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Presence of *Escherichia coli* O157 and O157:H7 in raw milk and Van herby cheese

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Abstract

The Shiga toxin-producing *Escherichia coli* (STEC) strains are currently considered important emerging pathogens threatening public health. Among Shiga toxin-producing *Escherichia coli*, *E. coli* O157:H7 strains have emerged as important human pathogens. This study was conducted to determine the presence of *Escherichia coli* O157 and O157:H7 in raw milk samples and Van herby cheese samples. For this purpose, 100 samples of raw milk were collected and 100 samples of herby cheese sold for consumption in Van province in Turkey were obtained from grocers and markets in order to detect the presence of *Escherichia coli* O157 and O157:H7. The method of *E. coli* O157 and O157:H7 isolation proposed by the Food and Drug Administration (FDA) was used. *E. coli* O157 in raw milk and herby cheese samples was found in 11% and 6% of samples respectively, and *E. coli* O157:H7 was found in 2% of herby cheese samples. No *E. coli* O157:H7 was detected in raw milk samples. This study showed that raw milk was contaminated with *E. coli* O157 and herby cheese was contaminated with both *E. coli* O157 and *E. coli* O157:H7; therefore, herby cheese poses a serious risk to public health.

Keywords: *E. coli* O157, *E. coli* O157:H7, raw milk, herby cheese.

Introduction

Escherichia coli O157:H7 belongs to a group of enterohaemorrhagic *E. coli* strains, which are recognised as a pathogen that spreads from food to humans and causes considerable epidemics, as has been seen in recent years. Toxins produced by the serogroup cause gastroenteritis, which can lead to death in humans. *E. coli* O157:H7 is one of the most significant foodborne pathogenic serogroups in *E. coli* strains (14, 23).

E. coli O157:H7 was first considered a foodborne pathogen in the states of Oregon and Michigan (USA) at the beginning of 1982 after two substantial epidemics, which were caused by contaminated burgers (26). The pathogen generally spreads from faeces and foods like meat and milk, especially from dairy cattle. In several studies carried out in the USA, it was found out that among *E. coli* O157:H7 infections in humans, contaminated food is the most frequent (67%) source (14, 19, 23). Many pathogenic microorganisms grow readily in raw milk. The foodborne pathogens isolated from raw milk cause infections in humans. *E. coli* O157:H7 was first detected in raw milk in the USA in 1986 and was reported to be responsible for the development of haemolytic-uraemic syndrome (HUS) in two infants after raw milk consumption (14). Later, many *E. coli* O157:H7 infections caused by raw milk were reported (2, 7, 9, 25). Similarly, this microorganism was isolated from different cheese types (1, 2, 13, 17).

In previous studies on milk in Turkey, it was reported that *E. coli* O157:H7 was not isolated from raw milk samples (8, 17, 28). However, in other studies, *E. coli* O157:H7 was isolated from 2% (2) and from 3% (1) of raw milk samples. Studies carried out on the isolation of the *E. coli* O157 serogroup demonstrated that the serogroup could be isolated from 1% (22) and 2% (8) of raw milk samples. Tolun *et al.* (28) did not isolate *E. coli* O157:H7 from white cheese;

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however, it was isolated from just such in 1% (1), 2% (2), and 3.33% (17) in other studies performed in different regions of Turkey. *E. coli* O157 and *E. coli* O157:H7 were isolated from white cheese samples at the levels of 1% (8) and 4% (22) respectively.

Van herby cheese, one of the most important traditional Turkish cheeses, is widely consumed in Eastern Anatolia, and has increasing popularity in Turkey's large cities. It is a semi hard, dry salted cheese mainly manufactured artisanally from whole unpasteurised ewe's and cow's milk, and/or a mixture of both without any addition of starter cultures. It contains 0.1%–1.5% (w/w) herb or mixture of herbs (*Allium*, *Thymus*, and *Ferula* sp.), which is added to the curd. No herby cheese manufacturing procedure has been standardised commercially yet. Herby cheese is rindless, white in colour, salty, and piquant in flavour (27).

In the study carried out in Van province, *E. coli* O157 was isolated from 4.66% of minced beef samples and from 2% of minced lamb samples (3). In another study, *E. coli* was found in 62% of herby cheese samples offered for sale in Van and Hakkari provinces, while no *E. coli* O157:H7 was isolated from these samples (27).

The study aimed to determine the prevalence of *E. coli* O157 and 0157:H7 strains in raw milk and ripened herby cheese for sale in Van province. These strains are important foodborne pathogens, which pose a risk to public health.

Material and Methods

Material. Raw cow milk samples and herby cheese samples were used and numbered 100 in each case. The samples were obtained from markets in Van city. Samples were collected into sterile glass jars in aseptic conditions and brought into the laboratory in an unbroken cold chain (4°C) within a maximum of 2 h. They were analysed on the day of receipt (18).

Method. Enrichment of the raw milk and herby cheese samples and *E. coli* O157:H7 isolation was carried out in line with the method recommended by the Food and Drug Administration (16). The samples (25 g each) were put into 225 mL of a modified Tryptone Soya Broth medium, to which VCC Selective Supplement (Oxoid, part of Thermo Scientific, UK) was added. This mixture was homogenised in a 2373/400 laboratory paddle blender (IUL, Spain) and then incubated for 24 h at 37 ± 0.5°C. After the incubation, the samples were streaked on Sorbitol MacConkey Agar (Oxoid) and incubated for 18–24 h at 35–37°C. Five typical colonies were transferred into Tryptone Soya Agar (TSA) (Oxoid) containing 0.6% Yeast Extract (YE) (Oxoid) for colony purification and again incubated for 18–24 h at 35°C.

The pure colonies were inoculated to Sulphide Indole Motility (SIM) medium (Oxoid) and indole positive colonies were transferred into Eosine Methylene Blue Agar (EMB Agar) (Oxoid) and TSA+YE. Then, a ColiComplete disc (BioControl, USA) including 4-methylumbelliferyl-β-D-glucuronide (MUG) was placed on the streak zone aseptically. The colonies were incubated for 24 h at 37°C. The same procedure was also applied to the reference *E. coli* strain (*E. coli* ATCC 25922) that is known to be MUG positive. The tested colonies and reference strains were subjected to ultraviolet light (4W/366 nm UV lamp, Merck), which allows detection of MUG positive *E. coli* strains. Additionally, typical colonies were observed in EMB Agar. Colonies which were indole positive or β-glucuronidase negative were assayed with a Dryspot *E. coli* O157 latex test kit (Oxoid), *E. coli* H antiserum H7 (BD Difco, Beckton, Dickinson, USA), and agglutination test. Colonies that displayed agglutination in the Dryspot *E. coli* O157 latex test kit were considered to be *E. coli* O157, while colonies that displayed agglutination with *E. coli* H antiserum H7 were considered to be *E. coli* O157:H7 (16, 21).

Results

The analysis showed that 11% of raw milk and 6% of herby cheese samples were contaminated with *E. coli* O157. No *E. coli* O157:H7 was isolated from raw milk samples, however, the strain was found in 2% of herby cheese samples. In the present study, 8.5% and 1.0% of all samples were positive for *E. coli* O157 and *E. coli* O157:H7 respectively (Table 1).

Table 1. Distribution of *E. coli* O157 and O157:H7 in raw cow's milk and herby cheese samples

Sample types	Number of samples	*E. coli* O157		*E. coli* O157:H7	
		Number of positive samples	%	Number of positive samples	%
Raw cow's milk	100	11	11.0	0	0.0
Herby cheese	100	6	6.0	2	2.0
Total	200	17	8.5	2	1.0

The *E. coli* O157 serogroup was isolated more frequently than the *E. coli* O157:H7 subgroup in all the examined samples. In contrast, the *E. coli* O157:H7 subgroup was not isolated from raw milk samples and only two samples of cheese were found positive (Table 1).

Discussion

Pathogenic microorganisms can spread directly to milk from the udder while other sources of contamination are barn surfaces during and after milking, air, devices and equipment, and personnel. *E. coli* serogroups such as *E. coli* O157 and O157:H7 can spread to milk and dairy products from faeces and cause severe infections (14, 15). Using generally unpasteurised ewe's, cow's, and/or goat's milk in the production of herby cheese and production of this cheese under unhygienic conditions increase the risk of infection (27).

In the study, the isolation rate (11%) of *E. coli* O157 in raw cow's milk was found to be higher than the isolation rate obtained in other studies (10, 22). *E. coli* O157:H7 was not found in raw cow's milk samples. This finding is similar to results of other studies (8, 17, 28) while the contamination with *E. coli* O157:H7 in our study was lower than that found by other researchers (1, 2, 7, 13, 24).

The *E. coli* O157 isolation rate in herby cheese samples (6%) was higher than in the findings of other studies (8, 10, 11, 27) but it was similar to results obtained by Oksuz *et al.* (22). On the other hand, the *E. coli* O157:H7 isolation rate obtained from herby cheese samples (2%) was found to be the same as the findings of Aksu *et al.* (2) and higher than the results seen by Tolun *et al.* (28) and Akkaya *et al.* (1).

The presence of *E. coli* O157 and O157:H7 in raw milk and herby cheese may be caused by unhygienic production and sale conditions in Van province (15, 27). The isolation of *E. coli* O157 from herby cheese is very important for public health, because the cheese is widely consumed in Eastern Anatolia and major Turkish cities (27).

The frequency of the *E. coli* O157:H7 serogroup isolated in our study is similar to results obtained in Turkey and other countries (1, 25, 27). The presence of *E. coli* O157:H7 in herby cheese can be caused by the production of this cheese from sheep milk, because generally this is the animal whose milking takes place under the least hygienic conditions. Hygiene is also compromised by the ripening of the cheese underground, and it is sold for consumption under unhygienic conditions. The primitive production processes of the cheese may be a factor that increases its contamination with *E. coli* O157:H7 (1, 15, 25, 27, 28).

Some studies reveal that *E. coli* O157:H7 may survive even in the last periods of maturation (20, 25, 30). Isolation of *E. coli* O157:H7 from herby cheese reminds us that these cheeses pose a risk in terms of public health even when they are consumed after ripening. It is reported that the agent has the ability to adapt to acidic conditions and thus can persist for a long time even in fermented dairy products (29). Vernozy-Rozand *et al.* (30) reported that *E. coli* O157:H7 can survive even on the 42nd d of maturation of cheese made from raw goat milk. *E. coli* O157:H7 is eliminated from Castellano cheeses, which are produced from raw sheep milk and ripened for almost a year, but three Shiga-like toxin-producing serogroups may persist (9). In a study on *E. coli* O157:H7's ability to survive in Galotyri cheese, it is reported that survival of the pathogen depends on the type of cheese, pH value, salt concentration, lactic acid concentration, and metabolic activity of lactic acid flora. The researchers also demonstrated that fresh acid-curd cheeses were higher risk than cheeses consumed after ripening (20).

In studies conducted in different countries, it was reported that *E. coli* O157:H7 was detected in two (0.76%) of 264 milk samples (7), in one (0.33%) of 100 each of sheep, cow, and goat milk samples (13), and in two (2%) of 100 raw milk samples (24). In studies carried out on cheeses in different countries, *E. coli* O157:H7 was isolated in three (3.16%) of 95 samples (12). High amounts of *E. coli* were found in cheeses produced from raw milk but no *E. coli* O157 was isolated in any of the samples studied (10). In a study performed in Italy, verotoxin-producing *E. coli* O157 (VTEC) was isolated from 0.43% of minced meat, while the strain was not isolated from any dairy products (11). In research on Shiga-like toxin-producing *E. coli* serogroups, in 360 unpasteurised sheep and goat milk, 103 fresh cheese curd, and 39 different cheese type samples, the pathogen was isolated from 10.8% of milk samples, 3.9% of fresh cheese curds, and 5% of different cheese types. Strains isolated were composed of nine serogroups and only one milk isolate was identified as *E. coli* O157:H7 (25).

The isolation of VTEC was reported in Germany, Austria, and Latvia at levels of 1.9%–4.4% in milk samples in 2005 but VTEC O157 was detected in raw cow's milk samples only in Latvia. VTEC was isolated from cheese at levels between 0.2% and 2.3% in studies performed in Italy, Slovakia, and Germany; however, VTEC O157 was detected only in Italy and Slovakia (4). In 2008, the levels of VTEC contamination in different food categories were low, which was similar to findings recorded in 2005, 2006, and 2007, except for raw milk from cows, where a three-fold increase in positive samples was observed compared to 2007. VTEC was isolated from 2.7% of samples of cheese made from cow's milk and from 3.3% of samples of cheese made from goat's milk (5). Raw cow's milk was examined in Bulgaria, Germany, Hungary, and Slovakia in 2010. VTEC was detected at a moderate level (17.6%) in Germany. VTEC-positive samples (2.6%) of soft and semi-soft cheeses made

from raw or low-heat-treated cow's milk were also found in Germany (4, 6).

Although *E. coli* O157:H7 is isolated in lower amounts only from cheese samples, small infective doses of the pathogen contaminating raw milk and cheese may always pose a risk of infection (1, 14, 23).

Our study showed that raw milk was contaminated with *E. coli* O157 and herby cheese was contaminated with both *E. coli* O157 and *E. coli* O157:H7. Therefore, consumption of the cheese may cause severe health problems. Thus, it is necessary to prevent contamination risks by the application of Hazard Analysis Critical Control Points (HACCP) rules.

Conflict of Interests Statement: The authors declare that there is no conflict of interests regarding the publication of this article.

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