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PREVALENCE OF *E. coli* O157:H7 ISOLATED FROM HUMAN AND ANIMAL SOURCE IN KIRIKKALE PROVINCE

Kırıkkale Yöresinde İnsan ve Hayvan Kaynaklarından İzole Edilen E. coli O157:H7'nin Prevalansı

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ABSTRACT

Objective: *Escherichia coli* O157:H7 strain is a cause of hemorrhagic colitis and may give rise to severe outbreaks even at a low concentration. Transmission may occur through fecal-oral route with contaminated food but direct transmission from personal contact is also possible. Presence of *E. coli* O157:H7 was investigated in humans, cattle, animal feed, and ground beef over a one-year year period in order to determine the prevalence in the Kırıkkale region.

Material and Methods: All samples were transferred to the microbiology laboratory as rapidly as possible under appropriate and sterile conditions. The isolation of *E. coli* O157:H7 was performed by serotyping with Dynabeads and ELISA methods in stool specimens in 89 patients with gastroenteritis, 108 cattle, 69 different animal broth samples, and 84 samples from ground beef after culture using classical methods. Minced meat samples were kept at -70 degrees until working tests.

Results: *E. coli* O157:H7 was not detected in humans or animals or sources including animal feed and beef carcasses.

Conclusion: Our results indicate that the meat chain from cattle to humans is safe with respect to the *E. coli* O157:H7 strain. On the other hand, other food or water sources may be potential sources for this microorganism.

Keywords: *Escherichia coli, seroprevalence, human, cattle, ELISA*

ÖZ

Amaç: *Escherichia coli* O157:H7 suşu hemorajik kolite neden olmakta ve çok düşük konsantrasyonlarda bile ciddi salgınlara yol açabilmektedir. Bulaşma kontamine yiyeceklerin fekal-oral yol ile alınması ile gerçekleşmekte fakat doğrudan insan teması ile de görülebilmektedir. Ülkemizde *E. coli* O157:H7 prevalansı genel olarak bilinmemekle beraber Kırıkkale bölgesinde de bilinmemektedir. *E. coli* O157:H7 prevalansını belirlemek amacı ile Kırıkkale bölgesinde insan, besi hayvanı, yem ve et örnekleri bir yıl süre ile araştırıldı.

Gereç ve Yöntemler: Bütün örnekler mümkün olan en kısa sürede doğru ve steril şartlarda laboratuvara getirildi. *E. coli* O157:H7 izolasyonunda 89 gastroenteritli hastanın dışkı örneği, 108 sığır dışkısı, 69 değişik hayvan yemi ve 84 et (kıyma) örneği klasik kültürü takiben Dynabead ve ELISA metotları kullanılarak belirlendi. Kıyma örnekleri çalışma testleri yapılıncaya kadar -70 derecede bekletildi.

Bulgular: *E. coli* O157:H7 araştırılan insan ve hayvanlarda, yem veya et (kıyma) örneklerinde tespit edilemedi.

Sonuç: Bulgularımız ahırdan insana uzanan besin zincirinin *E. coli* O157:H7 suşu açısından güvenli olduğunu göstermiştir. Öte yandan diğer besin ve su kaynakları bu mikroorganizma için potansiyel kaynaklar olabilir.

Anahtar Kelimeler: *Escherichia coli, seroprevalans, insan, sığır, ELISA*

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INTRODUCTION

Enterohemorrhagic *Escherichia coli* (EHEC) remains a health care problem in many parts of the world. Recently, a serious outbreak concerning one of the serotypes of this bacterium emerged in developed countries, resulting in fatal outcomes (1,2). *E. coli* O157:H7 is a well-known member of this family that was first determined in Michigan in 1982 during two food-borne outbreaks and was defined thereafter (3,4). An estimated 75.000 cases of *E. coli* O157:H7 infections occur annually in the United States (5). This microorganism, which is linked to 23% of food-borne illnesses, is responsible for life-threatening hemolytic-uremic syndrome, colonizes in ruminants and is released in feces (6). Contamination is possible during slaughtering of cattle and by infection via consumption of raw or undercooked meat or by-products. Cattle and especially cows have been defined as reservoir hosts for *E. coli* O157:H7 (7).

In view of the close association between water resources, the food chain and possible fecal contamination, we concentrated on patients with gastroenteritis and cattle. In order to determine the incidence of this serotype in our region, we investigated broth, meat samples before marketing and fecal disposals.

MATERIALS AND METHODS

Microbiologic Analysis of Human and Animal Specimens

A total of 89 human and 108 calf stool samples were investigated. In human samples, especially bloody stools were obtained from children and elderly patients who admitted to Kırıkkale University Faculty of Medicine with gastroenteritis. All samples were transferred to the microbiology laboratory as rapidly as possible under appropriate and sterile conditions. Samples were suspended in saline and then transferred to sorbitol MacConkey (SMAC), Eosine Methylene Blue (EMB) and bloody agar plates. They were incubated in selenite-F broth for 6-8 hours (h), and then transferred to the S*almonella-Shigella* (SS) broth. Colonies were transferred to the SMAC after biochemical controls were performed for *E. coli*. In order to differentiate, *E. coli* EDL 931, VT1, 2 (+) strain (Japan) was used for positive, and American type culture collection (ATCC) 25922 strain was used for negative controls, respectively. Enrichment and identification were performed according to classical methods (8).

Immunomagnetic Separation (IMS) Technique for E. coli O157:H7

The principle of this method was adhesion of attached antibodies on magnetic or super paramagnetic carriers to the target microorganism (Dynabeads anti-*E. coli* O157: Dynal AS, Norway). In order to determine *E. coli* O157, Vancomycin (8mg/L), Cefixime (0.05 mg/L), and Cefsulodin (10 mg/L) added broths were used for pre-enrichment of human or calf feces samples and were incorporated with anti- *E. coli* Dynabeads particles to confirm aggregates. These aggregates were then transferred to SMAC broth to produce sorbitol-negative colonies, as described elsewhere in detail (9).

ELISA for E. coli O157

Samples were stored at -70°C until analyses with Carry-Blair. All samples were thawed and studied on the same day. The supernatant obtained after centrifugation was distributed as 100 µL in each hole and incubated for 30 minutes. Enzyme conjugate (red solution) was added after rinsing three times and incubated at room temperature for another 30 min. The rinsing procedure was repeated and 100 µL chromogene substrate was added. Then the sample was incubated in the dark for 10 min. Stop solution 100 µL was added to each hole. Optic densities were determined using ELISA reader (SEAC). Optic densities higher than the reference value were accepted as positive and those which were lower than the

reference value remained negative. Values near the reference were repeated.

Beef Carcasses

A total of 84 minced meat samples were taken from 47 butchers and markets. The samples (200 g each) were transferred to the laboratory under aseptic conditions and analyzed for *E. coli* O157.

Animal Broth Samples

A total of 69 calf broth samples obtained from different sale points were studied. The composition of the broths included barley, wheat, draw, cracked wheat, corn, corn gluten, corn pulp, sunflower pulp, hazelnut pulp, marble powder, cotton, open pellet, and minced pellet.

RESULTS

E. coli O157:H7 seropositivity was not detected in any human stool specimens investigated using Dynalbead, latex tests, and conventional culture methods. The distribution of patients according to age and gender is displayed in Table 1.

Table 1. Age and gender distribution of patients.

Age	Male n (%)	Female n (%)	Total n (%)
1-4	5 (5.6)	6 (6.7)	11 (12.35)
5-9	8 (9)	9 (10.1)	17 (19.1)
10-14	2 (2.2)	3 (3.4)	5 (5.6)
15-19	1 (1.1)	2 (2.2)	3 (3.3)
20-24	2 (2.2)	1 (1.1)	3 (3.3)
25-29	1 (1.1)	2 (2.2)	3 (3.3)
30-34	0 (0)	0 (0)	0 (0)
35-39	1 (1.1)	1 (1.1)	2 (2.2)
40-44	0 (0)	0 (0)	0 (0)
45-49	3 (3.3)	2 (2.2)	5 (5.6)
50-54	4 (4.5)	7 (7.9)	13 (14.6)
> 55	11 (12.3)	18 (20.2)	29 (32.6)
Total	38 (42.7)	51 (57.3)	89 (100)

In cattle stool specimens, *E. coli* O157:H7 seropositivity was not determined after being searched by classical culture method, Dynabead and latex tests. Age and gender distribution for the cattle are shown in Table 2.

Seropositivity for *E. coli* O157:H7 was determined neither in beef carcasses nor in animal feed samples. The test results obtained by different methods with various samples are summarized in Table 3.

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Table 2. Age and gender distribution of cattle.

Age	Male n (%)	Female n (%)	Total n (%)
1	0 (0)	0 (0)	0 (0)
2	14 (13)	19 (17.6)	33 (30.5)
3	17 (15.7)	16 (14.8)	33 (30.5)
4	15 (13.8)	13 (12)	28 (25.8)
5	3 (2.8)	3 (2.8)	6 (5.6)
6	2 (1.9)	1 (0.9)	3 (2.8)
7	2 (1.9)	3 (2.8)	5 (4.6)
Total	53 (49.2)	56 (51.8)	100 (100)

Table 3. Test results for *E. coli* O157:H7 obtained from various samples.

	Laboratory tests	**Test (n)**	**Positivity (n)**	**%**
Human stool specimens	Dynal bead method	89	0	0
	O157 with latex test	89	0	0
	H7 with latex test	89	0	0
	E. coli with EMB	89	87	97.8
	SMAC culture	89	81	91.0
	MacConkey with MUG	89	35	39.3
	O157 with ELISA	89	0	0
Cattle stool specimens	O157 with latex test	108	0	0
	H7 with latex test	108	0	0
	E. coli with EMB	108	104	96.3
	SMAC culture	108	98	90.7
	MacConkey with MUG	108	67	62.0
Cattle meat specimens	Dynal bead method	84	0	0
	O157 with latex test	84	0	0
	H7 with latex test	84	0	0
	E. coli with EMB	84	64	76.7
	SMAC culture	84	79	94.0
	MacConkey with MUG	84	56	66.7
Animal feed specimens	Dynal bead method	69	0	0
	O157 with latex test	69	0	0
	H7 with latex test	69	0	0
	E. coli with EMB	69	2	2.8
	SMAC culture	69	1	1.4
	MacConkey with MUG	69	0	0

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DISCUSSION

Human studies addressing *E. coli* O157 strain in our country are rare. Food borne infections may be major health concerns in developing countries including Turkey. There is no data for *E. coli* O157:H7 searched before from Kırıkkale. Our results indicate that the etiology of gastroenteritis in patients could not be attributed to *E. coli* O157:H7. Similarly, Hasçelik et al. evaluated 677 pediatric patients in Ankara at 1994 and reported that all were negative for *E. coli* O157:H7 (10). The pathologic strain of *E. coli* is a chronic problem in developed countries. The incidence was reported as 6.3% in infant patients with acute gastroenteritis in north-west Italy (11). In Switzerland, non-O157 shiga toxin-producing *E. coli* was isolated in 97 cases between 2000 and 2009 (12). It also seems to be a critical heath care problem in underdeveloped parts of the world. Multi-drug resistance of *E. coli* O157 isolated from stool specimens and surface waters in Nigeria was observed, indicating dissemination of the transferable plasmids encoding resistance to the other enterobacterial species (13).

Detection of bacteria or toxin in beef carcasses indicates a possible contamination from disposals during the slaughtering of cattle or sheep, which may produce a more important health care problem due to possible infection during consumption. Again, the samples from beef carcasses were free of *E. coli* O157:H7 in the present study. In contrast to our results, Inat et al. reported that 52 sample was found to be positive from 200 slaughtered cattle using immune-magnetic separation technique. Fourty-nine were *E. coli* O157 and three samples were O157:H7 strain from 52 positive sample of carcasses in Samsun (14). Ahmed et al. detected 54 O157:H7 strains isolated from 1600 food samples (800 meat products and 800 dairy products) collected from butchers, retail markets, and slaughterhouses in Egypt (15). Hessain AM et al. evaluated a study which was carried out to evaluate the prevalence of *E. coli* serotype O157:H7 recovered from raw meat and meat products collected from Saudi Arabia. Three-hundred and seventy meat samples were collected from abattoirs and markets located in Riyadh, Saudi Arabia. The samples were taken from 200 raw meat and 170 meat products. Bacteriological analysis of the meat samples and serotyping of the isolated *E. coli* revealed the isolation of 11 (2.97%) strains of *E. coli* O157:H7 (16). These results indicate the importance of close monitoring and of following strict rules to prevent contamination in local slaughterhouses. Abdissa et al. detected *E. coli* O157: H7 in 1.89% of fecal samples, 0.81% of intestinal mucosal swab samples, 0.54% of skin swab samples and 0.54% of carcass internal swab samples (17). The prevalence of *E. coli* O157 in the carcass surface from the UK at butcher shops in South Yorkshire was found to be 2.9%, (29/1877 samples) for lamb products. However, the prevalence in beef products at the same butcher shops tended to be lower (1.1%, 36/3216 samples of beef products) (18). We did not observe carcass contamination. Our data indicate that none of the animals were positive for *E. coli* O157:H7 strain also in cattle stool specimens. Our study suggests the need for large scale on-farm studies to determine the prevalence of *E. coli* O157:H7 in Kırıkkale. In our study *E. coli* O157 was not detected in the carcass samples. Nevertheless differences in prevalences could have been due to the limited sample size in our and several of the other studies.

Animal broth is another possible source of infection that may lead to contamination of the cattle. We searched various types of cattle feed samples for the pathogenic *E. coli* O157:H7 strain. However, all were free from this form of microorganism. Investigations in subjects by controlling the infection through broth enrichment might be promising. The presence of lactose in broth may influence *E. coli* adherence to the epithelial cells in vitro (19).

Recent studies have focused on auto-transporter proteins, which are essential for promoting biofilm

formation (20). Auto-signaling molecules are capable of promoting bacterial colonization and could be controlled by altering these communications in animal reservoirs of cattle (21).

In conclusion, we were unable to detect the *E. coli* O157:H7 strain in patients with gastroenteritis, beef carcasses, dairy products or animal/human stool or broth specimens. Nevertheless, other sources of contamination including, water or plants that are consumed freshly should be closely observed.

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