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RESEARCH ARTICLE

Molecular Prevalence of *Escherichia coli* O157:H7 in Cattle in Edirne Province, Türkiye

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ABSTRACT

Escherichia coli O157:H7 is a zoonotic bacterium that resides in the intestinal flora of cattle, which serve as reservoirs, and contaminates food and water through the spread of their feces. The causative agent can be transmitted by direct contact or indirect intermediaries and can cause serious diseases in humans. Therefore, it is important to investigate the carrier status of cattle to prevent possible transmission. This study aimed to investigate the presence of *E. coli* O157:H7 in cattle raised in the Edirne Province (Türkiye) by Real-Time PCR. The study material consisted of a total of 400 fresh fecal samples collected from semi-extensive (n=59) and intensive (n=20) farms located in villages of Edirne province and its districts. These samples were obtained from asymptomatic (n=260) and symptomatic (n=140) cattle based on clinical status, and from calves (n=120), heifers (n=80), and adult cattle (n=200) based on age group. The samples were analyzed for *E. coli* O157:H7 by Real-Time PCR. *E. coli* O157:H7 positivity was detected in a total of two (0.5%) samples, one of which was a calf with malaise, anorexia, and mild bloody diarrhea from an intensive farm and one asymptomatic adult cattle from a semi-extensive farm. Although no statistically significant relationship was found between *E. coli* O157:H7 positivity and factors such as breeding type, clinical condition, and age in cattle ($p>0.05$), their potential role as reservoirs with different characteristics and varying reservoir rates suggests they may influence the epidemiology of the pathogen. However, the limited number of positive samples may have reduced the power to detect significant differences, so these findings should be interpreted with caution.
Keywords: *Escherichia coli* O157:H7, Bovine fecal samples, Reservoir, Molecular diagnostics

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Edirne İlindeki Sığırlarda *Escherichia coli* O157:H7'nin Moleküler Prevalansı

ÖZ

Escherichia coli O157:H7, rezervuarı olduğu sığırların bağırsak florasında bulunan ve dışkılarının yayılmasıyla gıda ve suyu kontamine eden zoonotik bir bakteridir. Direkt temas veya indirekt aracılarla bulaşabilen etken insanlarda ciddi hastalıklara sebep olabilmektedir. Bu nedenle, sığırların etken taşıyıcılıklarının araştırılması olası bulaşların önlenmesi açısından önem arz etmektedir. Bu çalışmada, Edirne İlinde yetiştirilen sığırlarda *E. coli* O157:H7'nin varlığının Real-Time PCR ile araştırılması amaçlanmıştır. Çalışmanın materyalini, Edirne il ve ilçelerindeki köylerde bulunan yarı ekstansif (n=59) ve entansif (n=20) işletmelerden alınan toplam 400 taze dışkı örneği oluşturdu. Bu örnekler, klinik duruma göre asemptomatik (n=260) ve semptomatik (n=140) hayvanlardan; yaş grubuna göre ise buzağı (n=120), düve (n=80) ve erişkin sığırdan (n=200) alınmıştır. Dışkı örneklerinin *E. coli* O157:H7 yönünden analizi Real-Time PCR yöntemi ile gerçekleştirildi. Biri entansif işletmeye ait halsizlik, iştahsızlık ve hafif kanlı sulu ishalli bir buzağı ve biri yarı ekstansif bir işletmeye ait asemptomatik ergin bir sığır olmak üzere toplam iki (%0,5) örnekte *E. coli* O157:H7 pozitifliği tespit edildi. Sığırlarda yetiştirme türü, klinik durum ve yaş gibi faktörlerle *E. coli* O157:H7 pozitifliği arasında istatistiksel olarak anlamlı bir ilişki bulunmamasına rağmen ($p>0,05$), farklı karakteristikleriyle ile değişen oranlarda rezervuarlık yaptıkları *E. coli* O157:H7'nin epidemiyolojisini etkileyebilecekleri düşündürmektedir. Ancak, pozitif numunelerin sınırlı sayısı, anlamlı farklılıkları tespit etme gücünü azaltmış olabilir, bu nedenle bu bulgular dikkatle yorumlanmalıdır.
Anahtar kelimeler: *Escherichia coli* O157:H7, Sığır dışkı örnekleri, Rezervuar, Moleküler tanı

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INTRODUCTION

Escherichia coli O157:H7 is a serotype of *Escherichia coli* that colonizes the intestines of cattle without causing symptoms in the host (Zaheer et al. 2017). Producing Shiga-like toxins, it is a significant food-borne pathogen responsible for severe illnesses in humans, including hemorrhagic colitis, hemolytic uremic syndrome (HUS), thrombotic thrombocytopenic purpura, and in some cases, death (Mohseni et al. 2023). This pathogen can be transmitted to humans primarily through contaminated food or water. Infection typically occurs via undercooked beef, unpasteurized milk, fecally contaminated water, raw vegetables, or through direct contact with infected or carrier animals (Gambushe et al. 2022). Human infections, which increase especially during the summer months, have been reported with high prevalence in some African countries (<11.1%) and European countries (2.801.000 serious infections worldwide, with an incidence rate of 43.1 cases per 100.000 person years), and the USA (>265.000) (Gambushe et al. 2022; ECDC 2024; Ameer et al. 2025; Somda et al. 2025, Wirth et al., 2025). In Türkiye, *E. coli* O157:H7 was identified in humans (fecal samples) (Ağralı et al. 2021) and in various samples (milk, meat and fecal samples) of animal species such as cattle, sheep and buffalo raised for food production (Aksoy et al. 2007; Seker and Yardımcı 2008; Birdal and Ak 2018; Bekar 2019).

Cattle are important asymptomatic reservoirs of *E. coli* O157:H7. The presence of *E. coli* O157:H7 is influenced by various factors such as geography, season, husbandry practices, diet, age, breed, and health status. Seasonal changes and weaning in young animals are associated with higher positivity rates, whereas infections in adults are typically subclinical (Çiçek 2008; Caprioli et al. 2005; Moxley and Smith 2010). Although intensive farming is often linked to increased prevalence, some studies report higher rates in adult, asymptomatic cattle raised under extensive conditions (Birdal and Ak 2018; Hunduma et al. 2024). Given that *E. coli* O157:H7 can be detected at concentrations ranging from 10^2 to 10^5 CFU/g in cattle feces (Bach et al. 2002), monitoring its presence is critical for early detection and prevention of environmental contamination. In particular, screening known reservoir animals, such as domestic ruminants (e.g., cattle, sheep, goats) and wild animals (e.g., deer, wild boars), plays a key role in reducing the risk of zoonotic transmission.

A number of conventional, serologic and molecular methods are used in the diagnosis of *E. coli* O157:H7. Conventional methods include culturing on selective and chromogenic agars, immunomagnetic separation, biochemical tests, serologically agglutination tests with antisera, and molecular tests based on the polymerase chain reaction (PCR). There are several PCR types, such as conventional PCR, Real-Time PCR, and Digital PCR, widely used in the diagnosis of *E. coli* O157:H7 due to their advantages, such as high diagnostic accuracy, the ability to distinguish species, subspecies and variants, and the ability to perform direct agent analysis from clinical materials as well as bacterial culture (Bai et al. 2022).

This study aimed to investigate the presence of *Escherichia coli* O157:H7 in cattle raised in farms in Edirne province and its districts by Real-Time PCR.

MATERIALS and METHODS

Study Material

The study was conducted between September and December 2022 on samples taken from 59 semi-extensive and 20 intensive type family and private enterprises located in the center, districts, and villages of Edirne Province, Türkiye. The study material consisted of fresh fecal samples taken from 400 animals, including 120 calves, 80 heifers, and 200 adult cattle raised in these enterprises. Of the animals from which fecal samples were collected, 140 were symptomatic (fever, diarrhea, or malaise) and 260 were asymptomatic. The fecal samples (several grams) were collected in sterile plastic sample containers (Fıratmed, Turkey) under aseptic conditions, immediately transported to the laboratory under cold chain, and analyzed promptly.

Pre-enrichment

For this purpose, the European standardized method, ISO/TS13136:2012, was used to detect VTEC in food and similar materials, including the five most common serogroups (O157, O26, O111, O103, O145). Twenty-five grams each of the fecal samples to be analyzed were transferred to sterile plastic bags and 225 mL of Modified Tryptone-Soy Broth (mTSB) was added. The samples were homogenized in a stoamacher for two minutes and incubated at 41.5 °C for 12-18 hours (ISO, 2012).

Molecular Identification of *E. coli* O157

DNA extraction

One hundred µl of the samples enriched in mTSB were added to 365 µl STLB (Stool lysis buffer), 35 µl PEGN (Polyethylene glycol) and 10 µl OHT (Dead cell buffer). The samples were vortexed for 2 minutes and then centrifuged for 1 minute. Total DNA was extracted from the samples using a RINA M14 nucleic acid extraction robot (Bioeksen, RINA M14). DNA extracts were kept at -20 °C until analysis.

Real-Time PCR analysis of E. coli O157

Identification of *E. coli* O157 from enriched stool samples was performed by Real-Time PCR using Biospeedy Real-Time PCR Kit (Bioeksen, Türkiye). Real-Time PCR components and thermal cycle are presented in Table 1. Target *E. coli* O157 was read in the FAM channel, Inhibition and Internal control (IC)

were read in the HEX channel. Two amplification curves were consistently observed in positive samples in contrast to negative samples, which displayed only internal control (IC) results. All samples underwent duplicate analysis. A lack of amplification or a cycling threshold (Ct) value equal to or exceeding 27 was interpreted as a negative result according to the kit manual.

Molecular Identification of *E. coli* O157:H7

Molecular identification of *E. coli* O157:H7 was performed only on stool samples positive for *E. coli* O157 by the Biospeedy Real-Time PCR Kit.

DNA extraction

For the integrity of the PCR, DNA was re-extracted from positive samples in accordance with the kit manual (iQ-Check *E. coli* 157:H7 kit (Bio-Rad, USA)). Stool samples were homogenized by vigorously vortexing for 10 minutes in 10 mL of preheated Buffered Peptone Water. Homogenized samples were incubated at 41.5 °C for 8-24 hours. DNA extraction was then performed using the Easy II Extraction method, as outlined in the iQ-Check *E. coli* 157:H7 kit manual. The DNA extracts were kept at -20 °C until analyzed.

Real-Time PCR analysis of E. coli O157:H7

Confirmation of *E. coli* O157:H7 was performed by Real-Time PCR using the iQ-Check *E. coli* 157:H7 kit (Bio-Rad, USA). Real-Time PCR components and thermal cycle are presented in Table 2. Target *E. coli* O157:H7 was read in the FAM channel and Inhibition and Internal control (IC) were read in the HEX channel. Two amplification curves were consistently observed in positive serum samples, in contrast to negative samples, which displayed only internal control (IC) results. All samples underwent duplicate analysis. A lack of amplification or a cycling threshold (Ct) value equal to or exceeding 28 was interpreted as a negative result according to the kit manual.

Statistical Analysis

Statistical analysis of the data obtained from the analysis of *E. coli* O157:H7 in fecal samples using Real-Time PCR was conducted using Fisher's Exact Test in the SPSS 26 program.

Table 1. Biospeedy Real-Time PCR components and thermal cycle.

Biospeedy Real-Time PCR components			
Content	**Inhibition and Reagent Control**	**Negative Control**	**Positive Control**
2x Prime Script Mix	5 µl	5 µl	5 µl
E. coli O157-Oligo Mix	3 µl	3 µl	3 µl
Negative Control	-	2 µl	-
Inhibition and Reagent Control	1 µl	1 µl	1 µl
DNA Extract	2 µl	-	-
Positive Control	-	-	2 µl
Total Reaction Volume	11 µl	11 µl	11 µl
Biospeedy Real-Time PCR thermal cycling			
Steps	**Number of cycles**	**Temperature**	**Time**
Reverse Transcription	1 cycle	52 °C	3 min
Initial Denaturation	1 cycle	95 °C	10 sec
Denaturation	40 cycles	95 °C	10 sec
Annealing and Elongation		55 °C	30 sec

Table 2. iQ-Check Real-Time PCR components and thermal cycle.

iQ-Check Real-Time PCR components			
Content	**Inhibition and Reagent Control**	**Negative Control**	**Positive Control**
Probes (Reagent B)	5 µl	5 µl	5 µl
Amplification mix (Reagent C)	40 µl	40 µl	40 µl
Negative Control	-	5 µl	-
Positive Control	-	-	5 µl
DNA Extract	5 µl	-	-
Total Reaction Volume	50 µl	50 µl	50 µl
iQ-Check Real-Time PCR thermal cycling			
Steps	**Number of cycles**	**Temperature**	**Time**
Reverse Transcription	1 cycle	55 °C	2 min
Initial Denaturation	1 cycle	95 °C	10 sec
Denaturation	40 cycles	95 °C	10 sec
Annealing and Elongation		55 °C	20 sec

RESULTS

The Overall Positivity

The distribution of age, clinical signs, breeding type, and farming locations of the animals sampled is presented in Table 3. *E. coli* O157 positivity was detected in two (0.5%) of the 400 cattle fecal samples analyzed with the Biospeedy Real-Time PCR Kit. Analysis of these two positive samples by the iQ-Check Real-Time PCR identified that both samples were positive for *E. coli* O157:H7 (Figure 1).

The detected *E. coli* O157:H7 positivity belonged to i) an intensively reared calf showing clinical signs such as lethargy, loss of appetite, and mild bloody watery diarrhea, and ii) a semi-intensively reared adult cow without clinical signs (Table 3).

Enterprise-Level Positivity

Real-Time PCR analyses were performed on fecal samples collected from 79 cattle enterprises, including 59 semi-extensive and 20 intensive holdings. *E. coli* O157:H7 was detected in 2 enterprises (2.53%) overall. One semi-extensive enterprise (1.69%) located in İpsala and one intensive enterprise (5%) in Lalapaşa tested positive (Table 3). There was no statistically significant association between *E. coli* O157:H7 positivity and the type or location of the enterprises ($p>0.05$).

Animal-Level Positivity

Fecal samples were also collected from 400 animals: 120 calves, 80 heifers, and 200 adult cattle. Real-Time PCR analysis detected *E. coli* O157:H7 in two samples (0.5%) in total. One positive result was found in a calf (0.83%) and the other in an adult cow (0.5%). No positivity was detected in samples from heifers (Table 3). The differences in positivity rates among the age groups were not statistically significant ($p>0.05$).

Clinical Status-Based Positivity

Fecal samples were collected from 140 symptomatic animals (showing fever, diarrhea, or malaise) and 260 asymptomatic animals. Real-Time PCR analysis revealed *E. coli* O157:H7 positivity in two samples (0.5%) overall. One positive result (0.71%) was found among symptomatic animals, and one (0.38%) among asymptomatic animals (Table 3). No statistically significant difference was found between clinical status and *E. coli* O157:H7 positivity ($p>0.05$).

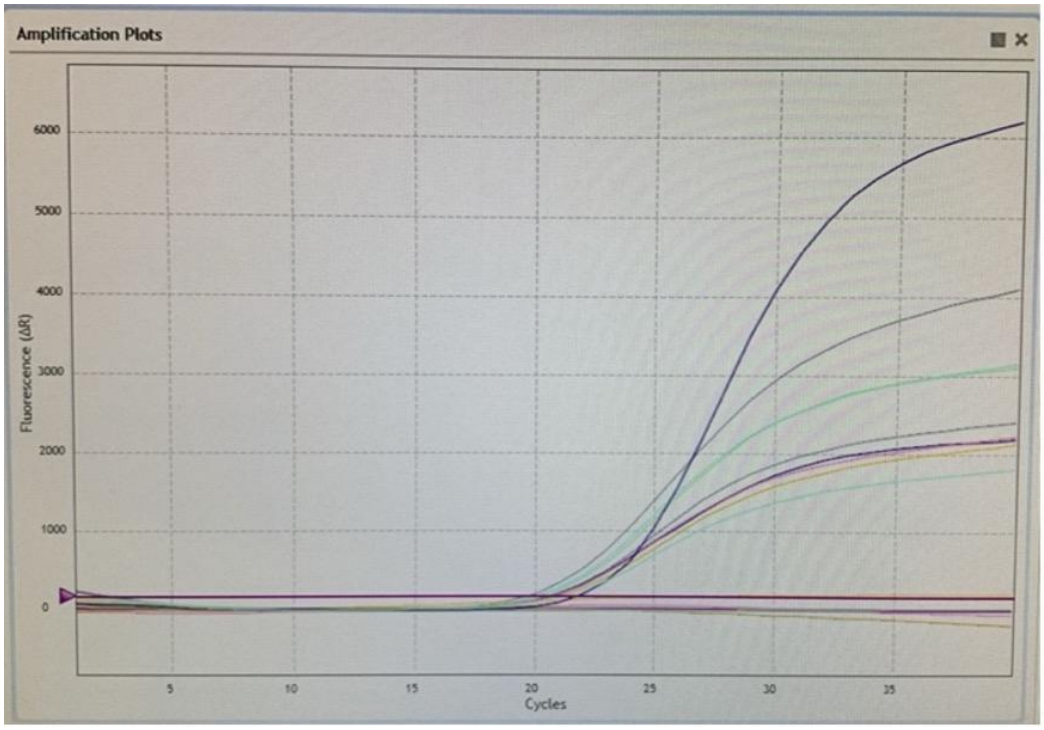

Figure 1. Fecal sample detected positive (*E. coli* O157:H7) by iQ-Check Real-Time PCR.

Table 3. Distribution of sampled animals according to age, clinical signs, breeding type and farming locations.

Location[a]	Number of *E. coli* O157-*E. coli* O157:H7 positive samples/Total number of samples (positivity%)								p-value
	Number of Enterprises[b]	Calf[c]		Heifer[c]		Adult cattle[c]		Total		
		Asymptomatic	Symptomatic	Asymptomatic	Symptomatic	Asymptomatic	Symptomatic	Asymptomatic[d]	Symptomatic[d]	
Center	0/6	0/6	0/4	0/5	0/2	0/17	0/3	0/28	0/9	
Enez	0/5	0/4	0/6	0/6	0/2	0/10	0/5	0/20	0/13	
Keşan	0/13	0/7	0/13	0/10	0/5	0/21	0/9	0/38	0/27	
İpsala	**1/18 (5.56%)**	0/8	**1/12 (8.33%)**	0/13	0/2	0/25	0/10	0/46	**1/24 (4.17%)**	
Uzunköprü	0/12	0/6	0/14	0/9	0/3	0/28	0/7	0/43	0/24	$p>0.05^{a,b,c,d}$
Meriç	0/9	0/6	0/9	0/6	0/2	0/19	0/6	0/31	0/17	
Havsa	0/5	0/3	0/2	0/4	0/1	0/17	0/3	0/24	0/6	
Lalapaşa	**1/6 (16.67%)**	0/4	0/6	0/3	0/2	**1/7 (14.29%)**	0/3	**1/14 (7.14%)**	0/11	
Süloğlu	0/5	0/6	0/4	0/4	0/1	0/6	0/4	0/16	0/9	
Subtotal	**2/79 (2.53%)**	0/50	**1/70 (1.43%)**	0/60	0/20	**1/150 (0.67%)**	0/50	**1/260 (0.38%)**	**1/140 (0.71%)**	
Total		**1/120 (0.83%)**		0/80		**1/200 (0.5%)**		**2/400 (0.5%)**		

a, b, c, d = There was no statistical significance between *E. coli* O157:H7 positivity and these variables (p>0.05).

DISCUSSION

E. coli O157:H7 can cause infections such as bloody diarrhea, hemorrhagic colitis, HUS, and TTP, and can be fatal in humans. Meat and various meat products from cattle, raw milk, and dairy products are the main sources of infection for humans. *E. coli* O157:H7 contaminates soil and water through excretion in the feces of infected or healthy animals and then infects other living organisms. Many mammalian and poultry species are reservoirs for *E. coli* O157:H7. Among these, cattle are known to be important reservoirs and may facilitate the spread of the agent into the environment and possible zoonotic transmission (Chapman et al. 1993; Caprioli et al. 2005; Gambushe et al. 2022).

Cultural methods are frequently used for the identification of *E. coli* O157:H7 from various food and clinical samples and feces. These methods, which are considered a gold standard, have many disadvantages, such as being laborious, time-consuming and costly, and can transform into viable but nonculturable (VNBC) forms (Taskin 2017; FDA 2020). Therefore, several molecular methods that provide more sensitive and rapid results are employed in the diagnosis of *E. coli* O157:H7 (Nitecki et al., 2015; Bai et al., 2022). Both individual and comparative tests have demonstrated the diagnostic superiority of Real-Time PCR for the detection of *E. coli* O157:H7 in various samples, including feces in animals (Guy et al. 2014; Noll et al. 2018; Bosilevac et al. 2019). Real-Time PCR, which can measure a wide range of concentrations, makes it easier to understand the dynamics and risks of transmission with *E. coli* O157:H7. In this study, the prevalence of *E. coli* O157:H7 in cattle in semi-extensive and intensive farms in Edirne province (Türkiye) and its districts was investigated by Real-Time PCRs with high diagnostic capabilities.

Studies on cattle fecal samples have shown that the prevalence of *E. coli* O157:H7 varies significantly by region. While this rate was reported between 1-5% in the USA (Zhao et al. 1995; Sargeant et al. 2000), it was reported between 1-15.7% in Europe (Chapman et al. 1993; Blanco et al. 2004). The highest rate in Europe was found in England, where *E. coli* O157:H7 positivity was reported as 15.7% in 1995-1996 and 12.9% in 2001 (Chapman 2000). In Türkiye, *E. coli* O157:H7 positivity in clinically healthy cattle was reported as 1.04% in the Marmara Region (Birdal and Ak 2018), 1.28% in Van Province (Çabalar et al. 2001), 1.3% in the Aegean Region (Çiçek 2008) and 3.93% in Istanbul Province (Yılmaz et al. 2002). In a study conducted in Balıkesir, *E. coli* O157 culture positivity was obtained in 28.75% of clinically healthy calf fecal samples, while *E. coli* O157:H7 PCR positivity was determined in 2.5% (Babacan 2024). In Afyonkarahisar, *E. coli* O157:H7 culture positivity was detected in 3.1% of fecal samples collected from cattle, with 2.3% belonging to cows, 2.6% to healthy calves, and 10.6% to calves with diarrhea (Kuyucuoğlu et al., 2011). In this study conducted in cattle in Edirne Province, *E. coli* O157:H7 positivity was obtained at values very close to the lower limit of both global and national reports. Considering that cattle excrete 20-50 kg of feces per day and these reservoirs shed significant amounts of *E. coli* O157:H7 into the farm environment (most of them shed the agent at a concentration of $<10^2$ CFU/g, while a few shed at high levels such as $\geq 10^4$ CFU/g) (Omisakin et al. 2003; Pearce et al. 2004), this positivity has epidemiological importance in terms of the risk of raw milk, carcass and environmental contamination.

The prevalence of *E. coli* O157:H7 in cattle is influenced by factors such as season, age, immune status, and farming practices. While higher positivity is commonly reported in young, weaned animals during summer and in intensive farming systems, some studies have also found increased rates in adult, asymptomatic, and extensively raised cattle (Çiçek 2008; Aslantaş et al. 2006; Caprioli et al. 2005; Moxley and Smith 2010; Suardana et al. 2017; Birdal and Ak 2018; Ashenaf et al. 2020; Hunduma et al. 2024). In the current study, one of the samples in which positivity was detected belonged to a calf showing clinical symptoms such as weakness, loss of appetite and slightly bloody watery diarrhea, and this animal was raised intensively, quarantined due to symptoms and general treatment was started; the other fresh fecal sample with positive results belonged to an adult cattle without clinical findings, and this animal was raised semi-extensively and did not go to pasture frequently due to the season. Therefore, due to the limited number of positive samples, it is not possible to draw strong conclusions about the relationship between the *E. coli* O157:H7 prevalence and variables such as season, age, clinical condition, or farming practices, although, similar to other studies, the pathogen positivity appears not to be clearly associated with these factors. Moreover, since *E. coli* O157:H7 is often asymptomatic in cattle, the presence of clinical signs in one of the positive animals may suggest co-infection with other enteric pathogens (Coura et al. 2015). As no parallel testing for other common causes of calf diarrhea (e.g., *Salmonella* spp., rotavirus, coronavirus, *Cryptosporidium* spp., *Giardia* spp.) was performed, any causal relationship between *E. coli* O157:H7 and the observed symptoms cannot be definitively established and should be interpreted with caution.

It is thought that the *E. coli* O157:H7 positivity variances between districts, villages, or enterprises, which are frequently detected in studies carried out to represent a geography, may be due to local geographical features, feeding habits of animals and variability of hygiene rules between enterprises, which are not statistically significant, as in many studies (Suardana et al. 2017; Hunduma et al. 2024). A similar situation was detected in the current study. While *E.*

coli O157:H7 positivity was detected in enterprises belonging to only 2 (2.53%) of the 9 districts of Edirne Province, İpsala and Lalapaşa, no statistical significance was found between this positivity and locations ($p>0.05$). However, the limited number of positive samples in this study may have reduced the statistical power to detect significant differences between locations, and thus these findings should be interpreted with caution.

CONCLUSION

In this study, the molecular prevalence of *E. coli* O157:H7 in cattle from farms in Edirne Province (Türkiye) was determined to be 0.5% (2/400). In this context, commercially available real-time PCR kits (Biospeedy Real-Time PCR kit and iQ-Check *E. coli* O157:H7 kit) proved to be practical tools for identifying the agent. Although no statistically significant association was found between *E. coli* O157:H7 positivity and farming method, clinical status, or age groups, relatively higher positivity rates were observed in calves and animals exhibiting symptoms such as weakness, anorexia, and mild bloody watery diarrhea. Based on current literature, the overall prevalence of *E. coli* O157:H7 in cattle feces appears low; however, cattle of different ages, clinical conditions, and management practices may still contribute to the eco-epidemiology of this pathogen by serving as reservoirs.

Conflict of interest: The authors have no conflicts of interest to report.

Authors' Contributions: YEK and FB contributed to design and execution of the study. YEK and FB contributed to the acquisition of data. YEK and FB analysed the data. YEK and FB drafted and wrote the manuscript. FB reviewed the manuscript critically. All authors have read and approved the finalized manuscript.

Ethical approval: This study was carried out at Afyon Kocatepe University Research Animals Application Center. This research was approved by the Animal Experiments Local Ethics Committee of the Kafkas University (KAÜ-HADYEK, Ref No: 2022/133).

Explanation: The content of this study has been presented as a poster (16th National Veterinary Microbiology Congress (with International Participation), 25-28 October 2024, İzmir, Türkiye) and an oral presentation (5th International Food, Agriculture and Veterinary Congress, 17-19 February 2023, Kars, Türkiye) in congresses.

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