

The prevalence, virulence factors and antibiotic resistance of *Escherichia coli* O157 in feces of adult ruminants slaughtered in three provinces of Turkey

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ABSTRACT

In the present study the prevalence, presence of *Stx1*, *Stx2*, *EhlyA* and *eaeA* virulence genes and antibiotic resistance of *Escherichia coli* O157 strains isolated from the feces of 417 adult ruminants slaughtered in three provinces of Turkey were investigated. A total of 16 (3.8%) *E. coli* O157 strains were isolated from 417 fecal samples. Among these strains 9 (3.3%), 4 (7.8%), 2 (2.4%) and 1 (7.1%) were obtained from 269 cattle, 51 water buffaloes, 83 sheep and 14 goats, respectively. All strains were screened for the presence of *rjbO157*, *fliCH7*, *Stx1*, *Stx2*, *eaeA* and *EhlyA* genes by PCR. The *rjbO157* gene was determined in all strains, while 7 (43.8%) of 16 strains harbored *fliCH7*, *Stx2*, *EhlyA* and *eaeA* genes. *eaeA* gene was obtained from 11 (68.8%) strains, 4 (25.0%) of these were alone. The *Stx1* gene was not determined in any of the 16 strains and 5 (31.2%) strains were also negative for *fliCH7*, *Stx2*, *EhlyA* and *eaeA* genes. High resistance rates were determined against ampicillin (68.7%), neomycin (68.7%), tetracycline (68.7%), trimethoprim/sulfamethoxazole (62.5%) and amoxicillin/clavulanic acid (56.2%) in 16 *E. coli* O157 strains isolated in this study. The present study investigated the presence of *E. coli* O157 serotype, its major virulence genes and antibiotic resistance in the strains isolated from the feces of different slaughtered ruminants, including cattle, water buffalo, sheep and goats for the first time in Turkey, and showed the water buffaloes, sheep and goats, like cattle, may be a potential reservoir of *E. coli* O157:H7 infections for humans.

Key words: abattoir; antibiotic resistance; *E. coli* O157; ruminant; virulence genes

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Introduction

Escherichia coli O157:H7/H- is the zoonotic agent most frequently isolated from food-borne outbreaks and severe human diseases such as hemorrhagic colitis (HC), hemorrhagic or non-hemorrhagic diarrhea, hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) worldwide (KARMALI et al., 2010). Healthy cattle are the main asymptomatic reservoir for *E. coli* O157:H7/H- (CHAPMAN et al., 2001), but this serotype has also been isolated from asymptomatic sheep, goats (MERSHA et al., 2010) and water buffaloes (ŞEKER and YARDIMCI, 2008). Transmission of *E. coli* O157:H7/H- from animals to humans occurs principally via contamination of foods by animal feces. Contact with animal feces is also accepted to be a strong risk factor for sporadic *E. coli* O157:H7/H- cases (KARMALI et al., 2010).

Shiga toxin 1 and 2 (encoded by the genes, *Stx1* and *Stx2*), intimin (encoded by the gene, *eaeA*) and the plasmid-encoded enterohemolysin (encoded by the gene, *EhlyA*) have been reported to be significant virulence determinants in the pathogenesis of *E. coli* O157:H7/H- infections (KARMALI et al., 2010). While Shiga toxins are responsible for the inhibition of protein synthesis, the death of host cells and the development of HC and HUS (BERGAN et al., 2012), intimin, which is a protein, is associated with attaching and effacing (A/E) lesions in the intestinal mucosa (DEAN-NYSTROM et al., 1997). Although the influence of enterohemolysin in the pathogenesis remains unclear, it has been considered that enterohemolysins may be used as the epidemiological marker for Shiga toxin-producing *E. coli* (STEC) strains (FARROKH et al., 2013).

Antimicrobial agents are routinely used for disease prevention, in addition to their therapeutic usage. However, antibiotic usage for treating STEC O157:H7/H- infections is still controversial among researchers. While some authors consider that antimicrobials may cause increased expression of Shiga toxin genes *in vivo* (WONG et al., 2000; ZHANG et al., 2000), other researchers suggest that some antibiotics applied in the early period of infection may prevent disease progression (IKEDA et al., 1999; SHIOMI et al., 1999).

Research into the ecology, prevalence of major virulence genes and antibiotic resistance of *E. coli* O157:H7/H- strains isolated from domestic ruminants slaughtered is limited in Turkey and has focused only on cattle (ASLANTAŞ et al., 2006; INAT and SIRIKEN, 2010; KALENDER, 2013). Therefore, we investigated the occurrence, *Stx1*, *Stx2*, *EhlyA* and *eaeA* virulence genes and antibiotic resistance of *E. coli* O157 in the feces of different ruminants slaughtered in three provinces of Turkey.

Materials and methods

Collection of samples. A total of 417 fecal samples were collected from ruminants slaughtered in three provinces of Turkey during the period from September 2013 to August 2015, to investigate the presence of *E. coli* O157:H7/H-. Of the samples examined 203

(n = 152 cattle, n = 51 water buffaloes) were obtained from Afyonkarahisar province, 117 (cattle) from Antalya province and 97 (n = 83 ewes and n = 14 goats) from Burdur province (Table 1). The sampled animals were apparently healthy and non-diarrheic. Commercial abattoirs were visited twice a month. Immediately after slaughter, rectal content samples were aseptically collected from the animals. All sampled animals were randomly selected in this study. Fecal samples were transferred into single-use plastic containers and transported to the laboratory in a cool box on ice.

Table 1. Distribution of samples according to the sampled province and ruminant

Province	Cattle	Water Buffalo	Sheep	Goat	Total
Afyonkarahisar	152	51	-	-	203
Burdur	-	-	83	14	97
Antalya	117	-	-	-	117
Total	269	51	83	14	417

Isolation and identification of E. coli O157:H7/H-. A pre-enrichment stage and inoculation onto selective differential medium were performed to isolate *E. coli* O157:H7/H- from fecal samples. One gram of feces from each animal was transferred in 9 mL modified Tryptone Soy Broth (mTSB) (Oxoid Ltd., Basingstoke, Hampshire, UK) containing Novobiocin (20 mg/L) (Oxoid Ltd., Basingstoke, Hampshire, UK) and homogenized by vortexing for 5 min at 120 rpm. The mixture was incubated at 37 °C for 6 h. 0.1 mL was taken from pre-enrichment broth and inoculated onto Cefixime-Tellurite (Oxoid Ltd., Basingstoke, Hampshire, UK) added Sorbitol MacConkey agar (CT-SMAC) (Oxoid Ltd., Basingstoke, Hampshire, UK) containing 5-bromo-4-chloro-3-indoxyl-b-d-glucuronide (BCIG). Following overnight incubation, sorbitol fermentation and β -glucuronidase enzyme activity were examined in colorless colonies, and these suspected colonies confirmed using standard biochemical tests. For this purpose, Gram staining, motility, oxidase, indole, methyl red, Voges Prouskauer, citrate, urease, hydrogen sulphide, glucose, sucrose, lactose and cellobiose tests were made (ŞEKER and YARDIMCI, 2008). For serological confirmation, isolates were examined by latex agglutination using the *E. coli* O157 antiserum (Oxoid Ltd., Basingstoke, Hampshire, UK) followed by H7 antisera (Remel Inc, KS, USA). In all tests, EHEC O157:H7 strain EDL 933 and *E. coli* ATCC 25922 (Oxoid Ltd., Basingstoke, Hampshire, UK) were used as the positive and negative control strains, respectively.

Detection of virulence genes by PCR. Bacterial DNAs were extracted from control and test strains using the boiling method. For this purpose, all strains were inoculated onto Trypticase Soy Agar. After incubation, fresh colonies were suspended in 500 μ L of DEPC-treated water (DNase-RNase free). The suspension was held in a 100°C water bath for 10 min. After centrifugation at 10 000 rpm for 5 min, the supernatant containing

bacterial DNA was used as a template for the subsequent PCR mixture. All strains were screened for the presence of *rfbO157*, *fliCH7*, *Stx1*, *Stx2*, *eaeA* and *EhlyA* genes by PCR. The primers described by DESMARCHELIER et al. (1998) for *rfbO157*, GANNON et al. (1997) for *fliCH7*, OTAWA et al. (2004) for *Stx1* and *Stx2*, OSEK (2003) for *eaeA* and *EhlyA* were used in the present study (Table 2). Multiplex PCR was performed for the detection of *fliCH7*, *Stx1*, *Stx2* and *EhlyA* genes and duplex PCR was performed for the detection of *rfbO157* and *eaeA* genes. Two microliters of the extracted DNA was used as a template in a 50 μ L PCR mixture containing 10XPCR buffer, 2.5 mmol/L MgCl₂, 0.2 mmol/L of each dNTP, 0.25 μ mol/L of each primer, 2U Taq DNA polymerase (MBI Fermentas, Vilnius, Lithuania) and deionized water. EHEC O157:H7 strain EDL 933 was used as the positive control and *E. coli* ATCC 25922 was used as the negative control. The multiplex PCR amplification conditions of *fliCH7*, *Stx1*, *Stx2* and *EhlyA* genes consisted of an initial denaturation step at 95 °C for 4 min, and 30 cycles of 95 °C for 30 s, 57 °C for 30 s, 72 °C for 30 s and a final step 72 °C for 7 min. The amplification cycles of *rfbO157* and *eaeA* genes were programmed for 4 min at 95 °C for initial denaturation; 30 cycles, 30 s at 95 °C, 1 min at 45 °C, 1 min at 72 °C; and a 7 min final extension step at 72 °C. PCR products were analyzed by electrophoresis on 1.5% agarose gel and visualized by ethidium bromide staining. Molecular size markers (100-bp DNA ladder; Fermentas, Vilnius, Lithuania) were included in each agarose gel.

Table 2. Oligonucleotide primers used in this study

Gene	Primer	Oligonucleotide Sequence (5'→3')	Amplicon size (bp)
<i>rfb</i> _{O157}	O157-F O157-R	AAGATTGCGCTGAAGCCTTG CATTGGCATCGTGTGGACAG	497
<i>fliC</i> _{H7}	H7-F H7-R	GCGCTGTCTGAGTTCTATCGAGC CCACGGTGACTTTATCGCCATTCC	625
<i>Stx1</i>	Stx1-F Stx1-R	TGTAAGTGGAAAGGTGGAGTATACA GCTATTCTGAGTCAACGAAAAATAAC	210
<i>Stx2</i>	Stx2-F Stx2-R	GTTTTTCTTCGGTATCCTATTCC GATGCATCTCTGGTCATTGTATTAC	484
<i>eaeA</i>	Int-F Int-R	GGGATCGATTACCGTCAT TTTATCAGCCTTAATCTC	837
<i>EhlyA</i>	hlyA-F hlyA-R	GCATCATCAAGCGTACGTTCC AATGAGCCAAGCTGGTTAAGCT	534

Antibiotic susceptibility test. The antibiotic resistance of strains was determined using the Kirby-Bauer disc diffusion test on Mueller Hinton agar (Oxoid Ltd., Basingstoke, Hampshire, UK) according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) (ANONYM, 2013). For this purpose, ampicillin (10 μ g), amoxicillin/clavulanic acid (30 μ g), imipenem (10 μ g), aztreonam (30 μ g), cephalothin (30 μ g),

cephazolin (30 µg), cefoxitin (30 µg), ceftriaxone (30 µg), ceftiofur (30 µg), streptomycin (10 µg), gentamicin (10 µg), kanamycin (30 µg), neomycin (30 µg), tobramycin (10 µg), tetracycline (30 µg), chloramphenicol (30 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), norfloxacin (10 µg) and trimethoprim/sulfamethoxazole (25 µg) antibiotic discs (Oxoid Ltd., Basingstoke, Hampshire, UK) were used.

Results

Isolation and identification findings. A total of 16 (3.8%) *E. coli* O157 strains were isolated from 417 fecal samples collected from ruminants slaughtered in three provinces of Turkey. Seven of 16 strains were shown by positive reaction to H7 antisera. Among these strains 9 (3.3%), 4 (7.8%), 2 (2.4%) and 1 (7.1%) were obtained from 269 cattle, 51 water buffaloes, 83 sheep and 14 goats, respectively (Table 3).

Table 3. The origin of animals and distribution of genes detected by PCR in this study

No. positive samples / No. animals sampled (%)	Genes		
	<i>fliC_{H7}</i> , <i>Stx2</i> , <i>EhlyA</i> , <i>eaeA</i>	Alone <i>eaeA</i>	Gene negative
Cattle 9/269 (3.3)	5	3	1
Water buffalo 4/51 (7.8)	1	1	2
Sheep 2/83 (2.4)	1	-	1
Goat 1/14 (7.1)	-	-	1
Total 16/417 (3.8)	7 (43.8)	4 (25.0)	5 (31.2)

PCR findings. All 16 strains were positive for the *rfbO157* gene. Seven (43.8%) of the 16 strains were found to be positive *fliCH7*, *Stx2*, *EhlyA* and *eaeA* genes. The *Stx1* gene was not obtained from any of the 16 strains, and five (31.2%) strains were also negative for *fliCH7*, *Stx2*, *EhlyA* and *eaeA* genes. Intimin was found to be the predominant virulence factor. It was determined in a total of 11 (68.8%) strains, and four (25.0%) of these were alone. Distribution of *fliCH7*, *Stx2*, *EhlyA* and *eaeA* genes detected by PCR according to sampled animals is presented in Table 3. Amplification of *rfbO157* (497 bp) and *eaeA* (837 bp) genes is shown in Fig. 1 and *fliCH7* (625 bp), *Stx2* (484 bp) and *EhlyA* (534 bp) genes is shown in Fig. 2.

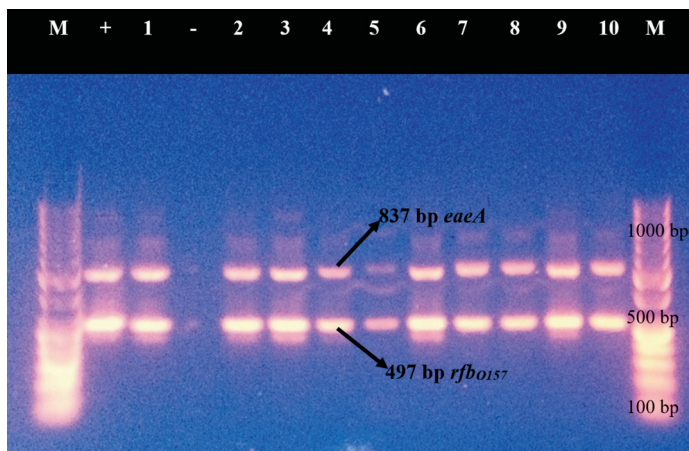


Fig. 1. Detection of *rfbO157* and *eaeA* genes by duplex PCR. M: 100 bp DNA ladder (Fermentas, Vilnius, Lithuania); +: positive control (EHEC O157:H7 strain EDL 933); -: negative control (*E. coli* ATCC 25922); line 1, 2-10: specific bands of *rfbO157* and *eaeA* genes

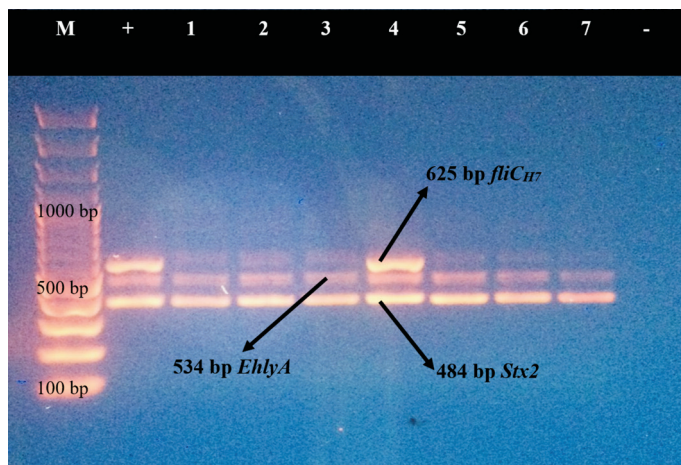


Fig. 2. Detection of *fliCH7*, *Stx2* and *EhlyA* genes by multiplex PCR. M: 100 bp DNA ladder (Fermentas, Vilnius, Lithuania); +: positive control (EHEC O157:H7 strain EDL 933); line 1-7: specific bands of *fliCH7*, *Stx2* and *EhlyA* genes; -: negative control (*E. coli* ATCC 25922)

Antibiotic susceptibility test. According to the Kirby-Bauer disc diffusion results, high resistance rates were determined against ampicillin (68.7%), neomycin (68.7%), tetracycline (68.7%), trimethoprim/sulfamethoxazole (62.5%) and amoxicillin/clavulanic

acid (56.2%) in 16 *E. coli* 157 strains isolated in this study. Of 9 cattle isolates, 7 (77.8%) were resistant to neomycin and tetracycline, 6 (66.7%) were resistant to amoxicillin/clavulanic acid and 5 (55.5%) were resistant to ampicillin and trimethoprim/sulfamethoxazole. The resistance rate to ampicillin, chloramphenicol and tetracycline was found to be 75.0% in 4 water buffalo isolates. All the sheep isolates were resistant to ampicillin, while a 100% resistance rate to ampicillin, neomycin, tetracycline, ciprofloxacin and trimethoprim/sulfamethoxazole was observed in one goat isolate. The antibiotic resistance rates according to the sampled ruminants are shown in Table 4.

Table 4. Antibiotic resistance of *E. coli* O157 isolates obtained from ruminants

Antibiotic	Cattle (n = 9 isolates)		Water buffalo (n = 4 isolates)		Sheep (n = 2 isolates)		Goat (n = 1 isolate)		Total (n = 16 isolates)	
	n	%	n	%	n	%	n	%	n	%
AMP (10 µg)	5	55.5	3	75.0	2	100	1	100	11	68.7
AMC (30 µg)	6	66.7	2	50.0	1	50.0	-	0	9	56.2
IPM (10 µg)	-	0	-	0	-	0	-	0	-	0
ATM (30 µg)	-	0	-	0	-	0	-	0	-	0
KF (30 µg)	4	44.4	1	25.0	-	0	-	0	5	31.2
KZ (30 µg)	2	22.2	-	0	-	0	-	0	2	12.5
FOX (30 µg)	2	22.2	1	25.0	-	0	-	0	3	18.7
CRO (30 µg)	2	22.2	1	25.0	-	0	-	0	3	18.7
EFT (30 µg)	1	11.1	1	25.0	-	0	-	0	2	12.5
S (10 µg)	4	44.4	1	25.0	1	50.0	-	0	6	37.5
CN (10 µg)	4	44.4	1	25.0	1	50.0	-	0	6	37.5
K (30 µg)	3	33.3	1	25.0	-	0	-	0	4	25.0
N (30 µg)	7	77.8	2	50.0	1	50.0	1	100	11	68.7
TOB (10 µg)	2	22.2	1	25.0	-	0	-	0	3	18.7
TE (30 µg)	7	77.8	2	50.0	1	50.0	1	100	11	68.7
C (30 µg)	3	33.3	3	75.0	-	0	-	0	6	37.5
NA (30 µg)	2	22.2	1	25.0	1	50.0	-	0	4	25.0
CIP (5 µg)	3	33.3	2	50.0	-	0	1	100	6	37.5
NOR (10 µg)	3	33.3	2	50.0	1	50.0	-	0	6	37.5
SXT (25 µg)	5	55.5	3	75.0	1	50.0	1	100	10	62.5

AMP: Ampicillin, AMC: Amoxicillin/clavulanic acid, IMP: Imipenem, ATM: Aztreonam, KF: Cephalothin, KZ: Cephazolin, FOX: Cefoxitin, CRO: Ceftriaxone, EFT: Ceftiofur, S: Streptomycin, CN: Gentamicin, K: Kanamycin, N: Neomycin, TOB: Tobramycin, TE: Tetracycline, C: Chloramphenicol, NA: Nalidixic acid, CIP: Ciprofloxacin, NOR: Norfloxacin, SXT: Trimethoprim/sulfamethoxazole

Discussion

The present study investigated the prevalence, major virulence genes and antibiotic resistance of *E. coli* O157 isolated from the feces of adult ruminants slaughtered in three provinces of Turkey.

Cattle are well-known as a major source human pathogenic *E. coli* O157:H7/H- strains. Other food animals, such as water buffaloes, sheep and goats, may also be significant contributing sources (CHAPMAN et al., 2001; ISLAM et al., 2008; MERSHA et al., 2010; ŞEKER et al., 2010). Although a higher prevalence of this serotype in ruminants on farms has been reported in published research, studies on slaughtered ruminants have suggested that fewer than 15% of ruminants are shedders of *E. coli* O157 (HEUVELINK et al., 1998; CHAPMAN et al., 2001; ISLAM et al., 2008; MERSHA et al., 2010; JACOB et al., 2013). *E. coli* O157 was isolated from 10.6% of adult cattle and 3.8% of ewes in the Netherlands (HEUVELINK et al., 1998), 12.9% of cattle and 1.4% of sheep in the UK (CHAPMAN et al., 2001), 14.4% of buffaloes, 7.2% of cows and 9.1% of goats in Bangladesh (ISLAM et al., 2008), 3.3% of goats and 5.4% of sheep in Ethiopia (MERSHA et al., 2010), 11.1% of goats in the US (JACOB et al., 2013). In Turkey, investigations have generally only been conducted on slaughtered cattle, and the prevalence rates in these studies ranged between 3.3% and 25% (ASLANTAŞ et al., 2006; INAT and SIRIKEN, 2010; KALENDER, 2013). Unlike other studies in Turkey, we investigated the fecal carriage rate of *E. coli* O157 in the different slaughtered ruminants. A total of 16 (3.8%) *E. coli* O157 strains, isolated using conventional methods, had the *rfbO157* gene and 7 of these harbored the *fliCH7* gene. The prevalence of this serotype was found to be 3.3% (n = 9), 7.8% (n = 4), 2.4% (n = 2) and 7.1% (n = 1) in 269 cattle, 51 water buffaloes, 83 sheep and 14 goats, respectively. When compared to the results of authors from Turkey and other countries, the different carriage rates obtained from this study may be explained by the number and diversity of animals sampled, and the sampling method and geographical variations. Similarly, many researchers emphasized that several factors, such as the sensitivity of the isolation methods, study design, sample size, geographical origin, age and sex of animals and/or abattoir conditions, may affect the isolation rates of *E. coli* O157 (CHAPMAN et al., 2001; ISLAM et al., 2008; MERSHA et al., 2010; KALENDER, 2013).

The pathogenicity of *E. coli* O157:H7/H- is associated with various virulence factors, such as Shiga toxins, intimin and enterohemolysin (KARMALI et al., 2010; BERGAN et al., 2012). Shiga toxins are known to be associated with HC and HUS, while intimin is responsible for the formation of attaching/effacing (A/E) lesions on intestinal epithelial cells. It has been considered that enterohemolysins may be used as the epidemiological marker for STEC strains (FARROKH et al., 2013). Although the low and high prevalence rates of the genes encoding these virulence factors were reported in *E. coli* O157:H7/H-

strains isolated from the feces of slaughtered ruminants, it was usually shown that the prevalence of *Stx2* gene was higher than *Stx1* gene (JOHNSEN et al., 2001; McEVOY et al., 2003; ASLANTAŞ et al., 2006; ISLAM et al., 2008; KALENDER, 2013). BLANCO et al. (2001) emphasized that while *Stx2* or both *Stx1* and *Stx2* genes were predominant in healthy animals, the *Stx1* gene had a significantly higher prevalence among STEC strains isolated from diarrheic animals. In the present study, 7 (43.8%) of 16 strains were found to be positive *Stx2*, *EhlyA* and *eaeA* virulence genes, of these, 5, 1 and 1 were obtained from cattle, buffalo and sheep isolates, respectively (Table 3). JACOB et al. (2013) suggested that small ruminants more frequently carried *Stx1* or *Stx1* and *Stx2* than *Stx2* alone. However, the number of strains isolated from sheep and goats in our study was already limited. The *Stx1* gene was not obtained from any of the strains in our study. This finding was consistent with the opinion of BLANCO et al. (2001), because the animals sampled in this study were not diarrheic. FERDOUS et al. (2015) also reported that *Stx*-negative *E. coli* O157:H7/H- strains should be thought to have lost the *Stx* phages or to be a progenitor of STEC *E. coli* O157:H7/H-. Similarly, NIELSEN and SCHEUTZ (2002) suggested in their study that phage loss occurs in the intestinal tract of the animal instead of outside the host, or during routine laboratory culturing. Numerous researchers have emphasized that *Stx2* and *eaeA* are clinically important virulence genes and a strong association has been found between the carriage of these genes and the severity of human disease, especially for HUS (FRIEDRICH et al., 2002; BEUTIN et al., 2004). Similar to other authors' findings (ASLANTAŞ et al., 2006; ISLAM et al., 2008; JACOB et al., 2013), intimin was the predominant virulence factor in this study. It was determined in a total of 11 (68.8%) strains, and 4 (25.0%) of these were alone. In our study, 5 (31.2%) strains were negative for *Stx2*, *EhlyA* and *eaeA* genes. In this instance, *E. coli* O157 strains harboring the major virulence genes may be considered as more virulent for humans than those without them in this study. Nevertheless, it was reported by some authors that the production of major virulence genes was not essential for pathogenesis, because a number of sporadic cases of HUS were caused by *Stx* and *eaeA*-negative strains (SCHMIDT et al., 1999; KARCH et al., 2005; KO et al., 2016).

Antimicrobial resistance has been recognized as a global health problem for many decades. Food animals are considered to be key reservoirs of antibiotic resistant bacteria because some of the antibiotic resistance genes identified in the bacteria of food of animal origin have also been identified in humans (FOUNOU et al., 2016). The use of antibiotics in the treatment of *E. coli* O157:H7/H- infections is controversial because of the differing opinions of investigators (IKEDA et al., 1999; WONG et al., 2000; ZHANG et al., 2000). In studies related to the antibiotic resistance of *E. coli* O157:H7/H- strains isolated from the feces of slaughtered ruminants, the researchers emphasized that the isolates showed high resistance rates and/or multiple drug resistance (MANNA et al., 2006; RAHIMI and NAYEBPOUR, 2012; KALENDER, 2013; DULO et al., 2015). MANNA et al. (2006)

reported that of 3 isolates from slaughtered cattle, two were resistant to nitrofurantoin, tetracycline and co-trimoxazole. In a study by RAHIMI and NAYEBPOUR (2012), it was found that gentamycin resistance was the most common (56.0%), followed by ampicillin (48.0%) (48.0%), erythromycin (40.0%), amoxicillin (16.0%), tetracycline (12.0%), chloramphenicol (8.0%), nalidixic acid (8.0%) and streptomycin (4.0%), among slaughtered ruminant isolates. In another study from the Somali region of Ethiopia, it was reported that resistance to erythromycin and ampicillin were the most common resistance profiles identified among slaughtered goat isolates (DULO et al., 2015). In Turkey, the studies concerning the antibiotic resistance of *E. coli* O157 strains isolated from ruminant feces samples are limited (KALENDER, 2013). KALENDER (2013) from Turkey reported that all 18 *E. coli* O157 strains obtained from the feces of slaughtered cattle were resistant to penicillin, clindamycin, tiamulin and tilmicosin, while 6 STEC O157 isolates were resistant to chlortetracycline and sulphadimethoxine. In the present study, overall, the occurrence of resistance was most frequent against ampicillin (68.7%), neomycin (68.7%), tetracycline (68.7%), trimethoprim/sulfamethoxazole (62.5%) and amoxicillin/clavulanic acid (56.2%) in 16 *E. coli* 157 strains. High resistance rates were determined to neomycin and tetracycline (77.8%), amoxicillin/clavulanic acid (66.7%), ampicillin and trimethoprim/sulfamethoxazole (55.5%) among 9 cattle isolates, while the resistance rate to ampicillin, chloramphenicol and tetracycline was found to be 75.0% in 4 water buffalo isolates. All the sheep isolates were resistant to ampicillin, while a 100% resistance rate to ampicillin, neomycin, tetracycline, ciprofloxacin and trimethoprim/sulfamethoxazole was also observed from one goat isolate. Ampicillin, neomycin, tetracycline, trimethoprim/sulfamethoxazole and amoxicillin/clavulanic acid are the most frequently used antibiotics in Turkey and as expected, the prevalence of resistance was high against them. Geographical variations, differences in preferred antibiotics and the origins of strains may also affect the resistance rates to antibiotics.

Conclusions

In the present study, the presence of the *E. coli* O157 serotype, its major virulence genes and antibiotic resistance in the strains isolated from the feces of slaughtered ruminants including cattle, water buffalo, sheep and goats, were investigated for the first time in Turkey. The food products obtained from these animals have an important consumer market in Turkey. Fecal contamination due to poor hygiene is a risk factor in the contamination of these products. Therefore, it should not be ignored that water buffaloes, sheep and goats, like cattle, can be a potential reservoir of *E. coli* O157:H7 infections for human. Although the use of antibiotics in the treatment of *E. coli* O157:H7/H-infections is controversial, monitoring of antibiotic resistance in the strains is still useful for epidemiological purposes. Moreover, resistance factors may be transferred from food

animals harboring the resistant bacteria to the susceptible population by food handling and/or consumption.

References

- ANONYMOUS (2013): Performance standards for antimicrobial susceptibility testing; Twenty Third informational supplement. (Clinical and Laboratory Standards Institute) CLSI document M100-S23. Wayne, PA.
- ASLANTAŞ, O., S. ERDOĞAN, Z. CANTEKIN, I. GULAÇTI, G. A. EVRENDILEK (2006): Isolation and characterization of verocytotoxin-producing *Escherichia coli* O157 from Turkish cattle. *Int. J. Food Microbiol.* 106, 338-342.
DOI: 10.1016/j.ijfoodmicro.2005.08.005
- BERGAN, J., A. B. D. LINGELEM, R. SIMM, T. SKOTLAND, K. SANDVIG (2012): Shiga toxins. *Toxicon* 60, 1085-1107.
DOI: 10.1016/j.toxicon.2012.07.016
- BEUTIN, L., G. KRAUSE, S. ZIMMERMANN, S. KAULFUSS, K. GLEIER (2004): Characterization of shiga toxin-producing *Escherichia coli* strains isolated from human patients in Germany over a 3-year period. *J. Clin. Microbiol.* 42, 1099-1108.
DOI: 10.1128/JCM.42.3.1099-1108.2004
- BLANCO, J., M. BLANCO, J. E. BLANCO, A. MORA, M. P. ALONSO, E. A. GONZALEZ, M. I. BERNARDEZ (2001): Verocytotoxigenic *Escherichia coli*. In: *Epidemiology of Verocytotoxigenic Escherichia coli (VTEC) in Ruminants.* (Duffy, G., P. Garvey, D. A. McDowell, Eds.), Food&Nutrition Press Inc., Trumbull, CT, USA, pp. 113-148.
- CHAPMAN, P. A., A. T. CERDÁN MALO, M. ELLIN, R. ASHTON, M. A. HARKIN (2001): *Escherichia coli* O157 in cattle and sheep at slaughter, on beef and lamb carcasses and in raw beef and lamb products in South Yorkshire, UK. *Int. J. Food Microbiol.* 64, 139-150.
DOI: 10.1016/S0168-1605(00)00453-0
- DEAN-NYSTROM, E. A., B. T. BOSWORTH, W. C. CRAY, H. W. MOON (1997): Pathogenicity of *Escherichia coli* O157:H7 in the intestines of neonatal calves. *Infect. Immun.* 65, 1842-1848.
- DESMARCHELIER, P. M., S. S. BILGE, N. FEGAN, L. MILLS, J. C. VARY, P. I. TARR (1998): A PCR specific for *Escherichia coli* O157 based on the *rfbE* locus encoding O157 lipopolysaccharide. *J. Clin. Microbiol.* 36, 1801-1804.
- DULO, F., A. FELEKE, B. SZONYI, R. FRIES, M. P. O. BAUMANN, D. GRACE (2015): Isolation of multidrug-resistant *Escherichia coli* O157 from goats in the Somali region of Ethiopia: a cross-sectional, abattoir-based study. *Plos One* 10: e0142905.
DOI: 10.1371/journal.pone.0142905
- FARROKH, C., K. JORDAN, F. AUVRAY, K. GLASS, H. OPPEGAARD, S. RAYNAUD, D. THEVENOT, R. CONDRON, K. DE REU, A. GOVARIS, K. HEGGUM, M. HEYNDRIKX, J. HUMMERJOHANN, D. LINDSAY, S. MISZCZYCHA, S. MOUSSIEGT, K.

VERSTRAETE, O. CERF (2013): Review of shiga-toxin-producing *Escherichia coli* (STEC) and their significance in dairy production. *Int. J. Food Microbiol.* 162, 190-212.

DOI: 10.1016/j.ijfoodmicro.2012.08.008

FERDOUS, M., K. ZHOU, A. MELLMANN, S. MORABITO, P. D. CROUGHS, R. F. DE BOER, A. M. D. KOOISTRA-SMID, J. W. A. ROSSEN, A. W. FRIEDRICH (2015): Is Shiga toxin-negative *Escherichia coli* O157:H7 enteropathogenic or enterohemorrhagic *Escherichia coli*? Comprehensive molecular analysis using whole-genome sequencing. *J. Clin. Microbiol.* 53, 3530-3538.

DOI: 10.1128/JCM.01899-15

FOUNOU, L. L., R. C. FOUNOU, S. Y. ESSACK (2016): Antibiotic resistance in the food chain: a developing country-perspective. *Front. Microbiol.* 7, 1881.

DOI: 10.3389/fmicb.2016.01881

FRIEDRICH, A. W., M. BIELASZEWSKA, W. L. ZHANG, M. PULZ, T. KUCZIUS, A. AMMON, H. KARCH (2002): *Escherichia coli* harboring Shiga toxin 2 gene variants: frequency and association with clinical symptoms. *J. Infect. Dis.* 185, 74-84.

DOI: 10.1086/338115

GANNON, V. P., S. D'SOUZA, T. GTAHAM, R. K. KING, K. RAHN, S. READ (1997): Use of the flagellar H7 gene as a target in multiplex PCR assays and improved specificity in identification of enterohemorrhagic *Escherichia coli* strains. *J. Clin. Microbiol.* 35, 656-662.

HEUVELINK, A. E., F. L. A. M. VAN DEN BIGGELAAR, E. DE BOER, R. G. HERBES, W. J. G. MELCHERS, J. H. J. HUIS IN'T VELD, L. A. H. MONNENS (1998): Isolation and characterization of verocytotoxin-producing *Escherichia coli* O157 strains from Dutch cattle and sheep. *J. Clin. Microbiol.* 36, 878-882.

IKEDA, K., O. IDA, K. KIMOTO, T. TAKATORIGE, N. NAKANISHI, K. TATARA (1999): Effect of early fosfomycin treatment on prevention of hemolytic uremic syndrome accompanying *Escherichia coli* O157:H7 infection. *Clin. Nephrol.* 52, 357-362.

INAT, G., B. SIRIKEN (2010): 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. *J. Vet. Sci.* 11, 321-326.

DOI: 10.4142/jvs.2010.11.4.321

ISLAM, M. A., A. S. MONDOL, E. DE BOER, R. R. BEUMER, M. H. ZWIETERING, K. A. TALUKDER, A. E. HEUVELINK (2008): Prevalence and genetic characterization of Shiga toxin-producing *Escherichia coli* isolates from slaughtered animals in Bangladesh. *Appl. Environ. Microbiol.* 74, 5414-5421.

DOI: 10.1128/AEM.00854-08

JACOB, M. E., D. M FOSTER, A. T. ROGERS, C. C. BALCOMB, M. W. SANDERSON (2013): Prevalence and relatedness of *Escherichia coli* O157:H7 strains in the feces and on the hides and carcasses of U.S. meat goats at slaughter. *Appl. Environ. Microbiol.* 79, 4154-4158.

DOI: 10.1128/AEM.00772-13

- JOHNSEN, G., Y. WASTESON, E. HEIR, O. I. BERGET, H. HERIKSTAD (2001): *Escherichia coli* O157:H7 in faeces from cattle, sheep and pigs in the southwest part of Norway during 1998 and 1999. *Int. J. Food Microbiol.* 65, 193-200.
DOI: 10.1016/S0168-1605(00)00518-3
- KALENDER, H. (2013): Isolation, virulence genes and antimicrobial susceptibilities of Shiga toxin-producing *Escherichia coli* O157 from slaughtered cattle in abattoirs and ground beef sold in Elazığ. *Kafkas Univ. Vet. Fak. Derg.* 19, 461-467.
DOI: 10.9775/kvfd.2012.8040
- KARCH, H., P. I. TARR, M. BIELASZEWSKA (2005): Enterohaemorrhagic *Escherichia coli* in human medicine. *Int. J. Med. Microbiol.* 295, 405-418.
DOI: 10.1016/j.ijmm.2005.06.009
- KARMALI, M. A., V. GANNON, J. M. SARGEANT (2010): Verocytotoxin-producing *Escherichia coli* (VTEC). *Vet. Microbiol.* 140, 360-370.
DOI: 10.1016/j.vetmic.2009.04.011
- KO, H., H. MAYMANI, C. ROJAS-HERNANDEZ (2016): Hemolytic uremic syndrome associated with *Escherichia coli* O157:H7 infection in older adults: a case report and review of the literature. *J. Med. Case Rep.* 10, 1-4.
DOI: 10.1186/s13256-016-0970-z
- MANNA, S. K., M. P. BRAHMANE, C. MANNA, K. BATABYAL, R. DAS (2006): Occurrence, virulence characteristics and antimicrobial resistance of *Escherichia coli* O157 in slaughtered cattle and diarrhoeic calves in West Bengal, India. *Lett. Appl. Microbiol.* 43, 405-409.
DOI: 10.1111/j.1472-765X.2006.01975.x
- McEVOY, J. M., A. M. DOHERTY, J. J. SHERIDAN, F. M. THOMSON-CARTER, P. GARVEY, L. MCGUIRE, I. S. BLAIR, D. A. MCDOWELL (2003): The prevalence and spread of *Escherichia coli* O157:H7 at a commercial beef abattoir. *J. Appl. Microbiol.* 95, 256-266.
DOI: 10.1046/j.1365-2672.2003.01981.x
- MERSHA, G., D. ASRAT, B. M. ZEWSW, M. KYULE (2010): Occurrence of *Escherichia coli* O157:H7 in faeces, skin and carcasses from sheep and goats in Ethiopia. *Lett. Appl. Microbiol.* 50, 71-76.
DOI: 10.1111/j.1472-765X.2009.02757.x
- NIELSEN, E. M., F. SCHEUTZ (2002): Characterisation of *Escherichia coli* O157 isolates from Danish cattle and human patients by genotyping and presence and variants of virulence genes. *Vet. Microbiol.* 88, 259-273.
DOI: 10.1016/S0378-1135(02)00107-4
- OSEK, J. (2003): Development of a multiplex PCR approach for the identification of Shiga toxin-producing *Escherichia coli* strains and their major virulence factor genes. *J. Appl. Microbiol.* 95, 1217-1225.
DOI: 10.1046/j.1365-2672.2003.02091.x

OTAWA, K., M. SATO, T. SASAKI, H. SASAKI, J. NONAKA, K. ITO, T. KUROKI, Y. NAKAI (2004): Genetic analysis of shiga-toxigenic *Escherichia coli* isolates from cattle in a limited region. *Anim. Sci. J.* 75, 261-269.

DOI: 10.1111/j.1740-0929.2004.00185.x

RAHIMI, E., F. NAYEBPOUR (2012): Antimicrobial resistance of *Escherichia coli* O157:H7/NM isolated from feces of ruminant animals in Iran. *J. Cell Anim. Biol.* 6, 104-108.

SCHMIDT, H., J. SCHEEF, H. I. HUPPERTZ, M. FROSC, H. KARCH (1999): *Escherichia coli* O157:H7 and O157:H- strains that do not produce Shiga toxin: phenotypic and genetic characterization of isolates associated with diarrhea and hemolytic-uremic syndrome. *J. Clin. Microbiol.* 37, 3491-3496.

ŞEKER, E., H. YARDIMCI (2008): First isolation of *Escherichia coli* O157:H7 from faecal and milk specimens from Anatolian water buffaloes (*Bubalus bubalus*) in Turkey. *J. S. Afr. Vet. Assoc.* 79, 167-170.

DOI: 10.4102/jsava.v79i4.267

ŞEKER, E., Y. KUYUCUOĞLU, B. SAREYYÜPOĞLU, H. YARDIMCI (2010): PCR detection of Shiga toxins, enterohaemolysin and intimin virulence genes of *Escherichia coli* O157:H7 strains isolated from faeces of Anatolian water buffaloes in Turkey. *Zoonoses Public Health* 57, e33-e37.

DOI: 10.1111/j.1863-2378.2009.01285.x

SHIOMI, M., M. TOGAWA, K. FUJITA, R. MURATA (1999): Effect of early oral fluoroquinolones in hemorrhagic colitis due to *Escherichia coli* O157:H7. *Pediatr. Int.* 41, 228-232.

DOI: 10.1046/j.1442-200X.1999.4121038.x

WONG, C. S., S. JELACIC, R. L. HABEEB, S. L. WATKINS, P. I. TARR (2000): The risk of hemolytic-uremic syndrome after antibiotic treatment of *Escherichia coli* O157:H7 infections. *N. Engl. J. Med.* 342, 1930-1936.

DOI: 10.1056/NEJM200006293422601

ZHANG, X., A. D. McDANIEL, L. E. WOLF, G. T. KEUSCH, M. K. WALDOR, D. W. ACHESON (2000): Quinolone antibiotics induce Shiga toxin-encoding bacteriophages, toxin production, and death in mice. *J. Infect. Dis.* 181, 664-670.

DOI: 10.1086/315239

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SEKER, E., F. S. KUS: Prevalencija, čimbenici virulencije i otpornost na antibiotike bakterije *E. coli* O157 u izmetu odraslih preživača zaklanih u tri turske provincije. *Vet. arhiv* 89, 107-121, 2019.

SAŽETAK

Cilj ovoga istraživanja bio je utvrditi prevalenciju, prisutnost gena virulencije *Stx1*, *Stx2*, *EhlyA* i *eaeA* te otpornost na antibiotike sojeva *E. coli* O157 izoliranih iz izmeta 417 odraslih preživača zaklanih u tri turske

provincije. Ukupno je izolirano 16 sojeva *E. coli* O157 (3,8 %). Od tih sojeva 9 sojeva (3,3 %) potjecalo je od 269 goveda, 4 soja (7,8 %) od 51 vodenog bivola, 2 soja (2,4 %) od 83 ovce i 1 soj (7,1 %) od 14 koza. Svi su sojevi analizirani metodom lančane reakcije polimeraze na prisutnost gena *rfbO157*, *fliCH7*, *Stx1*, *Stx2*, *eaeA* i *EhlyA*. Gen *rfbO157* dokazan je u svim sojevima, dok su u 7 (43,8 %) od 16 sojeva utvrđeni geni *fliCH7*, *Stx2*, *EhlyA* i *eaeA*. Gen *eaeA* utvrđen je u 11 sojeva (68,8 %), a u 4 (25,0 %) od tih 11 bio je samo taj gen. Gen *Stx1* nije pronađen ni u jednom od 16 sojeva, dok je 5 sojeva (31,2 %) bilo negativno za gene *fliCH7*, *Stx2*, *EhlyA* i *eaeA*. Utvrđena je visoka otpornost na ampicilin (68,7 %), neomicin (68,7 %), tetraciklin (68,7 %), trimetoprim/sulfametoksazol (62,5 %) i amoksicilin/klavulansku kiselinu (56,2 %) u 16 sojeva *E. coli* O157 izoliranih u ovom istraživanju. U ovom je istraživanju analizirana prisutnost serotipa *E. coli* O157, glavni geni virulencije i antibiotska otpornost u sojevima izoliranim iz izmeta nekoliko različitih vrsta preživača, uključujući goveda, vodene bivole, ovce i koze. Ovime je prvi put dokazano da navedene vrste životinja mogu biti potencijalni rezervoari infekcije bakterijom *E. coli* O157 za ljude u Turskoj.

Ključne riječi: klaonica; antibiotska otpornost; *E. coli* O157; preživač; geni virulencije
