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Incidence of *Escherichia coli* O157 in raw milk and white pickled cheese manufactured from raw milk in Turkey

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Abstract

In different countries numerous people have suffered from many infections caused by *Escherichia coli* O157:H7 within the last 20 years. This bacterium has been found in healthy bovine faeces. Therefore milk and milk products produced from such animals' milk may cause an infection risk if the milk has not been adequate pasteurised. In this research, 100 raw milk samples from different bovines and 50 white pickled cheeses manufactured from raw milk were examined for the presence of *E. coli* O157. Furthermore some physical and chemical properties were investigated. According to the analysis results, *E. coli* O157 was determined in 1% of the total raw milk samples and in 4% of the cheese samples. pH values were found to be higher than 4.50 in 80% of the total cheese samples. The main reason was that no lactic starters were used in this kind of cheese manufacturing process. Due to the low acidity of the cheese samples, *E. coli* O157 counts may increase and its survival time may be longer than in cheeses made using starter cultures. Thus, white pickled cheeses manufactured from unpasteurised milk have a potential infection risk as a result of *E. coli* O157 existence.

Keywords: *Escherichia coli* O157; Raw milk; White pickled cheese

1. Introduction

Escherichia coli, firstly identified by Dr. Theodor Escherich in 1885 (Levine, 1987), was accepted as a faecal contamination indicator because of its presence in the intestinal system of warm blooded organisms. In extensive research, many serotypes of it 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 in verocytoxin producing *Escherichia coli* (VTEC), was first identified as a pathogen due to bloody diarrhoea observed in 47 people who ate contaminated hamburgers (Su & Brandt, 1995). After its identification, it was isolated from cases of foodborne disease (Betts, 2000).

Although, it is reported that apple cider, lettuce, sprouted alfalfa and goats' milk have been identified as *E. coli* O157:H7-transmitting foods (Ackers et al., 1998), most of the foodborne outbreaks of *E. coli* O157:H7 have been associated with the consumption of foods originated from cattle, especially foods contaminated with cattle faeces. Because *E. coli* O157 has been found regularly in healthy cattle faeces (Mead & Griffin, 1998), this animal is known to be an asymptomatic transmitter (Heuvelink et al., 1998; Wang, Zhao, & Doyle, 1997; Zhao, Doyle, Shere, & Garber, 1995).

In the last 20 years, many people have been effected by this organism, which is why the interest in this bacteria has increased. Therefore many investigations about the different properties and the availability of this organism in different foods have been performed continuously. The infection risk is high because the infective dose of *E. coli* O157:H7 is low such as 10–20 cfu/g (Bolton, Crozier, & Willamson, 1996), 1–700 cfu/g (Tilden et al., 1996).

Since dairy cattle are asymptomatic carriers (Heuvelink et al., 1998; Wang et al., 1997; Zhao et al., 1995) of *E. coli* O157:H7, meat and milk products are thought to be risky foods from the point of view of this organism. Therefore insufficient heat-treatment of ground meat and raw milk forms a potential infection risk (Kuntz & Kuntz, 1999; Betts, 2000). The processing conditions for

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different milk products are very important from the standpoint of the organism's infection risk. Although it can be destroyed with pasteurisation, the survival rate in other processes is high, for example it can tolerate 8.5% salt concentration (Glass, Loeffelholz, Ford, & Doyle, 1992). Cheese made with unpasteurised milk is potential vehicle for transmission of *E. coli* O157 to the consumer. In Turkey, as in other countries some cheeses are manufactured from raw milk in modern plants, as well as in little family-owned plants where raw milk is commonly used. Additionally, milk producers sell their raw milk cheese unripened for consumption. The aim of this study was to determine *E. coli* O157 in raw milk and white pickled cheese manufactured from unpasteurised milk.

2. Materials and methods

2.1. Materials

A total of 150 samples consisting of raw milk (100 samples) and white pickled cheese (50 samples), manufactured from raw milk, were collected randomly from 10 villages of Tekirdag for examination.

2.2. Methods

pH was measured by a pH meter (Nel 890) equipped with a combined electrode. For the determination of the titratable acidities of the milk samples, 9 g of milk and 18 g of distilled water were weighed in a beaker and 0.5 ml of phenolphthalein was added into the beaker and titrated with 0.1 N NaOH to the first permanent colour change to pink. For cheese samples, 10 g of cheese was weighed in a beaker and mixed with 100 ml of distilled water using an electric mixer. Twenty-five gram of mixed sample was transferred into an Erlenmeyer flask and 5 ml of phenolphthalein was added into the Erlenmeyer and titrated with 0.1 N NaOH to the first permanent colour change to pink. All the titratable acidity values were expressed as percentage of lactic acid (Marshall, 1992). The moisture contents of the cheeses were determined using the oven drying method (at 100 ± 2 °C, 16 ± 0.5 h) (Marshall, 1992). Salt in the cheese samples was determined with Mohr titration method. According to this method, 10 g of cheese sample was mixed with 15 ml of warm water (50–55 °C) and stirred with magnetic stirrer. Twenty-five millilitre of distilled water was added and mixed until the sample is dispersed. Dispersed sample was transferred into a 100 ml volumetric flask and the volume was completed to 100 ml with distilled water. This was then filtered with a filter paper, the collected filtrate was approximately 50 ml. Twenty-five millilitre of filtrate was transferred into a clean flask and added 1 ml of potassium chromate indicator and then titrated with 0.1 N silver nitrate to the first visible pale red–brown colour lasting 30 s (Marshall, 1992).

The enumeration of different groups of bacteria was done by using modified violet red bile agar (mVRBA) for coliforms (at 32 °C, 48 h), standard method agar (SMA) for total mesophilic aerob bacteria (at 32 °C, 24 h), and lauryl tryptose MUG broth (LST/MUG) used in the most probable number technique (at 35 °C, 24 h) for *E. coli* (Marshall, 1992). For the detection of *E. coli* O157:H7 trypticase soy broth was supplemented with cefixime (0.05 mg/l), cefsulodin (10 mg/l) and vancomycin (8 mg/l) for pre-enrichment (37 °C). After the addition of the samples into the modified trypticase soy broth, shake-incubation was performed at 37 °C the enriched samples were plated onto sorbitol Mac-Conkey agar (SMCA) supplemented with 0.05 mg/l cefixime and potassium tellurite (2.50 mg/l) after 4 and 24 hours. Presumptive *E. coli* O157:H7 colonies (indole positive) were confirmed serologically using antibodies to the O157 antigen (*E. coli* O157:H7 latex test, Oxoid DR 260). According to these results, agglutination and indole positive colonies were recognised as *E. coli* O157 (AOAC, 1998).

3. Results and conclusion

Analysis results of the raw milk samples are given in Table 1. *Escherichia coli* O157 was determined only in one sample of 100 raw milk samples. According to this result, it can be thought that there is very low risk. But on the other hand, this bacterium can survive for more than 50 days in municipal reservoir and lake water (Wang & Doyle, 1998) and dairy cattle are asymptomatic carriers of this bacterium (Heuvelink et al., 1998; Wang et al., 1997; Zhao et al., 1995), thus increasing the risk for transmission through cattle to cattle, milk and milk products and to other foods. The titratable acidities of 5% of the milk samples were such that the samples could not resist pasteurisation while 1% were at coagulation point at room temperatures. In Turkey milk production is usually performed in crofts, where 10–20 kg milk is produced daily, thus hand milking is carried out. Both non-hygienic milking and inappropriate storage conditions can make the milk coagulate because of acid formation. Likewise another analysis results showed that raw milk samples for cheese making had high total aerobic bacterial counts of 2.4×10^7–1.6×10^8 cfu/ml (Öksüz, 1996), which has confirmed our results.

It is clearly seen in Table 2 that, 50 white pickled cheese samples made from raw milk contained total aerobic mesophilic bacteria at levels ranging from 10^3 to 10^8 cfu/g. The reason why 8% of the cheese samples had $\leqslant 10^4$ cfu/g was that starter culture was not used and small amount of milk was processed to cheese immedi-

Table 1
Some properties of raw milk samples

Analysis types	Values	%
pH value	6.70–6.79	13
	6.60–6.69	47
	6.50–6.59	7
	6.40–6.49	19
	6.30–6.39	12
	5.30	1
	5.10	1
Titratable acidity (% lactic acid)	0.14⩾	27
	0.15	27
	0.16	19
	0.17	8
	0.18	10
	0.19	4
	0.22	1
	0.25	1
	0.46	2
	0.54	1
E. coli O157	–	1

Table 2
Microbiological properties of white pickled cheese made from raw milk

Groups of bacteria	Cell count (colony/g)	No. of samples	%
Total aerobic mesophilic bacteria	3.0×10^3–5.0×10^3	2	4
	2.2×10^4–7.8×10^4	2	4
	1.0×10^5–9.0×10^5	11	22
	1.0×10^6–7.0×10^6	15	30
	1.0×10^7–9.0×10^7	7	14
	1.0×10^8–6.0×10^8	13	26
Coliform group bacteria	<1	3	6
	1.1×10^2–8.0×10^2	8	16
	1.1×10^3–6.0×10^3	8	16
	1.1×10^4–6.0×10^4	30	60
	10^5	1	2
E. coli	<1	20	40
	1.1×10^2–8.0×10^2	10	20
	1.1×10^3–6.0×10^3	11	22
	1.1×10^4–6.0×10^4	9	18
E. coli O157	+	2	4

ately after milking. Furthermore the hygienic conditions were appropriate since these samples showed a coliform group bacteria count of 10^2 cfu/g, which is considerably low. Additionally, coliform group bacteria were not detected in three samples containing total aerobic mesophilic bacteria ranging from 10^4 to 10^5 cfu/g.

According to the Turkish Regulations (Türk Standardı, 1995), coliform bacteria counts have to be maximum 100 cfu/g in white pickled cheese. Also Collins-Thompson et al. (1977) stated that coliform counts in cheese manufactured from raw milk should not exceed the limit of 10^3 cfu/g. Only 11 (22%) out of 50 samples were in accordance with the Turkish Regulations and the value stated by Collins-Thompson et al. (1977). *Escherichia coli* was detected in 60% of the cheese samples. In different studies about cheeses *E. coli* rates (not O157) were reported as follows: 58% in soft and semi-hard cheeses (Ansay & Kaspar, 1997), 32.8% in Damietta and 20.8% in Kareish cheese (Aman, Knappstein, & Hahn, 1998). The last two are made from raw milk and produced in Egypt. Turkish Regulations do not permit any *E. coli* presence in white pickled cheese.

Escherichia coli O157 was determined only in two cheese samples (4%). Inactivation of *E. coli* O157:H7 is increased with the increasing rate of salt between the pH values 4.1 and 4.7. It is stated that salt stimulates the inactivation (Guraya, Frank, & Hassan, 1998). Two cheese samples containing *E. coli* O157 had salt contents of 3.81%, 4.15% and pH values of 5.46, 5.0 respectively (Table 3). *Escherichia coli* O157:H7 survived for 30–40 days at pH values of 4.0–4.5 (McIngvale, Chen, McKillip, & Drake, 2000), 34–38 days at 5 °C at pH value of 4.4 and 158 days when it is added to Cheddar cheese milk at the rate of 10^3 cfu/ml (Reitsma & Henning, 1996). Considering these facts this bacterium can constitute a high risk in white pickled cheese. Regarding only salt, *E. coli* O157:H7 can tolerate NaCl concentrations as high as 8.5% (Glass et al., 1992). In other words, it is not possible to inactivate this bacterium only with increasing the sodium chloride amount in white pickled cheese. Besides, salt concentration is limited by standards. According to the Turkish standards for white pickled cheese, there should be maximum 4% salt in at

Table 3
Physical and chemical properties of white pickled cheese made from raw milk

Analysis types	Values	Sample no.	%
Moisture (%)	30–34	1	2
	35–39	1	2
	40–44	2	4
	45–49	6	12
	50–54	17	34
	55–60	19	38
	61⩽	4	8
Salt (%)	⩽1	5	10
	1.00–1.99	2	4
	2.00–2.99	12	24
	3.00–3.99	19	38
	4.00–4.99	7	14
	⩾5	5	10
pH	⩾5.51	5	10
	5.50–5.00	18	36
	4.99–4.50	17	34
	4.49⩾	10	20
Titratable acidity (%)	0.49⩾	13	26
	0.5–0.99	17	34
	1.00–1.50	15	30
	1.51⩽	5	10

least 40% dry matter base. Due to these statements both the pH value of the samples containing *E. coli* O157 and the low acidity value of 80% of the total samples prolong the inactivation period of *E. coli* O157 in white pickled cheese produced from raw milk. Furthermore, growth phase plays an important role in inactivation process. *Escherichia coli* O157:H7 being in late log phase (Benjamin & Datta, 1995) and stationary phase (Buchanan & Edelson, 1999) is most resistant to stress and lactic acid. Consequently, it can be said that inactivation of *E. coli* O157 in cheese with low acidity at the beginning of ripening process is faster when acid is formed after the early log phase. Conversely, if there is a contamination after acid formation (pH 4.4), the growth phase of the organism will determine the result. These data indicate that, for cheeses manufactured from raw milk, the milk production quantity and milking technique applied in Turkey, will result with the survival of *E. coli* O157. Therefore persons consuming such foods are at greater risk of *E. coli* O157 infection. For decreasing the risk, the process techniques should be changed and different starter cultures with more inhibitory effect on *E. coli* O157 should be investigated.

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