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# Detection of *Escherichia coli* O157 and *Escherichia coli* O157:H7 by the immunomagnetic separation technique and *stx1* and *stx2* genes by multiplex PCR in slaughtered cattle in Samsun Province, Turkey

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**This study was conducted to investigate the presence of *Escherichia* (*E.*) *coli* O157 and *E. coli* O157:H7 and *stx1* and *stx2* genes on cattle carcasses and in rectal samples collected from Samsun Province of Turkey. A total of 200 samples collected from cattle carcasses and the rectal contents of 100 slaughtered cattle from two commercial abattoirs were tested using the immunomagnetic separation technique and multiplex PCR methods. *E. coli* O157 and *E. coli* O157:H7 were detected in 52 of the 200 samples (26%) tested. Of the positive samples, 49 were *E. coli* O157 and three were *E. coli* O157:H7. The *E. coli* O157 strain was isolated from 24 carcasses and 25 rectal samples, while *E. coli* O157:H7 was isolated from two carcasses and one rectal sample. Of the 49 samples positive for *E. coli* O157, 32 were from the rectal and carcass samples of the same animal, while two *E. coli* O157:H7 isolates were obtained from rectal swabs and carcasses of the same animal. The *stx1* and *stx2* genes were both detected in 35 *E. coli* O157 isolates and one *E. coli* O157:H7 isolate, but the *stx2* gene was only detected alone in two *E. coli* O157 isolates. Overall, 16 carcasses tested positive for *E. coli* O157 and one carcass tested positive for *E. coli* O157:H7 based on both carcass and rectal samples. Overall, the results of this study indicate that cattle carcasses pose a potential risk to human health due to contamination by *E. coli* O157 and *E. coli* O157:H7 in the feces.**

**Keywords:** carcass, cattle, *E. coli* O157, *E. coli* O157:H7, rectum, *stx1*, *stx2*

## Introduction

Enterohemorrhagic *Escherichia* (*E.*) *coli* strains are a subset of the Shiga toxin-producing *E. coli* (STEC) that cause diseases in humans and pose a threat to public health worldwide [19,20]. Many environmental and food sources have caused *E. coli* O157 or *E. coli* O157:H7 infections, but they are primarily attributed to consumption of food of animal origin, especially cattle [36], or to direct or indirect contact with cattle or other farm animals [24]. Human infection by *E. coli* O157:H7 has been reported in over 30 countries, and cattle appear to be the chief source of infection. Indeed, many outbreaks have been linked to beef consumption [8,10,26]. Cattle and other ruminants have been established as major natural reservoirs of *E. coli* O157 [32] and play a significant role in the epidemiology of human infections [20]. Specifically, between 1% and 35.8% of cattle in the United Kingdom, and the United States were estimated to be contaminated with *E. coli* O157 [8,15,25,34].

*E. coli* O157 and *E. coli* O157:H7 are present in the intestines of cattle as a component of the native microbiota and they can contaminate both the meat and the slaughterhouse environment. As a result, contamination of both carcasses and the environment by *E. coli* O157 and O157:H7 from the intestinal contents of cattle during slaughter is one of the most significant risk factors in transmission to humans [26,31]. Hide removal operations potentially constitute another very important source of cattle carcass contamination. Therefore, feces and hide removal are considered to be the main sources of *E. coli* O157 and *E. coli* O157:H7 contamination of carcasses during slaughter [12,25]. Contamination of carcass meat with *E. coli* O157 and *E. coli* O157:H7 can occur during dressing, primarily during the skinning, but also during the evisceration phase. Once the *E. coli* is transferred to the carcass surface, handling and trimming operations can spread the pathogen to the beef trimmings [17].

The pathogenicity of *E. coli* O157 and *E. coli* O157:H7, including STEC, is associated with several virulence factors. The main factor contributing to their pathogenicity is their capacity to produce two potent phage-encoded

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cytotoxins called Shiga-toxins (namely, Stx1 and Stx2). Shiga toxins cause diseases such as hemorrhagic colitis and hemolytic uremic syndrome through cytopathic effects on the vascular endothelial cells of the kidneys, intestines, central nervous system and other organs [16]. In addition to the production of toxins, another virulence-associated factor expressed by STEC is a protein called intimin, which is encoded by the *eae* gene and responsible for the intimate attachment of STEC to the intestinal epithelial cells. The role of other virulence genes through the production of enzymes such as enterohemolysin, an extracellular serine protease, and a catalase/peroxidase in causing infection appears to be minor [23].

According to studies reported from various parts of the world, cattle carcasses carry a potential risk of the presence of *E. coli* O157 and *E. coli* O157:H7 through fecal contamination [5,7,22,25,35,38,39]. There have been many studies conducted to determine *E. coli* O157 and *E. coli* O157:H7 in only carcass or rectal samples at abattoirs worldwide, including Turkey [5,7,22,41]. However, to the best of our knowledge, there has been no attempt to detect *E. coli* O157 and *E. coli* O157:H7 in both rectal and carcass samples from the same animal. Therefore, this study was conducted to investigate the presence of both *E. coli* O157 and *E. coli* O157:H7 and to detect the presence of the *stx1* and *stx2* genes in isolates from both cattle carcasses and their rectal samples obtained from two commercial abattoirs located in the Samsun Province of Turkey.

## Materials and Methods

In this study, a total of 200 swab samples obtained from 100 slaughtered cattle were tested to investigate the presence of *E. coli* O157 and *E. coli* O157:H7, as well as to detect the presence of *stx1* and *stx2* genes in the isolates. Swab samples were taken from 100 cattle carcasses and their rectal contents at two commercial abattoirs located in Samsun Province, Turkey between December 2007 and March 2008. Samples were collected immediately after removal of the hide (dressing step) from the carcass. Each carcass surface sample consisted of two pooled neck and rump subsamples. An area of 200 $cm^2$ of the neck and rump regions of the carcass was swabbed (100 $cm^2$ - 10 × 10 cm - of each region) using two different sterile cotton swabs [37]. For isolation, the swabs were placed in a modified tryptone soy broth (mTSB-Oxoid-CM 989; Basingstoke, England) supplemented with novobiocine (20 mg/L, N1628; Sigma, USA) in 10 mL tubes and then incubated at 41.5$^o$C for 24 h [35]. The two tubes from one carcass were mixed and evaluated as one carcass sample. A total of 100 rectal samples were collected and processed in the same manner as the carcass samples. Immunomagnetic separation (IMS) was conducted using immunomagnetic beads coated with an anti-*E. coli* O157 antibody (Dynabeads anti-*E. coli* O157; Dynal A.S., Norway) according to the manufacturer's instructions. The 50 µL IMS bead complex finally recovered was spread onto tellurite (2.5 mg/L) - cefixime (0.05 mg/L) - sorbitol MacConkey (TC-SMAC) agar (Oxoid-CM 813, Supl.SR 172 E; Basingstoke, England) and incubated at 37$^o$C for 24 h. Up to five colonies exhibiting typical presumptive positive characteristics of *E. coli* O157 colonies were identified, subcultured onto yeast extract-tripticase soy agar (Oxoid-CM 131-L21; Basingstoke, UK), incubated for 24 h at 37$^o$C and subjected to the confirmatory tests described below:

### Confirmatory testing for *E. coli* O157 and *E. coli* O157:H7

The pinpoint indol test was conducted, after which indol test positive colonies were streaked onto 4-methylumbelliferly-β-D-glucuronide sorbitol MacConkey (MUG-SMAC) agar (Oxoid-BR 071 E; Basingstoke, England) and incubated overnight at 37$^o$C. Colonies displaying no fluorescence when illuminated with 366 nm UV light were assumed to be exhibiting typical characteristics of *E. coli* O157. The cellobiose test was subsequently conducted in purple broth base (Difco-0227-01-6; Difco, USA). Only colonies that tested negative to the cellobiose fermenting test were selected for further processing.

All sorbitol non-fermenting, indole-positive, MUG-negative, cellobiose-negative colonies were cultured on TSA and incubated at 37$^o$C for 24 h. The isolates were then examined by latex agglutination with *E. coli* O157 (#210753; Denka-Sheiken, Japan) and O157:H7 antisera (#211057; Denka-Sheiken, Japan). Colonies exhibiting a positive precipitation reaction with the O157 antiserum

**Table 1.** Oligonucleotide primers sequences used in multiplex PCR for amplification of *stx1* and *stx2* genes

| Target gene | Sequence (5´-3´) | Amplification product size (bp) | References |
|---|---|---|---|
| *stx1*F | ATAAATCGCCATTCGTTGACTAC | 180 | [30] |
| *stx1*R | AGAACGCCCACTGAGATCATC | | |
| *stx2*F | GGCACTGTCTGAAACTGCTCC | 255 | [30] |
| *stx2*R | TCGCCAGTTATCTGACATTCTG | | |

were identified as *E. coli* O157, while colonies showing a positive precipitation reaction with the H7 antiserum were identified as *E. coli* O157:H7.

## Determination of virulence genes (*stx1* and *sxt2*)

Multiplex PCR was conducted to detect the presence of the shiga-toxin genes (*stx1* and *stx2*) in *E. coli* O157 and *E. coli* O157:H7 isolates were trated according to a modified version by using multiplex PCR assays [14,30]. The primers and PCR conditions used in this study are shown in Table 1. *E. coli* O157:H7 ATCC 43895 was used as a reference strain.

DNA extractions of strains were conducted using the boiling method. Briefly, each positive colony was inoculated on TSA and incubated for 24 h at 37°C. Two colonies were then selected and suspended separately in 500 µL of sterile distilled water in microcentrifuge tubes, after which they were incubated at 95°C for 10 min in a water bath (Memert, Germany). The tubes were then centrifuged (Hettich-Universal-320R unit) at 9,503 × g for 10 min, after which the supernatant containing the DNA to be used as the template DNA was transferred into Dnase/Rnase-free microcentrifuge tubes. The extracted DNA samples were stored at −20°C until use.

To detect the *stx1* and *stx2* genes, 10 µL of extracted DNA was used as a template in a reaction mixture with a final volume of 50 µL that contained 200 mM of each deoxynucleoside triphosphate (dNTP), 250 nM *stx1* primer, 250 nM *stx2* primers, 1U of Taq DNA polymerase in 1× PCR buffer and 2 mM of $MgCl_2$. The amplification of DNA (MJ Mini; BioRad, USA) was conducted as follows using a thermocycler (MJ Mini-PTC-1148; BioRad, USA): initial denaturation at 94°C for 10 min, 35 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, extension at 72°C for 1 min, and final extension at 72ºC for 7 min [14].

For gel electrophoresis, the 20-µL amplicon mixtures were supplemented with 4-µL of loading dye and loaded onto a 2.0% agarose gel containing ethidium bromide (Gene choice). Electrophoresis (Power Pac-Basic; Bio-Rad, USA) was then conducted at 90 V for 1.5 h. A 100 ~ 1,000 bp molecular weight marker was used to identify the amplified products, which were visualized by UV illumination (WiseUV-Wuv-L50; Daihan Scientific, Korea). The nucleotide sequences and predicted product sizes of the primers are shown in Table 1.

# Results

In the present study, *E. coli* O157 or O157:H7 was detected in 52 of 200 samples tested (49 *E. coli* O157 and 3 *E. coli* O157:H7). The *E. coli* O157 strain was isolated from 24 carcasses and 25 rectal samples, while the *E. coli* O157:H7 strain was isolated from two carcasses and one rectal sample.

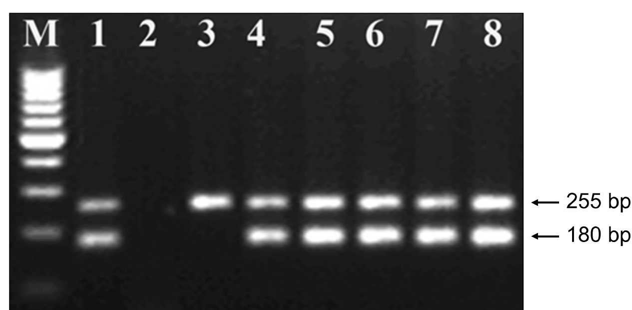


**Fig. 1.** Amplification products of *stx1* and *stx2* genes in *E. coli* O157 and O157:H7 identified by multiplex PCR. Lane M: Marker (100 ~ 1,000 bp).

**Table 2.** Isolation of *E. coli* O157 and *E. coli* O157:H7 strains from slaughtered cattle and detection of *stx1* and *stx2* genes

| Sampling site (No. of samples) | *E. coli* O157 n (%) | *E. coli* O157:H7 n (%) | *stx1* gene in *E. coli* O157 n (%) | *stx2* gene in *E. coli* O157 n (%) | Both *stx1* and *stx2* genes in same *E. coli* O157 n (%) | Both *stx1* and *stx2* genes in same *E. coli* O157:H7 n (%) |
|---|---|---|---|---|---|---|
| Carcass (n = 100) | 8 (8.0) | 1 (1.0) | 0 (0.0) | 2 (2.0) | 3 (3.0) | 1 (1.0) |
| Rectum (n = 100) | 9 (9.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 3 (3.0) | 0 (0.0) |
| Carcass and rectum (n = 200) | 16 (8.0) | 1 (0.5) | 0 (0.0) | 0 (0.0) | 13 (7.5)<br>*1 carcass, †2 rectum<br>(14 carcasses, 15 rectum) | 0 (0.0) |
| Total cattle carcass samples (n = 100) | 24 (24.0) | 2 (2.0) | 0 (0.0) | 2 (2.0) | 17 (17.0) | 1 (1.0) |
| Total rectal samples (n = 100) | 25 (25.0) | 1 (1.0) | 0 (0.0) | 0 (0.0) | 18 (18.0) | 0 (0.0) |
| Total number (n = 200) | 49 (24.5) | 3 (1.5) | 0 (0.0) | 2 (1.0) | 35 (17.5) | 1 (0.5) |

**stx1* and *stx2* genes present in *E. coli* O157 isolated from carcass alone. †*stx1* and *stx2* genes present in *E. coli* O157 isolated from rectum alone.

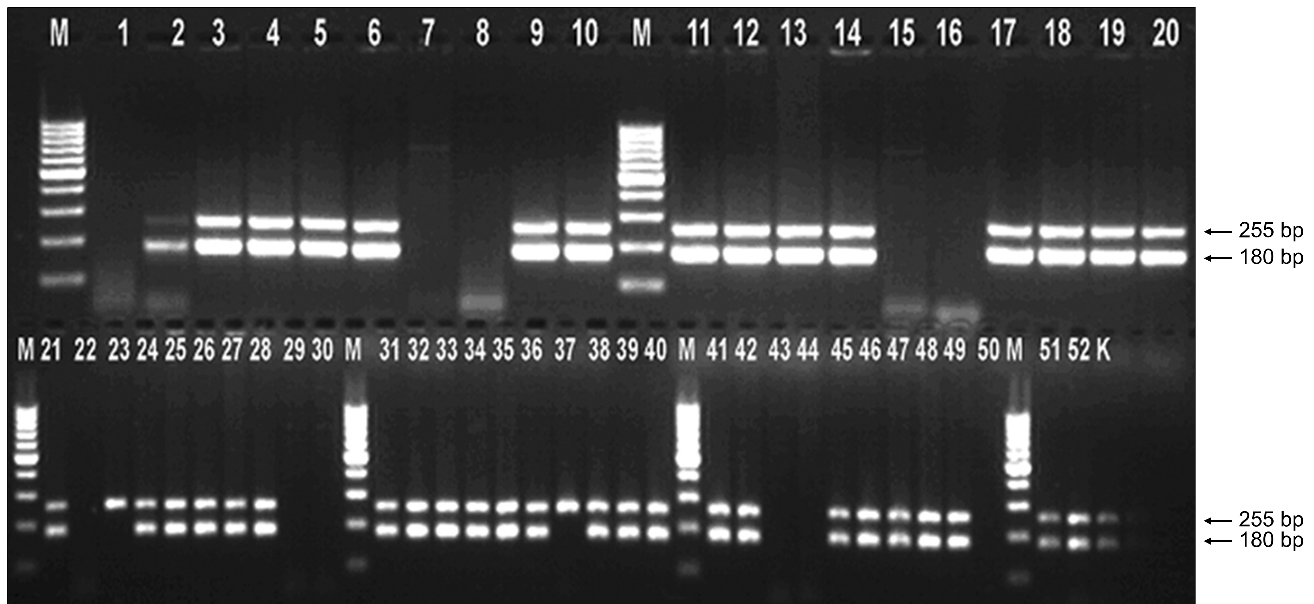


**Fig. 2.** The determination of *stx1* and *stx2* genes of *E. coli* O157 and O157:H7 by multiplex PCR. Lane M: Marker (100～1,000 bp), Lane K: positive control (*E. coli* O157:H7 ATCC 43895).

Of the 49 samples that contained *E. coli* O157, 32 were from the rectal and carcass samples of the same animal, while the other 17 isolates were all from different cattle. Specifically, eight were from carcasses and the remaining nine were from rectal samples. Two of the *E. coli* O157:H7 isolates were obtained from the carcass and rectal swabs of the same animal, while the remaining isolate was obtained from a carcass sample (Table 2).

The results of multiplex PCR for the detection of *stx1* and *stx2* genes are shown in Fig. 1. While both the *stx1* and *stx2* genes were detected in 35 *E. coli* O157 isolates from 17 carcass and 18 rectal samples, they were detected in only one *E. coli* O157:H7 isolate from a carcass sample. The *stx2* gene alone was detected in only two *E. coli* O157 isolates and the *stx1* gene was not detected alone in any isolates. Neither of these genes was detected in the 12 *E. coli* O157 and two *E. coli* O157:H7 isolates from carcass and rectal samples (Figs. 1 and 2).

## Discussion

In Turkey and other parts of the world, although there have been many studies conducted to determine the presence of *E. coli* O157 and *E. coli* O157:H7 in either carcass or rectal samples at abattoirs, the authors of the present study are unaware of any attempts to detect *E. coli* O157 and *E. coli* O157:H7 in both the rectal and carcass samples of the same animal. Therefore, the present study was conducted to investigate the contamination of carcasses and their rectal contents with *E. coli* O157 and *E. coli* O157:H7 at two abattoirs in Samsun Province of Turkey because the majority of food-borne *E. coli* O157 and *E. coli* O157:H7 infections in humans occur after the consumption of contaminated beef and cattle products [31].

Isolation rates of *E. coli* O157 and *E. coli* O157:H7 from bovine carcasses and feces ranging from low (0.39%) to high (17.0%) have been reported for Mexico, Ireland, Belgium, England, France, Poland, Germany, the United States, Turkey and other countries. *E. coli* O157 or *E. coli* O157: H7 isolates obtained from cattle carcasses or feces have also been found to contain at least one of the *stx1*, *stx2*, *eaeA*, *hylA* and $fliC_{h7}$ genes [5,7,22,25,29,35,38,39, 41]. In contrast to other countries, there has been only one study of carcasses reported in Turkey [21]. The results of that study revealed that 3.9% and 2.4% of the bovine carcasses were contaminated by *E. coli* O157 and *E. coli* O157:H7, respectively. There have been a few studies of cattle feces conducted in Turkey [1,3,9,41], the results of which revealed that *E. coli* O157 or *E. coli* O157:H7 were present in 1.28% and 13.6% of the samples, respectively. It has also been reported that at least one virulence gene (*stx1*, *stx2* or *eae* genes) was detected in the *E. coli* O157 or *E. coli* O157:H7 isolates from feces samples [1,3,41].

In the present study, the *E. coli* O157 strain was isolated from 24 carcasses (24%) and 25 rectal samples (25%), while the *E. coli* O157:H7 strain was isolated from two carcasses (2%) and one rectal sample (1%). These values for *E. coli* O157 were higher than those of previously conducted studies. However, previously conducted studies show a wide range of isolation ratios for *E. coli* O157 and *E. coli* O157:H7. This variation may be due, at least in part, to the sensitivity of the method, diverse geographical

origins of cattle, numbers of cattle, study design, number of herds and cattle, sex and age of cattle, season, abattoir conditions and treatment with antimicrobial substances during the process [6,7,39]. It has also been reported that the prevalence of *E. coli* O157 and *E. coli* O157:H7 varies with the seasons, generally increasing in the warm months of March-September in the northern hemisphere [4,6, 7,25,39]. Another important factor influencing the identification of individual strains of *E. coli* is the isolation method. Indeed, the detection of *E. coli* O157:H7 from cattle fecal samples is known to be very difficult due to their low concentration. Therefore, direct inoculation of samples onto plates is not sensitive enough. Several enrichment culturing methods and isolation methods have been developed to counter this problem [38]. One of the more sensitive methods is the IMS technique [28,38,40], which is why enrichment/IMS procedures were employed in the present study. Another study found that the IMS technique was superior to the classic culture technique for *Salmonella* isolation (unpublished data). In the present study, swab samples were collected from the rectum of the cattle instead of direct feces samples, because the recto-anal junction mucosa has been identified as the primary site of *E. coli* O157:H7 colonization in cattle [18,33,35]. In attempting to manage *E. coli* O157 and *E. coli* O157:H7 contamination in abattoirs, it is crucial to consider cross contamination during slaughter. *E. coli* O157 and *E. coli* O157:H7 have been reported to spread easily onto carcass surfaces from the hide or during evisceration [2,12,21]. The results of the present study support that contention, with many rectal and carcass samples of the same animal being positive.

Epidemiological studies in cattle indicate that the horizontal transmission of *E. coli* O157 or *E. coli* O157:H7 occurs in groups of animals, and that contaminated water may facilitate its spread and persistence within herds [13,15]. Therefore, control of the spread of *E. coli* O157 and *E. coli* O157:H7 at the farm level becomes very important. Moreover, cross contamination may occur during the slaughter of cattle and other processes at abattoirs.

The results of the present study indicate that meat from cattle poses a risk to human health in Turkey because of potential *E. coli* O157 and *E. coli* O157:H7 contamination. To minimize the risk to public health, implementation of the HACCP system in abattoirs is recommended.

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