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Characterization and Antibiotic Resistance Profile of E. coli O157:H7 Isolates in Water Buffalo Carcasses

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IN this study, from the buffaloes slaughtered in slaughterhouses in Giresun and Samsun provinces, seasonally skin, rectoanal mucosal swab (RAMS) and carcass swab samples were collected. In the breadth of this study, a total of 600 samples were examined for the presence of Escherichia coli O157:H7. E. coli O157:H7 prevalence is 2.5% in RAMS, 3.5% in skin samples and 2% in carcass samples, with an average of 2.66%. Some virulence gene expressions of the isolates are distributed as follows; 28% (7/25) for the stx1 gene, 56% (14/25) for stx2, 68% (17/25) for the hlyA gene and 100% (25/25) for eaeA. The rate at which the stx1 and stx2 genes are found at the same time is determined to be 5/25 (20%). The antibiotic resistance rate of isolates have been identified as ampicillin at 24%, cephalothin at 20%, trimethoprim/sulfamethoxazole at 16%, gentamicin at 16%, streptomycin at 16%, tetracycline at 8%, amoxicillin at 4% and ciprofloxacin at 4%. It can be concluded that water buffalos, like cattle, are reservoirs of E. coli O157:H7, because of cross-contamination as a result of infections from feces to the carcass, feces to the skin, and skin to the carcass. The isolates containing the genes stx1, stx2, eaeA, and hlyA in actively contaminated water buffalo meat present the risk that major infection and death can occur in humans. Also resistance of isolates to antibiotics rates also indicates the unnecessary and uncontrolled use of antibiotics in the field of Veterinary Medicine.

Keywords: Water Buffalo Carcass; E. coli O157:H7; Virulence Gene; Antibiotic Resistance.

Introduction

Buffaloes serve a dual purpose, being utilized for their meat and milk as well as their strength in agricultural activities. They are continentally and regionally raised in North Africa, South America, Australia, Southeast Asia, the Balkans, in some Central European nations and in the Mediterranean. Ninety-six point four percent (96,4%) of the world's buffalo population is found on the Asian continent, and they are mainly raised using traditional and conventional methods. Fifty-five percent (55%) of the world's buffaloes are present in India, 17% in Pakistan and 13% in China. These three countries make up 85% of the world's buffalo production. [1,2]. Worldwide; 1.605.905 tons of buffalo meat were made in 1980; and in 2019; 4.290.212 tons of buffalo meat were produced and the amount of production is increasing year by year [3].

Water Buffalo (called "Su Mandası" in Turkish) are named as Camış, Dombay, Kömüş and Camız according to the geographical areas where they are being produced [4]. According to the 2022 data of TUİK, while there were 366,150 buffaloes in the country in 1991, in 2010 the number of buffaloes decreased to 84,726 and in 2021, 185,574 buffaloes were recorded [5]. The number of buffalo slaughtered in Turkey and the amount of buffalo meat production in 2020 was reported as 40,929 buffalo and 8,424 tons of meat [6].

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If the growth acceleration of the human population in the world continues at this rate, in 2050, the human population may be between 9-10 billion. The need for food will increase by 50% compared to today and is foreseen to increase even more. Naturally, production and consumption would increase in animal products [3]. Due to this need, problems arise in obtaining food in more sustainable ways. Chief among these is, the failure to implement hygiene measures that should be in food production steps cause human food sources to be contaminated with various food-borne pathogens that threaten human health. Generally, when poor hygienic conditions are present and the carcasses of cattle are slaughtered, foodborne gastrointestinal pathogens are contaminated primarily by agents such as Salmonella spp., Escherichia coli serotype O157:H7 and Clostridium perfringens [7, 8].

Detection of the agent in their stools indicates that the source of this serotype is cattle and that it is effective in its dispersal and therefore cattle are found to be the primary reservoir for E. coli O157:H7 infections almost everywhere in the world [9]. To date, isolation of an average of 500 Enterohemorrhagic Escherichia coli (EHEC) serotypes with bovine origins has been completed. The E. coli serotype O157:H7 has seven lineages that have genetically been identified. These are Ia, Ib, Ic, I/II, IIa, IIb, and IIc [10, 11]. People of all ages may be affected by E. coli O157:H7 and are susceptible to infections but children, the elderly and those with compromised immune systems have higher sensitivity [12]. E. coli O157:H7 causes hemorrhagic uremic syndrome (HUS), hemorrhagic colitis (HC) and thrombotic thrombocytopenic purpura (TTP) [13-15]. Although there are many subspecies of enterohemorrhagic E. coli (EHEC), shiga toxin 1 (stx1) and shiga toxin 2 (stx2) have two separate pathogenic properties [16, 17]. The pathogenicity of STEC depends on the production ability of shiga toxins (encoded by the stx1 or stx2 genes) and additionally acquired virulence characteristics [18]. Three toxins belonging to the stx1 subtype stx1a, stx1c and stx1d have been identified, many toxins including stx2a, stx2b, stx2c, stx2d, stx2e, stx2f, stx2g, stx2h and stx2i have been identified in the stx2 subtype [19-22]. However, there are at least two additional subspecies that are not yet fully accepted which are the toxins stx2j and stx2k [23, 24].

Widespread use of antibiotics in the treatment of human and animal diseases causes pathogens like Listeria spp., Salmonella spp. and many important bacterial strains, including E. coli O157 to gain resistance to antibiotics [25]. The use of antibiotics to treat the infections STEC's cause is controversial. Because the use of antibiotics can increase the production of toxins. For example, the use of small inhibitory doses of sulfonamides, quinolones and fluoroquinolone has been shown to result in increased production of (stx) shiga toxin. For this reason, supportive treatments should be the main course of treatment [26, 27]. Our study aimed to assess the presence of Escherichia coli O157:H7, a pathogen of public health concern, in water buffalo carcasses, and to perform isolate characterization and determine their antibiotic resistance patterns.

Material and Methods

A total of 600 samples, including 200 RAMS, 200 skin, and 200 carcass samples, were collected from 200 animals slaughtered in the slaughterhouses of Samsun and Giresun between February and November 28, 2021, across all four seasons (Winter, Spring, Summer, and Autumn).

Sample collection and determination of E. coli O157:H7 isolates by classical culture technique

Samples were collected as 200 pieces of skin, RAMS and carcass swab samples from Samsun (41° 17′ 25“ N, 36° 20′ 1“ E) and Giresun (40°34′47“N, 38°35′40“E) provinces for 1 year with 50 buffaloes (40 samples Samsun, 10 samples Giresun) each season and a total of 600 samples were analyzed for the presence of E. coli O157:H7. Samples; after slaughter, skin and RAMS samples were collected before the skinning stage, and carcass swab samples were collected just after the evisceration stage. Carcass sponge swab samples are taken from the carcass before the cooling step after skinning with a sterile sponge. Round (1), flank (2), breast (3) and groin (4) samples were taken from four different areas as a result of applying equal pressure with 10 vertical and 10 horizontal rubbing movements. For every carcass sample, two sponges were used, and then the sponges were then put inside 25 mL of Maximum Recovery Saline Broth (MRD-Maximum Recovery Dilutent, Oxoid-CM0733) is to be brought to the laboratory. Two sponge samples belonging to the same animal carcass and 25 mL of MRD 10 mg/L novobiocin (Novobiocin Selective Supplement, Oxoid SR0181) was added 225 mL of Modified Tryptone Soya Broth (mTSB, Oxoid CM0989) was also added for dilution.

The skin samples cover an area of 400 cm^2 and are taken from the area with the highest contamination which is from the breast to the abdomen using a sponge that is wet with 10 mL of MRD that has been collected by pressing the sponge 10 times vertically and 10 times horizontally, and then later diluted in 10 mL MRD and a sponge sample of 90 mL mTSB. RAMS samples were taken by inserting a 3-5 cm sterile foam swab after the animal has been slaughtered and the swabs were diluted in 5 ml MRD and 45 ml mTSB. After all of the samples were mixed, it was incubated at 37°C for 18 to 24 hours in ovens [28-31]. After the incubation period of skin, RAMS and carcass samples, 50 µl suspension, cefixime 0.05 mg/L, tellurite 2.5 mg/L mixed SMAC-Sorbitol MacConkey agar (Oxoid-CM 813, Supl. SR 172 E) were passed and plates were incubated for 24 hours at 37ºC [32, 33]. Following the incubation, 5 colorless colonies that did not ferment sorbitol grown on the plates were selected and sub cultured in Yeast extract-Trypticase Soy Agar (TSA-YE) (Oxoid-CM 131-L21), and the plates were incubated at 37ºC for 24-48 hours [32, 33]. In the mean while that the suspected isolates are administered indole and motility tests, and the sample is passed on to MUG-SMAC agar (SMAC-MUG Suppl. Oxoid-BR 071 E) containing 4-Methylumbelliferyl- β-D-glucuronide is incubated at 37ºC for 24 hours, and then it is determined that colonies that do not give out blue or green light when exposed to UV light with a wavelength of 366 nm are MUG negative. Later, the isolates are passed in a purple broth base (Difco-0227-01-6) containing cellobiose and left to incubate for 24 hours at 37ºC. Finally, samples that could not ferment the sorbitol and were colorless were picked and these colonies went through an agglutination test with the O157 anti-serum (DR0620M; Oxoid, UK). The colonies that have been determined to have positive agglutination and also have been determined to be E. coli O157 are later put in H7 (Denka Seiken, 211057, Tokyo, Japan) antiserum to test for agglutination, and the colonies that test positive have been identified as E. coli O157:H7 [28, 29, 31]. Isolates that have been identified using conventional methods have had their DNA identified using the DNA boiling method. The DNA has been stored at -20ºC until the PCR testing has been performed.

Genetic analysis of E. coli and E. coli O157:H7 isolates using multiplex PCR to detect stx1, stx2, eaeA and hlyA genes

E. coli O157 and the E. coli serotype O157:H 7 have been completed and confirmation of the presence of shiga toxin 1 (stx1), shiga toxin 2 (stx 2), hemolysin (hlyA), and intimin (eaeA) were completed using the method Maurer et al. [34], and Fratamico et al [35]. The primers list used in this study are illustrated in Table 1. The E. coli serotype O157:H7- ATCC 43895 and the E. coli serotype O157:H7- ATCC 35150 were used as reference control strains.

TABLE 1. Primer sequences used in this study [34, 35].

Primers		Sequences (5'- 3')	PCR product (Base pair)
rfbO157 F	rfbO157 R	CGTGATGATGTTGAGTTG AGATTGGTTGGCATTACTG	420 bp
fliCh7 F	fliCh7 R	GCGCTGTCGAGTTCTATCGAGC CAACGGTGACTTTATCGCCATTCC	625 bp
stx1 F stx1R		T G T A A C T G G A A A G G T G G A G T A T A C A GCTATTCTGAGTCAACGAAAAATAAC	210 bp
stx2 F	stx2 R	GTTTTTCTTCGGTATCCTATTCC GATGCATCTCTGGTCATTGTATTAC	484 bp
eaeA F	eaeA R	ATTACCATCCACACAGACGGT ACAGCGTGGTTGGATCAACCT	397 bp
hlyA F	hlyA R	ACGATGTGGTTTATTCTGGA CTTCACGTCACCATACATAT	166 bp

For the purposes of gene amplification, 1 X PCR buffer with a 50 µL volume, 1.5 mM MgCl2, 0.1 mM dNTP, 0.5 U Taq-Polymerase, 1 µM and 5 µl of DNA for every primer were prepared. Gene amplifications of rfbO157, fliCh7, stx1, stx2, eaeA and hlyA were completed in the Thermal Cycler (Bio-Rad MJ mini Gradient CA-USA). Electrophoresis of the obtained amplicons was performed in 2% agarose at 90 V for 90 minutes (Biorad-Powerpac.basic).

Antibiotic Resistance Tests

In this study, antibiotic resistance of isolates against trimethoprim-sulfamethoxazole, amoxicillin, tetracycline, chloramphenicol, gentamicin, streptomycin, ampicillin, cephalothin, ofloxacin and ciprofloxacin was investigated. The disc diffusion method was used to measure the region diameters around the antibiotic discs and then compared with EUCAST standards to determine whether the isolates were resistant, intermediate or susceptible. In addition, the E-test method was used to determine the MIC values of the isolates [36].

Results

Between February 1, 2021 and November 28, 2021; and between a total of four seasons (Winter, Spring, Summer, and Fall) 200 carcasses slaughtered in the slaughterhouses in Samsun and Giresun have had samples of RAMS (n:200), skin (n:200) and carcasses (n:200) for a total of 600 received samples that were run. In 200 RAMS samples, 10 (5%); 200 skin samples, 9 (4.5%); 200 carcasses samples, 6 (3%); and in the grand total, out of 600 samples, 25 of them (4.16%) have been detected to have been positive for E. coli O157 (Table 2).

The isolate distribution of seasonal E. coli is as follows; out of 6 E. coli positive isolates from carcass samples 4 of them are E. coli O157:H7 positive (%66,6), also out of 10 isolates obtained from RAMS 5 of them are positive (50%) and out of the 9 samples from skin samples 7 of them are E. coli O157:H7 positive (77.7%), for a total of the 25 samples in the whole experiment, 16 of them are E. coli O157:H7 positive (64%). In a general sense, E. coli O157:H7 positivity is 2.5% according to RAMS samples, 3.5% according to skin samples, 2% according to carcass samples and the total positivity rate of all of the samples is found to be 2.66%. (Table 3).

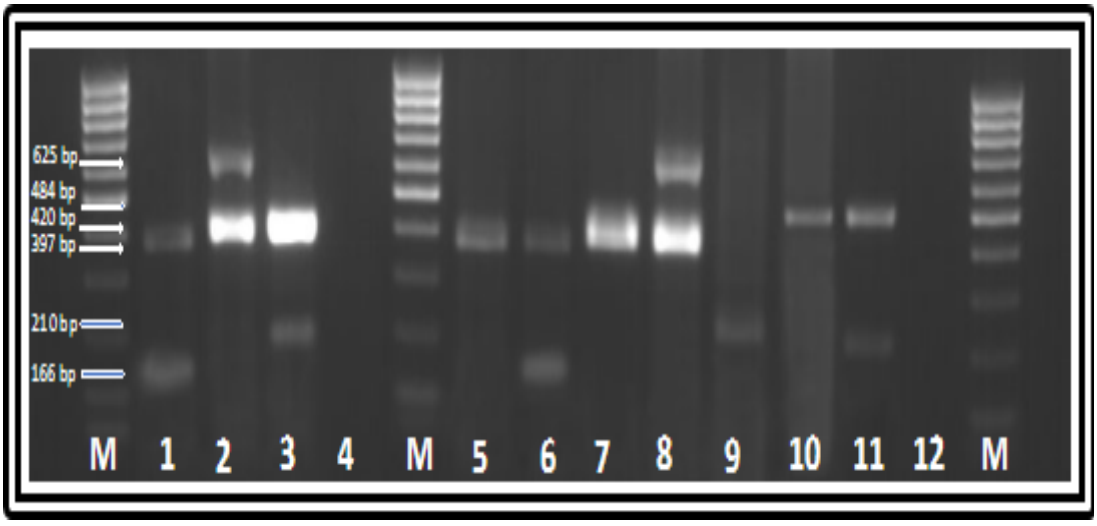

Fig. 1. Electrophoretic results that belong to the genes O157, H7, stx1, stx2, eae and hlyA were obtained using multiplex PCR from RAMS, skin and carcass isolates obtained from buffaloes.

M: DNA marker (50 bp), Lane 1: positive control isolate of the eaeA and hlyA genes (E. coli O157:H7 ATCC 35150), Lane 2: positive control isolate of the O157 and H7 genes (E. coli O157:H7 ATCC 43895), Lane 3: positive control isolate of the stx1, stx2 genes (E. coli O157:H7 ATCC 35150; ATCC 43895), Lane 4: Negative control (ionized water), Lane 5: positive isolate of the eaeA gene, Lane 6: positive isolate of the eaeA and hlyA genes, Lane 7: O157 positive isolate, Lane 8: O157 and H7 positive isolate, Lane 9: positive isolate of the stx1 gene, Lane 10: positive isolate of the stx2 gene, Lane 11: positive isolate of the stx1, stx2 genes, Lane 12: Negative control (ionized water).

TABLE 2. Seasonally Analyzed Samples and the Number of E. coli O157 Positive Samples.

TIME PERIOD	Number of Samples Taken			Number of E. coli O157 positive samples
	CARCASS	RAMS	SKIN	
WINTER (01-07 February 2021)	13	13	13	*1 Carcass,1 RAMS
WINTER (08-14 February 2021)	12	12	12	-
WINTER (15-21 February 2021)	13	13	13	1 Carcass, 1 RAMS, 1 Skin
WINTER (22-27 February 2021)	12	12	12	1 RAMS
SPRING (03-09 May 2021)	13	13	13	1 RAMS, 1 Skin
SPRING (10-16 May 2021)	12	12	12	1 RAMS, 1 Skin
SPRING (17-23 May 2021)	12	12	12	-
SPRING (17-23 May 2021)	13	13	13	1 RAMS, 1 Skin
SUMMER (31 May- 06 June 2021)	12	12	12	1 Carcass
YAZ (07- 13 June 2021)	13	13	13	1RAMS, 1 Skin
YAZ (14- 20 June 2021)	13	13	13	1 Skin
YAZ (21- 26 June 2021)	12	12	12	1 Skin
FALL (11-17 October 2021)	13	13	13	**2 Carcass,1 RAMS, 1 Skin
FALL (18-24 October 2021)	12	12	12	***1 Carcass,1 RAMS, 1 Skin
FALL (15-21 November 2021)	12	12	12	1 RAMS
FALL (22-28 November 2021)	13	13	13	-
SAMPLE COUNT	200	200	200	200/6 Carcass- 3% 200/10 RAMS- 5% 200/9 Skin- 4.5%
GRAND TOTAL		600		600/25 Samples, 4.16%

*Carcass and RAMS samples that belong to the same animal.
** Carcass and RAMS samples that belong to the same animal.
*** Skin and RAMS samples that belong to the same animal.

When examining the virulence features of the E. coli O157 and H7 isolates, the following gene presence rates were observed: rfbO157a was present in all 25 isolates (100%), fliCh7 was present in 16 of the 25 isolates (64%). The presence of stx1 was detected in 7 of the 25 isolates (28%), while stx2 was present in 14 of the 25 isolates (56%). The eaeA gene was present in all 25 isolates (100%), and the presence of hlyA was detected in 17 of the 25 isolates (68%). Both stx1 and stx2 genes were present in 5 of the 25 isolates (20%). The genes stx1-stx2-eaeA were all present together in 5 of the 25 isolates (20%). The genes stx1-stx2-eaeA-hlyA were all present together in 5 of the 25 isolates (20%). The genes stx2-eaeA-hlyA were all present together in 14 of the 25 isolates (56%). All isolates that harbored the fliCh7 gene also possessed the stx2-eaeA-hlyA genes (Table 4).

Using the disk diffusion method, the antibiotic resistance rates of the isolates have been determined to be 6/25 (24%) for ampicillin,

TABLE 3. Distribution of Seasonal E. coli Isolates.

Sample (n)	Season	*E. coli* O157 Positive Isolate Count/Isolate Code	*E. coli* O157: H7 Positive Isolate Count/Isolate Code
CARCASS (n:200)	Winter	**2** [1]**KK-2a,** KK-29b	**2** [1]**KK-2a,** KK-29b
	Spring	-	-
	Summer	**1** KY-110a	-
	Fall	**3** KS-161a, [2]**KS-162b,** KS-172a	**2** [2]**KS-162b,** KS-172a
RAMS (n:200)	Winter	**3** [1]**RK-2a,** RK-33a, RK-43b	**2** [1]**RK-2a,** RK-33a
	Spring	**3** Rİ-56b, Rİ-73a, Rİ-88a	**1** Rİ-88a
	Summer	**1** RY-119a	-
	Fall	**3** [2]**RS-162a,** [3]**RS-170a,** RS-185a	**2** [2]**RS-162a,** [3]**RS-170a**
SKİN (n:200)	Winter	**1** DK-30a	-
	Spring	**3** Dİ-51a, Dİ-75b, Dİ-94b	**3** Dİ-51a, Dİ-75b, Dİ-94b
	Summer	**3** DY-122a, DY-133a, DY-140a	**2** DY-122a, DY-140a
	Fall	**2** DS-160a, [3]**DS-170b**	**2** DS-160a, [3]**DS-170b**
TOTAL (n:600)		**25**	**16**

[1]In the wintertime, carcass number 2 (KK-2a) ve RAMS number 2 (RK-2a) obtained their isolates from the same animal.

[2]During the fall, carcass number 162 (KS-162b) ve RAMS number 162 (RS-162a) obtained their isolates from the same animal.

[3]During the fall, RAMS number 170 (RS-170a) ve skin number 170 (DS-170b) obtained their isolates from the same animal.

TABLE 4. E. coli O157: H7 virulence characteristics of positive isolates.

Sample/ Positive Isolate Count (n)	Isolate Code	E. coli O157/ E. coli O157:H7 Positive Isolate		Virulence Gene			
		E. coli O157 (rfbO157a)	H7 (fliCh7)	stx1	stx2	eaeA	hlyA
CARCASS (n:6)	[1]KK-2a	+	+	-	+	+	+
	KK-9b	+	+	+	-	+	+
	KY-110a	+	-	-	-	+	-
	KS-161a	+	-	+	-	+	+
	[2]KS-162b	+	+	+	+	+	+
	KS-172a	+	+	-	+	+	+
RAMS (n:10)	[1]RK-2a	+	+	-	+	+	+
	RK-33a	+	+	-	+	+	+
	RK-43b	+	-	-	-	+	-
	Rİ-56b	+	-	-	-	+	-
	Rİ-73a	+	-	-	-	+	-
	Rİ-88a	+	+	+	+	+	+
	RY-119a	+	-	-	-	+	-
	[2]RS-162a	+	+	+	+	+	+
	[3]RS-170a	+	+	-	+	+	+
	RS-185a	+	-	-	-	+	-
SKIN (n:9)	DK-30a	+	-	-	-	+	+
	Dİ-51a	+	+	-	+	+	+
	Dİ-75b	+	+	+	+	+	+
	Dİ-94b	+	+	-	+	+	+
	DY-122a	+	+	+	+	+	+
	DY-133a	+	-	-	-	+	-
	DY-140a	+	+	-	-	+	-
	DS-160a	+	+	-	+	+	+
	[3]DS-170b	+	+	-	+	+	+
TOTAL (n:25)		25	16 (64%)	7 (28%)	14 (56%)	25 (100%)	17 (68%)

[1]In the winter, isolates belonging to the samples carcass number 2 (KK-2a) and RAMS number 2 (RK-2a) have been obtained from the same animal.

[2]In the fall, isolates belonging to the samples carcass number 162 (KS-162b) and RAMS number 162 (RS-162a) have been obtained from the same animal.

3Also in the fall, isolates belonging to the samples RAMS number 170 (RS-170a) and skin number 170 (DS-170b) have been obtained from the same animal.

TABLE 5. The E test (MIC-µg/ml) profile of E. coli O157 and H7 isolates with phenotypical resistance.

SAMPLE ORIGIN		Isolate Code and Type E. coli O157/H7	E TEST (MIC-µg/ml)							
			Trimethoprim-Sul. (TRS) MIC: 0.002-32	Amoxicillin (AMO) MIC: 0.016-256	Tetracycline (TET) MIC: 0.016-256	Gentamicin (GEN) MIC: 0.016-256	Streptomycin (STR) MIC: 0.064-1024	Ampicillin (AMP) MIC: 0.016-256	Cephalothin (CEF) MIC: 0.016-256	Ciprofloxacin (CIP) MIC: 0.002-32
CARCASS		[1]KK-2a O157:H7	-	-	-	-	2	-	12	-
		KK-9b O157:H7	-	-	-	2	-	-	-	-
		KY-110a O157	1	-	-	-	-	16	-	-
		[2]KS-162b O157:H7	-	-	0.25	2	1	-	-	-
		KS-172a O157:H7	-	-	-	-	-	32	-	-
RAMS	RAMS	[1]RK-2a O157:H7	-	-	-	-	0.5	-	12	-
		Rİ-73a O157	-	-	-	-	-	-	8	-
		Rİ-88a O157:H7	1	-	-	-	-	32	-	-
		[2]RS-162a O157:H7	-	-	0.5	2	2	-	-	-
SKIN	Deri	DK-30a O157	-	-	-	-	-	32	-	-
		Dİ-51a O157:H7	1	-	-	-	-	-	4	-
		DY-122a O157:H7	-	-	-	6	-	8	-	-
		DY-133a O157	1	-	-	-	-	-	4	-
		DY-140a O157:H7	-	8	-	-	-	-	-	-
		DS-160a O157:H7	-	-	-	-	-	32	-	2

[1]In the winter, samples belonging to carcass number 2 (KK-2a) and RAMS (RK-2a) have been obtained from the same animal.

[2]In the fall, samples belonging to carcass number 162 (KS-162b) ve RAMS (RS-162a) have been obtained from the same animal.

5/25 (20%) for cephalothin, 4/25 (16%) for trimethoprim-sulfamethoxazole, 4/25 (16%) for gentamicin, 4/25 (16%) streptomycin, 2/25 (8%) for tetracycline, 1/25 (4%) for amoxicillin and 1/25 (4%) for ciprofloxacin. The evaluation of the data has been completed using the E-test method and is shown in the (Table 5) below.

Discussion

In the research literature, there is limited research about the presence of E. coli in buffalo carcasses, wastes, meats obtained from their skin and their products, and its milk and its products. The research for isolate characterization and the determination of antibiotic resistance profiles are also limited.

Upon looking at the studies done in Turkey about buffalo carcasses, skin and RAMS samples for the presence of E. coli O157:H7; it can be seen that in Afyonkarahisar, 300 buffalos have had RAMS samples collected, and the E. coli serotype O157:H7 positivity rate is found to be 11/300 (3.7%) [37]. Out of 300 fecal samples of clinically healthy Anatolian buffalo, 11/300 (%3.66) of the buffalo samples contained the E. coli serotype O157:H7. Out of the isolated 11 E. coli serotype O157:H7 samples, 3 (27.3%) of them had both the stx1 and stx2 genes. The results that the researchers have obtained is closely relevant to this study [38]. Out of 1000 fecal samples in Samsun, 38 of them (3.8%) were positive for E. coli O157:H7, while 400 of them were negative for E. coli O157:H7. This adds up to a total of 438 samples (43.8%) with E. coli that were isolated and identified. The results that the researchers obtained are similar to the results that we have obtained in our own study in that the rates of occurrence of the serotype O157:H7 in the studies are similar [39]. 417 fecal content samples were collected from Afyonkarahisar (152 cattle, 51 buffalo), Antalya (117 cattle), and Burdur (83 sheep, 14 goats). E. coli O157 positivity was 16/417 (3.8%) and O157:H7 positivity is found to be 7/417 (1.67%). Among buffalo samples, the rate is determined to be 4/51 (7.8%). The rate that the isolates contain the genes fliCH7, stx2, eaeA and ehlyA is 7/16 (43.8%), while the rate that they contain eaeA is 11/16 (68.8%), and the rate that they contain stx1 is 0/16 (0%). According to the comparison results, we have proportional differences between the virulence genes. The differences in our studies can be attributed to differences in geographical location, the differences of the animal species we were working with, the number of samples we were working with, and differences in data collection methods [40].

When looking at studies of carcass, skin and RAMS samples of buffalo conducted in different countries for the existence of E. coli O157 and the serotype O157:H7; we see that In India, 348 samples were collected, including 153 buffalo meat samples, 99 buffalo kebab samples and 96 buffalo shami kebab samples. The E. coli O157 positivity rate was found to be 2/348 (0.57%). The 2 isolated obtained that were infected with O157 were both buffalo shami kebab samples. When their study is compared to our study, their study has an O157 positivity rate that is a lot lower than ours. This difference can be explained by the steps of processing the meat has gone through to become a product, the heat treatment it has gone through, the variety of the type of samples of the buffalo, and the difference in the number of samples that are being worked with [41]. In Italy, from a herd of 65 buffaloes, 289 fecal samples were taken from healthy buffalo, and the E. coli O157 positivity rate was found to be 42/289 (14.5%). These results were found to be higher than the values in our study. This difference is thought to be explainable by climate, different geographical regions, and the number of samples in our trial versus their trial [42]. In Brazil, the prevalence of shiga-toxin producing E. coli can range anywhere from 0% to 64% based on the farm where the sample is taken from [43]. From 98 farms in Vietnam, 568 healthy domestic animals (buffaloes, cattle and goats), the STEC and intimin positive samples are at 27% for buffaloes, 23% for cattle, and 38.5% for goats. 70% of the buffalo farms, 60% of the cattle farms and100% of the goat farms have been determined to have tested positive for STEC (shiga-toxin producing E. coli). As a result of the studies of we see among that animals that live in Vietnam natively, buffaloes are an important reservoir for the epidemiology of STEC is based on the data presented by this study [44]. In Bangladesh, 174 buffalo, 139 cattle and 110 goats fecal matter have been collected. As a result of the study, STEC O157 positivity was determined as 25/174 (14.4%) in buffalo samples [45]. 360 fecal content samples have been observed in Iran and the O157 positivity rate is %3.8 [46]. In 150 buffalo meat samples in India (30 ground, 30 muscle parts, 30 lungs, 30 intestines and 30 livers), the O157 positivity rate is determined to be 3/150 (2%) [47].

Upon looking at buffalo skin, carcass, and RAMS tests done in different countries about the virulence factors of the serotype O157:H7, the study that was conducted in Italy by where fecal samples belonging to 289 healthy buffaloes were collected to derive 42 E. coli O157 isolates and the rate that both the stx1 and stx2 genes show at the same time is 3/42 (7.14%7). The rate that the stx2 gene was found in 39/42 (92.8%) and the gene eaeA is found at 42/42 (100%) [42]. In Brazil, the genetic profiles that contain the stx1, stx2, iha, ehxA, saa, and ehxA, iha, stx2, saa in STEC isolates are found to be more common. Out of the 20 different serotypes that are completed, more than 50% amount for serotypes that cause human infection [43]. The STEC E. coli prevalence of 568 Vietnamese farm animals (buffalos, cattle and goats) were researched in this study. Of 170 STEC samples, 99 of them had both the stx1 and stx2 genes, 36 carried the stx2 gene, and 35 of them only carried the stx1 gene. The eaeA genes were detected in six goats, could not be detected in buffalo and cattle isolates. Out of 173 samples of E. coli (170 STEC and 3 intimin-positive), 110 have been determined to have the ehxA gene, and 106 to have the saa gene [44]. In Bangladesh, 174 buffalo feces samples were collected and the rate that the stx1 and/or the stx2 being found is determined to be 143/174 (82.2%). The rate that the stx1 gene is found individually is 15/174 (8.6%), the rate that the stx2 gene found individually is determined to be 12/174 (6.9%). When this study is compared with our study, the occurrence of the stx1 and/or stx2 in their study was higher than in our study, however, the rate stx1 and stx2 being found separately are lower than in our studies [45]. In Iran, in 360 fecal content samples collected from buffaloes, stx1 gene presence was 42.3%, stx2 gene presence was 34.6%, stx1 and stx2 genes were found at the same time as 23%, hly gene presence was 23% and eae gene presence was 7.6%. When the results of the study are compared with our study, we see that their study results are similar to ours in that they both have similar rates of stx1 and stx2 occurrence. The rates of occurrence of the genes stx1, stx2, eae and hly do not match our study and their study [46]. In Iran, cattle (n:85), camels (n:50), sheep (n:62), goats (n:60) and buffaloes (n:8) were collected to have a total of 295 raw meat samples, and out of the 14 O157 isolates that have been calculated, 1/14 (7.14%) stx1, 4/14 (%28.57) stx2, 2/14 (14.28%) eaeA and 1/14 (7.14%) ehlyA has been detected [48]. In Italy, out of 220 E. coli isolates that have been obtained from 314 Mediterranean buffalo calves, all ETEC isolates have been determined to be labile toxin-positive and stable toxin-negative. All STEC isolates are also all stx and intimin positive, and the gene ratios for stx1 were 80% and 27% for stx2 as expressed by the research [49]. In Malaysia, the meat of 52 Indian buffaloes and 56 native cattle were obtained for a total of 108 samples from which isolates derived from it had a of stx1-stx2 occurrence and/or only the occurrence of stx2, which was 21/108 (19.44%). The ratio of stx1 gene was determined as 0/108 (0%). There seem to be some parallels between our study and this study because the rate of stx1 and stx2 occurring at the same time is similar to both of our studies [50]. Out of 80 buffalo RAMS samples from Pakistan, the rate that the isolates all contain stx1, stx2, eae and hlyA is 1/80 (1.25%) [51].

When looking at studies about antibiotic resistance of the E. coli serotype O157: H7 by looking at carcass, skin, RAMS and animal product samples from animals like buffalo, cattle, and other animals; In Italy, 220 E. coli isolates were obtained from 314 Mediterranean buffalo calves, 11 antibiotics were tested for effectiveness and the E. coli showed strong resistance rates against antibiotics (>30%). This and our study used the same antibiotics and when they listed high rates of resistance to low rates of resistance, they are as follows: ampicillin (81.8%), oxytetracycline (74%), sulphamethoxazole/ trimethoprim (45.9%) and gentamicin (31.2%) [49]. 363 isolates have been obtained from 165 fecal matter samples from buffaloes in India and the antibiotic resistance rates have been found to be the following: 95.8% erythromycin, 62.5% cephalothin, 54.2% amikacin and 4.2% amoxicillin. The results of this study show that cephalothin and amoxicillin are consistent with our study in regard to antibiotic resistance, and amoxicillin is consistent with our study proportionally [52]. In Malaysia, a total of 108 samples were collected from 56 native cattle and 52 Indian buffalo meats, among the antibiotics we used, the resistance rate for ampicillin was 4/5 (80%), the resistance rate for amoxicillin was 4/5 (80%), and the resistance rate for trimethoprim-sulfamethoxazole was 2/5 (40%). Although the resistance status of the antibiotics in their studies that we use in common are similar, the resistance rates are not similar. Tetracycline and

ciprofloxacin resistance rates are in parallel with our study [50].

Conclusion

The study data show that the digestive systems of buffaloes are an important reservoir for E. coli serotype O157:H7, as in cattle and it shows that the contamination from faeces to carcass, faeces to skin and skin to carcass is caused by the lack of hygiene conditions during the slaughter of buffaloes. The obtained isolates contain the genes stx1, stx2, eaeA, and hlyA, which are determining factors for the virulence of O157:H7 and this means that buffalo meat can cause HC, HUS, TPP infections and can cause O157:H7 related deaths. Resistance of isolates to antibiotics rates also indicates the unnecessary and uncontrolled use of antibiotics in the field of Veterinary Medicine. As a result of all these analyzes we have carried out; we believe that prevention of E. coli serotype O157:H7 contaminations, foodborne infections caused by E. coli O157:H7 and resistance to antibiotics will be possible by making the existing Veterinary Authority much stronger and more effective.

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Conflicts of interest

All authors declare that they have no conflicts of interest.

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