Prevalence and numbers of *Escherichia coli* O157:H7 in minced beef and beef burgers from butcher shops and supermarkets in the Republic of Ireland

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Abstract

This study investigated the prevalence and numbers of *Escherichia coli* O157:H7 in minced beef and beef burgers in supermarkets and butcher shops in the Republic of Ireland. Fifteen samples were collected quarterly from each of 26 counties over a 13-month period. All samples (*n* = 1533) were (1) directly plated on SMAC, and (2) enriched in mTSB with novobiocin, extracted by immunomagnetic separation (IMS), plating onto SMAC-CT agar and finally confirmed by PCR. Overall, *E. coli* O157:H7 was recovered from 43 samples (2.80%) with counts ranging from 0.52–4.03 log\textsubscript{10} cfu g\textsuperscript{-1}. Of the positive samples, 2.70% (32/1183) were purchased from supermarkets and 3.14% (11/350) from butcher shops. Only one product type (fresh unpacked burgers from supermarkets) was negative for *E. coli* O157:H7. Of the products containing the pathogen, fresh packaged burgers from supermarkets had the highest prevalence of 4.46% (7/157) while fresh unpackaged mince purchased from supermarkets had the lowest prevalence of 2.01% (6/299). Of the 43 isolates recovered, 41 possessed verotoxin-producing genes (*vt*\textsubscript{1} and *vt*\textsubscript{2}), *E. coli* attaching and effacing gene (*eae*\textsubscript{A}), haemolysin gene (*hly*\textsubscript{A}), 60-MDa plasmid or *rfd* gene cluster that encodes for the biosynthesis of the O-antigen (pO157) and flagellar H7 antigen encoding gene (*fliC*\textsubscript{h7}). The remaining 2/43 isolates contained only one of the verotoxin-producing genes (*vt*\textsubscript{1} or *vt*\textsubscript{2}) and all the other genes named.

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1. Introduction

*Escherichia coli* O157:H7 has emerged as an important foodborne pathogen of considerable public health concern, because of the severity of infection which it causes and an infectious dose which may be as low as 10 organisms (Coia, 1998). In an outbreak study reported by Willshaw et al. (1994) contamination levels in an implicated product were reportedly as low as 2 cells per 25 g. This pathogen has been implicated in a number of high-profile outbreaks in the USA (Bell et al., 1994), Scotland (Ahmed and Donaghy, 1998) and Japan (Michino et al., 1998) as well as in many sporadic cases of infection. Cattle are still regarded as one of main reservoirs of *E. coli* O157:H7, with the pathogen occurring in the faeces (Chapman et al., 1997), rumen (Van Donkersgoed et al., 1999), hide (Elder et al., 2000), and derived carcasses (Elder et al., 2000). At the other end of the human food chain, numerous studies have reported the presence of *E. coli* O157:H7, usually at low prevalences, on retail meats. A recent study in France (Vernozy-Rozand et al., 2002) reported 0.12% (4/3450) samples positive for *E. coli* O157:H7 in large-scale processed minced beef, while studies of butcher’s shops in a range of countries have reported higher values i.e. 3.8% (6/160) in Argentina (Chinen et al., 2001); 2.3% (5/211) in Switzerland (Fantelli and Stephan, 2001); and 1.1% (36/3216) in the UK (Chapman et al., 2000). In the
respectively (NDSC, 2001). Most of these cases were with 76, 51, 42, and 52 cases from 1998 to 2001, the ROI have remained relatively small in recent years in minced beef samples from six stores in Seattle, USA. With the exception of the study of Vernozy-Rozand et al. (2002) the above studies merely determined presence or absence of E. coli O157:H7, usually after enrichment, and in general, little data are available on the numbers of this pathogen in retail meat products.

In one study, E. coli O157:H7 numbers in beef burgers and in minced beef were estimated using a most probable number (MPN) technique and ranged from $<0.3–4\text{ cfu g}^{-1}$ ($-0.5–0.6\log_{10}\text{cfu g}^{-1}$) (Bolton et al., 1996), although one sample was reported to contain 2300 cfu g$^{-1}$ ($3.36\log_{10}\text{cfu g}^{-1}$). The general pattern observed by Bolton et al. (1996) was confirmed by Tuttle et al. (1999) who used a MPN technique to determine that samples ($n=76$) of ground beef patties from a slaughter house epidemiologically linked to an outbreak at a fast-food outlet, contained the pathogen at concentrations ranging from $<0.3$ to $15\text{ cfu g}^{-1}$ ($-0.52–1.18\log_{10}\text{cfu g}^{-1}$). Studies in other foods have established counts of $<10^2\text{cfu ml}^{-1}$ in raw milk, and $<10^5\text{cfu g}^{-1}$ in raw-milk cheeses (Coia et al., 2001). Because of the low infectious dose of the pathogen, these reported concentrations of the pathogen are of potential public health significance.

A number of small scale or localised investigations of E. coli O157:H7 numbers in meat in the Republic of Ireland (ROI) have been previously reported. These include a study in the southern region of the ROI (Munster), which detected only 1/586 meat samples positive for E. coli O157:H7 (Walsh et al., 1997). Another small study of retail fresh and processed meats ($n=120$) from the Dublin area, recovered the pathogen in 3/56 salami samples (at concentrations of approximately $1.5\log_{10}\text{cfu g}^{-1}$) and in 1/31 cooked ham products (at a concentration of approximately $0.5\log_{10}\text{cfu g}^{-1}$) (Duffy et al., 1998).

The numbers of clinical cases of E. coli O157:H7 in the ROI have remained relatively small in recent years with 76, 51, 42, and 52 cases from 1998 to 2001, respectively (NDSC, 2001). Most of these cases were sporadic, involving children between 1 and 4 years old. A number of suspect foods were reported by cases but as the majority of these were sporadic cases it was not possible to epidemiologically link most of them to an infection source (NDSC, 2001). Considering the severity of the disease caused by this organism, and the population section most frequently infected, it is important to establish the role of beef in the transmission of E. coli O157:H7 in Ireland. Such investigation necessitates a nation-wide study, to obtain qualitative and quantitative data on the presence of E. coli O157:H7 in Irish retail meat products, which would provide an accurate baseline for accurate risk analysis and assessment, to underpin the development of adequate means of controlling this dangerous pathogen in such products.

The aim of this study was to determine the incidence and numbers of E. coli O157:H7 in minced beef (unpackaged or packaged by modified atmosphere packaging (MAP) or air), and beef burgers—frozen and fresh (unpackaged or packaged by MAP or air) sold in Irish butcher and supermarket outlets over a period of 13 months in the 26 counties in the ROI.

2. Materials and methods

2.1. E. coli O157:H7

Two toxigenic strains of E. coli O157:H7 (Ent C9490 and ATCC 43895, Protect™ Stock Culture Bead) and a non-toxigenic strain of E. coli O157:H7 (NCTC 12900) were maintained on Protect™ Stock Culture Beads (Protect, Technical Consultants Limited, UK) at $-18\degree\text{C}$. Beads with each strain were resuscitated, cultured on Sorbitol MacConkey agar (SMAC) (Oxoid) and included as controls in the analysis of each batch of samples examined.

2.2. Sample plan, locations and types

Samples ($n=1533$) were collected (15 samples from each of 26 counties, every 3 months between March 21st 2001 and April 12th 2002). Samples were drawn on a rolling basis within each period so that counties were sampled and resampled in a fixed order. During each visit to each county, 15 samples were collected from five different premises (three supermarkets/two butcher shops). In each county, the five sampled premises were in two large towns (four premises) and one small town (one premise). Not all sample locations could provide all sample types. The numbers of each type of sample and their sources are presented below.

- Beef burgers (fresh, packaged, brand name, supermarket) ($n=157$)
- Minced beef (fresh, unpackaged, butcher shop) ($n=211$)
- Beef burgers (frozen, packaged, brand name, supermarket) ($n=206$)
- Minced beef (fresh, packaged, brand name, supermarket) ($n=457$)
- Beef burgers (fresh, unpackaged, butcher shop) ($n=140$)
- Minced beef (fresh, unpackaged, supermarket) ($n=299$)
Beef burgers (fresh, unpackaged, supermarket) \((n = 63)\)

At each location, three samples were randomly purchased from the range available, immediately placed into cooler boxes at \(4 \pm 1^\circ C\), transported back to the laboratory, within 32 h, held at \(1 \pm 1^\circ C\), and examined within 64 h.

2.3. Sample analysis

*E. coli* O157:H7 were (1) enumerated in samples by direct plating and/or (2) detected by enrichment, IMS recovery and plating.

2.3.1. Enumeration

Samples of minced beef or beef burgers (10 g) were stomached (230 rpm) for 1 min in 90 ml of EC Broth (Becton Dickinson Microbiology Systems, USA). Aliquots (0.1 or 1.0 ml), of undiluted, stomached (230 rpm) for 1 min in 90 ml of EC Broth were plated in duplicate and in triplicate onto SMAC agar. All plates were incubated at 37°C for 22 h and examined for typical *E. coli* O157:H7 colonies, i.e. colourless, smooth, circular and entire edge colonies with brown centres. Five typical (suspect) colonies from each duplicate plate were identified, subcultured onto Nutrient agar (Oxoid), and subjected to confirmatory tests as described below.

2.3.2. Enrichment and IMS recovery of *E. coli* O157:H7

Samples were analysed using the method described in ISO 16654. In brief, samples (25 g) of minced beef or beef burgers were added to 225 ml volumes of modified Tryptone Soya Broth (mTSB, Medical Supply Company, (MSC) Mulhuddart, Dublin) supplemented with 1.0 ml of a 0.45% novobiocin solution (Sigma, Tallaght, Dublin). The samples were stomached (230 rpm) for 2 min and incubated for 24 h at 41.5°C. Immunomagnetic separation (IMS) was conducted after 6 and 24 h using immunomagnetic beads coated with an anti-*E. coli* O157 antibody (Dynabeads® anti-*E. coli* O157, Dynal A.S., Oslo, Norway). Each recovered IMS bead complex was spread in duplicate onto Sorbitol MacConkey agar (SMAC) (Oxoid, Basingstoke, UK) supplemented with Cefixime tellurite Selectavial™ (MSC) using a sterile cotton swab, and incubated at 37°C for 22 h. Five colonies exhibiting typical presumptive positive characteristics from each duplicate plate were identified, subcultured onto Nutrient agar (Oxoid), and subjected to confirmatory tests as described below.

2.4. Confirmatory tests

Typical colonies from SMAC and Nutrient agar plates were directly streaked onto Levine’s Eosin Methylene Blue (EMB) agar (Oxoid) and Phenol Red Sorbitol agar with 4-methylumbelliferyl-B-D-Glucuronide (PRS-MUG) (Oxoid) agar, and incubated overnight at 37°C. Colonies displaying a green metallic sheen on EMB and no fluorescence on PRS-MUG illuminated with UV light, were identified as colonies exhibiting typical characteristics of *E. coli* O157 species. These colonies were emulsified in 5 ml of sterile saline solution, inoculated onto an API 20E strips (bioMérieux, Basingstoke, UK), and incubated at 37°C for 24 h. Comparison with the results obtained, with the API numerical database, along with a number of additional biochemical tests, i.e. sorbitol, indole and oxidase tests allowed identification of *E. coli* O157 colonies.

2.5. O157 and H7 antigen determination

All sorbitol non-fermenting, indole positive, MUG-negative colonies were examined by latex agglutination (Wellcolex, Merseyside, UK). These beads are coated with antibodies which bind to any O157 or H7 antigens on the test organisms, forming a visible antigen-antibody precipitate (De Boer and Heuvelink, 2000). Colonies giving a precipitation reaction were confirmed as *E. coli* O157:H7 positive.

2.6. Detection of virulence factor genes by polymerase chain reaction (PCR) analysis

Confirmed *E. coli* O157:H7 positives colonies were subject to PCR analysis to detect the presence of the verotoxin genes \((vt_1, vt_2)\), the attaching and effacing gene \(eae\), enterohaemorrhagic *E. coli* hlyA gene and the O-antigen encoding region of pO157 gene. DNA was extracted from latex positive colonies using a DNeasy extraction kit (Qiagen™, Crawley, UK). The DNeasy protocol for animal tissues was used, and all DNA extractions were performed according to the manufacturers’ instructions, with minor adjustments as follows. Each positive colony was inoculated in 10 ml of Brain Heart Infusion (BHI) broth (Oxoid) and incubated for 24 h at 37°C. Bacterial cells were recovered from each culture by centrifugation of 1.5 ml of inoculum at 7500 rpm for 10 min. The supernatant was discarded and procedure repeated. The recovered cells were resuspended in 180 μl of Tissue Lysis Buffer (ATL) and 20 μl of proteinase K was added instead of 40 μl and lysed by incubation at 55°C in a water bath for 3 h. Bacterial DNA was recovered by a series of further washes as described in manufacturer’s handbook.

The primers and PCR conditions used in this study (Assays I–III) are detailed in Table 1. Assays I and II were modified versions of the (Paton and Paton, 1998) multiplex PCR (Fitzmaurice, 2003). Assay III is a modified version of the (Fratamico et al., 2000) multiplex PCR assay. Assay IV, which used all the above
primers in a single PCR assay, was only used if the other assays (I, II or III) yielded unclear or ambiguous results. In Assay I, 10 μl of genomic DNA was used in each 50 μl PCR reaction. The primer concentrations were as follows: vt1F 10 pmol/μl; vt1R 10 pmol/μl; vt2F 12.5 pmol/μl and vt2R 25 pmol/μl (MWG—Biotech AG, Ebersberg, Germany).

For Assay I, 5 μl of genomic DNA was used in each 50 μl PCR reaction. The primer concentration for p0157 was 12.5 pmol/μl. For each assay the PCR mixture consisted of 50 mM KCl (Promega, Madison, Wisconsin, USA), 2.0 mM MgCl₂ (Promega), 400 μM (each) of the four deoxynucleoside tri-phosphates (dNTPs) (Promega), 2.5 U Taq DNA polymerase, thermophilic DNA polymerase 10X buffer, magnesium free (Promega). Samples from Assays I and II were subjected to 35 PCR cycles, each consisting of 1 min denaturation at 94°C and 2 min of annealing at 65°C for the first 10 cycles. Then decreasing to 60°C by cycle 15; and 1.5 min of elongation at 72°C, increasing to 2.5 min from cycles 25–35.

In Assay III, 5 μl of genomic DNA was used in each 50 μl PCR reaction. The primer concentration for fliC7 (MWG—Biotech AG) in a 50 μl reaction was 25 pmol. The primer mixture consisted of 50 mM KCl (Promega), 3.0 mM MgCl₂ (Promega), 400 μM (each) of the four deoxynucleoside tri-phosphates (dNTPs) (Promega), 2.5 U Taq DNA polymerase, thermophilic DNA polymerase 10X buffer, magnesium free (Promega). The PCR samples were then subjected to 94°C for 2 min and then 35 cycles of denaturation at 94°C for 20 s, annealing at 57°C for 1 min, and extension at 72°C for 1 min following the 35 cycles a final extension for 10 min at 72°C was carried out.

In Assay IV, the PCR mixture per 50 μl reaction consisted of 3 μl of genomic DNA, 1 μl of each primer pair at a final concentration of 0.1–0.5 μM and 25 μl of Taq PCR Master Mix (Qiagen™). The PCR samples were then subjected to an initial denaturation period at 94°C for 2 min and then an additional cycle of 94°C for 1 min, followed by annealing at 62°C for 1 min and elongation at 72°C for 3 min. Excluding the initial denaturation the programme was repeated for 35 cycles with a final extension of 72°C for 8 min.

All assays included negative controls (in which isolate DNA was replaced with sterile distilled water) and positive controls (in which isolate DNA was replaced with DNA from known positive control organisms).

### 2.7. Electrophoretic analysis

PCR products were detected by gel electrophoresis, on a 2.0% agarose gel containing 2.0 μl of ethidium bromide (Gibco, UK). A volume of 10 μl of each PCR mixture was supplemented with 2 μl tracking dye (Sigma) and loaded onto a well of the gel. A 100-bp DNA ladder molecular weight marker (Pharmica Biotech, UK) was included in each electrophoretic run to allow identification of the amplified products. PCR products were visualized under UV illumination and

### Table 1

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5’–3’)</th>
<th>Reference</th>
<th>Amplification product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>vt1F</td>
<td>ATAAATCGCCATTCGGTCTAC</td>
<td>Paton and Paton (1998)</td>
<td>180</td>
</tr>
<tr>
<td>vt1R</td>
<td>AGAACGCCACTGAGATCATC</td>
<td>modified by Fitzmaurice (2003)</td>
<td></td>
</tr>
<tr>
<td>vt2F</td>
<td>GCACCTGTCAAAATGCTCCC</td>
<td>Paton and Paton (1998)</td>
<td>255</td>
</tr>
<tr>
<td>vt2R</td>
<td>TCGCCATTATCTGACATTCTG</td>
<td>modified by Fitzmaurice (2003)</td>
<td></td>
</tr>
<tr>
<td>eaeAF</td>
<td>GACCCCGCACAAGGATAAGC</td>
<td>Paton and Paton (1998)</td>
<td>384</td>
</tr>
<tr>
<td>eaeAR</td>
<td>CCACCTGCAACACAAGGAGG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>hlyAF</td>
<td>GCATCATCAAGGCTACGTTCCT</td>
<td>Paton and Paton (1998)</td>
<td>534</td>
</tr>
<tr>
<td>hlyAR</td>
<td>AATGAGGCAAGGTGGTTAAGCT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0157F</td>
<td>CCGACATCCATGTGATATGG</td>
<td>Paton and Paton (1998)</td>
<td>259</td>
</tr>
<tr>
<td>0157R</td>
<td>TGGCCTATGTCAGCTAACCC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>fliCh7-F</td>
<td>GCGCTGTCGAGTTCTATCGAGC</td>
<td>Fratamico et al. (2000)</td>
<td>625</td>
</tr>
<tr>
<td>fliCh7-F</td>
<td>CAACGATGAATTTATCGCCATCCC</td>
<td></td>
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</tr>
</tbody>
</table>

bp—base pairs.
catalogued with a gel documentation system (Stratagene EE 2, Germany). The sizes of the PCR products were compared with the 100-bp marker DNA ladder (Promega) and the PCR products of the required size indicated a presence of the target gene.

2.8. VTEC screening of isolates

Those isolates confirmed by PCR as possessing the verotoxin genes (vt1, vt2), the attaching and effacing gene (eaeA), enterohaemorrhagic E. coli hlyA gene and the O-antigen encoding region of pO157 gene were cultured onto Brain Heart Infusion agar slopes. Then treated with Polymyxin B (Bacillus cereus supplement, Oxoid), to release membrane bound toxins, and examined for the presence of VT1 and VT2 toxins using the VTEC-RPLA reverse passive latex agglutination test (Oxoid).

2.9. Analysis of gas atmosphere in packaged minced beef and burger samples

A representative number (n=7) of each type of presumptive MAP packaged product was sampled to ascertain the gas composition in the packaging environment. Gas composition of each pack was analysed by gas chromatography (GC) (Gow-Mac Spectra 250, Gow-Mac Instrument Company) using a normalization method (Beggan, 2001). A CI-4100 Integrator (Milton Roy, Ireland) was used to calculate and plot the chromatographs and calculate the proportions of specific gases in the pack headspace from the areas under the curves.

2.10. Statistical analysis

The proportion of positive samples that were found in a particular product type was compared to determine if they were significantly different from each other. This was carried out by calculating the standard error of the differences between the proportions for each pair of products using the formula

\[ \text{difference between proportions} = \sqrt{\left(p_1\times q_1/n_1\right) + \left(p_2\times q_2/n_2\right)} \]

where \( p_1 \) = proportion positive in product 1, \( q_1 = 1 - p_1 \) and \( n_1 \) = number of samples taken of product 1 and \( p_2 \) = proportion positive in product 2, \( q_2 = 1 - p_2 \) and \( n_2 \) = number of samples taken of product 2.

At the 95% confidence level proportions were considered significantly different if they were separated by more than twice the calculated standard error of the difference between proportions.

In addition, the likely population prevalence of E. coli O157:H7 in all retail minced beef products sold at retail in Ireland was calculated from the total number of positive samples in the 1533 samples taken of all five product types. The presence or absence of E. coli O157:H7 in a sample can be considered to be a binomial process. Using a stochastic approach, the proportion of positive samples can be represented by a Beta distribution. Montecarlo software, @Risk™ was used to fit a Beta distribution to the positive proportion and the 95th percentile was calculated. This represented the maximum likely prevalence of E. coli O157:H7 at the 95% confidence level.

3. Results

This survey detected E. coli O157:H7 in 43/1533 (2.80%) samples. The effect of the type of minced beef products tested on the prevalence of positive samples was not significant at the 95% confidence level. Given the survey size and the stochastic analysis of the aggregate prevalence data, the mean prevalence of E. coli O157:H7 in minced beef products on retail sale in the ROI is likely to be less than or equal to 3.6% at the 95% confidence level.

There were period differences observed, with higher levels of positive samples 4.8% (17/356) in period 4, (January to April 2002) than in Period 1 (March to June 2001 and April 2002), period 2 (June to September 2001) or period 3 (October 2001 to January 2002) which, yielded 3.2% (13/404); 2.4% (9/383); and 1.0% (4/390) positive samples respectively. Fig. 1 shows the monthly breakdown of the 43 positive samples, 4.65% (8/172) in January, 4.68% (18/385) in March/April, and 4.35% (5/115) in August while the remaining (1.39%) 12/861 positive samples were detected over the remaining 8 months.

Tables 2–5 detail the products, location, and date on which the E. coli O157:H7 isolates were detected in each period and detail the number of the pathogen present and its virulence profile. The number of E. coli O157:H7 in 21 of the 43 positive samples ranged from 0.52–4.03 log10 cfu g\(^{-1}\) while in the remaining 22 positive samples, the pathogen was detectable by enrichment only i.e. <0.52 log10 units. The range of counts for positive samples was similar in all four periods with counts in period 1 (<0.52–3.04 log10 cfu g\(^{-1}\)), period 2 (<0.52–3.04 log10 cfu g\(^{-1}\)), period 3 (<0.52–0.81 log10 cfu g\(^{-1}\)) and period 4 (<0.52–4.03 log10 cfu g\(^{-1}\)), respectively.

E. coli O157:H7 was detected at numerous locations throughout the ROI with positives reported in 19 of the 26 counties sampled. There was some location and time grouping of positive samples in each sampling period generally around large towns or cities. In period 1 (April) a large group of five positive samples was recorded in two towns in county Meath [Navan town (n = 4) and Kells (n = 1)]. In period 2 (August) a group of three positive samples was recorded in county Cavan.
[Cavan town \((n=3)\) and in period four (March) a cluster of four positive samples was recorded in county Donegal [Letterkenny \((n=3)\) and Bundoran \((n=1)\)].

Of the positive samples 2.70% (32/1183) were purchased from supermarkets and 3.14% (11/350) from butcher shops.

Table 6 summarizes by product type the samples containing \(E.\ coli\ O157:H7\). Only one product type (fresh unpacked burgers from supermarkets) had no detected \(E.\ coli\ O157:H7\). Of the products containing the pathogen, fresh packaged burgers from supermarkets had the highest prevalence of 4.46% (7/157)
Table 3
Summary of location, month, premises, product type and *E. coli* O157:H7 count (log10 cfu g⁻¹) and virulence profile for isolates recovered from Irish mince beef in Period 2

<table>
<thead>
<tr>
<th>County</th>
<th>Town</th>
<th>Month</th>
<th>Premises type</th>
<th>Product type</th>
<th>Count</th>
<th>Veroctotxin genes</th>
<th>Verotoxin production</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>vt1</td>
<td>vt2 VT1 VT2</td>
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<tr>
<td>Kilkenny</td>
<td>Kilkenny city</td>
<td>July</td>
<td>Supermarket</td>
<td>Mince fresh (map)</td>
<td>E</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Kilkenny</td>
<td>Kilkenny city</td>
<td>July</td>
<td>Supermarket</td>
<td>Mince fresh (map)</td>
<td>3.04</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Kildare</td>
<td>Kildare town</td>
<td>July</td>
<td>Butcher</td>
<td>Mince fresh (u)</td>
<td>2.30</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Dublin</td>
<td>Ballyfermot</td>
<td>July</td>
<td>Supermarket</td>
<td>Burgers fresh (map)</td>
<td>2.07</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Leitrim</td>
<td>Carrick-on-shannon</td>
<td>August</td>
<td>Supermarket</td>
<td>Mince fresh (map)</td>
<td>0.60</td>
<td>+</td>
<td>+</td>
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<td>Leitrim</td>
<td>Ballinamore</td>
<td>August</td>
<td>Butcher</td>
<td>Mince fresh (u)</td>
<td>1.65</td>
<td>+</td>
<td>+ + +</td>
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<td>Cavan</td>
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<td>August</td>
<td>Supermarket</td>
<td>Burgers fresh (map)</td>
<td>0.95</td>
<td>+</td>
<td>+ + +</td>
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<td>Cavan</td>
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<td>August</td>
<td>Supermarket</td>
<td>Mince fresh (map)</td>
<td>E</td>
<td>+</td>
<td>+ + +</td>
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</table>

u = unpackaged; map = modified atmosphere packaging; E = detectable only by enrichment; ++ = strong agglutination reaction; +++ = very strong agglutination reaction. All isolates contained *hylA*, *eaeA*, *pO157* and *fliC*.

Table 4
Summary of location, month, premises, product type and *E. coli* O157:H7 count (log10 cfu g⁻¹) and virulence profile for isolates recovered from Irish mince beef in Period 3

<table>
<thead>
<tr>
<th>County</th>
<th>Town</th>
<th>Month</th>
<th>Premises type</th>
<th>Product type</th>
<th>Count</th>
<th>Veroctotxin genes</th>
<th>Verotoxin production</th>
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<td></td>
<td>vt1</td>
<td>vt2 VT1 VT2</td>
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<tr>
<td>Sligo</td>
<td>Sligo town</td>
<td>January</td>
<td>Supermarket</td>
<td>Mince fresh (map)</td>
<td>E</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sligo</td>
<td>Sligo town</td>
<td>January</td>
<td>Supermarket</td>
<td>Mince fresh (map)</td>
<td>0.81</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Limerick</td>
<td>Limerick city</td>
<td>January</td>
<td>Supermarket</td>
<td>Burger frozen (p)</td>
<td>E</td>
<td>+</td>
<td>+ + + + +</td>
</tr>
<tr>
<td>Cork</td>
<td>Cork city</td>
<td>December</td>
<td>Supermarket</td>
<td>Burger fresh (map)</td>
<td>E</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

p = packaged, map = modified atmosphere packaging; E = detectable only by enrichment; ++ = strong agglutination reaction; +++ = very strong agglutination reaction. All isolates contained *hylA*, *eaeA*, *pO157* and *fliC*.

Table 5
Summary of location, month, premises, product type and *E. coli* O157:H7 count (log10 cfu g⁻¹) and virulence profile for isolates recovered from Irish mince beef in Period 4

<table>
<thead>
<tr>
<th>County</th>
<th>Town</th>
<th>Month</th>
<th>Premises type</th>
<th>Product type</th>
<th>Count</th>
<th>Veroctotxin genes</th>
<th>Verotoxin production</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>vt1</td>
<td>vt2 VT1 VT2</td>
</tr>
<tr>
<td>Wexford</td>
<td>Wexford town</td>
<td>January</td>
<td>Supermarket</td>
<td>Mince fresh (u)</td>
<td>3.43</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Waterford</td>
<td>Waterford city</td>
<td>January</td>
<td>Supermarket</td>
<td>Mince fresh (map)</td>
<td>4.03</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Kilkenny</td>
<td>Thomastown</td>
<td>January</td>
<td>Supermarket</td>
<td>Burger frozen (p)</td>
<td>0.51</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Kilkenny</td>
<td>Thomastown</td>
<td>January</td>
<td>Supermarket</td>
<td>Mince fresh (u)</td>
<td>1.69</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Clare</td>
<td>Shannon</td>
<td>February</td>
<td>Supermarket</td>
<td>Burger frozen (p)</td>
<td>E</td>
<td>+</td>
<td>+ + + + +</td>
</tr>
<tr>
<td>Meath</td>
<td>Ashbourne</td>
<td>February</td>
<td>Supermarket</td>
<td>Burger frozen (p)</td>
<td>E</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Roscommon</td>
<td>Ballahadreen</td>
<td>February</td>
<td>Supermarket</td>
<td>Mince fresh (u)</td>
<td>E</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Donegal</td>
<td>Letterkenny</td>
<td>March</td>
<td>Supermarket</td>
<td>Burgers fresh (map)</td>
<td>E</td>
<td>+</td>
<td>+ + + + + +</td>
</tr>
<tr>
<td>Donegal</td>
<td>Letterkenny</td>
<td>March</td>
<td>Supermarket</td>
<td>Mince fresh (map)</td>
<td>E</td>
<td>+</td>
<td>+ + + + + +</td>
</tr>
<tr>
<td>Donegal</td>
<td>Letterkenny</td>
<td>March</td>
<td>Supermarket</td>
<td>Burgers fresh (map)</td>
<td>E</td>
<td>+</td>
<td>+ + + + + +</td>
</tr>
<tr>
<td>Donegal</td>
<td>Bundoran</td>
<td>March</td>
<td>Butcher</td>
<td>Burgers fresh (u)</td>
<td>E</td>
<td>+</td>
<td>+ + + + + +</td>
</tr>
<tr>
<td>Tipperary</td>
<td>Tipurles</td>
<td>March</td>
<td>Supermarket</td>
<td>Mince fresh (u)</td>
<td>E</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tipperary</td>
<td>Thurlers</td>
<td>March</td>
<td>Supermarket</td>
<td>Burgers fresh (map)</td>
<td>E</td>
<td>+</td>
<td>+ + + + +</td>
</tr>
<tr>
<td>Cork</td>
<td>Cork city</td>
<td>March</td>
<td>Supermarket</td>
<td>Burgers frozen (p)</td>
<td>E</td>
<td>+</td>
<td>+ + + + + +</td>
</tr>
<tr>
<td>Cavan</td>
<td>Baileborough</td>
<td>April</td>
<td>Butcher</td>
<td>Mince fresh (u)</td>
<td>E</td>
<td>+</td>
<td>+ + + + + +</td>
</tr>
<tr>
<td>Offaly</td>
<td>Birr</td>
<td>April</td>
<td>Butcher</td>
<td>Mince fresh (u)</td>
<td>E</td>
<td>+</td>
<td>+ + + + + +</td>
</tr>
</tbody>
</table>

p = packaged; u = unpackaged; map = modified atmosphere packaging; E = detectable only by enrichment; ++ = strong agglutination reaction; +++ = very strong agglutination reaction. All isolates contained *hylA*, *eaeA*, *pO157* and *fliC*.
while fresh unpackaged mince purchased from supermarkets had the lowest prevalence of 2.01% (6/299).

Of the 43 *E. coli* O157:H7 positive samples, 20 were modified atmosphere packaged (MAP) in approximately 80% O2 and 20% CO2. This group included the samples with the highest *E. coli* O157:H7 counts in each period i.e. (Period (P) 1, fresh packaged burgers (3.04 log10 cfu g⁻¹); P2, fresh packaged mince (3.04 log10 cfu g⁻¹); P3, fresh packaged mince (0.81 log10 cfu g⁻¹) and P4, fresh packaged mince (4.03 log10 cfu g⁻¹) (Tables 2–5).

Tables 2–5 presents the genes detected in, and the toxins expressed by the 43 *E. coli* O157:H7 positive samples. All of these samples contained the eaeA, hyLA, pO157 and flfC_h7 genes. Forty-one of the samples contained both genes encoded for verotoxins (tt1 and vt2) with two samples missing one of the verotoxin genes. Of the 43 isolates, 20 produced VT1 or VT2 toxins, or both.

### 4. Discussion

Of the 1533 minced beef samples collected over a 13-month period, a total of 43 (2.80%) were positive for *E. coli* O157:H7. This result is higher than noted in previous studies i.e. 0.17% (1/586, Walsh et al., 1997) and 1.4% (4/282, Duffy et al., 1998). However, taking sample size into account, estimates of the population mean prevalence at the 95% confidence level was 3.6% in this current study and 3.2% and 0.8% in the studies of Duffy et al. (1998) and Walsh et al. (1997), respectively. Direct comparison of results is difficult due to differences in the study methodologies, such as sampling over a full year and the sampling of all regions in the country. While there is some evidence that *E. coli* O157:H7 may be increasingly common in beef production systems (McDowell and Sheridan, 2001), the detection of higher proportions of *E. coli* O157:H7 in more recent studies is more probably associated with the wider use of more sensitive detection methods such as IMS (Chapman et al., 2001).

In comparison to other countries, the prevalence reported in this study is higher than values reported in studies conducted in France (0.12%) (Vernozy-Rozand et al., 2002) and the UK (1.1%) (Chapman et al., 2000), similar to a Swiss study (2.3%) (Fantelli and Stephan, 2001) and lower than reported in a study in Argentina (3.8%) (Chinen et al., 2001).

In approximately half (21/43) of the positive samples, *E. coli* O157:H7 was present in numbers which were below the detection limit of direct plating methods i.e. 0.52 log10 units. In 22/43 samples, direct counts, ranging from 0.52–4.03 log10 cfu g⁻¹ were obtained. Four out of 43 positive samples, had counts > 3.00 log10 cfu g⁻¹. Such high values suggest point source contamination during primary meat production and processing, and/or subsequent temperature abuse of the meat and meat products within the production/retail chain. The counts obtained in this study are on average higher than the <0.3–4 cfu g⁻¹ (~0.5–0.6 log10 cfu g⁻¹) reported in a previous study in the UK by Bolton et al. (1996) although these authors did report one sample to contain, 2300 cfu g⁻¹ (3.36 log10 cfu g⁻¹). Considering the very low infective dose of this pathogen, its detection at such concentrations in retail beef products poses significant public health risks. While it is to be hoped that most retail beef products will be adequately cooked before consumption, leading to the destruction of the pathogen, the presence of contaminated meats at retail and consumer levels places consumers at risk. *E. coli* O157:H7 may persist in undercooked minced beef or beef burgers (Bell et al., 1994) or be transferred from such raw meats to cooked products, or products that do not receive heat treatments prior to domestic consumption, e.g. salad items, by cross contamination of hands, utensils or surfaces (Little and de Louvois, 1998; Field et al., 1977) during domestic food preparation.

*E. coli* O157:H7 was detected in samples from the majority (19/26) of the counties of the ROI. The
proportions of positive samples showed some grouping in relation to location and sampling occasion as detailed in Tables 2–5. However, further investigations (not shown) failed to establish any links between positive samples, and individual abattoirs and suppliers in the beef supply chain and this may be due to the transient and sporadic nature of this pathogen.

There was a seasonal effect observed in this study. The monthly breakdown of the 43 positive samples showed that 4.65% (8/172) in January, 4.68% (18/385) in March/April, and 4.35% (5/115) in August while the remaining (1.39%) 12/861 positive samples were detected over the remaining 8 months (Fig. 1). A previous study in the ROI on the prevalence of E. coli O157:H7 on beef carcasses also reported the highest prevalence of the pathogen in spring and late summer (McEvoy et al., 2001). In the UK, Chapman et al. (2000) also observed seasonal distribution in the prevalence of E. coli O157:H7 on retail meats with the prevalence highest in summer (July) at 4.0%, and lowest in winter (December) at 0.2%.

Of the positive samples 2.70% (32/1183) were purchased from supermarkets and 3.14% (11/350) from butcher shops. This is similar to that reported by Heuvelink et al. (1999).

E. coli O157:H7 was found in almost all products types examined including fresh unpackaged mince and burgers, fresh packaged mince and burgers and also frozen burgers as detailed in Table 6. These results confirm reports in the literature that neither freezing (Ansay et al., 1999) or MAP (Uyttendaele et al., 2001) prevent the survival of E. coli O157:H7 in minced beef and burgers. This confirms previous studies (Schluter et al., 1994) which reported that MAP can extend the shelf life of meat products but has little effect on pathogen survival.

All 43 positive samples contained the eaeA, hylA, pO157 and fliC\textsubscript{H7} genes. The genes encoding for verotoxins (vt1 and vt2), that determine the virulence potential of the organism which are essential in the establishment of the disease (Schmidt et al., 2001), were present in 41 of the samples tested. These findings are supported by a study in an Irish abattoir on the presence of E. coli O157:H7 on beef carcasses (McEvoy et al., 2001) which noted that more than 97% of isolates contained both vt genes, with a very small proportion of isolates carrying only one vt gene. Isolates from a study by Chinen et al. (2001) contained both the vt1 and vt2 genes, but none produced the verotoxins. The prevalence of E. coli O157:H7 in retail minced beef products in the ROI as calculated in this study (2.8%) and the observation of gross levels of contamination (> 3.00 log\textsubscript{10} cfu g\textsuperscript{-1}) in a number of samples pose a significant risk of transmission of verocytotoxigenic E. coli to Irish consumers. However, while this study has focused on the detection and/or enumeration of E. coli O157:H7 at near consumer levels, effective action to reduce or eliminate the risks posed by this organism will involve diverse and co-ordinated actions at a number of stages of the food chain. These include the incorporation and consistent application of Good Agricultural practice (GAP), Good Manufacturing practice (GMP), and Hazard Analysis of Critical Control Points (HACCP) at every stage of the beef supply chain, from the farm, through the abattoir, to the retailer, and those involved with the handling and processing of such raw meat products in the home environment (Attenborough and Matthews, 2000). In addition, suitable intervention measures may be necessary to eliminate the pathogen in food reaching the consumer.

Acknowledgements

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References


