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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 were collected from the colon immediately after slaughter, and carcass samples were taken following the excision method described by the Commission Decision of 8 June 2001 (2001/471/EC). Four tissue samples, representing a total of 20 cm^2, were obtained from the carcass after dressing but before chilling by using a sterile blade to cut 5-cm^2 slices from the rump, flank, brisket, and neck. Each pool of samples representative of one carcass were placed aseptically into a plastic dilution bag, and 25 ml of Cary Blair transport medium was added. All specimens were cooled at 4ºC and transported to the laboratory.

Isolation and identification of *E. coli* O157. The samples were examined for the presence of *E. coli* O157 using the MFLP-80 (isolation of *E. coli* in foods) and MFLP-90 (identification of *E. coli* by DynaBeads anti-*E. coli* O157) procedures of the Bureau of Microbial Hazards, Food Directorate (Health Canada). This procedure is fully described in [http://www.hc-sc.gc.ca/fn-an/securit/ill-intox/ecoli/ecoli_0157_e.html]. Briefly, a 25-g sample of feces or beef was enriched in 225 ml of modified tryptic soy broth with novobiocin (mTSB-n) and incubated at 42ºC for 24 h. After enrichment, 1 ml of culture broth was treated by inmunomagnetic separation (IMS) (Dynabeads anti-*E. coli* O157, Dynal, Oslo, Norway) following the manufacturer's instructions. Two plates of sorbitol MacConkey agar (SMAC) and modified hemorrhagic coli agar (mHC) with tellurite, cefsulodin, and MUG were seeded with IMS-enriched material. The plates were incubated at 42ºC for 24 h. Up to ten typical *E. coli* colonies were selected, especially those sorbitol-nonfermenting and β-glucuronidase negative, subcultured and tested for indole production, cellobiose fermentation, and urea. The selected colonies were subjected to slide agglutination with latex particles sensitized with *E. coli* O157 antibodies (Dry Spot *E. coli* O157, Oxoid, UK). The agglutinating cultures were confirmed biochemically as *E. coli* with the API 20E system (BioMérieux, France).

Serotyping and phage typing. O and H antigens were determined by the method of Guinée et al. [21], employing O157 and H1 to H56 antisera. Phage typing was done according to the method of Khakria et al. [26], using phages provided by The National Laboratory for Enteric Pathogens (Laboratory for Disease Control, Ottawa, Ontario, Canada). The 16 different phages used in the assay were able to identify 90 phage types.

PCR *stx_1*, *stx_2*, *ehxA*, *eae*, *O157 rfbE* and *fliCh7* genes. All *E. coli* O157 isolates were tested as described elsewhere [5,6] with primers specific for the genes encoding Stx_1 and Stx_2 toxins (*stx_1* and *stx_2* genes) [5,6], EHEC-hemolysin (*ehxA* gene) [35], intimin (*eae* gene and *eae*-γ1 variant gene) [7–9], O157 (O157 *rfb*E gene) [17], and H7 (*fliC*h7 gene) [18] antigens (Table 1). Bacteria were harvested from tryptone soy agar, suspended in 250 µl of sterile water, incubated at 100ºC for 5 min to release the DNA, and centrifuged. The supernatant was used in the PCR reaction as described below. Bacterial DNA was amplified using 30-µl volumes containing 7 µl of the prepared sample supernatant; 150 ng of oligonucleotide primers; 0.2 mM (each) dATP, dGTP, dCTP, and dTTP; 10 mM Tris-HCl (pH 8.8); 1.5 mM $MgCl_2$; 50 mM KCl, and 1 U of Biotaq DNA polymerase (Bioline, UK). The PCR conditions were 94ºC for 2 min for initial denaturation of DNA within the sample followed by 35 cycles of 94ºC for 1 min (denaturation), 55ºC for 1 min (primer annealing), and 72ºC for 1 min (DNA synthesis). PCR was carried out with a thermal cycler (model PCR express, Hybaid, UK) and the amplified products were visualized by standard submarine gel electrophoresis using 10 µl of the final reaction mixture on a 2% agarose gel in TBE buffer (89 mM Tris, 89 mM boric acid, 2.5 mM EDTA). The samples were electrophoresed for 20–40 min at 130 V. Amplified DNA fragments of specific sizes were located by UV fluorescence after staining with ethidium bromide. Molecular size markers (*Hae*III digest of ϕx174DNA) (Promega) were included in each gel.

Pulsed-field gel electrophoresis. PFGE was done as described in [32]. PFGE allowed the relatedness and diversity among a representative group of 32 *E. coli* O157:H7 strains to be established. Isolates with different PCR profiles from individual samples were included for subtyping. When two or more colonies of *E. coli* O157 with similar PCR profile were isolated from individual samples, two isolates representative of each pathotype were chosen.

Sequencing of *eae* genes. A fragment of the 3′ variable region of the *eae* gene of five *Escherichia coli* O157:H7 isolates lacking genes encoding Shiga-toxins was amplified with universal *eae* primers EAE-F and EAE-RB and then sequenced. The nucleotide sequences of the amplification products were analyzed by the dideoxynucleotide triphosphate chain-termination method of Sanger, with the BigDye Terminator v3.1 Cycle Sequencing Kit and an ABI 3100 Genetic Analyzer (Applied Bio-Systems). The *eae*-γ1 sequences of the isolates were deposited in the European Bioinformatics Institute (EMBL Nucleotide Sequence Database) and assigned accession numbers AJ829719 to AJ829723.

***Escherichia coli* control strain.** STEC strain EDL-933, which is positive for the genes *stx_1*, *stx_2*, *ehxA*, *eae*-γ1, O157 *rfb*E, and *fliC*h7, was used as control.

Table 1. Primer sequences and lengths of PCR amplification products

Gene	Primer	Oligonucleotide sequence (5´–3´)	Fragment size (bp)	Reference
stx$_1$	VT1-A VT1-B	CGCTGAATGTCATTCGCTCTGC CGTGGTATAGCTACTGTCACC	302	Blanco et al. [5]
stx^2	VT2-A VT2-B	CTTCGGTATCCTATTCCCGG CTGCTGTGACAGTGACAAAACGC	516	Blanco et al. [5]
ehxA	HlyA1 HlyA4	GGTGCAGCAGAAAAAGTTGTAG TCTCGCCTGATAGTGTTTGGTA	1551	Schmidt et al. [35]
eae[a]	EAE-1 EAE-2	GGAACGGCAGAGGTTAATCTGCAG GGCGCTCATCATAGTCTTTC	346	Mora et al. [30]
eae[b]	EAE-F EAE-RB	ATTACTGAGATTAAGGCTGAT ATTTATTTGCAGCCCCCCAT	682	Blanco et al. [5]
eae-γ1	EAE-FB EAE-C1	AAAACCGCGGAGATGACTTC AGAACGCTGCTCACTAGATGTC	804	Blanco et al. [5]
O157 *rfb*E	O157-AF O157-AR	AAGATTGCGCTGAAGCCTTTG CATTGGCATCGTGTGGACAG	497	Desmarchelier et al. [17]
*fli*Ch7	H7-F H7-R	GCGCTGTCGAGTTCTATCGAGC CAACGGTGACTTTATCGCCATTCC	625	Gannon et al. [18]

[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.

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 *rfb*E gene. In addition, 88 of those 96 *E. coli* O157 isolates were positive by PCR for the *fli*Ch7 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 *fli*Ch7 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 *stx*$_1$, *stx*$_2$, *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*$^+$ *stx*$^+$ and *E. coli* O157:H7 *eae*$^+$ *stx*$^-$. Table 2 shows the prevalence of the two pathotypes. Among the 88 *E. coli* O157:H7 strains isolated, 48 carried the *stx*$_2$ gene, 37 were negative for both genes, and three strains were both *stx*$_1$ and *stx*$_2$. The eight sorbitol-fermenting β-glucuronidase positive *E. coli* O157:H12 isolates were negative for all virulence genes (*stx*$_1$, *stx*$_2$, *eae*, *ehxA*).

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

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

Fecal samples			
STEC O157:H7	*eae*+ *stx*+	9/45	20%
E. coli O157:H7	*eae*+ *stx*$^-$	3/45	6.7%
Carcass samples			
STEC O157:H7	*eae*+ *stx*+	2/45	4.4%
E. coli O157:H7	*eae*+ *stx*$^-$	3/45	6.7%

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/It2867, RO/It2939) 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 3. Characteristics of *E. coli* O157:H7 isolates

Date	Farm/animal code	Origin	Isolate code	*stx* genes	Intimin type	Phage types	PFGE profile
22/01/02	RAa/It..6150	Carcass	1,2	stx_1 stx_2	γ1	PT8	VII
22/01/02	PI/Fr..0073	Feces	3	stx_1 stx_2	γ1	PT8	VII
18/02/02	MN/Fr..1133	Feces	4		γ1	PT54	II
06/05/02	MO/Fr..1274	Feces	5,6		γ1	PT54	V-A
		Carcass	7,8		γ1	PT54	V-A
06/05/02	MO/Fr..9264	Feces	9	stx_2	γ1	PT 32V	I-A
			10	stx_2	γ1	PT 32V	I-B
		Carcass	11,12		γ1	PT54	V-A
06/05/02	MO/Fr...8397	Feces	13	stx_2	γ1	PT 32	I-C
		Feces	14	stx_2	γ1	PT 32V	I-A
		Feces	15	stx_2	γ1	PT NT	VI
		Carcass	16,17		γ1	PT54	V-A
07/10/02	VRa/Fr..7007	Feces	18,19	stx_2	γ1	PT 32V	III-C
07/10/02	RAb/It..2867	Feces	20,21	stx_2	γ1	PT 32V	III-A
		Feces	22,23		γ1	PT 32V	III-B
25/11/02	VRb/Fr..6563	Feces	24,25	stx_2	γ1	PT54	V-B
		Carcass	26,27	stx_2	γ1	PT54	V-B
25/11/02	RO/It..9099	Feces	28,29	stx_2	γ1	PT 8	IV-C
25/11/02	RO/It..8829	Feces	30	stx_2	γ1	PT 8	IV-A
25/11/02	RO/It..2939	Feces	31	stx_2	γ1	PT 8	IV-B
			32	stx_2	γ1	PT 8	IV-A

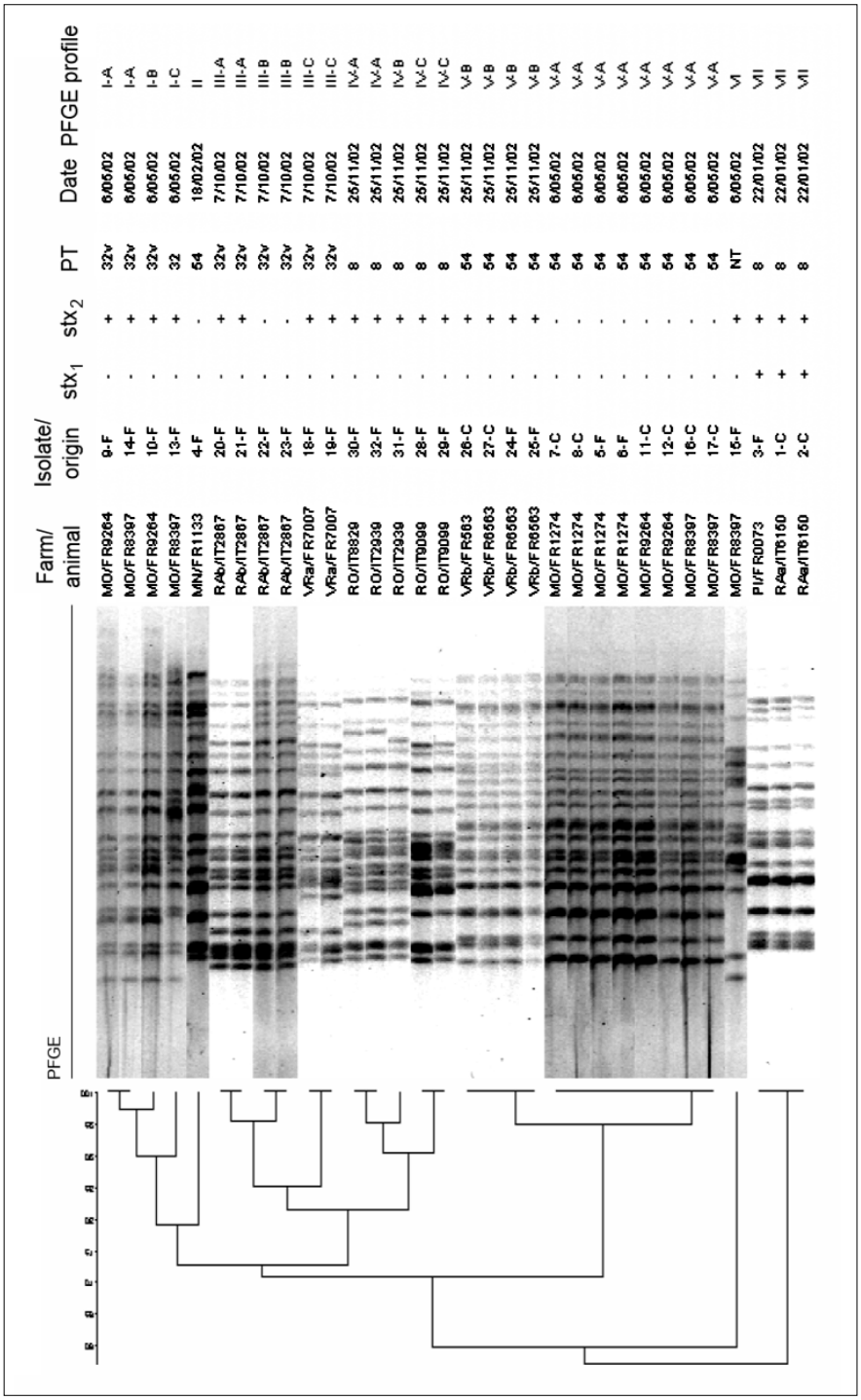

Fig. 1. Dendrogram generated by Bionumeric software, showing the relationships between *E. coli* O157:H7 isolates generated restricted with *Xba*I. The phenogram was constructed using the Dice coefficient and UPGMA analysis. The degree of similarity (%) is shown on the scale.

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/κ/β2O, γ1, θ/γ2, ε1, νR/ε2, ζ, η1, η2, ι1, μR/ι2, λ, μB, νB, and ξB, o [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 *rfb*E and *fliC*h7 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 stx_1 and stx_2 genes, 16 only stx_2, and six only stx_1. 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 stx_2 gene, 37 were negative for both genes (but *eae*-γ1 positive), and three strains were positive for both stx_1 and stx_2 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, Conedera 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 Analyis and Critical Control Points) programs at abattoirs.

The same PFGE-phage-type *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 *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 *E. coli* O157:H7, can enter the retail food chain, the safety of meat and other foods depends on the ability to trace pathoges 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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