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COMPARISON OF PREVALENCE AND GENETIC DIVERSITY OF *ESCHERICHIA COLI* O157:H7 IN CATTLE AND SHEEP

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ABSTRACT

In this study the prevalence of *Escherichia coli* O157:H7 was detected by immunomagnetic separation (IMS) based cultivation technique and polymerase chain reaction (PCR) in feces and/or colon tissue of cattle (n= 282) and sheep (n= 218) at slaughterhouse. The major virulence genes, intimin variants, Shiga toxin variants and antibiotic resistance genes of the isolates were examined by PCR and genomic diversity of the cattle and sheep *E. coli* O157:H7 isolates were assessed using pulsed field gel electrophoresis (PFGE). In the present study the prevalence of *E.coli* O157:H7 was found higher in sheep (6.4 %) than in cattle (3.9 %). All the *E.coli* O157:H7 isolates were detected as positive for at least one *stx* gene and positive for other virulence genes. Twelve (29.3 %) and one (2.4 %) of the cattle isolates carried stx_2 and stx_1 gene, respectively. However 11 (17.7 %) of the sheep *E. coli* O157:H7 isolates carried stx_2 and five (8.1 %) of the isolates harbored stx_1 gene only. At least one antibiotic resistance gene was detected from 35 isolates. *E. coli* O157:H7 isolates from four sheep and three cattle harbored *tetB* gene. From three cattle and one sheep samples *strA* carrying *E. coli* O157:H7 were isolated. Among them, isolates from 2 cattle and one sheep samples were carried both *tetB* and *strA*. Isolates were grouped into six different clusters. From a cattle and a sheep, two different *E. coli* O157:H7 which have different PFGE patterns, were isolated. It can be concluded that sheep pose a risk as cattle for STEC O157:H7 contamination in Turkey.

Keywords: *E. coli* O157:H7; virulence genes; *stx* variants; antibiotic resistance; PFGE

INTRODUCTION

Escherichia coli O157:H7 has emerged as a pathogen of considerable public health importance which causes a spectrum of illnesses ranging from hemorrhagic colitis to hemolytic-uremic syndrome (HUS) worldwide. Gastro-intestinal system of the ruminants is the primary reservoir of this organism **(Meng *et al.*, 2001)**. Many studies showed that both dairy and beef cattle were the primary source and the carrier of the bacterium **(Chapman *et al.*, 1993; Byrne *et al.*, 2003; Shin *et al.*, 2014)**. Limited number of studies reported sheep as a reservoir of this emerging pathogen **(Söderlund *et al.*, 2012)**.
Several virulence factors have been described in *E. coli* O157:H7, the major ones are known as Shiga toxins (stx_1 and stx_2) which are responsible for life threatening illnesses like HC and HUS. *E. coli* O157:H7 produces putative virulence genes such as intimin (encoded by *eaeA*) which is necessary for attaching and effacing adhesion on host cell membrane, and hemolysin (encoded by *hly*). There are several intimin variants identified and they were shown to affect tissue tropism and colonization site **(Mundy *et al.*, 2007)**. In addition, *espA* (*E. coli* secreted protein A) and *lpf* (Long polar fimbria) are the virulence genes which are important in tropism, attachment, persistence and virulence of *E. coli* O157:H7, were identified in recent years **(Torres *et al.*, 2009)**.
Antibiotic treatment in *E. coli* O157:H7 infections in human is not always possible because of lysis of the cells and increased expression and release of the Shiga toxins (Stx) in the intestinal tract **(Wong *et al.*, 2000)**. However using some antimicrobials in the early stage of infection may be protective against heamolytic uremic syndrome (HUS) progression **(Ikeda *et al.*, 1999)**. Due to extensive use of antibiotics in veterinary medicine for prophylaxis or growth promotion in animal production in several studies, resistant *E. coli* O157:H7 strains were reported to various antibiotics **(Schroeder *et al.*, 2004; Goncuoglu *et al.*, 2010)**. In a report 79.8% of the *E. coli* O157:H7 isolates were found to carry one or more antibiotic resistance genes **(Srinivasan *et al.*, 2007)**.
The objectives of this study were to determine the prevalence of *Escherichia coli* O157:H7 by immunomagnetic separation (IMS) based cultivation technique and PCR in feces and/or colon tissue of cattle and sheep, to detect the major virulence genes, intimin variants, Shiga toxin variants and antibiotic resistance genes of the isolates by polymerase chain reaction (PCR) and to determine the genomic diversity using pulsed field gel electrophoresis (PFGE).

MATERIAL AND METHODS

Sample collection

A total of 282 cattle consisting 207 beef and 75 dairy cattle and 218 sheep (with a total of 500 animals) feces and/or colon tissue samples were collected from a slaughterhouse within 28 visits in Ankara province. Samples were taken into sterile filtered bags and taken into laboratory in an ice box and analyzed within 2 hours.

Microbiological analysis

IMS based cultivation technique was used for the isolation of *E. coli* O157 **(Byrne *et al.*, 2003)**. Ten grams of samples were weighed to a sterile bag and suspended with 90 ml EC broth (Oxoid CM0853, Hampshire, UK) containing novobiocin (20 µg/L; Sigma N-1628, St. Louis, USA) and incubated at 37°C at 100 rpm/min for 18 h in a shaking incubator (Bellco Shel Lab Shaking Incubator S16R, Oregon, USA). Then, IMS was performed with 20 µl of magnetic beads (Dynabeads anti *E. coli* O157, Dynal, Norway) according to the manufacturer's protocol.
Following to IMS procedure, 100 µl of resuspended suspension was plated on Cefixime-tellurite (Oxoid SR0172) supplemented Sorbitol MacConkey Agar (Oxoid CM0813) and incubated at 42°C for 24 h. After incubation, sorbitol negative colonies were tested for the O157 antigen by latex agglutination (Oxoid DR0620) and up to five positive colonies were picked for PCR analysis.

PCR analysis for the detection of virulence genes

DNA extraction was performed by Chelex-100 (Bio-Rad, Hercules, CA, USA) resin based technique, using proteinase K (20 mg/ml; AppliChem GmbH, Darmstadt, Germany). Virulence genes including; stx_1, stx_2, *eaeA*, *hly* and $fliC_{h7}$ **(Fratamico *et al.*, 2000)** were detected by multiplex PCR; *espA* **(McNally *et al.*, 2001)** and *lpfA*1-3 **(Torres *et al.*, 2009)** genes were detected by PCR. *E. coli* O157:H7 ATCC 43895 (stx_1^+, stx_2^+, $eaeA^+$, hly^+, $lpfA1\text{-}3^+$, $espA^+$) was used as positive control.

Detection of intimin and Shiga toxin variants of *E. coli* O157:H7 isolates

Intimin variants α1, α2, β, β1, β2, γ1 and γ2/θ were tested in *eaeA* gene detected intimin harboring isolates by previously published primer pairs and PCR conditions **(Blanco *et al.*, 2004)**. *E. coli* O157:H7 ATCC 43895 ($eae\gamma_1^+$) was used as positive control.
E. coli O157:H7 isolates were subjected to consecutive multiplex and conventional PCR assays for determination of stx_1 variants (stx_{1c} **[Zhang *et al.*, 2002]** and stx_{1d} **[Bürk *et al.*, 2003]**) and/or stx_2 variants (stx_{2c}, stx_{2d}, stx_{2e}, stx_{2f} **[Osek, 2003]** and stx_{2g} **[Leung *et al.*, 2003]**). *E. coli* O157:H7 ATCC 43895 (stx_{1c}^+), strains *E. coli* O157:NM 137/98 (stx_{2c}^+), *E. coli* O62:H⁻ 551/98 (stx_{2d}^+), *E. coli* O139:K12 107/86 (stx_{2e}^+), *E. coli* O⁻:H18 214/125 (stx_{2f}^+) and *E. coli* O2:H25 S86 (stx_{2g}) were used as positive controls.

Detection of antibiotic resistance genes by multiplex PCR

Antimicrobial resistance genes encoding for the tetracycline efflux pump (*tetA*, *tetB*, *tetC*, *tetD*, *tetE*, and *tetG*); streptomycin phosphotransferases (*strA* and *strB*); aminoglycoside adenyltransferase (*aadA*); chloramphenicol transporter nonenzymatic chloramphenicol-resistance protein (*cmlA*); florfenicol export protein (*floR*); dihydropteroate synthetase type I (*sulI*); dihydropteroate synthetase type II (*sulII*); and beta-lactamase-ampicillin resistance (*ampC*) in *E. coli* O157:H7 isolates were determined by PCR according to **Srinivasan *et al.*, (2007)**.

Genomic characterization by PFGE

Escherichia coli O157:H7 isolates were sub-typed by PFGE technique of CHEF electrophoresis described by **Harsono *et al.*, (1993)**. Genomic DNA was digested in agarose plugs with *XbaI* (Promega) as recommended by the manufacturer. The resulting DNA fragments were resolved by CHEF- PFGE with a CHEF-DR III apparatus (Bio-Rad Laboratories, CA, USA) at 200 V for 19 h at 14°C and switch times from 1 to 60 s. Low-range lambda concatemers (Promega) were used as DNA size standards. The fragments were visualized by a gel documentation system (Syngene Ingenius). GeneTools software (version 3.08.01; Syngene, United Kingdom) was used for processing the gel image. PFGE results were ascertained by the presence, absence and similarity of restriction fragments and the subtypes were coded as A, B, C, D, E and F.

RESULTS AND DISCUSSION

Fourteen of 218 sheep and 11 of 282 cattle were determined as positive for *E. coli* O157:H7. The prevalence of *E.coli* O157:H7 was found in sheep as 6.4 % and in cattle as 3.9 %. Among cattle samples, dairy cattle prevalence of *E. coli* O157:H7 was higher than beef cattle samples with a ratio of 5.3 % and 3.4 %, respectively. Most of the isolates (20/25, 80 %) were determined in warm months (spring and summer). *E. coli* O157:H7 was isolated from 54.5 % (6/11) of all positive cattle samples in the July. Other one and four *E. coli* O157:H7 positive cattle samples were recovered in May and October, respectively. Seasonal distribution of *E. coli* O157:H7 is similar in sheep with cattle samples. Ten out of 14 (71.4 %) *E. coli* O157:H7 positive samples were taken in summer; three positive samples were detected in spring. Only in one (9.1 %) sheep winter sample *E. coli* O157:H7 was found (Tab 1).

Table 1 Seasonal distribution of *E. coli* O157:H7 in cattle and sheep feces and/or colon tissue samples

Season	Months	Number of visits	Number of samples	Number of positive samples	% Seasonal distribution
Spring	April	3	5C+9S	2S	6.3%C, 16.7%S
	May	4	11C+9S	1C+1S	
Summer	June	5	30C+68S	-	6.1%C, 5.9%S
	July	6	54C+50S	6C+3S	
	August	4	15C+52S	7S	
Autumn	September	2	25C	-	6.6%C
	October	2	36C	4C	
Winter	December	3	61C+30S	1S	3.3%S
	January	1	35C	-	
	February	1	10C	-	

C: Cattle; **S:** Sheep

In order to find the *E. coli* O157:H7 contamination with multiple strains, up to five colonies were picked from the positive samples for further molecular characterization. For this purpose a total of 103 colonies (41 cattle and 62 sheep isolate) were isolated from these positive animals (14 sheep and 11 cattle). All of the 103 colonies carried at least one *stx* gene and 74 of them (71.8 %) were found to carry both of toxin genes. Additionally, all *E. coli* O157:H7 isolates carried *hly*, *eaeA*, *lpf* , *espA* genes and harbored *eae-γ1* as an intimin variant (Tab 2).
When we compared the cattle and sheep isolates, 12 (29.3 %) of the cattle isolates carried stx_2 and one (2.4 %) stx_1 gene only. However 11 (17.7 %) of the sheep *E. coli* O157:H7 isolates carried stx_2 and five (8.1 %) harbored stx_1 gene only. In the study, 28 of 41 (68.3 %) cattle *E. coli* O157:H7 colonies and 46 of 62 (75.4 %) sheep *E. coli* O157:H7 colonies harbored stx_1 and stx_2. Six (5.8 %) and 23 (22.3 %) of 103 isolates carried stx_1 and stx_2, respectively. In stx_1 and stx_2 positive isolates stx_{1c} and stx_{2c} variants were detected. In general, colonies that were isolated from the same sample harbored the same toxin profile except a cattle (coded as C3 in Tab 2) and a sheep (coded as S4 in Tab 2) sample. In sample C3 5 *E. coli* O157:H7 were picked during isolation; although 4 of them had both stx_{1c} and stx_{2c} Shiga toxin genes, one colony had only stx_{2c}. Similarly in sample S4, 4 of the colonies had both stx_{1c} and stx_{2c}, but one colony had only stx_{2c}. This means a sheep and cattle that we sampled, carried at least two different *E. coli* O157:H7 strains.

Table 2 Virulence and antibiotic resistance gene distribution and genomic diversity of the *E. coli* O157:H7 sheep and cattle isolates

Sample no [a] (number of colonies)	H7	*hly*	*lpf*	*espA*	stx_1 variant	stx_2 variant	Intimin variant *eae-γ1*	Antibiotic resistance genes	PFGE group
S1 (5)	+	+	+	+	stx_{1c}	stx_{2c}	+	*tetB*	A
S7 (4)	+	+	+	+	stx_{1c}	stx_{2c}	+		A
S8 (4)	+	+	+	+	stx_{1c}	stx_{2c}	+		A
S9 (5)	+	+	+	+	-	stx_{2c}	+		A
S10 (5)	+	+	+	+	stx_{1c}	stx_{2c}	+	*tetB, strA*	A
S12 (2)	+	+	+	+	stx_{1c}	stx_{2c}	+	*tetB*	A
C4 (4)	+	+	+	+	-	stx_{2c}	+	*tetB, strA*	A
S3 (5)	+	+	+	+	stx_{1c}	stx_{2c}	+		B
S5 (5)	+	+	+	+	stx_{1c}	**-**	+		B
S13 (4)	+	+	+	+	stx_{1c}	stx_{2c}	+		B
C2 (5)	+	+	+	+	-	stx_{2c}	+		B
C5 (2)	+	+	+	+	-	stx_{2c}	+		B
C1 (5)	+	+	+	+	stx_{1c}	stx_{2c}	+	*tetB, strA*	C
S2 (5)	+	+	+	+	stx_{1c}	stx_{2c}	+	*strA*	D
S4 (4)	+	+	+	+	stx_{1c}	stx_{2c}	+		E

S4 (1)	+	+	+	+	-	stx_{2c}	+		-
S14 (5)	+	+	+	+	-	stx_{2c}	+		E
C3 (4)	+	+	+	+	-	stx_{2c}	+	*strA*	E
C3 (1)	+	+	+	+	stx_{1c}	stx_{2c}	+	*tetB*	-
C6 (5)	+	+	+	+	stx_{1c}	stx_{2c}	+		E
C7 (5)	+	+	+	+	stx_{1c}	stx_{2c}	+		E
C8 (5)	+	+	+	+	stx_{1c}	stx_{2c}	+		E
C9 (1)	+	+	+	+	stx_{1c}	stx_{2c}	+		E
C10 (1)	+	+	+	+	stx_{1c}	-	+		E
C11 (3)	+	+	+	+	stx_{1c}	stx_{2c}	+		E
S6 (4)	+	+	+	+	stx_{1c}	stx_{2c}	+		F
S11 (4)	+	+	+	+	stx_{1c}	stx_{2c}	+	*tetB*	F

[a] **S:** Sheep, **C:** Cattle.

Thirty five of 103 isolates (34 %) carried at least one antibiotic resistance gene. *E. coli* O157:H7 isolates from four sheep and three cattle harbored *tetB* gene. From three cattle and one sheep samples *strA* carrying *E. coli* O157:H7 were isolated. Among them, isolates from 2 cattle and one sheep samples were carried both tetB and strA genes. From *tetB* and *strA* were the only detected antibiotic resistance genes from the positive samples. Within the 14 antibiotic resistance genes carrying isolates (9 cattle and 5 sheep) both *tetB* and *strA* were determined while from 12 (1 cattle and 11 sheep) and nine *E. coli* O157:H7 (4 cattle and 5 sheep) only *tetB* or *strA* were detected, respectively (Tab 2). Representative PCR results are shown in the figure 1.

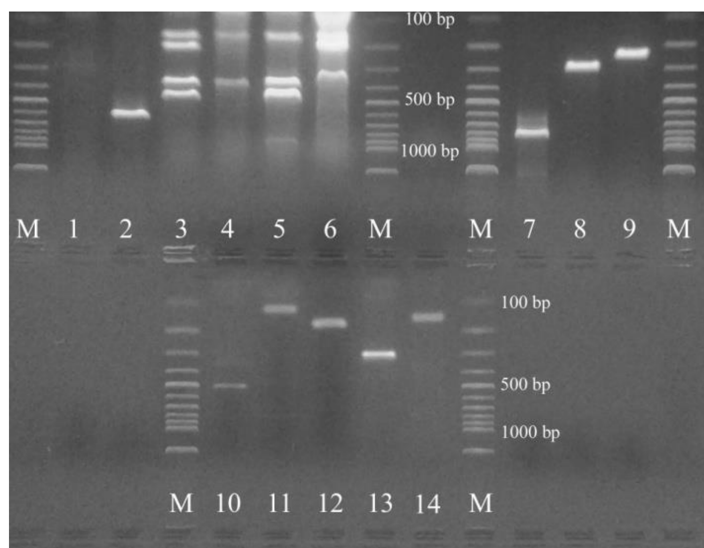

Figure 1 Representative PCR gel electrophoresis of virulence genes detected positive control strains and isolates

Lane M: 100 bp DNA marker; **Lane 1:** Negative control; **Lane 2:** *E. coli* O157:H7, ATCC 43895 (*fliC*$_{h7}^{+}$); **Lane 3:** *E. coli* O157:H7, ATCC 43895 (stx_1^+, stx_2^+, $eaeA^+$, hly^+); **Lane 4:** *E. coli* O157:H7, NCTC 12900 (stx_1^-, stx_2^-, $eaeA^+$, hly^+); **Lane 5:** *E. coli* O157:H7 isolate (stx_1^-, stx_2^+, $eaeA^+$, hly^+); **Lane 6:** *E. coli* O157:H7 isolate (stx_1^+, stx_2^-, $eaeA^+$, hly^+); **Lanes 7:** *E. coli* O157:H7 isolate ($eae\gamma_1^+$); **8:** *E. coli* O157:H7 isolate ($espA^+$); **9:** *E. coli* O157:H7 isolate ($lpfA1\text{-}3^+$); **Lane 10:** *E. coli* O157:H7, ATCC 43895 (stx_{1c}^+); **Lane 11:** *E. coli* O157:NM, 137/98 (stx_{2c}^+); **Lane 12:** *E. coli* O62:H$^-$, 551/98 (stx_{2d}^+); **Lane 13:** *E. coli* O139:K12, 107/86 (stx_{2e}^+): **Lane 14:** *E. coli* O$^-$:H18, 214/125 (stx_{2f}^+)

PFGE data indicated that there were six distinct restriction endonuclease digestion profiles (REDP) among the 103 isolates examined and coded as A, B, C, D, E and F (Tab 1). In pattern A, 29 isolates belonged to six sheep (25 isolates) and one cattle (4 isolates). Pattern A isolates were achieved from four different collection visits. Four sheep samples (18 isolates) that were from same collection date showed different toxin profile. Group B isolates (21 isolates) were belonged to five different animals (3 sheep and 2 cattle) and isolates showed quite distinct toxin profiles. Five isolates from one cattle and five isolates from one sheep were taken part in group C and D, respectively. Both isolates harbored two of the toxin genes.

In group E, nine (36 %) of the 25 positive cattle (7) and sheep (2) colonies showed the same genomic profile. Group E isolates were recovered from four different visits. A total of 35 isolates (25 from cattle and 10 from sheep) showed different toxin profiles some of them were harbored stx_1 and stx_2 together (23 isolates). One isolate was carried stx_1 while nine were stx_2. As it is shown in Table 2, remarkable results of the study were seen in group E. One isolate from cattle (coded as C3) and one from sheep (coded as S4) showed different genomic profiles from others 4 sub-colonies which were isolated the same samples. They had different toxin genes from their positive animal sub-colonies. The isolate of concern from cattle was detected as stx_1 and stx_2 positive on the other hand other colonies (4 colonies) from the same cattle harbored stx_2 only. Also this isolate carried *tetB* where the rest of the isolates from the same cattle carried *strA* as an antibiotic resistance gene. Likewise isolate of concern from sheep harbored only stx_2 although others (4 colonies) from the same sheep harbored both of the toxin genes together. These results indicated that this cattle and sheep were simultaneously contaminated with two different *E. coli* O157:H7.

Two sheep isolates (8 isolates) were grouped as F and isolates harbored both of the toxin genes. Four isolates from one sheep carried *tetB* and *strA* which the isolates from other positive sheep did not.

In this study which is an important data on the presence of *E. coli* O157:H7 in sheep in Turkey, the prevalence was found as 6.4 %. Lower than our results, 5.4 % of sheep faeces samples in Ethiopia **(Mersha *et al.*, 2010)** and 1.8 % of the Swedish sheep (**Söderlund *et al.*, 2012)** were found to carry *E. coli* O157:H7 while in North Wales in none of the sheep fecal samples this pathogen was detected **(Alhelfi *et al.*, 2013)**.

In the present study the prevalence of *E.coli* O157:H7 was found higher in sheep (6.4 %) than in cattle (3.9 %).**Van Donkersgoed *et al.*, (1999)** reported that prevalence of *E. coli* O157:H7 in fecal samples of cattle at slaughter level in Canada was 7.5 % by IMS. In another study from 1.5 % of fecal and/or tissue samples of healthy cattle in the USA *E. coli* O157:H7 was isolated **(Byrne *et al.*, 2003)**. Different from the present study only 3 of 1,300 (0.2 %) fecal samples collected from adult cattle using IMS technique in Norway were found to be contaminated with *E. coli* O157:H7 **(Johnsen *et al.*, 2001)** In the study, the PFGE analysis revealed two different PFGE profiles among 3 isolates. The toxin profiles between these groups were showed differences such as two isolates in the same group have stx_2, *eae* and *fliC*, the other isolate from the other group has both stx_1 and stx_2 with *eae* and *fliC* **(Hancock *et al.*, 1997)**.

In the study, the prevalence of *E. coli* O157:H7 in feces of healthy (3.7 %) and diarrheic cattle (4.3 %) were nearly the same. This can be explained that cattle can carry *E. coli* O157:H7 without showing any symptoms of disease **(Meng *et al.*, 2001)**. It was found that out of 207 beef cattle, and 75 dairy cattle samples, seven (3.4 %), and four (5.3 %) were found to be contaminated with *E. coli* O157:H7, respectively. However it is widely believed that dairy herds are the primary reservoirs of *E. coli* O157:H7 **(Hancock *et al.*, 1997)**.

The seasonal distribution of *E. coli* O157:H7 were 11.8, 5.9, 6.6 and 0.7 % during the spring, summer, autumn and winter, respectively. Similar to the previous studies **(Van Donkersgoed *et al.*, 1999; Johnsen *et al.*, 2001)** our results showed that the prevalence of *E. coli* O157:H7 in cattle and sheep in tested samples was higher in warm months (6.6 %) than in cold months (2.5 %).

In the present study, all 27 isolates from 15 sheep and 12 cattle which 25 of them were isolated from different samples and two of them have different toxin profile from the other sub-colonies isolated from same samples, were found to carry at least one toxin gene (stx_1 or stx_2). Eighteen (18/27; 66.7 %) of the isolates were positive both for stx_{1c} and stx_{2c}, seven of them (7/27; 25.9 %) were positive for only Shiga toxin 2 variant stx_{2c}, and two of them (2/27; 7.4 %) were positive for alone Shiga toxin 1 variant stx_{1c}. Importantly, it was reported that in most of human *E. coli* O157:H7 cases stx_2 gene was more important in generating illness than stx_1 gene harboring strains on the epidemiological study **(Boerlin *et al.*, 1999)**. Also in four different REDP, most of the isolates (72.7 %) were shown the same toxin profiles in the same genomic groups. In a study performed in Turkey, where differs from our results that 9 of the 13 *E. coli* O157:H7 isolates from cattle feces harbored only stx_2 and 2 of the isolates were found to carry stx_1 and stx_2 toxin genes **(Yilmaz *et al.*, 2006)**. In a previous study, presence of *stx1, stx2* and *stx2* variants in *E. coli* isolated from asymptomatic individuals or patients of clinical manifestations of either HUS or diarrhea without HUS was compared and they found out that the presence of stx_{2c} can more likely cause HUS while presence of stx_{2d} may manifest a milder case **(Friedrich *et al.*, 2002)**. In a different study, a higher *in vitro* cytotoxicity was also reported for stx_{2c} carrying *E. coli* O157:H7 than stx_1-stx_2 or stx_1-stx_{2c} carrying strains **(Lefebvre *et al.*, 2009)**. According to these studies it can be concluded that *E. coli* O157:H7 positive cattle and sheep were contaminated with highly virulent strains and this may pose potential public health risk.

In the present study, among the tested antibiotic resistance genes, only *tetB* and *strA* were detected. In a previous study, 25 (26.0 %) of the 96 *E. coli* O157:H7 cattle isolates harbored al least one antibiotic resistance gene. Twenty six out of 102 *E. coli* O157:H7$^+$/H7$^-$ (25.5 %) were carrying one or more tested tetracycline resistance genes. In the study, *tetC, tetA* and *tetB* were detected with a ratio of

14.7 %, 12.7 % and 4.9 % respectively. In addition to *tet* genes; *sulI*, *strA*, and *strB* were detected from 12.7 %, 4.9 % and 4.9 % of the isolates **(Ayaz *et al.,* 2015)**. **Srinivasan *et al.,* (2007)** reported that, 4 of 129 (3.1 %) *E. coli* O157:H7 isolates were resistance to tetracycline and all were harbored both *tetA-tetC* but other tetracycline resistance genes were not detected from the isolates. **Srinivasan *et al.,* (2007)** revealed that 8 of 9 streptomycin resistant *E. coli* O157:H7 carried *strA* and *strB* along with *aadA*. Also in a different study, it was reported that both *strA* and *strB* genes have to be present together in order to obtain functional streptomycin resistance **(Lanz *et al.,* 2003)**. Although in the present study detection of these resistance genes did not give information about the phenotypic antibiotic resistance of the isolates, it will be important to investigate the dissemination potential of the resistance genes to other pathogens and environment.

CONCLUSION

In the present study *E.coli* O157:H7 was more prevalent in sheep (6.4 %) than in cattle (3.9 %) therefore it can be concluded that, sheep pose a great risk as cattle for STEC O157:H7 contamination in tested samples. Most of the cattle and sheep feces and/or colon tissue *E. coli* O157:H7 isolates were carrying stx_{2c} gene which makes the isolates highly virulent for human. Also tetracycline and streptomycin resistance genes were detected from some of the isolates. In the study, *tetB* and *strA* genes were detected only a few of the cattle and sheep isolates. So due to the presence of antibiotic resistance genes, there was not any significant difference between cattle and sheep *E. coli* O157:H7 isolates. The presence of the same clone from different animals may represent the persistence of a certain strain in a limited geographical area. The PFGE results showed that, cattle and sheep is possible to contaminate with clonal *E. coli* O157:H7 isolates. On the other hand, different REDP of *E. coli* O157:H7 isolated from samples in different dates showed that no cross contamination and sort of contact was occurred between farms in Turkey.

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