Prevalence of Shiga Toxin-Producing O157 and Non-O157 *Escherichia coli* in Anatolian Buffaloes (*Bubalus bubalis*)

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ABSTRACT

Shiga toxin producing *Escherichia coli* (STEC) serotypes are recognized as potentially important food-borne pathogens for humans. Ingestion of *E. coli* contaminated food is largely known to originate from livestock. Cattle and sheep herds hold the majority of agricultural revenue in Türkiye but Anatolian water buffaloes have often been underestimated for foodborne pathogens. The aim of this study is to determine virulence genes harboring *E. coli* O157 and six major non-O157 STEC (O26, O45, O103, O111, O121, and O145) serotypes in feces of healthy Anatolian water buffaloes by using multiplex PCR (mPCR) method. Of the collected 458 fecal samples from healthy live animals, we have performed virulence and serotype targeting mPCR following direct DNA extraction from collected samples. Results indicate that there is 0.9% of O157 prevalence while six major non-O157 *E. coli* have not been identified. The characterization results of the virulence genes also showed that *eae* is most prevalent (5.7%) followed by *ehxA* (3.9%) and stx_1 (3.1%). In this study, we have shown Anatolian buffaloes might have a relationship with other O-type *E. coli* strains. Non-O157 STECs, which are often disregarded in both animals and humans, should be investigated. As a consequence, gaining regional or national data collection will allow to implement better effective diagnosis and treatment options.

Keywords: *E. coli* O157; non-O157; STEC; Anatolian Buffaloes.

INTRODUCTION

Escherichia coli (*E. coli*) consists of a diverse and large group of bacterial organisms residing within the gut of humans and animals (1). Although most *E. coli* variants are harmless or result in asymptomatic colonization, some pathogenic variants can cause gastroenteritis, including hemorrhagic colitis (HC) or extra-intestinal infections such as urinary tract infections, hemolytic uremic syndrome (HUS) or other severe systematic infections including pneumonia (2).

Diarrhea-causing pathogenic *E. coli* variants are identified under several groups according to their O-type serogroup classification and pathogenesis with clinical implications: entero-aggregative *E. coli*, entero-pathogenic *E. coli* (EPEC), diffuse-adherent *E. coli*, entero-invasive *E. coli,* and entero-haemorrhagic *E. coli* (3). Shiga toxin-producing *E. coli* (STEC) agents are mostly associated with the entero-haemorrhagic group of variants but several types of diarrheagenic *E. coli* pathovariants other than O157 can also produce shiga toxin (4). In a public outbreak that emerged in Germany in 2011, it was found that non-O157 shiga toxin-producing *E. coli* O104 was responsible for mass public infections. Among the infected patients (3842 cases) in Germany, tragedy 22.1% of people developed HUS, and the outbreak resulted in

1.4% mortality. A detailed investigation confirmed that the consumption of contaminated vegetable sprouts led to the infection (5). Many large sporadic outbreaks have emerged in the past between 1984 and 2009 but non-O157 STEC cases rarely have been reported due to the absence of identification and characterization of non-O157 STEC methods (6). In a different outbreak led by foodborne infection, it has been reported that 23 children from South Australia (1995) and 10 children from Norway (2006) developed HUS due to non-O157 STEC; O111 and O103 respectively (7, 8). As a result, we are now much more aware of the importance of both O157 and non-O157 STEC for public health issues due to unexpected sources of infection and outbreak.

E. coli strains may acquire virulence factors such as shiga toxin 1 and 2 (*stx₁, stx₂*) intimin (*eae*), and enterohaemolysin (*ehxA*). It was suspected that there was a close relationship between virulence factors and clinical manifestations. Shiga toxins are cytotoxins that inhibit protein synthesis and cause host cell death. Intimin is shared by STEC and EPEC implements which is tightly attach to epithelial cells. Therefore, causes attaching and effacing (A/E) lesions in the intestinal mucosa. Especially HC and HUS cases with severe diarrhea were determined to be closely related to STEC types carrying the *eae* gene (9). Enterohaemolysin is thought to provide an iron source to stimulate *E. coli* growth by lysis of erythrocytes and release of heme from hemoglobin (10). Hence, virulence genes incorporating *E. coli* strains or their combination with various virulence genes maybe closely associated with clinical symptoms of intestinal or extra-intestinal infections.

Foodborne STEC infections are caused mainly by contaminated water sources via close contact with fecal materials of farm animals, primarily cattle followed by goats, sheep, and pigs (11). Although most of the daily dietary needs of humans are primarily supplied from cattle, buffaloes are also considered as a source of meat and dairy products.

Türkiye continues breeding Anatolian buffaloes, descended from Mediterranean water buffalo, for its economic value and special aromas of dairy products. Previously, epidemiological investigation of O157 and non-O157 serogroup STEC isolation studies were carried out in farm animals including cattle and sheep in Türkiye, however there is less known about Anatolian water buffaloes as a potential source of STEC. Moreover, prevalence studies of STEC in Anatolian water buffaloes are limited. A few studies have been conducted in Anatolian water buffaloes showing the prevalence of O157:H7 STEC, but the presence of non-O157 STEC has not yet been elucidated (12-16). Besides, the importance of virulence genes harboring *E. coli* serotypes in relation to public health should also need to be highlighted.

The purpose of this study was to determine *E. coli* O157 and six major non-O157 (O26, O45, O103, O111, O121, O145) *E. coli* serotypes in feces of healthy Anatolian water buffaloes by characterization of encoded virulence genes: shiga toxin 1 (*stx₁*), shiga toxin 2 (*stx₂*), intimin (*eae*) and enterohaemolysin (*ehxA*) using a mPCR assay.

MATERIALS AND METHODS

Sampling

Sample collection was carried out at 83 different buffalo farms (animal numbers varies between 5 and 25) within Sivas city, Central Anatolia, Türkiye. A total of 458 fecal samples were directly taken from the rectum of healthy Anatolian buffaloes of 4 years and older using a disposable sterile glove per animal to eliminate cross contamination. Fecal samples were placed in sterile disposable plastic containers. Samples were kept in a car cooler fridge that maintained 4˚C inner temperature and then transferred to the laboratory.

Bacterial DNA isolation

DNA isolation was performed on samples immediately upon arrival to laboratory as described by the manufacturer of QIAamp DNA Stool Mini Kit (QIAGEN, Germany). A spectrophotometry absorbance-based method at A260/280 ratio was applied using NanoDrop (DeNovix, USA) to verify and quantify the amount (µg) of isolated DNAs. To verify the purity of DNA, the ratio of A260/280 around 1.8 was accepted as pure, otherwise samples were considered contaminated and extraction was repeated. The following controls were used in this study: O157 retrieved from American Typing Cell Culture (ATCC, 43894) and six non-O157 serotype reference strains retrieved from Staten's Serum Institute (Copenhagen, Denmark, SSI-95211 for O26; SSI-87256 for O45; SSI-82170 for O103; SSI-82118 for O111; SSI-82130 for O121; SSI-82280 for O145).ATCC 43895 strain also used as positive control for the *stx₁, stx₂, eae, ehxA* genes. DNA samples were labeled and stored at -20˚C until *E. coli* characterization.

Table 1. The list of primer pairs used in mPCR to identify O-type and virulence genes.

Target Gene	Primer ID	Nucleotide sequence (3'-5')	Product size (bp)	Tm(°C)	Ref
wzxO45	O45-F	GGGCTGTCCAGACAGTTCAT	890	65.5	(17)
	O45-R	TGTACTGCACCAATGCACCT		66	
wzxO103	O103F2	GCAGAAAATCAAGGTGATTACG	740	61.7	(17)
	O103R2	GGTTAAAGCCATGCTCAACG		63.3	
stx1	stx1-F	TGTCGCATAGTGGAACCTCA	655	64.8	(18)
	stx1-R	TGCGCACTGAGAAGAAGAGA		64.9	
wbqO121	O121-F2	TCAGCAGAGTGGAACTAATTTTGT	587	64.4	(17)
	O121-R2	TGAGCACTAGATGAAAAGTATGGCT		65.6	
wzxO145	O145F5	TCAAGTGTTGGATTAAGAGGGATT	523	63.9	(17)
	O145R5	CACTCGCGGACACAGTACC		65.6	
stx2	stx2-F	CCATGACAACGGACAGCAGTT	477	66.5	(18)
	stx2-R	TGTCGCCAGTTATCTGACATTC		64	
wzxO26	O26F4	AGGGTGCGAATGCCATATT	417	63.8	(17)
	O26R4	GACATAATGACATACCACGAGCA		64	
eae	eae-F	CATTATGGAACGGCAGAGGT	375	63.7	(18)
	eae-R	ACGGATATCGAAGCCATTTG		62.1	
rfbEO157	rfbE-F	CAGGTGAAGGTGGAATGGTTGTC	296	66.5	(19)
	rfbE-R	TTAGAATTGAGACCATCCAATAAG		60.6	
wzxO111	O111F2	TGCATCTTCATTATCACACCAC	230	62.6	(17)
	O111R2	ACCGCAAATGCGATAATAACA		62.9	
ehxA	ehxA-F	GCGAGCTAAGCAGCTTGAAT	199	64.7	(18)
	ehxA-R	CTGGAGGCTGCACTAACTCC		65.5	

E. coli serotype screening and STEC detection by mPCR

For the *E. coli* serotype screening, we performed multiplex PCR (mPCR) method consisting of both O-type and virulence gene targeting primer pairs listed as in Table 1. The sample screening for O-type and virulence genes was performed in two separated steps in all extracted DNA samples (n=458). In the first mPCR panel step, a mixture of O-type specific primer pairs was employed to determine O26, O45, O103, O111, O121, O145 and O157 types. In the second mPCR panel, a mixture of virulence genes targeting primer pairs was applied to identify *stx1, stx2*, *eae* and *ehxA* virulence traits in collected sample mixtures. The mPCR was accomplished in a total 50 µl mixture reaction volume that was constituted of the following molecules and reagents; 1XPCR buffer [75mM Tris-HCl, 20mM $(NH_4)_2SO_4$, 0.1% Tween–20; pH 8.8], 2.5 mM $MgCl_2$, 250 µM of each dNTP, 1.5 U Taq DNA polymerase (MBI, Therma Scientific Fermentas, Lithuania), 5 ρmol of each primer and 5 µl of isolated DNA (25 ηg). Reference strain DNA samples were also included to study for each run along with distilled water as a negative control. Thermal cycle (T100, BIORAD) conditions were set to single cycle at 94°C for 5 minutes; 35 cycles at 94°C for 30 seconds, 67°C for 80 seconds, then cooled down to 4°C. PCR products were run in 1.5% agarose by constant 100V pulsing voltage. The gel was soaked in 10mg/ml ethidium bromide for 30 minutes, and then visualized using UV transilluminator (Vilber Lourmat Quantum ST4, France). Low amplified or nonspecific bands showing samples were repeated to verify the results.

Statistical analysis

The chi-square test was used to reveal the statistical significance of the differences between PCR results of the shiga toxin positive *E. coli* O157 and non-O157 shiga

Table 2. Distribution of positive *E. coli* O serotypes and virulence genes in fecal samples of Anatolian buffaloes.

Sample no*	Farm**	O serotype***	*stx1*	*stx2*	*ehxA*	*eae*
5	Hayran	ND	–	–	+	–
6		ND	–	–	–	+
7		ND	–	–	+	+
8		ND	+	–	–	–
9	Pazar	ND	–	–	–	+
18	Catal	ND	+	–	–	–
21		ND	–	–	–	+
24		ND	–	–	–	+
25		ND	–	–	+	–
45	Guney	O157	+	–	+	–
46		O157	+	–	–	–
66	Alaca	ND	–	–	–	+
67		ND	–	–	+	–
70		ND	–	–	–	+
87	Mentes	ND	+	–	–	–
91		ND	–	–	–	+
96		ND	–	–	+	–
97		ND	–	–	–	+
118	Eken	O157	+	–	–	–
125		ND	–	–	–	+
144	Konak	ND	–	–	+	–
204	Hasdemir	ND	+	–	–	+
209		ND	–	–	–	+
219	Polat	ND	+	–	+	+
221		ND	–	–	+	+
228		ND	–	–	–	+
251	Akca	ND	+	–	+	+
252		ND	–	–	+	+
253		ND	–	–	+	–
256		**ND**	**–**	**–**	**–**	**+**
274	Sarica	O157	+	–	–	+
305	Bostan	ND	+	–	+	+
307		ND	–	–	+	+
308		ND	–	–	+	–
311		ND	–	–	–	+
345	Kara	ND	+	–	–	+
386	Pinar	ND	+	–	–	+
417	Bey	ND	+	–	+	+
440	Yayla	ND	–	–	–	+
443		ND	–	–	+	–
457	Soran	ND	–	–	+	–
41	**18**	**–**	**14**	**0**	**18**	**26**

*: Of the 458 fecal samples 41 were found PCR positive result for the investigated genes in this study. **: These 41 samples were fell within 18 of sampled 83 farms. ***: *E. coli* O26, O45, O103, O111, O121, O145 and O157 serotypes were examined and only four samples were found positive O157 but none of the samples were positive for other serotypes subjected in this study. *stx1*: shiga toxin 1, 14 samples were found positive for *stx1*. *stx2*: shiga toxin 2, none of the samples were found positive for stx2. *ehxA*: enterohaemolysin, 18 samples were found positive for *ehxA*. *eae*: intimin, 26 samples were found positive for *eae*. ND: Not determined. +: Gene present. –: Gene absent.

toxin positive samples and their distribution at the farm level. Values with p≤0.05 were considered statistically significant.

RESULTS

STEC prevalence

From 458 collected fecal samples from healthy Anatolian buffaloes, only 4 (0.87%) tested positive for O157 but none for non-O157 *E. coli* serotypes (O26, O45, O103, O111, O121, and O145). The *stx*$_1$ gene was detected in all O157 positive samples; however the *stx*$_2$ gene was not. Of the 458 samples, 10 individual samples were carrying *stx*$_1$ and none of them was found positive for seven major O serotypes being examined (Figure 1). The prevalence of O157 STEC and non-O157 STEC were estimated to be 0.87% and 2.18%, respectively, with a total STEC prevalence of 3.06% (Figure 2a). The difference between O157 and non-O157 STEC positivity rates was not significant (p>0.05). When the results were considered at the farm level, STECs were determined in 13.25% (11/83) of the herds. Within these 11 herds, the proportion of STEC positive samples found in range between 5% and 8%, with an overall proportion of 3.06% (14/458). The positivity of STEC O157 is 3.62% (3/83) while non-O157 STEC positivity is 9.64 % (8/83) at farm level (Figure 2b). The difference between these results were not significant (p>0.05).

Characterization of STEC

The O157 positive samples (n=4) all possessed *stx*$_1$ (100%) gene, while none of them were found positive for *stx*$_2$. It was also found that 10 out of 458 of the fecal samples were also positive for *stx*$_1$ but not for the seven major O serotypes examined. These samples were considered as non-O157 and not major six STEC serotypes which were out of subject of this study. These results were listed in Table 1. The prevalence of *eae* and *ehxA* were found around 5.7% and 3.9% of the fecal samples of Anatolian buffaloes, respectively. When non-STEC samples (n=27) were analyzed, results revealed that only 9 samples harbored the *ehxA* virulence gene alone while only 14 samples showed positivity for *eae*. Out of 27 non-STEC samples, only four samples possessed the combination of both *eae* and *ehxA* genes. Although we identified the presence of *ehxA* and *eae* virulence genes in non-STEC samples, we also detected those genes other than shiga toxins in STEC identified samples. Results indicated that, of the 14 STEC samples, only one sample was identified positive for *ehxA* (*stx*$_1$+*ehxA*) while *eae* determined alone in four samples (*stx*$_1$+*eae*). Among the shiga toxin-positive samples, we also determined the combination of virulence *eae* and *ehxA* genes in four samples (*stx*$_1$+*eae*+*ehxA*). Both virulence genes (*eae* and *ehxA*) were not found in the remaining five STECs.

DISCUSSION

Among the members of the global market, Türkiye was holding 4th place in terms of milk production from buffaloes in the early 2000's however production was reduced significantly due to modernization in agricultural revenue and substitution of Anatolian buffaloes with Holstein heifers (20). Although the majority of dairy products are supplied from cattle and sheep herds in Türkiye, Anatolian water buffaloes still have a significant economic impact due to the nutritional values of buffalo milk and their dairy products particularly mozzarella cheese. STEC isolation and identification in meat and dairy products of ruminants has been demonstrated however, there is less information known about STEC presence in buffaloes (21, 22). Although the presence of STEC O157:H7 in Anatolian buffaloes has been studied, non-O157 STEC and its characterization remains unqualified.

This study provides the first results of seven major STECs including the presence of virulence genes (*stx*$_1$, *stx*$_2$, *eae* and *ehxA*) from fecal samples of Anatolian water buffaloes reared in Central Anatolia, Türkiye. Several studies have shown the success of PCR techniques to characterize *E. coli* and STEC with 100% specificity and 98% sensitivity (17, 23); therefore, mPCR assay was employed for the detection of both virulence genes and *E. coli* O serotypes in the present study.

There is no known human STEC infection caused by meat or dairy products of Anatolian buffaloes in Türkiye but the presence of *E. coli* O157:H7 in Anatolian buffaloes in different regions was investigated (13-15). A study conducted in western Türkiye evidenced *E. coli* O157:H7 presence for the first time in raw milk and fecal samples of Anatolian water buffaloes, which showed 3.7%, and 1.4% positivity in fecal and milk samples, respectively (14). In a different study, 3.8% *E. coli* O157:H7 positivity was reported in Buffaloes residing in Northern Anatolia of Türkiye (15). In the current investigation, of the collected samples, we have identified

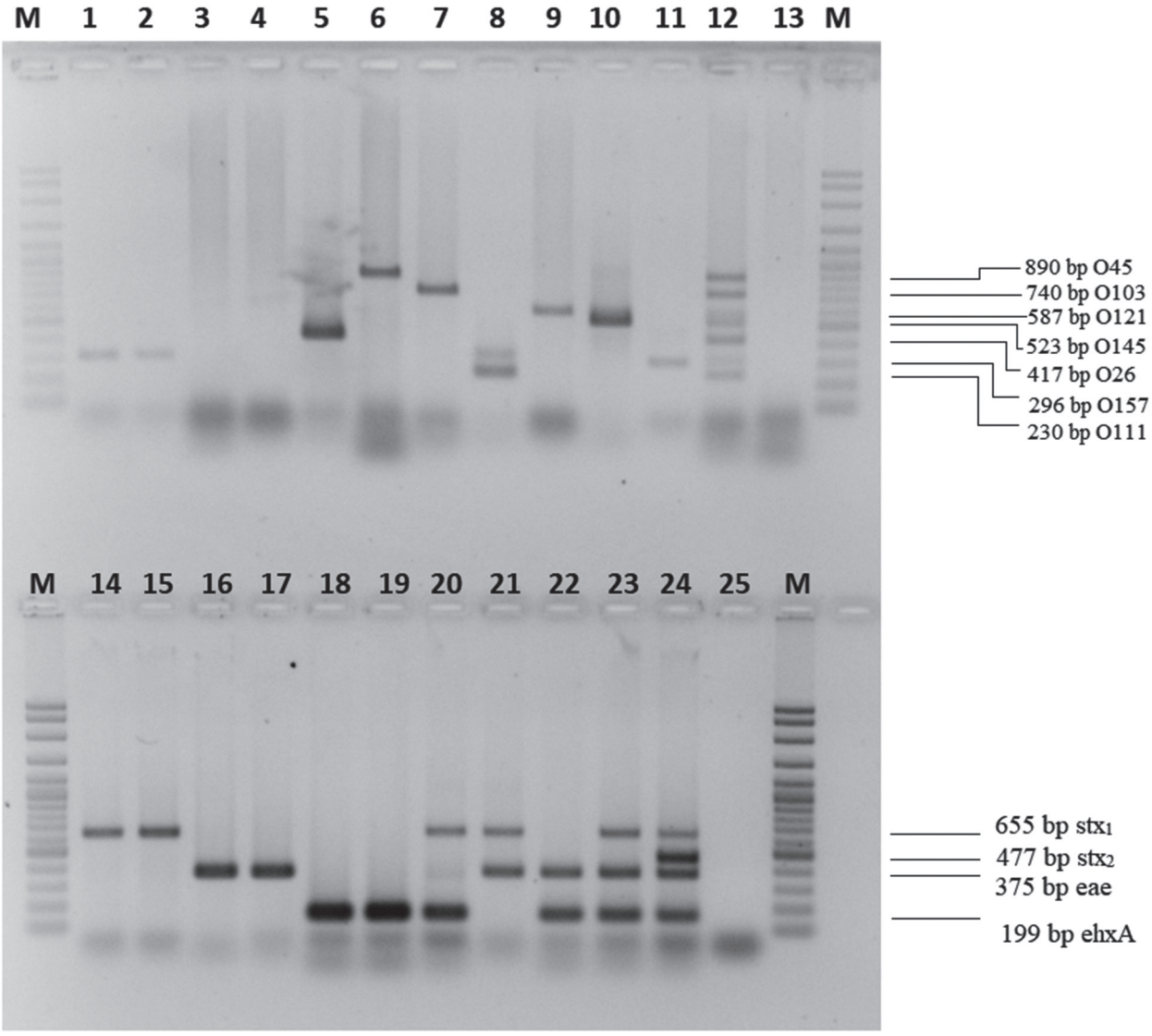

Figure 1: The multiplex PCR results of of DNA samples obtained from fecal samples of Anatolian buffaloes. The image representing the DNA amplicons in EthBr stained agarose gel electrophoresis.

Top line: Multiplex PCR results of *E. coli* O serotype. M: 100 bp DNA ladder, Lane 1-2: O157 positive field samples, Lane 3-4: Negative field samples. Lane 5: O26 positive control (SSI-95211), Lane 6: O45 positive control (SSI-87256), Lane 7: O103 positive control (SSI-82170), Lane 8: O111 positive control (SSI-82118), Lane 9: O121 positive control (SSI-82130), Lane 10: O145 positive control (SSI-82280), Lane 11: O157 positive control (ATCC 43895), Lane 12: Mixed DNA sample of all reference positive controls, Lane 13: Negative control (distilled water).

Bottom line: Multiplex PCR results of virulence genes. M: 100 bp DNA ladder, Lane 14-15: *stx1* positive field samples, Lane 16-17: *eae* positive field samples, Lane 18-19: *ehxA* positive field samples, Lane 20: *stx1+ehxA* positive field sample, Lane 21: *stx1+eae* positive field sample, Lane 22: *eae+ehxA* positive field sample, Lane 23: *stx1+eae+ehxA* positive field sample, Lane 24: *stx1+stx2+eae+ehxA* positive control (ATCC 43895), Lane 25: Negative control (distilled water).

0.87% prevalence for O157 but flagella antigen (H) was not studied. Besides, a study conducted on carcasses and rectal swab samples of buffalos to investigate the presence of *E. coli* O157:H7, none of the collected samples reacted in O157 and H7 agglutination assays, indicating no positivity (13). Such differences might be related to the chosen methods, bacterial load in animal (samples) or different geographical areas being investigated.

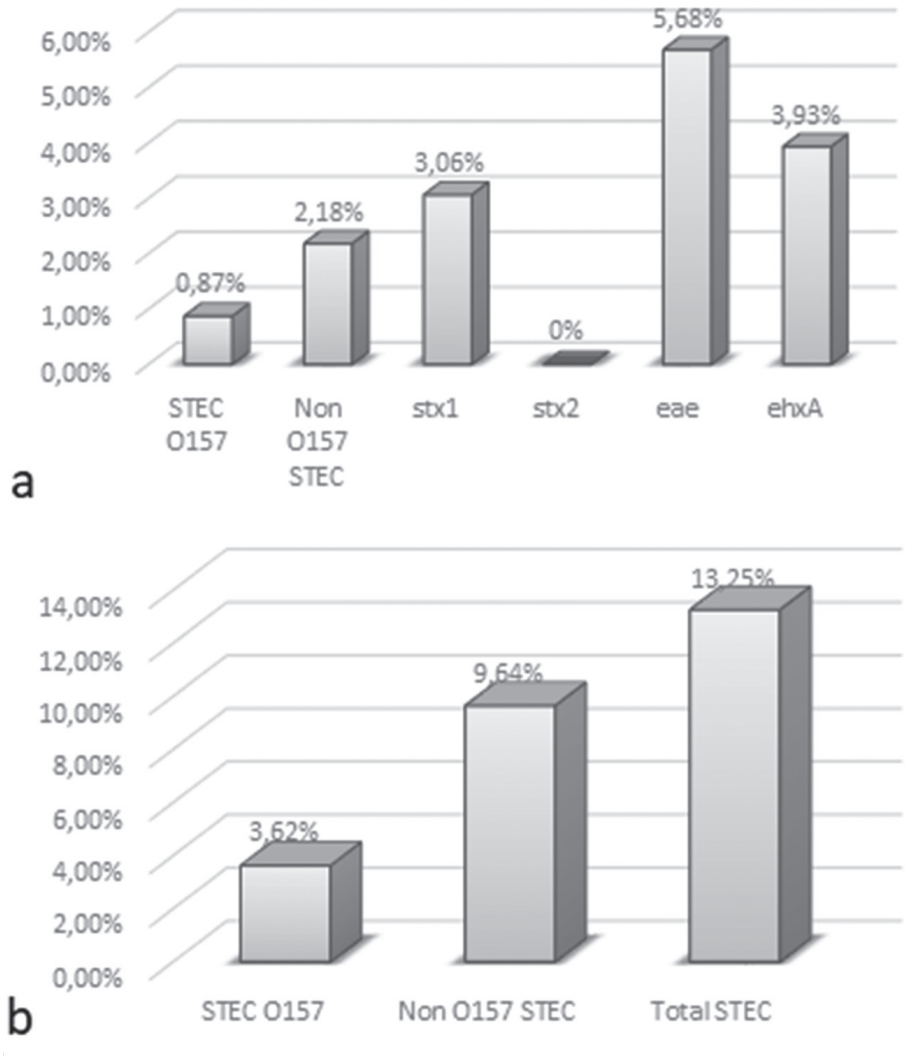

Figure 2: The prevalence of STECs and virulence genes identified in feces samples of field collected Buffaloes.

The bar graph (a) showing the prevalence of STECs and virulence genes in total collected samples (n=458) while second bar graph (b) representing the distribution of STECs in farms (n=83). *E. coli* O26, O45, O103, O111, O121, O145 serotypes were not detected and omitted in graph. There was no statistical difference between O157 and non O157 STEC prevalence of collected samples ($p>0.05$). The difference between O157 and non-O157 STEC positivity rates of farms was also not significant ($p>0.05$).

Our virulence gene screening results revealed that 14 samples out of 458 examined samples tested positive for *stx₁* (3.1%) virulence trait. None of the samples assayed were positive for *stx₂* whereas 5.7% and 3.94% of samples were carrying *eae* and *ehxA* respectively. We also showed the presence of *ehxA* or *eae* genes in non-STEC samples indicating that some *E. coli* strains carry at least one of the virulence genes other than shiga toxins. Therefore, those *eae* and *ehxA* carrying non-STEC strains can be considered as EPEC. It could be assumed that Anatolian water buffaloes or their dairy and meat products have a lower potential risk for public health when compared to other ruminants bred in Türkiye; nonetheless, they can play a role in foodborne infections when other non-O type STECs and EPECs are considered.

Water buffaloes are also considered important livestock; however, their products in several countries and economic values can vary. A study conducted in fecal samples of buffaloes in Italian rearing farms showed a 14.5% of STEC O157 prevalence, suggesting the water buffaloes should be

taken into account as a potential reservoir of STEC (24). In 2013, a STEC outbreak emerged and resulted in 22 cases of HUS in Italy due to shiga toxigenic O26 contamination, suspected from raw milk or vegetables, and this increased the awareness concerning non-O157 STECs (25). A recent study, carried out in different animal products has indicated that raw buffalo milk retained 35.71% pathogenic *E. coli* agents, which harbored *stx*$_1$, *stx*$_2$, *eae,* and *ehxA*. It was also shown in the same study that prevalence of O157, O26, and O111 was about 50%, 40%, and 10% respectively, while O45, O111, and O121 serotypes were not determined (26). A study from Bangladesh screened fecal samples of buffaloes and portion of STEC was about 11%, among which 7% and 5% of them possessed the *stx*$_1$ and s*tx*$_2$ respectively, suggesting buffaloes could pose an added public risk in rural areas in Bangladesh. However, O-type *E. coli* was not investigated in that study (27). In terms of O-type serogroup characterization, a study conducted in Southeastern Brazil showed a 37% prevalence of non-O157 STEC in fecal swab samples taken from buffaloes (28). Among the STEC positive samples, 38.5% and 22% harbored the *stx*$_1$ and *stx*$_2$ gene respectively, while none harbored the *eae* gene. Curiously, none of the samples tested positive for common O serogroups (O26, O103, O111, O145, O157) (28). Similarly, we also could not identify six major O-type *E. coli* (O26, O45, O103, O111, O121, and O145) in fecal samples of Anatolian water buffaloes. This finding could be explained by the prevalence of common O-type serogroups that can show a distinct and diverse relationship among animals at the species level.

Another study revealed that there was a strong relationship between *eae* gene with some certain O-type serogroups (O5, O26, O69, O84, O103, O111, O145, and O157 (29). As stx$_2$ causes more severe human infection than *stx*$_1$, the presence of other virulence genes could enhance clinical signs (30). Even though, virulence genes harboring STEC and non-STEC serogroups other than major O-types were not investigated in the present study, we have provided detailed investigation of major serotypes along with common virulence genes in terms of STEC prevalence in Anatolian water buffaloes in Türkiye. Future studies should be directed to characterize STECs and EPECs prevalence in a broad range spectrum of O-type antigens and virulence genes.

Although dairy products are plausibly contaminated during milking and production, animal carcasses are most commonly contaminated with pathogenic bacteria during slaughtering and dressing. In the current study, *E. coli* O157 and six non-O157 major serogroups (O26, O45, O103, O111,121, O145) were investigated by PCR in Anatolian water buffaloes for the first time and the presence of six major *E. coli* other than O157 which were not identified. Our findings revealed that the most prevalent virulence genes identified in healthy Anatolian water buffaloes were *eae* followed by *stx*$_1$. Even though most of the *E. coli* strains are known as harmless and sometimes referred to as commensal bacteria for mammals, acquirement of virulence traits through evolutionary processes can change their clinical manifestations. It has been shown that the presence of virulence genes alone or in combination with other virulence genes may enhance the pathogenicity of *E. coli*. Those acquirements include the attachment of *E. coli* to the intestinal wall by *eae* and increase in cytopathogenic abilities by shiga toxins (stx$_1$, stx$_2$). Furthermore, some animals infected with *E. coli* strains possessing *ehxA* showed clinical signs of hemolysis (10). It has been anticipated and discussed in detail that there is a close relationship between clinical outcomes and virulence genes. Some studies also showed virulence genes can contribute resistance to some antibiotics (31, 32).

To minimize foodborne O157 and non-O157 STEC human infections, it is highly crucial to determine which serotypes and virulence genes are most prevalent and what are the possible transmission routes to humans such as the consumption of meat, milk or vegetables. In this study, we observed that Anatolian buffaloes might have a relationship with other non-O157 STECs but they could not be determined due to current facilities. The epidemiology of STECs especially non-O157 STECs in Türkiye could be clarified by more comprehensive studies including other animal species as well. Therefore, it can lead to developing new control measures for the prevention of possible human outbreaks and to reduce the economic losses of food producers. Investigation of non-O157 STECs, which are mostly ignored in both animals and humans, should also be considered. Thus, obtaining regional or national data will provide more effective diagnosis and treatment opportunities.

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CONFLICT OF INTEREST STATEMENT

No potential conflict of interest was reported by the authors.

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