E. coli* O157

Detection of VTEC O157 in raw meat samples by immunomagnetic separation (IMS) was performed as has been described elsewhere (Wright et al., 1994). Raw meat samples (25 g) were inoculated into 225 ml of buffered peptone water supplemented with 8 mg l⁻¹ vancomycin, 0.05 mg l⁻¹ cefixime and 10 mg l⁻¹ cefsulodin (BPW-VCC) in a stomacher bag, and processed in a mechanical stomacher prior to incubation for 6 h at 37°C in air. A 1-ml aliquot of the broth and 20 μl of Dynabeads (Dynal, Oslo)
were added to an Eppendorf tube, and incubated with continuous mixing for 30 min at room temperature. A magnetic field was applied to the side of the tube, and the beads with any adherent VTEC O157 were drawn to the side of the tube. The culture supernatant was removed by aspiration, and the beads suspended in 1 ml of phosphate-buffered saline (PBS). This washing process was repeated twice. After the final wash step, the beads were resuspended in 100μl of PBS, following which the concentrated washed beads were inoculated onto sorbitol MacConkey agar supplemented with cefixime 0.05 mg l⁻¹ and tellurite 2.5 mg l⁻¹ (CT-SMAC). CT-SMAC plates were incubated for 18 h at 37°C in air following which the plates were examined for non-sorbitol fermenting (NSF) colonies.

NSF colonies were tested for agglutination with a commercial O157 latex test (Oxoid, Basingstoke), prior to biochemical identification using the API 20E system (Biomerieux, Basingstoke). Presumptive VTEC O157 isolates were forwarded to the E. coli O157 Reference Laboratory in Aberdeen for confirmation of identity and typing. Frequent control experiments with 25-g samples of meat, artificially inoculated with dilutions of broth cultures of clinical VTEC O157 isolates to yield final concentrations of 1–2 organisms g⁻¹, consistently demonstrated recovery of the organisms by this method.

Raw milks and raw-milk cheeses were processed in an analogous manner, however the IMS procedure was performed after 6-h incubation of the enrichment broth. After this time, the enrichment broth was incubated for a further 12 h. A 0.1-ml aliquot of the broth was then inoculated onto a CT-SMAC plate in duplicate and incubated for 18 h at 37°C in air. Any NSF colonies were processed as outlined above.

2.3. Measurement of pH

The pH of all cheese samples was measured using a surface-reading electrode as outlined in the manufacturer’s (Sentron, Netherlands) instructions.

2.4. Enumeration of E. coli

Total E. coli counts were determined for all raw cow’s milks and raw-milk cheeses according to the British Standard method (BS 5763).

2.5. Measurement of water activity

25 g samples of cheese were submitted to the Edinburgh City Analyst for measurement of water activity (A_w) according to the following scheme:

- Any sample found to be positive for VTEC O157.
- All samples with total E. coli counts greater than 10⁷ g⁻¹.
- At least five samples of each cheese where the E. coli count was less than or equal to 10² g⁻¹.

3. Results

3.1. Samples examined

A total of 2429 samples of foodstuffs were examined. Specimens comprised 1190 raw meats and meat products (829 beef, 233 lamb, 128 other/unidentified meats), 500 raw milks and 739 raw-milk cheeses.

3.2. Detection of E. coli O157

E. coli O157 was isolated from a specimen of beef sausage, and beef burger produced on the premises of the same retail butchers shop, and collected on the same day in July 1997. Both isolates were forwarded to the E. coli O157 Reference Laboratory in Aberdeen for confirmation of identity. The

<table>
<thead>
<tr>
<th>Cheese type</th>
<th>Total E. coli count (cfu g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample of Scottish blue cheese type 1</td>
<td>19500</td>
</tr>
<tr>
<td>Sample of Scottish blue cheese type 1</td>
<td>23300</td>
</tr>
<tr>
<td>Sample of Scottish blue cheese type 1</td>
<td>34900</td>
</tr>
<tr>
<td>Sample of Scottish blue cheese type 1</td>
<td>35800</td>
</tr>
<tr>
<td>Sample of Scottish blue cheese type 1</td>
<td>37200</td>
</tr>
<tr>
<td>Sample of Italian fontino cheese</td>
<td>41200</td>
</tr>
<tr>
<td>Sample of Scottish blue cheese type 2</td>
<td>42000</td>
</tr>
<tr>
<td>Sample of Scottish blue cheese type 2</td>
<td>45200</td>
</tr>
<tr>
<td>Sample of Scottish blue cheese type 2</td>
<td>136363</td>
</tr>
<tr>
<td>Sample of Roquefort cheese</td>
<td>230454</td>
</tr>
</tbody>
</table>
isolates were both phage type 32, and produced
vero cytotoxin 2 (VT2) alone.

A third sample of beef burger collected 3 days later from the same premises as part of a routine
d follow-up by EHO’s yielded an indistinguishable
organism. VTEC O157 were not isolated on any
other occasion from the study samples.

3.3. Enumeration of E. coli

Of the 495 raw milks for which a count was available, 479 (96.8%) had < 10^3 cfu ml^-1 present.
Of the 735 raw-milk cheeses for which a count was available, 725 (98.6%) had < 10^4 cfu g^-1 present.
Of the 102 cheese types sampled, 4 types accounted for the 10 specimens with counts greater than 10^4
cfu g^-1 (Table 1). The distribution of the counts is shown in Fig. 1.

3.4. Measurement of water activity

Of the 226 samples of cheese with a total E. coli count of < 10^2 cfu g^-1 for which A_w data were available, the average value was 0.946 (range 0.75–

1), with a median value of 0.95. Of the 32 cheese samples with a total $E. \text{coli}$ count of $10^2 \text{ cfu g}^{-1}$ for which $A_w$ data were available, the average value was 0.959 (range 0.92–0.98), with a median value of 0.96. The distribution of $A_w$ values amongst both groups of specimens is shown in Fig. 2.

3.5. Measurement of pH of cheeses

From the 734 raw-milk cheeses for which a result was available, the mean pH was 6.04 (range 4.0–8.5), and the median pH was 5.9. The average pH of the 663 samples of cheese with a total $E. \text{coli}$ count of $< 10^2 \text{ cfu g}^{-1}$ was 5.99 (range 4.0–8.5), with a median value of 5.9.

Of the 71 cheese samples with a total $E. \text{coli}$ count of $10^2 \text{ cfu g}^{-1}$ for which pH data were available, the average value was 6.5 (range 4.9–7.7), with a median value of 6.6. The distribution of pH values amongst both groups of specimens is shown in Fig. 3.

Fig. 3. Distribution of pH values for raw-milk cheeses.

4. Discussion

The current study has demonstrated a very low prevalence of contamination with VTEC O157 organisms in raw retail beef products (0.24%). $E. \text{coli}$ O157 was only isolated from a specimen of beef sausage, and a specimen of beef burger produced on the premises of the same retail butchers shop, and collected on the same day in July 1997.

$E. \text{coli}$ O157 was not detected in any other raw retail meat specimens, raw cow’s milks or raw-milk cheeses. Both isolations of VTEC O157 in the study were from minced beef products. Given the historically high local rates of infection with VTEC O157, the relatively common occurrence of sporadic cases, and the generally accepted view that contaminated meat is an important vehicle of infection, these findings were somewhat surprising.

Chapman et al. (1996) in a similar study performed in the north of England demonstrated a more than 5-fold higher level of contamination of raw beef.
products, and found almost 6% of lamb products yielded VTEC O157. However, other recent survey work in England and Wales (Little and de Louvois, 1998) demonstrated a much lower level of contamination of raw prepared meats (0.13%), although an IMS step was not incorporated as part of this protocol.

In a study of raw milks in the Netherlands, Heuvelink et al. (1998) did not isolate VTEC O157 from any of 1011 samples, even when IMS was employed. This data supports our own findings and suggests that although many European and North American studies show approximately 4% of cattle herds are positive for VTEC O157 (ACMSF, 1995; WHO, 1997; Coia, 1998), albeit with transient and sporadic carriage in individual animals, contamination of milk may be uncommon. Further indirect support for this hypothesis is provided by the results for total E. coli counts in the raw milk samples that showed that 96.8% had < 10^5 cfu ml^-1 present.

Although the results of the total E. coli counts in the ready-to-eat raw milk cheese samples were also encouraging, there is clearly still some scope for improvement. The draft revised PHLS guidelines for the microbiological quality of ready-to-eat foods suggest that for this type of product < 20 cfu g^-1 is satisfactory, < 10^2 cfu g^-1 is acceptable, 10^2–< 10^4 cfu g^-1 undesirable, and ≥ 10^4 cfu g^-1 unsatisfactory/potentially hazardous. According to these criteria, 90% of cheeses tested were acceptable, while only 10 samples (1.3%) would have been classified as potentially hazardous (Table 1), the remainder having counts in the undesirable range.

Of the 71 cheeses whose total E. coli counts would have been classified as undesirable and unsatisfactory under the new guidelines, sell-by date information was only given in 22 instances (31%), and all of those were “in-date”. Overall, sell-by date information was available for 223 (30%) of the cheese specimens.

Although the data suggest that there is increased water activity in those cheese samples with counts of 20 cfu g^-1 and above (Fig. 2), A_w results were only available for 36 samples in this category, as compared to 226 in the group with counts of < 20 cfu g^-1. Equally, although the pH distribution data for those specimens of cheese with ≥ 20 cfu g^-1 suggest that at least a subset of this group has higher values than those with < 20 cfu g^-1 (Fig. 3), the numbers are very small.

The only isolates of VTEC O157 in the study were of phage type 32. This type is not a common type causing human disease in Scotland (four human isolates in 1997, W.J. Reilly, personal communication). Although there were no cases identified locally in association with these isolates, it is perhaps of interest that a human case of phage type 32 infection was reported in the adjacent Fife health board area in the month following these isolations, although there was no apparent connection identified.

The low rates of VTEC O157 contamination, particularly in the raw retail meats, appears paradoxical in the context of the historically high levels of human infection in this area. However, a number of issues require further consideration in this regard.

The study interval coincided with a period of relatively low numbers of reported cases of human VTEC O157 infection in Scotland. The reasons for this decrease in cases is not immediately apparent. However, the study commenced in the wake of the very large Central Scotland outbreak, in which retail meat sources were implicated. Heightened awareness of the risks of faecal contamination on carcasses, and the potential risks of cross-contamination for transmission of these organisms would have been expected at this time. This “Pennington effect” may have been reflected in closer attention to appropriate hygienic practices on the farm, in abattoirs, and not least in butcher’s premises. Such changes in practice might be expected to have resulted in a direct influence on the level of contamination of retail meat products with VTEC O157.

Although our methodology employed an IMS step, for consistency with similar survey work being carried out elsewhere for the Department, BPW-VCC was used for enrichment. Some workers have questioned whether this may in fact be too inhibitory a medium for this purpose (Bolton et al., 1996), and have recommended the use of Tryptone Soy Broth (TSB) and other agents. This may have resulted in a lower yield than would otherwise have been the case. On the other hand, our control experiments suggested that lack of sensitivity is unlikely to have been a major problem.

The other factor that is worthy of further consideration is the role of alternative transmission routes.
Some authors have previously questioned the role of “conventional” routes of transmission of VTEC O157, such as contaminated undercooked ground beef products, in providing an adequate explanation for the levels of infection observed in Scotland (Sharp et al., 1994; Sharp et al., 1995).

A number of reports have highlighted the occurrence of infections associated with a range of other sources, including animal contact and water sources (Coia, 1998). This association with contact with animals and their by-products and water sources has also been supported by the results of a descriptive epidemiological study in Scotland (Coia et al., 1998). This hypothesis is currently being investigated further as part of a UK Department of Health-funded case control study.

This study has shown that contamination of the foodstuffs surveyed with VTEC O157 occurs at a very low level. This should provide some reassurance both to producers, retailers and consumers of these items in south-east Scotland.

Acknowledgements

This project was funded by a grant from the UK Department of Health (DH250). We would also like to thank the following for their assistance: the Scottish E. coli O157 Reference Laboratory, Aberdeen; the Scottish Office Agriculture and Fisheries Department; the Scottish Centre for Infection and Environmental Health; Dr. Paul Cook, Department of Health; City of Edinburgh Council; West Lothian Council; Midlothian Council; East Lothian Council; Scottish Borders Council.

References


