Investigating the presence and antibiotic susceptibilities of *Escherichia coli* O157 and *Listeria monocytogenes* in ruminant feces and feed in Balıkesir province

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ABSTRACT

The aim of this study is to determine the presence, virulence factors, and antibiotic susceptibilities of *E. coli* O157 and *L. monocytogenes* in ruminant feces and feed. This study was carried out for the first time in Balıkesir province. Feces, pellet feed, and silage samples were analyzed simultaneously for *E. coli* O157 and *L. monocytogenes* using feces of one gram and feed of twenty-five grams according to ISO 16654:2001/Amd 1:2017 and ISO 11290-1, respectively. 38 (38%) *E. coli* O157 strains were isolated and identified from a total of 100 ruminant feces. A total of 3 (3%) *E. coli* O157:H7 strains were detected by PCR from one hundred ruminant fecal samples. In the study, resistance to antibiotics increased, especially in *E. coli* O157 isolates. In this study, enterohaemolysin was the predominant virulence factor among the *E. coli* isolates, and it was thought that it was important for pathogenesis. The *Sxt1* gene was higher than the *Stx2* gene. A total of 24 *L. monocytogenes* strains were isolated from a total of 100 ruminant fecal samples and 50 silage samples. Three of these strains were isolated from silage samples taken from the farms, where *L. monocytogenes* was isolated from sheep feces. As a result, poor quality silage could be an important source of infection for listeriosis in Balıkesir province. Epidemiologically, poor quality silage was thought to be one of the sources of listeriosis. It was thought that ruminant feces played an important role as a reservoir in the spread and transmission of *E. coli* O157. The antibiotic resistance status of *E. coli* O157 and *L. monocytogenes* isolates should be monitored with epidemiological studies.

Introduction

Verotoxigenic *Escherichia coli* infections are one of the most frequently reported zoonotic diseases in the European Union countries, according to 2020 data from EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control) (19). *E. coli* O157:H7 is a member of the verotoxin-synthesizing *E. coli* group and was first identified in the 1970s (4, 9, 17, 23, 37, 49). *E. coli* O157:H7 has more than one virulence factor. The most important ones among them are Shiga toxin (Stx) 1 and 2 (encoded by the genes, *Stx1* and *Stx2*), intimin (encoded by the gene, *eaeA*), and the plasmid-encoded enterohaemolysin (encoded by the gene, *EhlyA*) (50). It is a zoonotic pathogen and causes various illnesses, such as Stx-producing *E. coli* (STEC). Stx is thought to be responsible for causing life-threatening conditions such as hemolytic uremic syndrome (HUS) and hemorrhagic colitis in humans (4, 9, 17, 23, 37, 49). Previous studies reported that the first *E. coli* O157:H7 outbreak occurred as a result of the consumption of beef and dairy products that did not undergo adequate heat treatment (5, 28).

It is well known that cattle are the most important reservoir of *E. coli* O157 (5, 11, 26, 46, 47). Other reservoir animals are sheep, goats, wild deer, pigs, and birds (2, 11, 43, 46). Infection occurs due to the oral consumption of contaminated cattle feces, and foods through direct contact with reservoir cattle (5, 25, 44, 46, 47). *E. coli* O157:H7 can survive in feces for approximately 50-99 days (16, 26). Feces are also used as a fertilizer. Silage could be contaminated with feces during the silage preparation and cause infections in

ruminants and humans (16). Drinking water contaminated with cattle feces as a result of consumption of contaminated products such as milk and meat plays a key role in contamination. Carcass contamination in slaughterhouses also causes transmission of *E. coli* O157 to humans (33, 35, 37). The bacteria that pass to the inner surfaces during the processing of meat can maintain their vitality when adequate heat treatment is not applied and can cause infections that are important for public health (20, 44, 47, 49).

The prevalence of the *E. coli* O157 disease increases during the summer months and at the beginning of autumn (2, 5, 11, 43, 46). Another important issue for this zoonotic bacterium is that it shows a wide variety of multi-antibiotic resistance with the complex interaction of different mechanisms (21).

Isolation of *E. coli* O157 can be detected from feces and contaminated materials with feces. Conventional and molecular methods are used for detection of *E. coli* O157. Conventional ones are immunomagnetic separation, culture, serological verification with antisera, and biochemical tests. CT-SMAC (cefixime tellurite-sorbitol MacConkey) agar and CHROM agar can be used as differential agars. In molecular methods, various gene regions of the bacterium and its toxin can be identified by polymerase chain reaction (PCR) (5).

Şeker and Kuş (49) isolated *E. coli* O157 in 16 of 417 fecal samples collected from adult ruminants. In addition, they found high resistance to ampicillin (68.7%), neomycin (68.7%), tetracycline (68.7%), trimethoprim/sulfamethoxazole (62.5%), and amoxicillin/clavulanic acid (56.2%) in these isolates. Birdal and Ak (8) reported that they isolated *E. coli* O157:H7 in 2 of 576 fecal samples collected from dairy cattle reared in several provinces in the Marmara region. McCabe et al. (42) isolated *E. coli* O157:H7 in 55 of 1317 rectal mucosal swab samples from dairy and beef cattle. Khalifa et al. (37) reported that they isolated 3 *E. coli* O157:H7 strains in fecal samples taken from 3 month-old calves with diarrhea, and these isolates were susceptible to amoxicillin and intermediate to cefotaxime and tetracycline. Umar et al. (53) reported that they isolated 4 *E. coli* O157:H7 strains in 50 fecal samples of cattle. Jacob et al. (32) reported that they isolated *E. coli* O157:H:7 in 33 of 296 goat fecal samples.

Listeria monocytogenes causes various infections in humans and animals (5, 7, 15, 18, 24). *L. monocytogenes* is found in the gastrointestinal tract of ruminants. Its fecal shedding is associated with the spread of the disease in the herd, especially in small ruminant farms (15, 27, 34, 55). For this reason, listeriosis can be found in soil, water, and associated food, primarily as a result of fecal contamination (1). *L. monocytogenes* cannot survive below pH 5. Cattle and sheep, which consume poorly prepared silage, may become infected as a result thereof (15). In addition, due to its psychrophilic character, it can grow in frozen foods (1). *L. monocytogenes* can be detected in feces. Following pre-, selective, and cold enrichment, *L. monocytogenes* can be isolated in both selective and differential agars. *L. monocytogenes* is identified through Gram stain, catalase, motion examination, aesculin, Voges-Proskauer tests, CAMP, and hemolysis tests. In addition, the agglutination test is performed serologically with O and H antisera (29, 53).

Oyinlore et al. (46) isolated a total of 176 colonies from 30 bovine fecal samples and identified 27 of these colonies as *L. monocytogenes*. Kalorey et al. (36) isolated *L. monocytogenes* in 8 of 50 fecal samples collected from 6 different mammals and one bird. Abay and Aydın (1) reported that they isolated *L. monocytogenes* from 27 of 400 fecal samples taken from healthy cattle and observed the highest isolation rate in January. Weis and Seeliger (56) isolated 15.2% of *L. monocytogenes* in 102 fecal samples collected from nature. Iida et al. (29) isolated *L. monocytogenes* from 189 of 9539 bovine large intestine contents.

In this study, which was carried out for the first time in Balıkesir province, it was aimed to determine the presence, virulence factors, and antibiotic susceptibilities of *E. coli* O157 and *L. monocytogenes* in ruminant feces and feed.

Materials and Methods

Sample collection: A total of 100 ruminant feces (80 from calves and 20 from sheep) and 100 animal feed samples (50 from silages and 50 from pellet feeds) were collected from 4 farms located in the Balıkesir province of Türkiye between January 2020 and May 2021 in order to examine the presence of *E. coli* O157 and *L. monocytogenes*. Fecal samples were collected from the animals' rectum by veterinarians working on these farms. The sampled calves were male and 11-15 month-old Charollois, Simmental, Blonde, and Limousin calves. The sampled sheep were female and 4-year-old Merino sheep, which are on their fiftieth day during the lactation period. All sampled animals and feeds were randomly selected from the farms. The animals were apparently healthy and did not suffer from diarrhea. Feces and animal feed samples were transferred into sterile boxes and transported to the laboratory in a cold chain (2-8 °C) and they were analyzed promptly in the laboratory.

Isolation and identification of E. coli O157 and L. monocytogenes: Each feces and animal feed sample were analyzed simultaneously for *E. coli* O157 and *L. monocytogenes* using one gram of feces and twenty-five grams of animal feed according to ISO 16654:2001/Amd 1:2017 and ISO 11290-1, respectively (30, 31). For feces and feed samples, 1/9 enrichment broth was used in both bacterial analyses according to ISO 16654:2001/Amd 1:2017 and ISO 11290-1.

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For *E. coli* O157, fecal and feed samples were transferred into modified Tryptone Soy Broth (mTSB) containing Novobiocin (20 mg/L) (Merck, Germany) and homogenized by using a mixer for one minute. The homogenate was incubated at 37°C for 6 h. After incubation, immunomagnetic separation (IMS) of *E. coli* O157 was done by using a Captivate Magnetic kit in accordance with the manufacturer's procedure (LabM, Germany). After the IMS procedure, the particles were streaked into the Cefixim-Tellurite Sorbitol MacConkey agar (CT-SMAC, LabM, Germany). Agar plates were incubated at 37°C for 18-24 h. After incubation, colonies were examined and confirmed using conventional biochemical tests such as Gram staining, motility, oxidase, indole, etc., according to Şeker and Yardımcı (51). Serological confirmation was done with latex agglutination using the *E. coli* O157 antisera (Microgen, Germany). In all tests, the *E. coli* O157:H7 strain (ATCC 43895) and the *E. coli* (ATCC 25922) strain were used as positive and negative control strains, respectively.

Feces, pellet feed, and silage samples were analyzed for *L. monocytogenes* according to ISO 11290-1 (30). For the pre-enrichment process, fecal and feed (pellet and silage) samples were put into the half-fraser broth (Merck, Germany) and incubated at 30 °C for 24 h. Then, 0.1 ml of half-fraser broth was put into fraser broth (Merck, Germany) and incubated in 37 °C for up to 48 h. Afterwards, enrichment cultures were streaked onto two selective agars-PALCAM (Oxoid, UK) and Rapid' *L. mono* (Bio-Rad, USA) and incubated at 37 °C for 24-48 h. Presumptive *L. monocytogenes* colonies were also confirmed through Gram staining, motility examination on a microscope, catalase, oxidase, and CAMP tests for *Staphylococcus aureus* (ATCC® 25923™) strain and aesculine test (1, 3, 6). All confirmed cultures of both *E. coli* O157 and *L. monocytogenes* were stored in beads (Cryobank, Mast) at-20 °C.

Antibiotic susceptibility tests: Antibiotic susceptibilities were investigated in the Mueller- Hinton agar (Merck, Germany) by using disc diffusion method stated by European Committee on Antimicrobial Susceptibility Testing (EUCAST) and in the Clinical and Laboratory Standards Institute (CLSI) standards (22).

Antibiotics, which were used in antibiogram tests for *E. coli* O157 and *L. monocytogenes,* were selected based on both previous studies (3, 40, 47) and antibiotics used for therapeutic purposes.

For *L. monocytogenes*, gentamicin (10 µg), streptomycin (10 µg), meropenem (10 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), amikacin (30 µg), ampicillin (10 µg), erythromycin (15 µg), trimethoprim/ sulfamethoxazole (25 µg), penicillin G (10 U), sulbactam/ ampicillin 1:1 (20 µg) and tetracycline (30 µg) antibiotic discs (Oxoid, UK, Liofilchem, Italy) were used.

For *E. coli* O157, ampicillin (10 µg), amoxicillin/ clavulanic acid (30 µg), ertapenem (10 µg), meropenem (10 µg), ceftriaxone (30 µg), cefotaxime (5 µg), cefotaxime/clavulanic acid (40 µg), gentamicin (10 µg), tobramycin (10 µg), tetracycline (30 µg), aztroneam (30 µg), oxacillin (5 µg), colistin sulphate (5 µg), doxycycline (5 µg), vancomycin (30 µg), erythromycin (15 µg), cefpodoxime (10 µg), ciprofloxacin (5 µg), and trimethoprim/sulfamethoxazole (25 µg) antibiotic discs (Oxoid, UK, Liofilchem, Italy) were used.

For *L. monocytogenes*, ampicillin, penicillin G, erythromycin, trimethoprim/sulfamethoxazole, and meropenem were evaluated according to EUCAST guidelines (22). The other antibiotics were evaluated according to Aksoy et al. (3) and CLSI guidelines (12, 13). For *E. coli* O157, antibiotic discs were evaluated according to Lukasova et al. (40) and international standards (14, 22). Oxacillin was evaluated according to the BSAC standard (11). Vancomycin was evaluated according to Oxoid manufacturer's guidance (45). In antibiograms, *E. coli* (ATCC ® 25922™) strain and *L. monocytogenes* strain (GeneBank Accession Number: MN496429) from Department of Veterinary culture collection of Balıkesir University Kepsut Vocational School were used as reference strains.

PCR tests of E. coli O157 strains: All *E. coli* O157 isolates were inoculated in Nutrient Broth (NB, Oxoid, UK) and incubated at 37°C for 18 hours to obtain pure cultures. After incubation, 1 mL of NB broth culture was centrifuged at 5000 g for 10 minutes. After centrifugation, the supernatant was removed and DNA extraction was performed using the pellet according to the GeneJET Genomic DNA Purification kit (Thermo Scientific, USA) and the DNA Purification Protocol for Gram-negative bacteria.

The presence of H7 serotype, Shiga toxin, intimin, and hemolysis was analyzed by PCR using *fliCH7, Stx1, Stx2, eaeA,* and *EhlyA* gene specific primers, which were previously used by Şeker and Kuş (49) (Table 1).

A multiplex PCR reaction mix for *fliCH7, Stx1, Stx2,* and *EhlyA* genes was prepared in a total volume of 50 µl. The PCR mix contained 2 µl of DNA extract and was prepared using a 35 µL DreamTaq PCR Master Mix (2X) Kit (Thermo Scientific, USA), 12.8 µL DEPC water, 0.1 µL Primer F (100 pmol/µL), and 0.1 µL Primer R (100 pmol/µL) (49). Amplification conditions were applied according to Şeker and Kuş (49).

A PCR reaction mix for the *eaeA* gene was prepared in a total volume of 50 µl. PCR mix contained 2 µl of DNA extract and was prepared with a 35 µL DreamTaq PCR Master Mix (2X) Kit (Thermo Scientific, USA), 12.8 µL DEPC water, 0.1 µL Primer F (100 pmol/µL), and 0.1 µL Primer R (100 pmol/µL) (49). Amplification conditions were applied according to Şeker and Kuş (49).

Table 1. Primer sequences, target genes, base pairs and references for *E. coli* O157 virulence genes.

Primers	Sequences	Target genes	Base pairs (bp)	Reference
H7-F	GCGCTGTCGAGTTCTATCGAGC	*fliCH7*	625	Şeker and Kuş (49)
H7-R	CCACGGTGACTTTATCGCCATTCC			
Stx1-F	TGTAACTGGAAAGGTGGAGTATACA	*Stx1*	210	Şeker and Kuş (49)
Stx1-R	GCTATTCTGAGTCAACGAAAAATAAC			
Stx2-F	GTTTTTCTTCGGTATCCTATTCC	*Stx2*	484	Şeker and Kuş (49)
Stx2-R	GATGCATCTCTGGTCATTGTATTAC			
Int-F	GGGATCGATTACCGTCAT	*eaeA*	837	Şeker and Kuş (49)
Int-R	TTTATCAGCCTTAATCTC			
hlyA-F	GCATCATCAAGCGTACGTTCC	*EhlyA*	534	Şeker and Kuş (49)
hlyA-R	AATGAGCCAAGCTGGTTAAGCT			

Table 2. Antibiogram results of *L. monocytogenes* isolates.

Antibiotic discs	*Listeria monocytogenes* strains (n:24)		
	S (Susceptible) (%)	I (Intermediate) (%)	R (Resistant) (%)
Amikacin (30 µg)	20 (83.33)	4 (16.67)	0 (0)
Ampicillin (10 µg)	21 (87.50)	-	3 (12.50)
Erythromycin (15 µg)	24 (100)	-	0 (0)
Gentamicin (10 µg)	23 (95.83)	1(4.16)	0 (0)
Chloramphenicol (30 µg)	21 (87.50)	1 (4.16)	2 (8.33)
Meropenem (10 µg)	24 (100)	-	0 (0)
Penicillin G (10 U)	20 (83.33)	-	4 (16.67)
Ciprofloxacin (5 µg)	19 (79.16)	4 (16.67)	1 (04.17)
Streptomycin (10 µg)	24 (100)	0 (0)	0 (0)
Sulbactam/ampicillin 1:1 (20 µg)	0 (0)	2 (8.33)	22 (91.67)
Tetracycline (30 µg)	24 (100)	0 (0)	0 (0)
Trimethoprim/sulfamethoxazole (25 µg)	24 (100)	-	0 (0)

S: Susceptible, I: Intermediate, R: Resistant.

All PCR amplicons were electrophoresed on a 1.5% agarose (Prona, USA) gel using Bluejuice dye (Thermo Scientific, USA) and DNA molecular weight marker (Gene Ruler 100bp DNA Ladder plus, Thermo Scientific, USA) and visualized on the gel imaging system (EBOX CX5 TS EDGE, Vilber).

Results

A total of 38 *E. coli* O157 (38%) strains were isolated from a total of 100 ruminant fecal samples. While 15 of them were isolated from sheep fecal samples, 23 of them were isolated from calf fecal samples.

A total of 18 *L. monocytogenes* (18%) strains were isolated from 100 ruminant fecal samples. While 13 of them were isolated from sheep fecal samples, 5 of them were isolated and identified from calf fecal samples.

L. monocytogenes was isolated from 6 (12%) of 50 silage samples. Three of these isolates were isolated from silage samples taken from the farms, where *L. monocytogenes* was isolated from sheep feces. *E. coli* O157 could not be isolated from a total of 100 silage and pellet feed samples.

All *L. monocytogenes* isolates were susceptible to trimethoprim/sulfamethoxazole, tetracycline, streptomycin, meropenem, and erythromycin. The highest resistance was detected to sulbactam/ampicillin (Table 2).

All *E. coli* O157 isolates were resistant to oxacillin and vancomycin. 3 isolates were resistant to gentamicin and 7 isolates were resistant to tobramycin. On the other hand, 21 isolates were resistant to erythromycin and 12 isolates were intermediate. Table 3 shows the detailed results.

According to PCR results, the *EhlyA* gene was found in 20 *E. coli* O157 isolates. Of these isolates, 4 were isolated from sheep fecal samples and 16 from calf fecal samples. The *Stx1* gene was detected in 5 *E. coli* O157 isolates, 1 from a sheep fecal sample and 4 from calf fecal samples. The *EhlyA* gene was also detected in all isolates with the *stx1* gene.

The *Stx2* gene was found in 3 *E. coli* O157 isolates, including 1 from sheep fecal samples and 2 from calf fecal samples. The *Sxt1* gene was found at a higher rate than the *Stx2* gene.

The intimin gene was found in 8 *E. coli* O157 isolates, including 2 from sheep fecal isolates and 6 from

calf fecal isolates. The *EhlyA* gene was detected in all isolates with the intimin gene (Table 4, Figure 1, Figure 2).

A total of 3 (3%) *E. coli* O157:H7 isolates were detected by a PCR test from 100 ruminant fecal samples. Two of them were isolated from calf fecal samples and one from sheep fecal samples. The intimin gene was also detected in the *E. coli* O157:H7 strain isolated from calf fecal samples (Table 4, Figure 1 and 2).

Table 3. Antibiogram results of *E. coli* O157 isolates.

Antibiotic Discs (µg)	*E. coli* O157 Cattle isolates (n:23)			*E. coli* O157 Sheep isolates (n:15)			Total *E. coli* O157 isolates (n:38)		
	S	I	R	S	I	R	S (%)	I (%)	R(%)
Cefpodoxime (10 µg)	23	-	-	15	-	-	38 (100)	-	-
Ciprofloxacin (5 µg)	23	-	-	15	-	-	38 (100)	-	-
Colistin sulphate (5 µg)	23	-	-	15	-	-	38 (100)	-	-
Ertapenem (10 µg)	23	-	-	15	-	-	38 (100)	-	-
Cephotaxime (30 µg)	23	-	-	15	-	-	38 (100)	-	-
Cephotaxime/clavulanic acid (40 µg)	23	-	-	15	-	-	38 (100)	-	-
Meropenem (10 µg)	23	-	-	15	-	-	38 (100)	-	-
Aztroneam (30 µg)	23	-	-	15	-	-	38 (100)	-	-
Ceftriaxone (30 µg)	23	-	-	15	-	-	38 (100)	-	-
Trimetoprim/sulphametoxazole (25 µg)	23	-	-	15	-	-	38 (100)	-	-
Oxacillin (5 µg)	-	-	23	-	-	15	-	-	38 (100)
Doxycycline (30 µg)	23	-	-	15	-	-	38 (100)	-	-
Vancomycin (30 µg)	-	-	23	-	-	15	-	-	38 (100)
Gentamicin (10 µg)	20	-	3	15	-	-	35 (92.10)	-	3 (7.90)
Ampisilin (10 µg)	23	-	-	15	-	-	38 (100)	-	-
Eritromycin (15 µg)	2	6	15	3	6	6	5 (13.16)	12 (31.58)	21 (55.26)
Tobramicin (10 µg)	19	-	4	12	-	3	31 (81.58)	-	7 (18.42)
Tetracycline (30 µg)	23	-	-	15	-	-	38 (100)	-	-
Amoxicillin/clavulanic acid (30 µg)	23	-	-	15	-	-	38 (100)	-	-

S: Susceptible, I: Intermediate, R: Resistant.

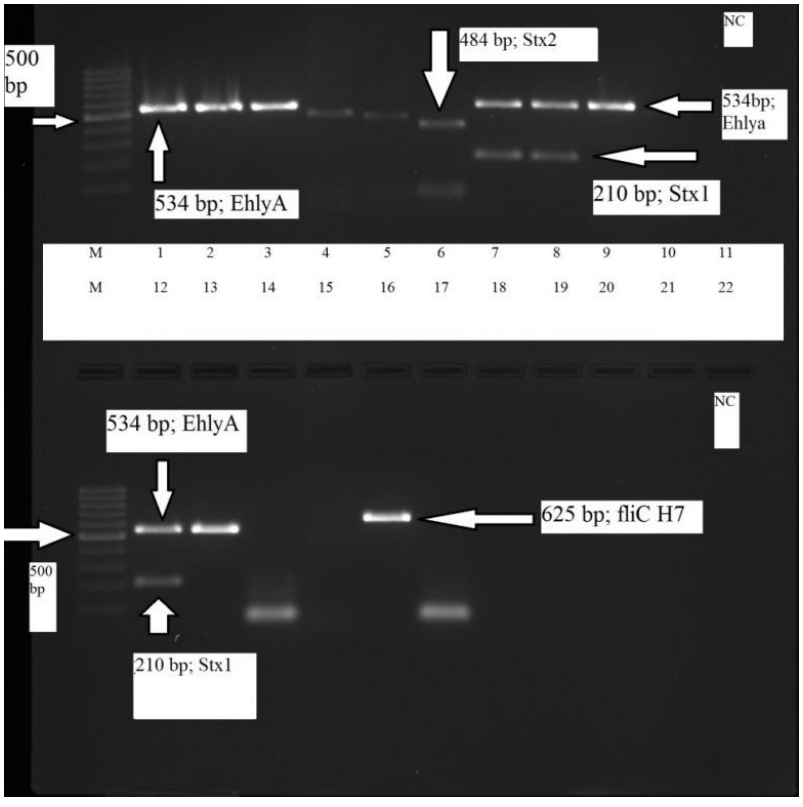

Figure 1. PCR image of virulence genes in *E. coli* O157 isolates.
M: Marker, Line 1,2,3: *E. coli* O157 isolates with *EhlyA* gene positive, Line 6: *E. coli* O157 isolate with *stx2* gene positive, Line 7,8,9: *E. coli* O157 isolates with *EhlyA* gene positive, Line 7,8,11: *E. coli* O157 isolates with *stx1* gene positive, Line 16: *E. coli* O157 isolate with *fliCH7* gene positive, Other lines: *E. coli* O157 isolates negative for the investigated virulence genes.

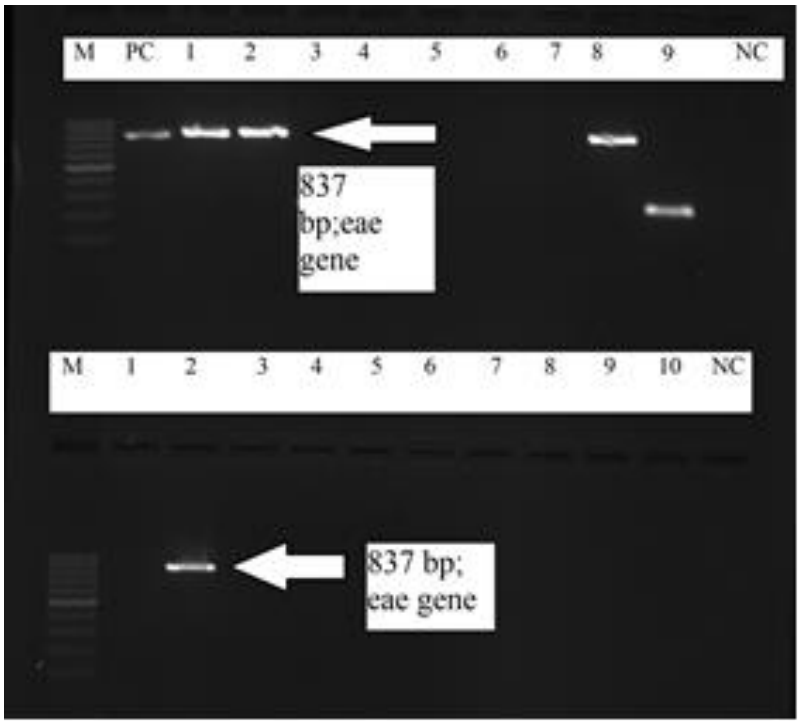

Figure 2. PCR image of *eae* (intimin) gene in *E. coli* O157 isolates.
M: Marker, PC: Positive Control, Line 2,3: *E. coli* O157 isolates with *eae* gene positive, Line 8: *E. coli* O157 isolate with *eae* gene positive, Bottom Line 2: *E. coli* O157 isolate with *eae* gene positive, Other Lines: *E. coli* O157 samples negative for the *eae* gene.

Table 4. PCR results of *E. coli* O157 virulence genes.

Virulence genes	*E. coli* O157 isolates from calf feces (n:23) (%)	*E. coli* O157 isolates from sheep feces (n:15) (%)	Total (n:38) (%)
fliCH7	2 (%8.69)	1 (%6.66)	3 (%7.89)
Stx1	4 (%17.39)	1 (%6.66)	5 (%13.15)
Stx2	2 (%8.69)	1 (%6.66)	3 (%7.89)
eaeA	6 (%26.08)	2 (%13.33)	8 (%21.05)
EhlyA	16 (%69.56)	4 (%26.66)	20 (%52.63)

Discussion and Conclusion

Cattle are the major reservoir of *E. coli* O157. Infection occurs due to the oral consumption of contaminated cattle feces and contaminated food and through direct contact with reservoir cattle (5, 11, 46, 47). *E. coli* O157 was much more intense in the feces of young cattle, especially after weaning, compared to adults (11, 16). In this study, it was thought that since the calves from which fecal samples were taken were 11-15 months old, the isolation rate might have increased. In addition, some studies reported that the number of *E. coli* O157 increased during the summer months (9, 11, 16). Since fecal samples were taken mostly during spring and summer in this study, it was thought that it might increase the isolation rate. Fecal contamination caused by poor hygiene is a risk factor in the contamination. Drinking water with fecal contamination is a suitable environment for this organism to live and grow and may cause cattle to get contaminated (11, 16). Apart from cattle, *E. coli* O157 has been found in domestic animals such as sheep, goats, pigs, dogs, and horses, as well as wild animals such as deer and birds. It is suggested that when domestic animals come into contact with wild animals, transmission between them may occur (5, 11, 16, 49).

In 2017, Alan et al. (4) detected *E. coli* O157 5.4% from two hundred and thirty seven cattle fecal samples. In this study, 15 *E. coli* O157 strains were isolated and identified from 20 sheep fecal samples. 23 *E. coli* O157 strains were identified from 80 calf fecal samples. 38 *E. coli* O157 (38%) strains were isolated from a total of 100 ruminant fecal samples.

When the results were compared, the isolation rates of *E. coli* O157 (38%) were increased as from 2017. The differences in the isolation rates of *E. coli* O157 between this study and previous studies were caused by factors such as the IMS method, age of the animals, geographical/seasonal conditions, hygiene conditions of farms, and contact with wild animals (5). Within the scope of one health concept, this result was thought to show the importance of ruminant feces and contaminated foods in human infections.

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In this study, the *EhlyA* gene was found in 20 *E. coli* O157 isolates with PCR. Of these isolates, 4 were isolated from sheep fecal samples and 16 from calf fecal samples. The *EhlyA* gene was also found in all the isolates with *Stx1* and intimin genes. Kuyucuoglu et al. (38) found the *eaeA* gene in 8 (57.1%) and the *EhlyA* gene in 13 (92.8%) of 14 *E. coli* O157:H7 isolates, and they also reported that enterohemolysin (*EhlyA*) was the dominant virulence factor. In this study, enterohaemolysin is the predominant virulence factor among the isolates, which is compatible with Kuyucuoğlu et al. (38)'s results.

Şeker and Kuş (49) reported that the *Stx2* gene was more common than the *Stx1* gene in *E. coli* O157 isolates. In this study, the *Sxt1* gene was found at a higher rate than the *Stx2* gene, which is opposite to the findings of Şeker and Kuş (49).

In 2011, Kuyucuoğlu et al. (39) reported that *E. coli* O157:H7 was detected at a rate of 3.1 % (14 out of 457 fecal samples) in calves and cattle. In this study, a total of 3 *E. coli* O157:H7 (3%) strains were detected from 100 ruminant feces by a PCR test. Isolation rates of *E. coli* O157:H7 were nearly the same compared to 2011. The intimin gene was also identified in *E. coli* O157:H7 strains isolated from calf feces.

Contamination of soil, water, and food with feces is of primary importance for the emergence of listeriosis (1). *L. monocytogenes* cannot survive below pH 5. Therefore, cattle and sheep which consume poorly prepared silage can develop various diseases as a result thereof (15). In 2019, Aydın et al. (6) detected *L. monocytogenes* 4% from 150 silage samples in Balıkesir province. In this study, six *L. monocytogenes* strains were isolated from 50 silage samples. Three of these isolates were isolated from silage samples taken from the farm, where *L. monocytogenes was* isolated from sheep fecal samples. Epidemiologically, poor quality silage was thought to be one of the sources of listeriosis. As a result, poor quality silage could be an important source of infection in Balıkesir province.

Unnerstad et al. (54) isolated *L. monocytogenes* from 6% of healthy dairy cow feces. Matto et al. (41) reported that there were identified clinically healthy dairy cows that shed *L. monocytogenes* via their feces, a situation previously described in studies conducted at dairy farms in other countries. Additionally, infected animals can shed *L. monocytogenes* via their feces. Also, fecal or soil contamination of silage is one of the sources of listeriosis (48, 52). In this study, *L. monocytogenes* was isolated from both feces and silage. The shedding of *L. monocytogenes* from clinically healthy cows shows that cattle feces could cause milk and carcass get contaminated and thus may contribute to foodborne listeriosis (54).

Resistance to antibiotics increased, especially in *E. coli* O157 strains isolated in the study. The antibiotic resistance status of *E. coli* O157 and *L. monocytogenes* isolates should be monitored with epidemiological studies.

As a conclusion, the presence and antibiotic susceptibility of *E. coli* O157 and *L. monocytogenes* were determined in this study, which was conducted for the first time in Balıkesir province of Türkiye. These results were presented as epidemiological data. It is thought that ruminant feces may be important as a reservoir in the spread and transmission of *E. coli* O157. Enterohemolysin was thought to be an important virulence factor in the pathogenesis of *E. coli* O157 infection. Contrary to the findings of other studies, the rate of the *Stx1* gene was higher than the rate of the *Sxt2* gene, especially in *E. coli* O157 isolates in this study, which is thought to be epidemiologically important. In addition, both feces and silage were an important source of listeriosis. The data of this study indicated that feces of calf and sheep are a potential source of infection and reservoir for both *E. coli* O157 infection and listeriosis in humans as well.

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Conflict of Interest

The author has no conflict of interest.

Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Ethical Statement

This study does not present any ethical concerns.

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