RESEARCH ARTICLE

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Fecal carriage of *Escherichia coli* O157:H7 and carcass contamination in cattle at slaughter in northern Italy

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Summary. Feedlot cattle slaughtered at a large abattoir in northern Italy during 2002 were examined for intestinal carriage and carcass contamination with *Escherichia coli* O157:H7. Carcass samples were taken following the excision method described in the Decision 471/2001/EC, and fecal material was taken from the colon of the calves after evisceration. Bacteria were isolated and identified according to the MFLP-80 and MFLP-90 procedures (Food Directorate’s Health Canada’s). Eighty-eight non-sorbitol-fermenting *E. coli* O157:H7 isolates were obtained from 12 of the 45 calves examined. In particular, *E. coli* O157:H7 isolates were found in 11 (24%) fecal and five (11%) carcass samples. PCR analysis showed that all 11 fecal samples and five carcass samples carried *eae*-γ1-positive *E. coli* O157:H7 isolates. In addition, genes encoding Shiga-toxins were detected in O157:H7 isolates from nine and two of those 11 fecal and five carcasses, respectively. A representative group of 32 *E. coli* O157:H7 isolates was analyzed by phage typing and DNA macrorestriction fragment analysis (PFGE). Five phage types (PT8, PT32v, PT32, PT54, and PT not typable) and seven (I–VII) distinct restriction patterns of similarity >85% were detected. Up to three different O157:H7 strains in an individual fecal sample and up to four from the same animal could be isolated. These findings provide evidence of the epidemiological importance of subtyping more than one isolate from the same sample. Phage typing together with PFGE proved to be very useful tools to detect cross-contamination among carcasses and should therefore be included in HACCP programs at abattoirs. The results showed that the same PFGE-phage type *E. coli* O157:H7 profile was detected in the fecal and carcass samples from an animal, and also in two more carcasses corresponding to two animals slaughtered the same day. [Int Microbiol 2007; 10(2):109-116]

Key words: *Escherichia coli* O157:H7 · Shiga-toxins · verotoxins · phage typing · abattoir · carcass contamination

Introduction

Shiga-toxin-producing *Escherichia coli* (STEC), also called verotoxin-producing *E. coli* (VTEC), comprise the most important recently emerged group of food-borne pathogens [2–5, 24,25,33,38]. These bacteria are a major cause of gastroenteritis that may be complicated by hemorrhagic colitis (HC) or the hemolytic uremic syndrome (HUS), which is the main cause of acute renal failure in children. Since its identification as a pathogen in 1982, STEC O157:H7 was found to be responsible for a series of outbreaks in Europe, Japan, and especially in North America. Many studies have indicated that cattle represent the main reservoir of STEC O157:H7 [4,6–8,28,39]. Transmission occurs through consumption of undercooked meat, unpasteurized dairy products, and veg-

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etables or water contaminated by feces of carriers, since STEC strains are found as part of the normal intestinal flora of animals. Person-to-person transmission has also been documented [4,6,12]. Most outbreaks and sporadic cases of HC and HUS have been attributed to strains of enterohemorrhagic serotype O157:H7 [2,25,26,29,38]. Unlike other E. coli strains, STEC O157:H7 does not ferment sorbitol and is β-glucuronidase negative. These differences simplify identification O157:H7 strains in clinical samples and food products [5,13].

STEC infections are not very common in Italy, and only two HUS outbreaks were reported between 1988 and 2000, one in 1992 in Lombardy and the other in 1993 in the Veneto region. These were associated with STEC belonging to serogroups O111 and O157, respectively. A third cluster of three cases associated with E. coli O26 occurred in Naples in 1997 [38]. Despite this apparently low incidence, STEC O157 has been isolated from cattle at slaughter [14], especially during warm seasons [10], and on cattle farms [15]. Contamination by this organism of cattle carcasses at slaughter [11], minced beef [16], and dairy products [16] has also been reported.

The aim of the present study, conducted in northern Italy, was to evaluate: (i) the prevalence of STEC O157:H7 in the intestinal content of cattle at slaughter and (ii) the rate of carcass contamination. The isolated strains were compared by phage typing and PFGE to trace dissemination of the bacteria during slaughtering procedures.

Materials and methods

Sample collection. Between December 2001 and January 2003, carcass and fecal specimens were collected from 45 feedlot cattle at a large abattoir located in Ravenna Province (northern Italy). Five calves randomly selected were sampled at each visit. From each animal, 25 g of fecal material and carcass contamination. The isolated strains were chosen.

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Results

Isolation of Escherichia coli O157:H7. Isolation and preliminary detection of *E. coli* O157 colonies were performed in the Department of Public Health and Animal Pathology, University of Bologna, Italy. *E. coli* O157 was isolated from 13 fecal and five carcass samples from 14 animals. The 96 *E. coli* O157 isolates obtained in Italy were further analyzed in the *E. coli* Reference Laboratory (LREC), University of Santiago de Compostela, Lugo, Spain, to establish O:H serotypes, virulence genes, phage types, and PFGE profiles. All 96 isolates were positive by PCR for the *O157 rfbE* gene. In addition, 88 of those 96 *E. coli* O157 isolates were positive by PCR for the *fliCh7* gene, and a selection of 40 representative O157 isolates with that gene were motile and expressed H7 antigen, as confirmed in serotyping studies. None of the 88 O157:H7 isolates obtained in this study fermented sorbitol after overnight incubation and all were β-glucuronidase negative, while the eight non-H7 strains (negative for *fliCh7* gene) were positive for those phenotypic characteristics. The eight H7 negative isolates were isolated from the feces of two animals. The H antigens of these eight isolates were established by testing against all 53 H antisera (H1 to H56). The results showed that they were serotype O157:H12. Eighty-eight *E. coli* O157:H7 isolates were recovered from 12 animals (27%); specifically, from 11 fecal samples (24%) and five carcasses (11%), of 45 calves.

Atypical biochemical features were observed in some of the *E. coli* O157:H7 isolates, i.e., ten isolates from two fecal samples (MO/FR9264 and MO/FR8397) produced urease. Genes *stx1, stx2, ehxA, eae, O157 rfbE and fliCh7*. PCR analysis showed that the 11 fecal samples and five carcass samples carried *eae*-γ1- and *ehxA*-positive *E. coli* O157:H7. In addition, genes encoding Shiga-toxins were detected in O157:H7 isolates from nine and two of those 11 fecal and five carcasses, respectively. The 88 *E. coli* O157:H7 isolates were therefore classified into two main groups with respect to their virulence genes: STEC O157:H7 *eae*γ1-*stx*+ and *E. coli* O157:H7 *eae*γ1-*stx*−.

Phage typing and PFGE. The results of phage typing and PFGE of 32 representative isolates (21 STEC O157:H7 *eae*γ1-*stx*+ and 11 *E. coli* O157:H7 *eae*γ1-*stx*−) are summarized in Table 3. The 32 isolates belonged to five different phage types: PT8, PT32v, PT32, PT54, and PTNT (not typable).

### Table 1. Primer sequences and lengths of PCR amplification products

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer</th>
<th>Oligonucleotide sequence (5´–3´)</th>
<th>Fragment size (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>stx1</td>
<td>VT1-A</td>
<td>CGCTGAATGTCATCGCTCCTGC</td>
<td>302</td>
<td>Blanco et al. [5]</td>
</tr>
<tr>
<td>stx1</td>
<td>VT1-B</td>
<td>GGGTATAGCGCTGCTGCTGCA</td>
<td>516</td>
<td>Blanco et al. [5]</td>
</tr>
<tr>
<td>ehxA</td>
<td>HlyA1</td>
<td>GGGTCAGCAGAAAGGTTGTAG</td>
<td>1551</td>
<td>Schmidt et al. [35]</td>
</tr>
<tr>
<td>eaeα</td>
<td>EAE-1</td>
<td>GGAACCGCACAGAGTTAATGCGG</td>
<td>346</td>
<td>Mora et al. [30]</td>
</tr>
<tr>
<td>eaeα</td>
<td>EAE-2</td>
<td>GCAGCCATCATGATGTTTAG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>eaeβ</td>
<td>EAE-F</td>
<td>ATTACTGAGTAAAGGCCCTGAT</td>
<td>682</td>
<td>Blanco et al. [5]</td>
</tr>
<tr>
<td>eaeβ</td>
<td>EAE-RB</td>
<td>ATTATATTGCAAGCCCAT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>eae-γ1</td>
<td>EAE-F</td>
<td>AAAACCGCAGAGTACCTTC</td>
<td>804</td>
<td>Blanco et al. [5]</td>
</tr>
<tr>
<td>eae-γ1</td>
<td>EAE-C1</td>
<td>AGGCCGTGCTCAGTAGTGT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O157</td>
<td>O157-AF</td>
<td>AAGATTGCGCTGAAAGCTTGGG</td>
<td>497</td>
<td>Desmarchelier et al. [17]</td>
</tr>
<tr>
<td>O157</td>
<td>O157-AR</td>
<td>GATGGCAGATTGCTGAGGCA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>fliCh7</td>
<td>H7-F</td>
<td>GCGCTGTGAGATGTCCAGGG</td>
<td>625</td>
<td>Gannon et al. [18]</td>
</tr>
<tr>
<td>fliCh7</td>
<td>H7-R</td>
<td>CAGCGGTGTACCTATCGGG</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Universal oligonucleotide primer pair EAE1 and EAE-2 with homology to the 5´ conserved region of the *eae* gene (detects all currently described *eae* variants). Used to detect the *eae* gene.

b Universal oligonucleotide primer pair EAE-F and EAE-RB with homology to the 3´ variable region of *eae* gene (detects all currently described *eae* variants). Used to sequence the *eae* gene.
Among those five, the most prevalent phage types were: PT8, detected in five animals from four fecal (six isolates) samples and one carcass sample (two isolates); PT54, detected in five animals from three fecal (five isolates) samples and four carcass samples (eight isolates); and PT32v, detected in four animals from four fecal samples (nine isolates).

The dendrogram produced by the UPGMA algorithm study revealed seven distinct restriction patterns (I–VII; 1–12 strains per group) of closely related (similarity >85%) PFGE patterns according to the Dice similarity index. Each group was subdivided into subgroups (A or B) based on the difference of one restriction fragment in the patterns used as criteria to discriminate between clusters. The total number of macrorestriction patterns was therefore 14 (Fig. 1; Table 3). Most isolates of the same phage type were clustered together, although differences were observed among each group. Thus, PT32v isolates presented two PFGE groups (I and III), showing 73% similarity; PT54 also presented two groups (II and V, 71% similarity) with 12 of the isolates grouped together (PFGE-V), and only one isolate in group II. The highest heterogeneity was observed among PT8 isolates, with two clusters clearly differentiated (PFGE-IV and PFGE-VII), showing 60% similarity.

Four fecal samples (animal code MO/FR9264, MO/FR8397, Rab/I2867, RO/I2939) carried *E. coli* O157:H7 isolates with different characteristics, and up to four isolates, each with a different PT-PFGE profile, were detected from the same animal (MO/FR8397). Fecal and carcass isolates from animals MO/FR1274 and VRb/FR6563 showed the same PT-PFGE profile, respectively. However, different PF-PFGE isolates were detected from fecal and carcass samples in animals MO/FR9264 and MO/FR8397, respectively (Table 3).

### Table 2. Prevalence of *E. coli* O157:H7

<table>
<thead>
<tr>
<th>STEC O157:H7</th>
<th>eae+ stx+</th>
<th>eae+ stx-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fecal samples</td>
<td>9/45 (20%)</td>
<td>3/45 (6.7%)</td>
</tr>
<tr>
<td>Carcass samples</td>
<td>2/45 (4.4%)</td>
<td>3/45 (6.7%)</td>
</tr>
</tbody>
</table>

### Table 3. Characteristics of *E. coli* O157:H7 isolates

<table>
<thead>
<tr>
<th>Date</th>
<th>Farm/animal code</th>
<th>Origin</th>
<th>Isolate code</th>
<th>stx genes</th>
<th>Intimin type</th>
<th>Phage types</th>
<th>PFGE profile</th>
</tr>
</thead>
<tbody>
<tr>
<td>22/01/02</td>
<td>RAa/I2.6150</td>
<td>Carcass</td>
<td>1,2</td>
<td>str1, str2</td>
<td>γl</td>
<td>PT8</td>
<td>VII</td>
</tr>
<tr>
<td>22/01/02</td>
<td>PI/Fr.0073</td>
<td>Feces</td>
<td>3</td>
<td>str1, str2</td>
<td>γl</td>
<td>PT8</td>
<td>VII</td>
</tr>
<tr>
<td>18/02/02</td>
<td>MN/Fr.1133</td>
<td>Feces</td>
<td>4</td>
<td></td>
<td>γl</td>
<td>PT54</td>
<td>II</td>
</tr>
<tr>
<td>06/05/02</td>
<td>MO/Fr.1274</td>
<td>Feces</td>
<td>5,6</td>
<td></td>
<td>γl</td>
<td>PT54</td>
<td>V-A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Carcass</td>
<td>7,8</td>
<td></td>
<td>γl</td>
<td>PT54</td>
<td>V-A</td>
</tr>
<tr>
<td>06/05/02</td>
<td>MO/Fr.9264</td>
<td>Feces</td>
<td>9,10</td>
<td>str1, str2</td>
<td>γl</td>
<td>PT32V</td>
<td>I-A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Carcass</td>
<td>11,12</td>
<td></td>
<td>γl</td>
<td>PT32V</td>
<td>I-B</td>
</tr>
<tr>
<td>06/05/02</td>
<td>MO/Fr.8397</td>
<td>Feces</td>
<td>13</td>
<td>str2</td>
<td>γl</td>
<td>PT32</td>
<td>I-C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Feces</td>
<td>14</td>
<td>str2</td>
<td>γl</td>
<td>PT32V</td>
<td>I-A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Feces</td>
<td>15</td>
<td>str2</td>
<td>γl</td>
<td>PTNT</td>
<td>VI</td>
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<tr>
<td></td>
<td></td>
<td>Carcass</td>
<td>16,17</td>
<td></td>
<td>γl</td>
<td>PT54</td>
<td>V-A</td>
</tr>
<tr>
<td>07/10/02</td>
<td>VRa/Fr.7007</td>
<td>Feces</td>
<td>18,19</td>
<td>str2</td>
<td>γl</td>
<td>PT32V</td>
<td>III-C</td>
</tr>
<tr>
<td>07/10/02</td>
<td>RAb/I2.2867</td>
<td>Feces</td>
<td>20,21</td>
<td>str2</td>
<td>γl</td>
<td>PT32V</td>
<td>III-A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Feces</td>
<td>22,23</td>
<td></td>
<td>γl</td>
<td>PT32V</td>
<td>III-B</td>
</tr>
<tr>
<td>25/11/02</td>
<td>VRb/Fr.6563</td>
<td>Feces</td>
<td>24,25</td>
<td>str2</td>
<td>γl</td>
<td>PT54</td>
<td>V-B</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Carcass</td>
<td>26,27</td>
<td>str2</td>
<td>γl</td>
<td>PT54</td>
<td>V-B</td>
</tr>
<tr>
<td>25/11/02</td>
<td>RO/I2.9099</td>
<td>Feces</td>
<td>28,29</td>
<td>str2</td>
<td>γl</td>
<td>PT8</td>
<td>IV-C</td>
</tr>
<tr>
<td>25/11/02</td>
<td>RO/I2.8829</td>
<td>Feces</td>
<td>30</td>
<td>str2</td>
<td>γl</td>
<td>PT8</td>
<td>IV-A</td>
</tr>
<tr>
<td>25/11/02</td>
<td>RO/I2.2939</td>
<td>Feces</td>
<td>31</td>
<td>str2</td>
<td>γl</td>
<td>PT8</td>
<td>IV-B</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Feces</td>
<td>32</td>
<td>str2</td>
<td>γl</td>
<td>PT8</td>
<td>IV-A</td>
</tr>
</tbody>
</table>
Sequencing of eae genes. A fragment of the 3′ variable region of the eae gene of five Escherichia coli O157:H7 strains lacking genes encoding Shiga-toxins was amplified with universal eae primers EAE-F and EAE-RB and then sequenced. The sequenced fragment was 645 bp long and had 100% homology with the variable region of the eae-γ1 gene of the reference strain STEC-EDL933 O157:H7 (AF071034).

Discussion

Hemolytic uremic syndrome is the most severe clinical manifestation of infections caused by STEC O157:H7 and other serotypes. Thus, the incidence of HUS represents a robust index of the total prevalence of these infections in a popula-
tion. In Italy, from May 1988 to December 2000, 342 cases of HUS were reported to the health-surveillance authorities. Twenty-four cases were part of two outbreaks that occurred in northern Italy in 1992 and 1993; these were associated with STEC O111 and O157 infections, respectively. A third cluster of three cases associated with the O26 serogroup occurred in 1997 in Naples, in southern Italy [38]. The incidence observed in Italy was the lowest among those reported in Europe for the same period. This finding, together with the rare occurrence of outbreaks, suggests that STEC infections are relatively uncommon in Italy. Note, however, that in northern regions of Italy, where most cattle farms are located, the average annual incidence was similar to that of central European countries in the same period [38].

Many studies have indicated that cattle is the main reservoir of STEC O157 [4,6,10]. In Italy, the presence of E. coli O157 in cattle farms ranged from 0.5% to 17% [11,15,20]. Tests for STEC O157 in samples of minced beef in Italy showed percentages ranging from 0.4% to a maximum of 9% of STEC-positive samples [14,16,27,37]. In the present study, the prevalence of fecal carriage was 24%; of these, 20% of the samples were carriers of O157:H7 eae-stx+ and 6.7% of O157:H7 eae-stx−. The incidence of carcass contamination was 11%, with 4.4% of the animals contaminated with O157:H7 eae-stx+ and 6.7% with O157:H7 eae-stx−. Similar results were reported by Bonardi et al. [10,11] in two studies conducted in northern Italy. In particular, STEC O157 was isolated from the feces of 17% of feedlot cattle in the first study (1997–1998) and in 17% of the intestinal contents during the second study (1998–1999). In the latter, the rate of carcass contamination was also examined, and STEC O157 was isolated from 12% of the samples.

Unlike other E. coli isolates, STEC O157:H7 strains are negative for sorbitol fermentation (SOR−) within 24 h of incubation and do not show β-D-glucuronidase activity (GUD−). This enables their efficient differential selection from clinical samples and food products on sorbitol-containing MacConkey agar (SMAC) [5,13]. However, phenotypic variants of non-motile STEC O157:H− SOR+ and GUD+ (mainly of phage types 23 and 88) have been isolated in Germany, the Czech Republic, and Finland [23,34]. Motile SOR− and GUD− atypical STEC O157:H7 strains have been isolated in the USA [22] and Japan [31]. In the current study, none of the 88 E. coli O157:H7 isolates studied fermented sorbitol after 24 h incubation and all were β-glucuronidase-negative, but atypical biochemical features were observed in ten E. coli O157:H7 isolates obtained from two fecal samples, in that all ten isolates were urease positive. Similarly, Bonardi et al. [10] detected two STEC O157 GUD+ and seven urease− strains isolated from fecal cattle samples.

Nineteen variants of the eae gene were identified by intimin-type-specific PCR assays using oligonucleotide primers complementary to the 3′ end of the specific intimin genes that encode for the intimin types and subtypes α1, α2, β1, ξR/β2B, δ/κβ2O, γ1, θ/γ2, ε1, νR/ε2, ζ, η1, η2, η1, μR/ξ2, λ, μB, νB, and ζB, δ [5–9, 19]. As in previous studies [2–9,28,29], intimin γ1 was detected in all O157:H7 eae-positive isolates. The 88 O157:H7 isolates also possessed O157 rfbE and fliC type 7 genes.

At least 90 phage types have been reported for STEC O157:H7 [26,28], but only seven of those (PT2, PT4, PT8, PT14, PT21/28, PT32, and PT54) account for the majority (>75%) of human strains isolated in Europe and Canada [28]. Phage types PT2, PT8, and PT14 were predominant in human STEC O157:H7 strains in Italy, as is the case in many other European countries, including Belgium, Finland, Germany, England, Scotland, and Spain; PT14 was the most frequently phage type isolated in Canada. Note that the majority of O157:H7 isolates in the present study belonged to the most common phage types associated with severe human illnesses [28].

The pathotypes and phage types detected in the present study were quite different from those reported by Bonardi et al. [11] in studies carried out in northern Italy. Thus, among the 32 STEC O157 strains isolated by Bonardi et al. [11], ten carried both the stx1 and stx2 genes, 16 only stx1, and six only stx2. The strains belonged to six different phage types, but the most prevalent were PT21 and PT33. In our study, among the 88 E. coli O157:H7 isolates, 48 carried the stx2 gene, 37 were negative for both genes (but eae-γ1 positive), and three strains were positive for both stx1 and stx2 genes. The most prevalent phage types were PT8 (five animals), PT54 (five animals), and PT32v (four animals). We detected a high prevalence of stx-negative O157:H7 isolates. In Italy, Coneder et al. [16] reported the detection of an E. coli O157 stx eae− strain isolated from raw-cow’s-milk cheese, in a survey of 3879 samples of foodstuffs from different origins. The role of E. coli strains belonging to other pathogenic groups cannot be overlooked, especially since stx-negative E. coli O157:H7/H− variants have been isolated from human cases of diarrhea and HUS [36].

We isolated three different O157:H7 strains from a fecal sample, and up to four strains from the same animal (fecal and carcass) could be distinguished. This finding provides evidence of the epidemiological importance of subtyping more than one isolate from the same sample. Used together, phage typing and PFGE proved to be very efficient tools to detect auto- and cross-contamination among carcasses. These techniques should therefore be included in HACCP (Hazard Analysis and Critical Control Points) programs at abattoirs.
The same PFGE-phage-type \textit{E. coli} O157:H7 profile (PT54-PFGE-VA) was detected in fecal and carcass samples from one animal (MO/FR1274), and also in two carcasses of two other animals (MO/FR9264, MO/FR8397) slaughtered the same day (6/05/02). Bonardi et al. [11] also used PT-PFGE profiles to confirm the dissemination of STEC O157 during the slaughter process. Similar to our findings, they detected globally high diversity among 25 STEC O157:H7 strains examined, with ten PFGE patterns. Heterogeneity was also observed by Avery et al. [1], even among 51 related \textit{E. coli} O157 isolates associated with beef cattle from a single-farm-to-single abattoir. These isolates produced 11 different PFGE profiles, which could be divided into four clonal groups. In our study, 32 strains produced 14 PFGE patterns clustered in seven groups.

Modern concepts of meat safety assurance depend on the reliable traceability of both animals and meats. Since food-borne pathogens, including \textit{E. coli} O157:H7, can enter the retail food chain, the safety of meat and other foods depends on the ability to trace pathogens implicated in food contamination. Thus, careful attention needs to be paid to the potential points of meat contamination (HACCP), i.e., hygiene and decontamination in abattoirs, storage time and temperature control, distribution centers, and final processing and handling of the food.

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