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Presence of enterotoxin and verotoxin in Turkish cheeses sold in İstanbul

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Abstract: The present study was conducted to determine the presence of enterotoxin and verotoxin levels in Turkish cheeses sold in retail stores in İstanbul, Turkey. For this purpose, 150 cheese samples (25 pieces of each: white pickled cheese, tulum cheese, mihaliç cheese, hellim cheese, örgü cheese, and civil cheese) were analyzed microbiologically for Staphylococcus *aureus*, Escherichia coli, and E. coli O157:H7, and the presence of the toxins was identified using an ELISA test (RIDASCREEN® Set A, B, C, D, E and Verotoxin). Of the examined samples, 40 (26.66%) were positive for S. *aureus* and 55 (36.66%) were positive for E. coli, whereas E. coli O157 was isolated in 3 of these cheeses but no E. coli O157:H7 was found. However, enterotoxins and verotoxins were detected in 25 and in 3 of the cheeses, respectively. As a result, it was concluded that even though the level of microorganisms in the cheese was not sufficient to cause disease, the presence of toxins could be considered a potential risk for public health.

Key words: Cheese, enterotoxin, verotoxin, S. *aureus*, E. coli O157:H7

Introduction

Cheese is an important milk product produced in almost every part of the world. It is widely consumed by the majority of people because of its high nutritional value and unique taste and flavor. Many varieties of cheese are produced in Turkey. There are more than 20 varieties of cheese, each differing in their production techniques. The most commonly produced cheese is white pickled cheese (60%), followed by varieties of cheese like kaşar cheese (17%), tulum cheese, mihaliç (kelle) cheese, and otlu cheese. In addition to these, different types of cheese originate from certain regions, like civil cheese, hellim cheese, çerkez cheese, abaza cheese, Urfa cheese, dil cheese, örgü cheese, and sıkma cheese. The production of cheese is not standardized; there are differences between regions or between dairies. Meanwhile, the production of cheeses, especially in small family-owned factories, can cause a health risk because of unhygienic manufacturing.

Staphylococcus aureus is considered one of the most common causes of disease worldwide (1). Staphylococcal food poisoning is caused by the ingestion of food containing one or more enterotoxins

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produced by *S. aureus* (2,3). The presence of *S. aureus* in foods constitutes a significant risk of contamination by food handlers and it can be also used as an indicator of cross-contamination (2). The US Food and Drug Administration established that effective doses of staphylococcal enterotoxin can be achieved when the population of *S. aureus* is greater than 10^5 organisms per gram of contaminated food (4).

Escherichia coli can also influence food safety and preservation because it is an indicator of fecal contamination, and the presence of *E. coli* in foods is a matter of concern because some strains may be pathogenic or can be propagated with other pathogenic organisms. The *E. coli* pathotypes have been associated with cases of mild and severe diarrhea in adults and children, mostly in developing countries (5). In extensive research, many serotypes of *E. coli* have been investigated for their pathogenic and enterohemorrhagic properties and grouped according to their virulence factors. *Escherichia coli* O157:H7 serotypes, identified as enterohemorrhagic *Escherichia coli* (EHEC) and grouped as verotoxin-producing *Escherichia coli* (VTEC), are recognized as the primary cause of hemorrhagic colitis (HC) and the diarrhea-associated form of hemolytic-uremic syndrome (HUS) (6,7).

Most foodborne outbreaks of *E. coli* O157:H7 have been associated with the consumption of foods that originated from cattle. The gastrointestinal tract of ruminants, in particular cattle, represents the natural reservoir of this pathogen (8). Therefore, meat and milk products are thought to be risky foods in terms of *E. coli* O157:H7. The infection risk is high because the infective dose of *E. coli* O157:H7 is low at 10-20 cfu/g (6).

Different cheese varieties have evidence of VTEC contamination. Cheese made with unpasteurized milk is particularly a potential vehicle for the transmission of *E. coli* O157 to the consumer. In addition, its survival rate in other processes is high; it can tolerate salt concentrations of 8.5% (9).

The present study was conducted to determine the presence of enterotoxins and verotoxins in Turkish cheeses sold in bazaars and supermarkets in İstanbul, Turkey, and to evaluate the magnitude of these pathogenic risks.

Materials and methods

Sample preparation

A total of 150 cheese samples (25 pieces of each: white pickled cheese, tulum cheese, mihaliç cheese, hellim cheese, örgü cheese, and civil cheese) were randomly collected from various markets and bazaars located in İstanbul. Samples (approximately 250 g) were transported under cold conditions from their place of collection to the laboratory. They were immediately analyzed microbiologically for *Staphylococcus aureus*, *Escherichia coli*, and *E. coli* O157:H7, and toxicologically for the presence of enterotoxins and verotoxins.

Microbiological analyses

A portion of cheese sample (25 g) was transferred to a sterile Stomacher bag with 225 mL of 0.1% peptone water (Oxoid CM0009, Basingstoke, UK) and homogenized for 2 min in a Stomacher (Lab Blender 400, Model BA 6021, Seward Laboratory Systems, London, UK). Serial decimal dilutions were prepared using the same diluents up to 10^{-6}.

The number of *S. aureus* organisms was defined on Baird-Parker agar (Oxoid CM0275) supplemented with egg yolk-tellurite emulsion (Oxoid SR0054). Spread plates were incubated at 35 °C for 24 h. Colonies with typical *S. aureus* morphology were subjected to Gram staining, examined microscopically, tested for catalase and coagulase reaction, confirmed with DNase agar (Oxoid CM0321), and then incubated at 35 °C for 18-24 h (10).

For the detection of *E. coli*, 25 g of cheese sample was added to 225 mL of sterile saline peptone water and homogenized. After that, 1 mL of aliquot was transferred to tryptone bile X-glucuronide agar (Oxoid CM0945) and incubated at 44 °C for 24 h (11).

For the determination of the *E. coli* O157:H7 serotype, 25 g of cheese sample was homogenized with 225 mL of modified EC broth containing 20 mg/L novobiocin (mEC+n) (Merck 14582, Darmstadt, Germany) and preenriched for a period of 24 h at 37 °C. The incubated broth culture was then streaked onto sorbitol MacConkey agar (Merck 109207, Germany) containing 0.05 mg/L cefixime and 2.5 mg/L tellurite. This was then incubated at 35-37 °C

for 24 h. After incubation, the plates were checked for the presence of sorbitol-negative, colorless colonies 1-2 mm in diameter. Subsequently, these presumptive colonies were confirmed serologically using an *E. coli* O157 latex agglutination test (Oxoid DR0620) and H7 antisera (Denka Seiken Co., Tokyo, Japan), as described by the manufacturers (12).

ELISA test procedures

Test procedure of S. *aureus* enterotoxins

RIDASCREEN® Set A, B, C, D, E (Art No.: R4101, R-Biopharm AG, Darmstadt, Germany) is an enzyme immunoassay for the detection of *S. aureus* enterotoxins A, B, C, D, and E in fluid and solid foods. According to the test kit manual, 10 g of cheese sample was minced and homogenized with 15 mL of phosphate buffered saline buffer and then shaken for 15 min. After centrifugation for 10 min at 3500 × *g* and 15 °C, sterile filtration of the resulting supernatant was applied. An aliquot (100 µL per well) of this solution was used in the test.

Added to the first 7 wells of the microtiter strip was 100 µL of sample, and 100 µL of the positive control was added to the last well. They were mixed gently and incubated for 1 h at room temperature (20-25 °C) in the dark. The liquid was dumped out of the wells into a sink to remove all of the remaining liquid from the wells. The wells were then each filled with 250 µL of washing buffer and the liquid was poured out again. The washing step was repeated 3 more times. The unbound conjugate was removed during washing. Subsequently, 100 µL of enzyme conjugate was added to each well and incubated for 1 h at room temperature (20-25 °C) in the dark after mixing gently. The liquid was dumped out of the wells into a sink and the wells were each filled with 250 µL of washing buffer. The liquid was poured out again and the wells were emptied to remove all of the remaining liquid. The washing step was repeated 3 more times again. Afterwards, 50 µL of substrate and 50 µL of chromogen solution was added to each well; the solution was mixed gently and incubated for 30 min at room temperature (20-25 °C) in the dark. Finally, 100 µL of the stop solution (1 M H_2SO_4) was added to each well with more gentle mixing. The absorbance was measured at 450 nm in an ELISA plate reader (ELX 800, BioTek Instruments, Bad Friedrichshall, Germany).

Test procedure of *E. coli* verotoxin

RIDASCREEN® Verotoxin (Art No.: R5701, R-Biopharm AG) is an enzyme immunoassay for the detection of verotoxin 1 and 2 (VT-I and VT-II) from an enrichment culture. For this purpose, 25 g of cheese sample was minced and homogenized in 225 mL of modified trypticase soy broth (mTSB; containing novobiocin) and incubated for 6 h at 37 °C. Subsequently, 1 mL of the preenrichment broth was added to 4 mL of mTSB (supplemented with mitomycin) and incubated overnight (16-18 h). After that, the culture was transferred in a centrifugal vial and centrifuged for 10 min at 3500 × *g* at room temperature (20-25 °C), as described in the test kit manual. An aliquot (100 µL per well) of this solution was used in the test.

For the detection of verotoxin-forming *E. coli* with the RIDASCREEN® Verotoxin test, the positive control contained in the kit and the negative control had to be run in duplicate wells in each assay. To separate duplicate wells, 100 µL of each control or prepared cheese sample was added and incubated for 1 h at room temperature (20-25 °C) in the dark. The liquid was poured out of the wells, the wells were then filled with 300 µL of washing buffer, and the liquid was poured out again. The washing step was repeated 4 more times. Subsequently, 100 µL of enzyme conjugate was added to each well and incubated for 30 min at room temperature (20-25 °C) in the dark. The liquid was poured out of the wells and the wells were filled with 300 µL of washing buffer, and then the liquid was poured out again (repeated 4 more times). Afterwards, 100 µL of substrate/chromogen solution was added to each well, mixed manually with caution, and incubated for 15 min at room temperature (20-25 °C) in the dark. Finally, 50 µL of the stop reagent (1 M H_2SO_4) was added to each well and mixed manually with caution again. The absorbance was measured at 450 nm in an ELISA plate reader (ELX 800, BioTek Instruments).

Results

A total of 150 cheese samples were examined for microbial populations. The number of positive cheese samples; count ranges and number/percentage of *S. aureus*, *E. coli*, and *E. coli* O157; and number/percentage of enterotoxins and verotoxins are given in Tables 1-3. The dispersion of enterotoxins is shown in Table 4.

Of the examined samples, 40 (26.66%) were positive for *S. aureus* and 55 (36.66%) were positive for *E. coli*, while no *E. coli* O157:H7 was found. On the other hand, *E. coli* O157 was detected in 3 of the examined cheese samples (2 white pickled cheeses and 1 tulum cheese), and enterotoxin and verotoxin were found in 25 and in 3 of the analyzed cheese samples, respectively.

Discussion

S. aureus and *E. coli* in cheese are frequently used as indicators of hygienic quality. *S. aureus* contamination can occur from raw milk produced from cows suffering from mastitis, food handlers who are carriers of *S. aureus*, or poor hygiene practices (13). Staphylococcal food poisoning is caused by ingestion of a heat-stable toxin produced by coagulase-positive *Staphylococcus aureus*. It is generally considered that the numbers of *S. aureus* need to be $>10^5$ cfu/g of cheese for the production of sufficient toxin to cause illness (1).

In this study, *S. aureus* was determined in 40 (26.66%) cheese samples, whereas 6 samples (4 white pickled cheeses, 1 tulum cheese, and 1 mihaliç cheese) equaled or exceeded 10^5 cfu/g. The incidence of *S. aureus* in different types of traditional cheeses has been reported in various studies by a number of researchers in Turkey. Gülmez et al. (14) emphasized that the mean *S. aureus* count was 4.0×10^4 cfu/g in unripened cheese, while it was 5.5×10^2 cfu/g in ripened cheese. Similarly, Gülmez and Güven (15) reported that the average *S. aureus* count was 4.9×10^2 cfu/g in civil cheese sold in Kars Province. In addition, Sağun et al. (16) determined that the average *S. aureus* count of 20 white and herby cheeses collected in Van were 0.64 $\log_{10}$ cfu/g and 0.99 $\log_{10}$ cfu/g, respectively. Tekinşen and Özdemir (17) investigated 50 unripened Van otlu cheese samples obtained in Van and Hakkari markets that had a mean *S. aureus* count of 6.1 log cfu/g. Tasci et al. (18) stated that 18 (36%) of 50 cheese samples were positive for the presence of *S. aureus* and had a mean count of 5.80 $\log_{10}$ cfu/g. In 3 (6%) of the examined samples, staphylococcal enterotoxins B, C, and E were determined with an *S. aureus* count of 2.6×10^5, 5.5×10^7, and 8.0×10^5 cfu/g, respectively. Likewise, Koluman et al. (19) reported that *S. aureus* was positive in 16 (10 white cheeses and 6 cheddar cheeses) of 75 cheese samples. They added that 1 of 25 (4%) Turkish white cheese samples were contaminated with staphylococcal enterotoxin B and 1 of 25 (4%) cheddar cheese samples was contaminated with staphylococcal enterotoxin A. Demirel and Karapınar (20) emphasized that *S. aureus* was found at a range of 1.0×10^1 to 3.0×10^5 cfu/g in 75 examined cheese samples. Staphylococcal enterotoxins were detected

Table 1. The number and percentage of positive microorganisms and toxins in analyzed cheese samples.

Microorganisms/ toxins	White cheese (n = 25)		Tulum cheese (n = 25)		Mihaliç cheese (n = 25)		Hellim cheese (n = 25)		Örgü cheese (n = 25)		Civil cheese (n = 25)		Total (n* = 150)	
	n^p	%	n^p	%	n^p	%	n^p	%	n^p	%	n^p	%	n^p	%
S. aureus	13	52	8	32	7	28	3	12	5	20	4	16	40	26.66
E. coli	14	56	12	48	9	36	2	8	11	44	7	28	55	36.66
***E. coli* O157**	2	8	1	4	-	-	-	-	-	-	-	-	3	2
***E. coli* O157:H7**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Enterotoxin	10	40	6	24	5	20	-	-	2	8	2	8	25	16.66
Verotoxin	2	8	1	4	-	-	-	-	-	-	-	-	3	2

n: number of analyzed samples, n^p: number of positive analyzed samples, n*: number of total analyzed samples.

Table 2. The results of microbiological analysis of cheese samples (n* = 150).

Varieties of cheese	n	*S. aureus* (cfu/g)			*E. coli* (cfu/g)		
		Min	Max	Average	Min	Max	Average
White pickled cheese	25	3.0×10^2	1.2×10^6	2.3×10^5	2.3×10^2	1.8×10^6	2.7×10^5
Tulum cheese	25	1.0×10^2	1.2×10^5	1.8×10^4	8.8×10^1	6.2×10^5	6.6×10^4
Mihaliç cheese	25	7.8×10^1	1.0×10^5	2.0×10^4	1.8×10^1	4.2×10^4	8.8×10^3
Hellim cheese	25	2.0×10^1	3.1×10^2	1.3×10^2	1.2×10^1	1.8×10^2	9.6×10^1
Örgü cheese	25	1.7×10^1	6.1×10^3	1.3×10^3	1.1×10^1	2.5×10^2	8.6×10^1
Civil cheese	25	1.8×10^1	3.2×10^3	7.5×10^2	1.0×10^1	2.1×10^4	4.8×10^3

n: number of analyzed samples, n*: number of total analyzed samples.

in 27 cheeses with a distribution of A in 19 samples (67.86%), B in 9 samples (32.14%), C in 2 samples (7.1%), D in 1 sample (3.6%), and E in 7 samples (25%).

Similarly, Ertas et al. (21) indicated that *S. aureus* was isolated from 60 (60%) sheep cheeses with an *S. aureus* distribution count of between 1×10^2 and 1×10^6 cfu/g. Additionally, they remarked that staphylococcal enterotoxins were determined in 7 (2.3%) out of 60 sheep cheese isolates using the ELISA technique, with a distribution count of 4 (1.3%) A, 2 (0.6%) B, and 1 (0.3%) D. Aside from these findings, staphylococcal enterotoxins were detected in 25 (16.66%) of the analyzed cheese samples in the present study. In 10 (40%) and in 6 (24%) of the staphylococcal enterotoxin-positive white pickled and tulum cheeses, enterotoxins A, B, C, and D were determined with a mean *S. aureus* count of 2.3×10^5 and 1.4×10^4 cfu/g, respectively; meanwhile, 5

Table 3. The number of microorganisms in analyzed cheese samples (n = 150).

Microorganisms	Range of microorganisms	White pickled cheese		Tulum cheese		Mihaliç cheese		Hellim cheese		Örgü cheese		Civil cheese	
		n^p	%	n^p	%	n^p	%	n^p	%	n^p	%	n^p	%
	<10	-	-	-	-	-	-	-	-	-	-	-	-
	10^1	-	-	-	-	1	14.28	2	66.67	2	40	2	50
	10^2	5	38.48	4	50	3	42.88	1	33.33	2	40	1	25
S. aureus	10^3	2	15.38	2	25	1	14.28	-	-	1	20	1	25
	10^4	2	15.38	1	12.5	1	14.28	-	-	-	-	-	-
	10^5	2	15.38	1	12.5	1	14.28	-	-	-	-	-	-
	10^6	2	15.38	-	-	-	-	-	-	-	-	-	-
	<10	-	-	-	-	-	-	-	-	-	-	-	-
	10^1	-	-	1	8.33	1	11.12	1	50	6	54.54	1	16.67
	10^2	4	28.58	4	33.33	3	33.33	1	50	5	45.46	1	16.67
E. coli	10^3	3	21.42	3	25	2	22.22	-	-	-	-	2	33.33
	10^4	3	21.42	2	16.67	3	33.33	-	-	-	-	2	33.33
	10^5	2	14.29	2	16.67	-	-	-	-	-	-	-	-
	10^6	2	14.29	-	-	-	-	-	-	-	-	-	-

n: number of total analyzed samples, n^p: number of positive analyzed samples.

Table 4. Presence of staphylococcal enterotoxins A, B, C, D, and E in analyzed cheese samples (n = 150).

Sample No.	White pickled cheese	Tulum cheese	Mihaliç cheese	Hellim cheese	Örgü cheese	Civil cheese
1	B	B, C	A	-	A	A
2	A	A	A, D	-	A	A
3	A, D	A	A	-	-	-
4	A, C	A, D	A	-	-	-
5	A	C, D	B	-	-	-
6	A	A	-	-	-	-
7	A, D	-	-	-	-	-
8	A	-	-	-	-	-
9	C, D	-	-	-	-	-
10	A	-	-	-	-	-

n: number of total analyzed samples.

(20%) of the mihaliç cheeses were positive for A, B, and D, with a mean count of 2×10^4 cfu/g. In spite of this, each of 2 (8%) örgü and civil cheeses were contaminated with only enterotoxin A and the mean *S. aureus* counts were 1.3×10^3 and 7.5×10^2 cfu/g, respectively.

Outside of Turkey, various results have been reported on the presence of *S. aureus* and its enterotoxins in cheeses (Table 5) (2,3,22).

In cheese, *E. coli* may indicate inadequate pasteurization, poor hygiene conditions during processing, or postprocessing contamination (13). Although most *E. coli* strains are harmless, EHEC strains are the most pathogenic strains among the VTEC (7) and are well recognized as the cause of severe disease in human beings.

In this study, *E. coli* was detected in 55 (36.66%) cheese samples, while no *E. coli* O157:H7 was found. However, *E. coli* O157 was identified in 3 of the examined samples (2 white pickled cheeses and 1 tulum cheese). Moreover, these 3 samples were positive for the presence of verotoxins. Tekinşen and Özdemir (17) stated that 50 Van otlu cheese samples had presumptive *E. coli* with a mean of 3.68 log cfu/g (62%), whereas none of the samples contained *E. coli* O157:H7. Similarly, *E. coli* O157:H7 was not isolated in 50 kaşar and 10 tulum cheeses by Aslantaş and Yıldız (23) or in 100 fresh cheeses by Gümüşsoy and Gönülalan (24), in agreement with our findings. However, the isolation of *E. coli* O157:H7 was reported in 6.6% (1/15) of tulum cheese (25), and in 2% (1/50) (26), 1% (1/100) (23), and 1% (1/100) (27) of white cheese samples. The level of *E. coli* was determined to range from <1 to 6.0×10^4 cfu/g in 50 white pickled cheeses by Öksüz et al. (28). Likewise, Gülmez and Güven (15) reported that *E. coli* was between <10 and 3.3×10^3 cfu/g (mean count: 2.8×10^2 cfu/g) in 30 civil cheeses. Vural et al. (29) emphasized that the contamination rates of *E. coli* and *E. coli* O157 were 65.71% (69/105) and 7.62% (8/105), respectively. Furthermore, Öksüz et al. (28) stated that *E. coli* O157 was determined in 4% (2/50) of white cheese samples. The above results show similarity with our findings, except that 16 cheese samples were higher than 10^4 cfu/g (Table 3). *E. coli* outbreaks implicating cheeses in other countries are summarized in Table 6 (2,22).

It was observed that all of the *E. coli* O157 positive samples in our study came from the same stall at the bazaar. The presence of various types of *E. coli* in the cheeses may indicate the inadequacy of hygienic practices undertaken throughout the milk and cheese production. On the other hand, the presence of verotoxins was rarely reported in cheese samples. De Reu et al. (7) indicated that only 1 of 71 cheeses was positive for verotoxin VT2, while Conedera et al. (8) determined that 1 raw cow's milk cheese out of 2948 dairy products possessed the "eae" gene and none harbored VT genes. Furthermore, Coia et al. (6)

Table 5. *S. aureus* incidence implicating various types of cheeses in other countries.*

Country	Year	Type of cheese	Mean *S. aureus* concentration (cfu/g)	Staphylococcal enterotoxin type	Prevalence (%)/ outbreak status
Canada	1980	Cheese curd	-	A, C	62
USA	1981	Pasteurized cheese	-	NS	16
England	1983	Pasteurized cheese	-	NS	2
England	1988	Stilton cheese	-	NS	155
Scotland	1984	Ewe cheese	-	A	27
France	1983	Farm ewe cheese	-	A, D	20
France	1981	Semihard cheese	3.0×10^7	A	-
France	1983	Semihard cheese	2.0×10^6	A, D	-
France	1985	Soft cheese	3.0×10^8	B	-
France	1986	Sheep's milk cheese	1.0×10^6	B	-
France	2000	Sheep's milk cheese	2.6×10^4	A	-
France	2001	Sliced soft cheese	$>1.5 \times 10^5$	A	-
France	2001	Semihard cheese	2.9×10^4	D	-
France	2002	Sheep's milk cheese	2.8×10^5	A	-
Brazil	1994	Unspecified cheese	-	H	7
Brazil	2001	Soft cheese	-	NS	20 ^{Pr}
Brazil	2002	Minas cheese	1.0×10^8	A, B, C	-
Germany	2002-2003	Goat cheese (soft)	6.3×10^3	C	8.0 ^{Pr}
Germany	2002-2003	Goat cheese (curd)	3.3×10^4	ND	8.0 ^{Pr}
Germany	2002-2003	Goat cheese (semihard)	2.9×10^5	A, C	5.4 ^{Pr}
Germany	2002-2003	Goat cheese (hard)	2.1×10^1	C	15.3 ^{Pr}
Italy	2000-2002	Unspecified cheese	-	A, C, D	61.5 ^{Pr}
Italy	2000-2002	Cheese curd	-	A, D	28.6 ^{Pr}
Italy	2000-2002	Ricotta cheese	-	A, D	83.3 ^{Pr}
Italy	1996	Soft cheese	-	NS	8.3 ^{Pr}
Italy	1996	Semisoft cheese	-	NS	18.9 ^{Pr}
Italy	1996	Mozzarella type cheese	-	NS	25.0 ^{Pr}
Belgium	2002	Raw milk cheese	$>10^4$	A, C	17 ^{Pr}
Egypt	2006	Stored soft cheese	-	NS	3.8 ^{Pr}

*Based on Kousta et al. (2), Kérouanton et al. (3), and De Buyser et al. (22).
ND: not detected, NS: not stated, ^{Pr}: prevalence.

and Erkan et al. (30) did not isolate verotoxin VT1 or VT2 in 739 raw-milk cheeses and 90 örgü cheeses, respectively.

It was concluded that even though the level of microorganisms in the cheese was not sufficient to cause disease, the presence of toxins could be considered a potential risk for public health. The presence of *S. aureus and E. coli* in the analyzed cheese samples seemed to be related to the use of raw milk and unhygienic production processes and storage conditions. Furthermore, it was reported by various researchers that cheeses had a low microbiological quality because of the poor hygienic conditions in small primitive production establishments, which are widespread in Turkey.

Table 6. *E. coli* incidence implicating various types of cheeses in other countries.*

Country	Year	Type of cheese	*E. coli*/*E. coli* serotype	Prevalence (%)/ outbreak status
USA	1983	Brie and Camembert cheeses made of pasteurized milk	*E. coli*	170
Netherlands and Sweden	1983	Brie from the same plant as for USA	*E. coli*	135
Scotland	1994	Farm cheese made of raw milk	*E. coli*	22
France	1992	Farm fromage frais made of raw milk	*E. coli*	4
France	1994	Farm fromage frais made of raw milk	*E. coli*	4
France	2004	Fresh unpasteurized goats' cheese	*E. coli* O157:H7	3
Canada	2002-2003	Unpasteurized Gouda cheese	*E. coli* O157:H7	13
UK	1997-1999	Raw milk cheese	*E. coli*	725 (98.6%) [Pr]
UK	1997-1999	Raw milk cheese	*E. coli* O157	1/71 [Pr]
UK	2004-2005	Raw or thermalized milk cheese	*E. coli*	37/1819
UK	2004-2005	Pasteurized milk cheese	*E. coli*	51/2618
Italy	2000-2001	Unpasteurized bovine cheese	*E. coli*	109/811 [Pr]
Belgium	2002	Raw milk cheese	*E. coli*	24/71 (33.8%) [Pr]
Belgium	2002	Raw milk cheese	*E. coli* O157	4/71 (5.6%) [Pr]

*Based on Kousta et al. (2) and De Buyser et al. (22).
[Pr]: Prevalence.

Since cheese is a ready-to-eat product, even a low incidence of contamination may pose a risk to consumer health, especially the presence of staphylococcal enterotoxins and verotoxins in dairy products, which can cause food poisoning outbreaks. Therefore, it is essential to ensure high safety standards such as raw milk quality, effective pasteurization processes, hygienic production and storage conditions, proper cleaning, and sanitation processes in production facilities.

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