

Investigation of *Escherichia coli* O157 in Turkish homemade white cheese

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Abstract

Verocytotoxin-producing *Escherichia coli* (VTEC) are important pathogens that cause serious public health risks such as food poisoning. Raw milk and cheeses made from raw or inadequately pasteurized milk remain a potential vehicle for VTEC serotype O157 infections. In this study, the occurrence of *E. coli* serotype O157 was investigated in a total of 245 Turkish homemade white cheeses, produced in rural areas, which were collected from various open public bazaars. Twenty-one non-sorbitol fermenting colonies were detected as suspicious *E. coli* O157 isolates on the sorbitol MacConkey Agar supplemented with cefixime and tellurite (CT-SMAC). Presumptive *E. coli* O157 isolates were tested for agglutination according to the manufacturers' instructions with a commercial *E. coli* O157 latex test. *E. coli* O157 was not detected in any of the 245 Turkish homemade white cheeses, popular food consumed by Turkish people. In conclusion, it is pleasing to note the absence of *E. coli* O157 in all of this popular food. However, it may be advisable to carry out a careful surveillance in such food in view of consumers' health and the possible presence of *E. coli* O157:H7, an important foodborne pathogen.

Keywords: Homemade white cheese, *Escherichia coli* O157, CT-SMAC, Foodborne infection, Latex test.

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Türk ev yapımı beyaz peynirlerde *Escherichia coli* O157'nin araştırılması

Özet

Verocytotoxin üreten *Escherichia coli* (VTEC) gıda zehirlenmeleri gibi ciddi halk sağlığı risklerine neden olan önemli patojendir. Çiğ süt ile çiğ süttten ya da yeterli derecede pastörize edilmemiş süttten yapılan peynirler VTEC serotip *E. coli* O157 enfeksiyonları için potansiyel bir kaynak teşkil etmektedir. Bu çalışmada, kırsal kesimde üretilen toplam 245 adet Türk ev yapımı beyaz peynir örneği çeşitli açık halk pazarlarından toplanmıştır. Toplanan örnekler *E. coli* serotip O157'nin varlığı açısından araştırılmıştır. Sefiksım-tellürite (CT) ihtiva eden sorbitol MacConkey (CT-SMAC) besiyerinden sorbitolü fermente etmeyen toplam 21 koloni *E. coli* O157 varlığı açısından şüpheli izolat olarak tespit edildi. *E. coli* O157 varlığı açısından şüpheli izolatlara ticari *E. coli* O157 lateks aglütinasyon test kitine üreticinin talimatları doğrultusunda tabi tutuldu. Türk halkı tarafından sevilerek tüketilen toplam 245 Türk ev yapımı beyaz peynirin hiçbirinde *E. coli* O157 varlığı tayin edilmedi. Sonuç olarak, bu sevilen gıdada *E. coli* O157'nin yokluğu sevindirici bir tespittir. Bununla beraber, gıdalarda tüketici sağlığı için önemli bir gıda kaynaklı patojen olan *E. coli* O157:H7'nin varlığına yönelik titiz incelemenin gerçekleştirilmesi tavsiye edilir.

Anahtar Kelimeler: Ev yapımı beyaz peynir, *Escherichia coli* O157, CT-SMAC, Gıda kaynaklı enfeksiyon, Lateks test.

Introduction

Escherichia coli is a common Gram-negative, nonspore-forming bacterium belonging to the family *Enterobacteriaceae*. It is a part of normal flora of the intestinal tract of humans and warm-blooded animals. Although usually harmless, some of them have potentially pathogenic characteristics and can cause intestinal and extra-intestinal illness. Furthermore, the presence of *E. coli* in foods indicates possible fecal contamination and the corresponding presence of intestinal pathogens responsible for various infectious diseases. Enterohemorrhagic is a group of diarrheagenic *E. coli* which has many serotypes. Certain Verocytotoxin-producing *Escherichia coli* (VTEC) of the serogroups O157, O26 and O111 are designated enterohemorrhagic *E. coli* (EHEC). VTEC serotype O157:H7 has been implicated in human foodborne illnesses of bloody diarrhea and the hemolytic uremic syndrome, which is the main cause of acute renal failure in children. This pathogen is capable of causing human illnesses by producing one or more related, potent toxins (Shiga-like toxin or verotoxin) that cause severe damage to the lining of the intestine (Karmali 1989; Ingham et al. 2000).

E. coli O157:H7 is the most common enterohemorrhagic serotype of *E. coli*, and has certain characteristic properties. It can survive at temperatures ranging between 7°C and 17°C (Heuvelink et al. 1998) and is destroyed by pasteurization. *E. coli* O157:H7 has a high degree of tolerance to acid and high NaCl concentrations, which facilitates its dissemination (Ingham et al. 2000; Bhunia 2008).

Ruminants, mainly cattle, which show no symptoms of disease (without causing disease), have been implicated as the principal reservoirs of STEC/VTEC (Scheutz and Strockbine 2005). The colonization of cattle and other domestic animals with STEC is generally asymptomatic

and transient (less than 1 month). Transmission of this foodborne pathogen occurs through direct contact with animals, consumption of undercooked meat, unpasteurized dairy products, vegetables or water contaminated by feces of ruminants. Also, person-to-person transmission has been documented (Karch et al. 1999).

The contamination of raw milk with *E. coli* O157:H7 has been reported in previous studies (Hancock et al. 1994; Reuben et al. 2002; Rey et al. 2006; Solomakos et al. 2009). If this bacterium is present in raw milk, the pathogen can survive during cheese production and processing. Since raw milk may be particular carrier of *E. coli* O157:H7, products made from raw milk are thought to be risky foods. The growth and survival ability of the pathogen in raw goat milk lactic cheeses (Vernozy-Rozand et al. 2005), Feta cheese (Govaris et al. 2002), Myzithra, Anthotyros and Manouri whey cheeses (Govaris et al. 2001), and soft cheeses (Ramsaran et al. 1998) have been well documented. Furthermore, some studies in Turkey have been reported for the presence of *E. coli* O157 in variety of cheeses as well as raw milk cheese (Öksüz et al. 2004; Erkan et al. 2007; Vural et al. 2010).

Raw milk and dairy products have been associated with outbreaks involving serotype O157:H7 strains including pasteurized milk (Upton and Coia 1994), raw milk (Anonymous 1996), fresh cheese curds (Durch et al. 2000), unpasteurized Gouda cheese (Honish et al. 2005), and unpasteurized goats' cheese (Espie et al. 2006). Although the numbers of reported cases of *E. coli* O157 infection are small in comparison to other enteric pathogenic bacteria such as *Salmonella* or *Campylobacter*, *E. coli* O157 has become an increasing public health problem because of a rise in its incidence and because of the severity of its complications (Jones 1999).

Turkish White cheese is a semisoft brined

cheese and a major dairy product in the Turkish market and public bazaars. It can be consumed while fresh, but it is generally eaten after ripening in a brine solution. It is produced under traditional and artisanal conditions, frequently from raw cow's milk with or without starter cultures and handled by cheese makers at various stages of manufacture. Thus, various types of microorganisms may enter the cheese during manufacture and subsequent handling (Turantaş et al. 1989).

Therefore, the aim of the present study was to investigate the presence of *E. coli* O157 in Turkish homemade white cheeses produced extensively in a rural area from raw cow's milk or inadequate pasteurized milk in order to estimate possible risks of consumption of cheese from the standpoint of public health and hygiene.

Materials and Methods

Cheese samples

A total of 245 Turkish homemade white cheese samples manufactured from raw milk or inadequate pasteurized milk in a rural area were examined for the presence of *Escherichia coli* and *E. coli* O157. The samples collected from various open-air public bazaars in Bolu (Western Turkey) were placed directly in sterile bags and transported at 4°C into cool boxes to the laboratory within 1 h on the day of collection. The microbiological analysis of samples was carried out immediately.

Detection of E. coli O157

A 25 g portion of cheese sample was added to 225 ml of buffered peptone water (pH 7.0) and homogenized. The homogenates were incubated for 3 h at 37°C. After these pre-enrichment steps, the samples were streaked for isolation on MacConkey Agar (MCA) plates and incubated aerobically overnight at 37°C. A minimum of five colonies of both lactose-fermenting and non-lactose-fermenting

isolates were selected. Identification of *E. coli* colonies grown on MCA plates was carried out using biochemical tests, including TSI reaction, oxidase, indole production, methyl red, Voges-Proskauer, Simmons citrate, urea hydrolysis, lysine and ornithine decarboxylase tests (Ewing 1986; Scheutz and Strockbine 2005). A total of 223 *E. coli* were isolated and identified from cheese. Each of them was plated onto Sorbitol MacConkey Agar (CT-SMAC) (Oxoid CM0813) supplemented with cefixime (0.05 mg/L) and potassium tellurite (2.5mg/L) (Oxoid SR0172) (Zadik et al. 1993). The CT-SMAC plates were incubated at 37°C for 18 h. At least three characteristic colonies from each plate, which were colorless, brown center on CT-SMAC, were picked from the plates. All presumptive colonies were screened serologically for the presence of the serogroup O157 using *E. coli* O157 latex test kit (Oxoid, DR0620M) according to the manufacturer's specifications. *E. coli* O157:H7 ATCC 43890 was used as a reference strain.

Results

A total of 245 Turkish homemade white cheese samples were analyzed for the presence of *E. coli* O157. Out of 223 *E. coli* only 21 (9.4%) presumptive non-sorbitol fermenting isolates were selected from Sorbitol MacConkey Agar supplemented with cefixime and tellurite. The result of this study showed that *E. coli* O157 was not detected in 21 (9.4%) presumptive non-sorbitol fermenting isolates serologically tested. Thus, all of the presumptive cheese isolates were considered as negative for *E. coli* O157. This result should be pleasurable for consumers, food producers, food researchers, stockbreeders and farmers.

Discussion

It is important to be aware of the presence of *E. coli* O157:H7 since the majority of human infections are related to the consumption of contaminated food products. *E. coli* O157:H7

can cause serious human diseases such as bloody diarrhea and hemolytic uremic syndrome (HUS). A number of studies have also reported outbreaks of hemorrhagic colitis and HUS associated with the consumption of milk and dairy products including cheeses made from cow's and goat's milk (Griffin and Tauxe 1991; Honish et al. 2005; Espie et al. 2006).

In Turkey, the incidence of *E. coli* O157:H7 in various cheese and milk samples has been documented previously. Aslantaş and Yıldız (2002) reported the occurrence of *E. coli* O157 was 2 (2%) in 100 raw milk and 1 (1%) in 100 white cheese. *E. coli* O157:H7 was found in 2 (4%) out of 50 white pickled cheeses made from raw milk and 1 (1%) out of 100 raw milk samples (Öksüz et al. 2004). A study of Erkan et al. (2007) stated that prevalence of *E. coli* O157:H7 was 4% in 50 herbed cheese samples tested. Only 1 (0.4%) *E. coli* O157:H7 was isolated from 234 blue cheese samples marketed in Konya (Akçamlı 2008). Vural et al. (2010) showed a high frequency of *E. coli* O157 with a rate of 7.6% in 105 Braided (Örgü) cheese sold in Diyarbakır.

In other countries, previous studies regarding prevalence of *E. coli* O157:H7 from milk and cheese have been also reported. Reuben et al. (2002) indicated that 2 isolates of *E. coli* O157:H7 were detected with (1.3%) from 150 raw milk samples. A total of 29 of 2005 raw milk tested, 950 bovine, 460 caprine, and 595 ovine, 21 were isolated from bovine (2.2%), 3 from caprine (0.7%) and 5 from ovine (0.8%), were found to be positive for *E. coli* O157 with a rate of 1.45% (Solomakos et al. 2009). *E. coli* O157 was detected in 1 out of 221 soft cheeses made from raw milk (Quinto and Cepeda 1997). Mora et al. (2007) showed 8 of 102 soft cheese samples were positive for *E. coli* O157.

Studies mentioned above show that raw milk and cheese made from inadequate pasteurized

milk may be a potential source for O157 infections (Espie et al. 2006). *E. coli* O157 isolates can survive the various stages during the cheese manufacture process, subsequent ripening and storage periods (Ramsaran et al. 1998; Vernozy-Rozand et al. 2005). Good manufacturing and sanitary practices should carefully be monitored and improved to eliminate routes of contamination in order to avoid the presence of foodborne pathogens such as *E. coli* O157:H7 in cheese (Govaris et al. 2001).

In our country, the absence of *E. coli* O157:H7 in milk and milk products has been announced in previous studies by a number of researchers as reported in our study. In a research by Aslantaş and Yıldız (2002) *E. coli* O157:H7 was not found in 50 Kashkaval cheese (Kaşar) and 10 Tulum cheese samples. Baz et al. (2003) did not isolate *E. coli* O157:H7 in any of 100 raw milk and 100 non-ripened Turkish white cheeses. In another study, Tekinşen and Özdemir (2006) reported that *E. coli* O157:H7 was not detected in 50 Van Herby (Otlu) cheese.

Also, the presence of *E. coli* O157:H7 in different types of cheese and milk samples has not been stated in previous studies conducted from various countries. Ansay and Kaspar (1997) indicated that *E. coli* O157:H7 was not isolated from varieties of soft and semi-soft cheeses dealing with Blue, Camembert, Brie, Colby, Havarti, Muenster and Monterey Jack sold in Madison. Enterohemorrhagic *E. coli* O157:H7 was not detected in 44 soft white cheese samples marketed in Rio de Janeiro, Brazil (Gonzalez et al. 2000). None of the 153 frozen samples of soft and semi-soft cheeses made from raw cow, ewe and goat milk was contaminated with *E. coli* O157 (Vivegnis et al. 1999). A study conducted by Caro et al. (2007) showed that O157 was not found in Castellano cheese which is hard or semi-hard Spanish cheese made from ewe's milk. Various studies on raw cows' milk performed

in the USA analyzing 42 samples (Ansay and Kaspar 1997), in the Netherlands analyzing 1011 samples (Heuvelink et al. 1998), in the UK (Scotland) analyzing 500 samples (Coia et al. 2001) and in Greece analyzing 100 samples resulted in no *E. coli* O157:H7 isolation (Dontorou et al. 2003).

In conclusion, *E. coli* O157 serotype was not isolated from any of the 245 homemade white cheeses in this study. It is satisfying to see that *E. coli* O157 exists in none of the homemade white cheese samples. Nevertheless, the presence of other *E. coli* in Turkish homemade white cheeses may raise a potential health hazard to consumers and emphasises the need for improved hygiene practice at all levels in the dairy products.

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