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Short Communication

Sheep as an important source of *E. coli* O157/O157:H7 in Turkey

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ABSTRACT

Escherichia coli O157:H7 is a globally important foodborne pathogen and has been mainly associated with cattle as the reservoir. However, accumulating data shows the importance of sheep as an *E. coli* O157:H7 vehicle. The presence of *E. coli* O157/O157:H7 in recto-anal mucosal swap and carcass sponge samples of 100 sheep brought to the slaughterhouse in Kirikkale were analyzed over a year. Molecular characteristics (*stx_1*, *stx_2*, *eaeA*, *hly*, *lpfA1-3*, *espA*, *eae-α1*, *eae-α2*, *eae-β*, *eae-β1*, *eae-β2*, *eae-γ1*, *eae-γ2/θ*, *$stx1_c$*, *$stx1_d$*, *$stx2_c$*, *$stx2_d$*, *$stx2_e$*, *$stx2_f$*, *$stx2_g$*, *bla_{ampC}*, *tet*(A), *tet*(B), *tet*(C), *tet*(D), *tet*(E), *tet*(G), *sul1*, *sul2*, *floR*, *cmlA*, *strA*, *strB* and *aadA*) of 79 isolates were determined and minimum inhibitory concentrations of 20 different antibiotics were investigated. *E. coli* O157/O157:H7 was found in 18% of sheep included in the study and was more prevalent in yearlings than lambs and mature sheep, and male than female sheep, though none of the categories (season, sex or age range) had significant effect on prevalence. Furthermore, Shiga-toxigenic *E. coli* (STEC) O157:H7 was determined in 2% and 8% of sheep feces and carcasses, respectively. Additionally, *lpfA1-3* and *eae-γ1* were detected in all isolates. None of the isolates showed resistance against investigated antibiotics, even though 4 sorbitol fermenting *E. coli* O157 isolates were positive for *tet*(A), *sul1* and *aadA*. This is the first study in Turkey that reveals the potential public health risk due to the contamination of sheep carcasses with potentially highly pathogenic STEC O157:H7 strains.

1. Introduction

Escherichia coli O157:H7 has emerged as the major etiological agent of hemorrhagic colitis and hemolytic uremic syndrome worldwide, following an outbreak that was traced back to undercooked burgers. While cattle have been associated with many human cases and are seen as the main source of the pathogen (Ayaz et al., 2014), prevalence in other healthy ruminants such as sheep should not to be underestimated as carriage rates have been found to vary between 0.4 and 40% (Chapman et al., 1997; Kudva et al., 1997; Blanco et al., 2003; Ogden et al., 2005).

As it covered 12.5% of the total 2,080,000 tons of meat production in 2010, meat of sheep origin has an important portion in Turkey. It even shows higher percentage of relative production than the 2010 sheep meat production leader (7.9%, UK) of 30 EU and EFTA countries (Anon, 2013). Being one of the major meat component of kebabs and meatballs, sheep meat is an important source of protein in Turkey, however, reports assessing *E. coli* O157:H7 presence in sheep have been very limited (Goncuoglu et al., 2010) and seemingly none have evaluated the prevalence of the pathogen on sheep carcasses. National reports by governmental authorities regarding the true incidence of *E. coli* O157:H7 infections in Turkey is lacking but there have been reports of sporadic cases of *E. coli* O157:H7 infections in humans associated with visits to Turkey (Erdogan et al., 2008), however the sources of the infections could not be determined.

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The current study was conducted to determine the presence of *E. coli* O157/O157:H7 in sheep feces and sheep carcasses over a one-year period and to assess the effect of categories such as season, age range and sex on the prevalence of the pathogen. Additionally, isolates were characterized for the presence of major virulence genes and gene variants, susceptibility toward various antibiotics and the presence of some most prevalent antimicrobial resistance genes to estimate the potential risk sheep meat presents for public health.

2. Materials and methods

2.1. Sampling design and sample collection

A total of 100 Akkaraman breed sheep brought to a small-scale slaughterhouse in Kirikkale between June 2012 and May 2013, over a year period, divided in 50 animals for each cold (November–April) and warm (May–October) season were included in the sampling (Table 1). The number of animals included in randomized sampling was achieved by a sampling of at least 50% of the sheep presented to slaughter in each occasional visit in the corresponding month and specifications such as; age range (lamb [L < 6 months], yearling [Y = 6–12 months] and mature [O > 12 months]) and sex (male and female) of sheep were recorded. A total of 200 samples (recto-anal mucosal swaps [RAMS] and carcass sponges [CS] from each sheep) were collected from 100 sheep (18 lambs [9 males and 9 females], 40 yearlings [34 males and 6 females] and 42 mature sheep [30 males and 12 females]) and analyzed for the presence of *E. coli* O157 by immunomagnetic separation (IMS) based cultivation technique.

RAMS samples were obtained by cotton swaps following bleeding and transferred to 3 ml Tryptone Soya Broth (TSB, Oxoid CM0129, Hampshire, UK) (Ayaz et al., 2014). CS samples were taken after evisceration, before chilling using a pre-moistened (10 ml buffered peptone water, BD Difco, New Jersey, USA) cellulose sponge-stick (3M, SSL100, Minnesota, USA), sampling a minimum of 700 cm^2 from flank, brisket and breast areas of each carcass sides (100 cm^2 each area) and ventral of the tail by horizontal, vertical and diagonal passes with pressure (Anon, 2001). All RAMS and CS samples were coded with same number for each sheep, kept in an ice bag and analyzed in less than 2 h.

Table 1
Distribution of the sampled sheep based on seasons.

Warm months	*n*	Cold months	*n*
May	16	November	6
June	10	December	9
July	–	January	7
August	8	February	12
September	7	March	12
October	9	April	4
Total:	50		50

2.2. Isolation of E. coli O157

Isolation of *E. coli* O157 was performed using IMS based cultivation technique as previously described (Ayaz et al., 2014) following enrichment of RAMS samples in 3 ml TSB that were thoroughly vortexed and incubated (Nüve EN120, Ankara, Turkey) at 37 °C for 18 h and CS samples that were incubated following addition of 90 ml EC broth (Oxoid CM0853) supplemented with novobiocin (20 mg/l; Sigma N-1628, St. Louis, MO, USA). Enriched cultures were subjected to IMS with anti-*E. coli* O157 Dynabeads (Invitrogen, Dynal AS, Cat no. 710-04, Oslo, Norway) and beads were plated on Cefixime-Tellurite (Oxoid SR0172) supplemented Sorbitol MacConkey (Oxoid CM0813) agar (CT-SMAC). Following incubation at 37 °C for 18 h, up to five suspected colonies were re-streaked on CT-SMAC for ensuring purity and tested by latex agglutination (*E. coli* Latex Test, Oxoid DR0620) for the presence of O157 antigen. Latex agglutination positive colonies were sub-cultured in TSB at 37 °C for 18 h and stored at −86 °C (Thermo Scientific, Revco Elite Plus 2586-6-v, USA) in cryovials supplemented with 20% sterile glycerol.

2.3. PCR analyses

For verification, identification, and virulence gene and antimicrobial resistance gene determination of *E. coli* O157/O157:H7 isolates, DNA was extracted using a method described previously (Goncuoglu et al., 2010) and 10 μl of these extracts were used as template for PCR amplifications that were carried out with Taq DNA polymerase set (Bioron GmbH, Ludwigshafen, Germany) using a thermal cycler (Eppendorf mastercycler gradient, Hamburg, Germany). *E. coli* ATCC43895 ($rfbE_{O157}^+$, $fliC_{h7}^+$, $stx1_c^+$, stx_2^+, *eae-γ1*$^+$, *hly*$^+$, *lpfA1-3*$^+$, *espA*$^+$), *E. coli* O157:H7 NCTC12900 ($rfbE_{O157}^+$, $fliC_{h7}^+$, stx_1^-, stx_2^-, *eae-γ1*$^+$, *hly*$^+$, *lpfA1-3*$^+$, *espA*$^+$), *E. coli* O157:NM 137/98 ($stx2_c^+$), *E. coli* O62:H$^-$ 551/98 ($stx2_d^+$), *E. coli* O139:K12 107/86 ($stx2_e^+$), *E. coli* O$^-$:H18 214/125 ($stx2_f^+$) and *E. coli* O2:H25 S86 ($stx2_g^+$) were used as positive controls for corresponding PCR analysis whereas sterile ultra pure water was used as negative control.

All of the primer pairs (Eurofins MWG Operon, Ebersberg, Germany) and corresponding references used for PCR analyses for verification of *E. coli* O157 ($rfbE_{O157}$), identification of *E. coli* O157:H7 ($fliC_{h7}$), determination of major virulence genes (*eaeA*, *hly*, stx_1 and stx_2), *lpfA1-3* and *espA*, *stx* variants ($stx1_c$ and $stx1_d$; $stx2_c$, $stx2_d$, $stx2_e$ and $stx2_f$ and $stx2_g$), and intimin variants (*eae-α1*, *eae-α2*, *eae-β*, *eae-β1*, *eae-β2*, *eae-γ1* and *eae-γ2/θ*) in this study are given in Table S1.

PCR analyses for determination of prevalent antimicrobial resistance genes (Table S1; bla_{ampC} [53 °C], *tet*(A), *tet*(B), *tet*(C), *tet*(D), *tet*(E), *tet*(G), *sul1* and *sul2* [58 °C], *floR* [55 °C], *cmlA* [62 °C], *strA* [56 °C], *strB* [54 °C] and *aadA* [60 °C]) were done in 50 μl and consisted of: 1× PCR buffer, 2 mM $MgCl_2$, 0.2 mM of each dNTP, 1 U of Taq DNA polymerase and 0.1 μM of each primer pairs. Following the initial denaturation at 94 °C for 5 min, 30 cycles of denaturation at 94 °C for 45 s, annealing (given above) for 45 s and extension at 72 °C for 45 s was applied with a

final extension for 5 min at 72 °C. Resultant PCR products were analyzed as described elsewhere (Ayaz et al., 2014).

2.4. MIC determination of antimicrobials on E. coli O157/O157:H7 isolates

MIC values of 20 different antimicrobials (amikacin [0.016–256 μg/ml], streptomycin [0.064–1024 μg/ml], tobramycin [0.064–1024 μg/ml], gentamicin [0.016–256 μg/ml], kanamycin [0.016–256 μg/ml], ampicillin [0.016–256 μg/ml], amoxicillin/clavulanic acid [0.016–256 μg/ml], aztreonam [0.016–256 μg/ml], cephalothin [0.016–256 μg/ml], cefaclor [0.016–256 μg/ml], cefoxitin [0.016–256 μg/ml], cefotaxime [0.016–256 μg/ml], ceftriaxone [0.016–256 μg/ml], norfloxacin [0.016–256 μg/ml], ciprofloxacin [0.002–32 μg/ml], nalidixic acid [0.016–256 μg/ml], sulfamethoxazole [0.064–1024 μg/ml], trimethoprim [0.002–32 μg/ml], chloramphenicol [0.016–256 μg/ml], tetracycline [0.016–256 μg/ml]) were determined using antibiotic test strips (Liofilchem MIC Test Strips, Roseto degli Abruzzi, Italy) by Epsilometer test (*E*-Test) on Mueller-Hinton agar (MHA, Oxoid CM0337) according to the manufacturer.

Twenty-four representative isolates (Table 2), showing the exact virulence gene distribution, originating either from RAMS or CS samples were grown overnight at 37 °C, diluted to 0.5 McFarland standard and spread on MHA with a cotton swap. Following the placement of test strips, plates were incubation at 37 °C for 18 h, inhibition ellipses were recorded and breakpoints were interpreted according to manufacturer's criteria.

2.5. Statistical analysis

Statistical analysis for determination of effect of season and age on *E. coli* O157/O157:H7 prevalence was performed using Pearson chi-square while the effect of sex on prevalence was analyzed using Fischer exact test (SPSS, version 14.01).

3. Results

3.1. Occurrence of E. coli O157/O157:H7 in sheep at slaughter

Out of 100 sheep that were brought to slaughter over a year period, 18 were found to be positive for *E. coli* O157 by IMS based cultivation technique. *E. coli* O157 was isolated from 10%, 4% and 4% of CS, RAMS and both CS and RAMS samples of sheep, respectively. A total of 75 non-sorbitol fermenting (NSF) *E. coli* O157 colonies were isolated from 14 CS and 8 RAMS samples. Furthermore, 4 suspected colonies (4CA-C and 5CA), isolated from CS samples of sheep 4 and 5, showing sorbitol fermentation (SF) on CT-SMAC in consecutive purification re-streaks were as well positive with O157 latex agglutination.

In all 79 (75 NSF and 4 SF) and 68 of 79 isolates $rfbE_{O157}$ and $fliC_{h7}$ genes were detected, respectively. Thus, 68 (86.1%) isolates were NSF *E. coli* O157:H7 and 11 (7 NSF and 4 SF) (13.9%) were *E. coli* O157. These results showed that 6% of sheep were positive for *E. coli* O157 (NSF from 4 sheep and SF from 2 sheep) whereas 12% were positive for *E. coli* O157:H7.

Table 2
Virulence gene and antibiotic resistance gene distribution of *E. coli* O157/O157/H7 sheep isolates.

Isolate[a]	*n*	Month[b]	Age[c]	Sex[d]	O157	H7	*eae*	*hly*	stx_1	stx_2	*lpf*	*espA*	Antimicrobial resistance gene
4CA[e]	1	June	L	F	+	–	γ_1	–	–	–	+	–	*tetA*, *sull*, *aadA*
4C (B, C)[e]	2	June	L	F	+	–	γ_1	+	–	–	+	–	*tetA*, *sull*, *aadA*
5CA[e]	1	June	L	F	+	–	γ_1	+	–	–	+	–	*tetA*, *sull*, *aadA*
16CA	1	August	Y	M	+	+	γ_1	+	–	–	+	+	
24R (A, B)	2	September	Y	M	+	+	γ_1	+	–	–	+	+	
26RA	1	September	Y	M	+	–	–	+	–	–	+	+	
30RA	1	October	Y	M	+	–	–	+	–	–	+	+	
30R (B, C)	2	October	Y	M	+	–	γ_1	+	–	–	+	+	
32R (A, B)	2	October	Y	M	+	–	γ_1	+	–	–	+	+	
34CA	1	October	Y	M	+	–	γ_1	+	–	–	+	+	
44C (A, B, C, D, E)	5	December	O	F	+	+	γ_1	+	–	*c*	+	+	
46C (A, B, C, D)	4	December	Y	M	+	+	γ_1	+	–	*c*	+	+	
46R (A, B, C, D, E)	5	December	Y	M	+	+	γ_1	+	–	*c*	+	+	
47C (A, B, C, D)	4	December	Y	M	+	+	γ_1	+	–	*c*	+	+	
47R (A, B, C, D, E)	5	December	Y	M	+	+	γ_1	+	–	*c*	+	+	
49C (A, B, C, D, E)	5	December	Y	M	+	+	γ_1	+	–	*c*	+	+	
51C (A, B, C, D, E)	5	January	O	M	+	+	γ_1	+	–	*c*	+	+	
71C (A, B, C, D, E)	5	March	O	M	+	+	γ_1	+	–	–	+	+	
71R (A, B, C, D, E)	5	March	O	M	+	+	γ_1	+	–	–	+	+	
72C (A, B, C, D, E)	5	March	O	M	+	+	γ_1	+	–	–	+	+	
72R (A, B, C, D, E)	5	March	O	M	+	+	γ_1	+	–	–	+	+	
95C (A, B, C, D, E)	5	May	Y	M	+	+	γ_1	+	–	*c*	+	+	
97C (A, B, C, D, E)	5	May	O	F	+	+	γ_1	+	–	*c*	+	+	
98C (A, B)	2	May	O	F	+	+	γ_1	+	–	*c*	+	+	

[a] Sampled sheep number; sample (C: carcass sponge; R: recto-anal mucosal swap); A–E: colony code.
[b] May–October (warm months); November–April (cold months)
[c] L: lamb (<6 months); Y: yearling (6–12 months); O: mature (>12 months).
[d] M: male; F: female.
[e] SF (sorbitol fermentation positive) colonies on CT-SMAC (see Section 3.1).

3.2. Influence of season, age range and sex on sheep E. coli O157/O157:H7 prevalence

Occurrence of *E. coli* O157/O157:H7 in sheep is given in Table 2. *E. coli* O157 was detected in 11 (22%) and 7 (14%) of sheep sampled during warm and cold seasons, respectively. When RAMS positivity and thus the carriage of *E. coli* O157 in sheep brought to slaughter was considered, 4 (8%) sheep were found positive in both seasons while 1 (2%) and 4 (8%) of them were positive for *E. coli* O157:H7, showing non-significant differences, respectively ($p > 0.05$). When CS positivity was considered, even though in both seasons *E. coli* O157 was detected from 7 (14%) of the sheep, *E. coli* O157:H7 was detected from 4 (8%) and 7 (14%) of the sheep carcasses sampled, respectively, and showed a non-significant difference ($p > 0.05$).

Out of 100 sampled animals, 2 (11%) of 18 lambs, 10 (25%) of 40 yearlings and 6 (14.2%) of 42 mature sheep were found to carry *E. coli* O157 in their RAMS and CS samples, while 0 (0%), 6 (15%) and 6 (14.3%) were positive for *E. coli* O157:H7, respectively (Table 2). *E. coli* O157 was not detected from RAMS samples of the lambs while 6 (15%) of the yearlings were positive for *E. coli* O157, 3 (7.5%) of which were positive for *E. coli* O157:H7, and 2 (4.8%) of mature sheep were positive for *E. coli* O157:H7. Presence of *E. coli* O157/O157:H7 in neither of the age groups showed significant differences ($p > 0.05$).

E. coli O157 was detected in 13 (17.8%) of 73 male sheep; 5 (6.8%) and 4 (5.5%) of which were detected from only CS or RAMS samples, respectively, while in remaining 4 (5.5%) from both CS and RAMS samples. However, 4 (5.5%) CS, 1 (1.4%) RAMS and 4 (5.5%) of the CS and RAMS samples of male sheep were found to be positive for *E. coli* O157:H7. In 27 female animals, *E. coli* O157 was detected from CS samples of only 5 (18.5%) animals while 3 (11.1%) of them were *E. coli* O157:H7. Sex of sheep did not show a significant influence on *E. coli* O157/O157:H7 prevalence ($p > 0.05$).

3.3. Virulence gene profiles of E. coli O157/O157:H7 isolated from sheep

Virulence gene profiles of 79 *E. coli* O157 isolates are given in Table 2. None of the isolates harbored stx_1 while 45 (57%) of the 68 *E. coli* O157:H7 isolates were positive for stx_2 and $stx2_c$. Furthermore, all isolates were *lpfA1-3*$^+$ and only one isolate (4CA) was *hly*$^-$. All SF *E. coli* O157 isolates were lacking the *espA* gene while the remaining isolates were *espA*$^+$ and all *eaeA*$^+$ isolates (77/79, 97.4%) were found to harbor *eae-γ1* variant. These results showed that 8 (8%) of 100 sheep carcasses were contaminated with Shiga-toxigenic *E. coli* (STEC) O157:H7 and only 2 sheep taken into sampling were carrying STEC O157:H7 (Table 2).

Six different virulence gene profiles were detected from 79 isolates: (i) *E. coli* O157:H7 (*eae-γ1*$^+$, *hly*$^+$, stx_1^-, $stx2_c^+$, *lpfA1-3*$^+$, *espA*$^+$) (45/79, 57%), (ii) *E. coli* O157:H7 (*eae-γ1*$^+$, *hly*$^+$, stx_1^-, stx_2^-, *lpfA1-3*$^+$, *espA*$^+$) (23/79, 29.1%), (iii) *E. coli* O157 (*eae-γ1*$^+$, *hly*$^+$, stx_1^-, stx_2^-, *lpfA1-3*$^+$, *espA*$^+$) (5/79, 6.3%), (iv) *E. coli* O157 (*eae-γ1*$^+$, *hly*$^+$, stx_1^-, stx_2^-, *lpfA1-3*$^+$, *espA*$^-$) (3/79, 3.8%), (v) *E. coli* O157 (*eaeA*$^-$, *hly*$^+$, stx_1^-, stx_2^-, *lpfA1-3*$^+$, *espA*$^-$) (2/79, 2.5%) and (vi) *E. coli* O157 (*eae-γ1*$^+$, *hly*$^-$, stx_1^-, stx_2^-, *lpfA1-3*$^+$, *espA*$^-$) (1/79, 1.3%).

3.4. Phenotypic and genotypic antibiotic resistance patterns of E. coli O157/O157:H7 isolates

Out of 24 representative *E. coli* O157/O157:H7 isolates, only 4 STEC O157:H7 isolates (47RA, 47CA, 49CA and 51CA) showed intermediate resistance against cephalothin while no other isolate showed phenotypic resistance against investigated antibiotics. Out of 14 tested antibiotic resistance genes, only *tet*(A), *sul1* and *aadA* were observed in SF *E. coli* O157 isolates (4CA, 4CB, 4CC and 5CA) (Table 2).

4. Discussion

As one of the most important foodborne pathogens worldwide, *E. coli* O157:H7 has been associated with many human cases that were linked to the asymptomatic cattle reservoir. Even though cattle have been recognized as the primary ruminant carrier of the pathogen in many countries, varying carriage rates between 0.4 and 40% were previously reported in sheep (Chapman et al., 1997; Kudva et al., 1997; Blanco et al., 2003; Ogden et al., 2005). *E. coli* O157 was detected from 18% of 100 sheep that were brought to slaughter over a year in this study. In 14%, carcasses were contaminated with *E. coli* O157, 8% being STEC O157:H7, whereas in 8%, the pathogen was detected from recto-anal mucosa and only 2% of the sheep were carrying STEC O157:H7. Cattle and cattle originating meat and meat products, in general, had been the primary focus of *E. coli* O157/O157:H7 in Turkey (Ayaz et al., 2014) and relatively less focus have been given to *E. coli* O157/O157:H7 presence in sheep and sheep-derived food products. Since this is the first study that reports *E. coli* O157/O157:H7 prevalence of sheep carcasses in Turkey, it indicates the alarming importance of sheep and sheep meat as the source of the pathogen for the public, as carcass prevalence was found much higher (14%) than previous reports from US (3.5%; Kalchayanand et al., 2007), Ireland (1.5%; Lenahan et al., 2007) or Australia (0.2%; Phillips et al., 2013) or than in cattle slaughtered at the same region of Turkey (7.1%; Ayaz et al., 2014). The prevalence of sheep, carrying *E. coli* O157 was also higher than in many studies reported around the globe (Heuvelink et al., 1998; Blanco et al., 2003; Ogden et al., 2005; Lenahan et al., 2007), as well as from countries or regions showing similarity in the season, climate or geographical location (Battisti et al., 2006; Goncuoglu et al., 2010). Since in most of these previous works, IMS-based isolation was used for detection of *E. coli* O157/O157:H7, these differences in carcass or sheep prevalence might have resulted from discrepancies in contamination levels of the pastures, hygienic practices and slaughtering processes in the slaughterhouses, as well as the age, sex or sampling season of the sheep included in previous investigations. Furthermore, it is important to point out that even though the prevalence of *E. coli* O157/O157:H7 in pelt or fleece of sheep was not in the scope of this study, detection of higher carcass prevalence compared to sheep prevalence might indirectly result from higher

contamination rates of these parts, as previously shown (Lenahan et al., 2007). Additionally, generally used manual pelt removal by the butchers rather than automatic pelt removal technology would be a contributing factor in transmission of bacteria originating from pelts to carcasses. Either way, nearly one out of ten sheep carcasses (8%) were contaminated with STEC O157:H7 and this finding directly suggested the poor technical and sanitary conditions, and hygienic precautions at the slaughterhouse. Hence, improvement of these factors by slaughterhouse executives and control of its implementation by governmental authorities will have utmost importance for reduction of public health risk that results from *E. coli* O157/O157:H7 contaminated sheep meat.

Another aim of this study was to investigate if categories such as season or sex, and age range of sheep have an influence on *E. coli* O157/O157:H7 prevalence. Occurrence of *E. coli* O157:H7 in cattle is known to be higher in summer than winter (Ayaz et al., 2014), however studies specifically investigating this phenomenon have been rather limited for sheep, and non-significant (Chapman et al., 1997) or significant (Kudva et al., 1997) higher prevalence of *E. coli* O157:H7 were reported in the summer season. No differences between warm and cold seasons were observed in carcass prevalence (8% vs. 8%) or sheep carriage rates (14% vs. 14%) in this study, which might have resulted from the relatively few number of sheep sampled. Higher prevalence of *E. coli* O157 was observed in weaned than suckling lambs (Battisti et al., 2006) and lambs than ewes (Heuvelink et al., 1998) previously. In this study however, *E. coli* O157 prevalence was non-significantly higher in yearlings (15%) than mature sheep (4.8%), while *E. coli* O157 was not detected from RAMS samples of the lambs younger than 6 months. Further work is needed to identify if this difference might be important in potential public health risk the yearlings possess, as husbandry of yearlings is one of the most preferred and economically privileged sheep husbandry style for the farmers. Influence of sex on *E. coli* O157 prevalence of cattle is controversial (Ayaz et al., 2014) and to my knowledge none of the previous studies have evaluated this category for sheep. Although it was not statistically significant due to the small number of female sheep sampled in this work, prevalence of *E. coli* O157 was higher in male (10.9%) compared to female (0%) sheep and further work, with emphasis on likewise numbers of sheep for each sex, is needed for clarification of this influence.

In none of the 79 *E. coli* O157/O157:H7 isolates was the stx_1 gene detected in accordance with some previous studies (Battisti et al., 2006) while contradicting some others (Lenahan et al., 2007). However, 45 (57%) of the isolates, detected from 8% of the sheep carcasses and 2% of the RAMS samples, were harboring $stx2_c$, suggesting their high potential to lead to the development of hemolytic uremic syndrome in humans (Friedrich et al., 2002). Studies investigating the intimin variants in *E. coli* O157/O157:H7 strains from sheep origin have been rather limited, but in accordance (Blanco et al., 2003), all of the eae^+ isolates were harboring *eae-γ1* variant in *E. coli* O157/O157:H7 isolates and all *eae-γ1* variant harboring *E. coli* O157/O157:H7 isolates were positive for *lpfA1-3* in accordance as previously shown to be associated (Torres et al., 2009). In 4 SF *E. coli* O157 isolates (4CA-C and 5CA) *espA* gene was not observed. This suggests the lack of potential in development of attaching and effacing lesions in these isolates (McNally et al., 2001), however, the coincidence of SF and lack of *espA* in these accidental isolates are of great interest and needs further work.

None of the isolates showed phenotypic resistance toward any of the investigated antibiotics, which could be resulting from relatively less preferred intensive breeding of sheep (Goncuoglu et al., 2010). It is clear that sheep originating *E. coli* O157/O157:H7 isolates have no or minor potential in transfer of antibiotic resistance traits.

5. Conclusion

In conclusion, being the first study in Turkey that reveals the potential public health risk due to the contamination of sheep carcasses with potentially highly pathogenic STEC O157:H7 strains, the current study shows the urgent need of improvement in slaughterhouses' hygienic and technological requirements. Since a high carriage rate of *E. coli* O157/O157:H7 was also found in sheep brought to slaughter, it is clear that the use of unprocessed sheep feces in agriculture and gardening as fertilizers pose a high risk for public health and thus, thorough hygienic precautions should be taken following contact with soil fertilized with sheep feces or pastures where sheep graze. Observation of higher prevalence of *E. coli* O157/O157:H7 in male and yearling sheep is an important finding and needs further investigation. Although no alarming antibiotic resistance was observed, presence of intermediate resistant isolates suggests further screening of this trend.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vetmic.2014.06.014.

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