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THE PRESENCE OF VEROTOXINOGENIC *E. COLI* IN SOME FOODS*

PRISUSTVO VEROTOKSINOGENE E.COLI U NEKIM NAMIRNICAMA

K. Bostan, O. Cetin, O. Ergun**

In this study 30 samples each of ready-to-cook meatballs and white cheese as well as 96 samples of various ready-to-eat foods, obtained from different sales outlets in Istanbul, were analysed for the presence of verotoxins (consequently verotoxigenic E. coli) E. coli with the aid of the enzyme immunoassay technique. Additionally, total coliform and chromogenic E. coli count were determined by cultural methods for all food samples. E. coli growth was detected in all ready-to-cook meatballs (100%), in 27 of the white cheese samples (90%) and in 69 of the other ready-to-eat food samples (71.9%). Verotoxins, however, could not be detected in any of the samples examined with the aid of the ELISA technique. The findings of this study indicate a low microbiological quality of the analysed meatball, white cheese and ready-to-eat food samples; a considerable part of them did not conform to legal standards. However, within the sensitivity limits of the method applied no verotoxinogenic E. coli could be detected.

Key words: Verotoxin, Escherichia coli, meatball, cheese, ready-to-eat food

Introduction / *Uvod*

Escherichia coli (E. coli), a member of the *Enterobacteriaceae*, are motile, non-spore-forming bacilli that stain Gram-negative. The bacterium lives commensally in the intestines of humans and warm blooded animals. Thus, the presence of *E. coli* in foods usually indicates a lack of hygiene in handling and production operations, and the possible presence of enteric pathogens. Many strains of *E. coli* bacteria are harmless. However, some strains are pathogenic and may cause serious illness in people [22]. Enterohaemorhagic *E. coli* (EHEC), which was first recognized as a cause of illness in 1982, has been held responsi-

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** K. Bostan, O. Cetin, O. Ergun, Istanbul University, Veterinary Faculty, Department of Food Hygiene and Technology, 34320, Avcilar, Istanbul, Turkey

ble for some cases of very severe poisoning that ended in death [6, 23, 9, 20, 3]. EHEC includes *E. coli* strains (*E. coli* O157:H7) that produce verotoxins (VT1 and VT2). Verotoxins have a cytotoxic effect and also produced by some other *E. coli* serotypes [15]. Verotoxin-producing *E. coli* (VTEC) are considered a major cause of gastrointestinal disease in developed countries. The nature of illness can range from a mild form of diarrhea to more severe forms known as hemorrhagic colitis and hemolytic uremic syndrome [30, 28]. Although the number of organisms required to cause disease is not known, it is suspected to be very small.

The main sources of infection are meat and meat products from cattle, sheep and goats. Among other known sources of infection are consumption of sprouts, vegetable salad, unpasteurized milk and juice. Insufficient cooking or raw consumption of contaminated products increases the risk of poisoning [36, 28]. The presence of verotoxigenic *E. coli* in various foods has been investigated by several researchers. The general finding was a low rate of incidence in animal based food [5, 26, 17, 13, 87]. There are various methods for isolation of verotoxigenic *E. coli* from food and other material. However, these methods are rather onerous and time consuming. In contrast, detection of toxins (verotoxin) from an enrichment culture using immunoenzymatic assay as a way of identifying the VTEC in foods is more easy, fast and sensitive [32, 14, 27, 25]. This study has been realised with the aim of determining with the aid of the ELISA technique the presence of verotoxinogenic *E. coli* in ready-to-cook meatballs, cheese and ready-to-eat foods marketed in Istanbul.

Materials and methods / *Materijal i metode rada*

Materials / *Materijal*

In this study a total of 156 food samples, which are composed of 30 ready-to-cook meatball, 30 white pickled cheese and 96 ready-to-eat food (traditional Turkish cold dishes) and marketed without packaging, have been analysed. The samples were obtained from various markets and other sales outlets in Istanbul. The samples were placed in sterile plastic bags and were transported immediately to the laboratory under hygienic conditions.

Microbiological analyses / *Mikrobiološke analize*

A 10 g sample of each food sample was homogenised 1:10 (w/v) with sterile 0.1 % peptone water for 2 min in a Stomacher Lab-Blender (Seward). Serial 10-fold dilutions were prepared from the same solution and inoculated in appropriate growth media [12].

Total coliform and *E. coli* count were determined on Chromogenic *E. coli* / Coliform Medium Agar (Oxoid, CM 0956) by using pour plate technique. The plates were incubated for 24 hours at 37°C and evaluated. Red to pink colonies were considered total coliform and dark-blue to violet colonies presumptive *E. coli* [10, 19].

Detection of verotoxin / *Ustanovljavanje verotoksina*

The presence of verotoxins was determined by ELISA-based Ridascreen Verotoxin Test Kit (Biopharm, R5701). Toxin detection was carried out after cultural enrichment. For this purpose, food samples (25 g) were added into 225 ml modified Tryptic Soy broth (mTSB, Oxoid CM129) supplemented with 1.0 ml of a 0.45% novobiocin (Biochemika 71882) and incubated overnight at 37°C. This was folloÓed by short centrifugation and the supernatant was directly applied into the assay. The assay was performed following the manufacturer's instructions to detect VT1 and VT2. The readings were taken at 450 nm wavelength using an automated micro plate reader (ELx-800, Universal Microplate Reader, BIO-TEK).

Results and discussion / *Rezultati i diskusija*

In this study, coliform and *E. coli* growth were detected in all ready-to-cook meatball samples (Tables 1 and 3). The average coliform and *E. coli* counts were high and determined as $1.2x10^5$ cfu/g and $3.4x10^4$ cfu/g, respectively. The high contamination rate and average counts may be explained as a sign of the low hygienic quality of the meatballs. Another reason for the high count of microorganisms may be due to the fact that they were collected during a relatively hot season (from May to August) of the year. Chapman *et al.* [7] who investigated the presence of *E. coli* in meat and raw meat products found microorganism counts in lamb and beef collected during July and August exceeding 10^4 cfu/g. The Turkish Food Codex Directive on prepared meat mixtures [31] sets a limit for *E. coli* of $5.0x10^3$ cfu/g. In 24 of the samples (80%), the *E. coli* count was above the legal limit.

Table 1. *Coliform counts of the cheese and ready-to-cook meatball samples (cfu/g)*
Tabela 1. Koliformna merenja uzoraka sira i polupripremljenih ćufti (cfu/g)

Sample / *Uzorak*	n / *n*	The lowest / *Najniže*	The highest / *Najviše*	Average / *Prosečno*
Meatball / *Mesna ćufta*	30	6.8x102	3.9x105	1.2x105
White pickled cheese / *Beli začinjeni sir*	30	<10 (3)	5.1x105	7.9x104

Of the cheese samples, only 3 (10%) were negative for coliform and *E. coli* growth (Tables 1 and 3). In twenty five samples the coliform and *E. coli* count exceeded 100 cfu/g. According to the cheese standard issued by the Institute for Turkish Standards [34], the coliform count in 1g of white cheese must not exceed 100, while *E. coli* must not be present at all. This means that 80% of the examined white cheese samples did not conform to that standard with respect to their coliform count while 90% failed to meet the *E. coli* condition. The average coliform count was found to be $7.9x10^4$ cfu/g while the average *E. coli* count was

$5.3x10^4$ cfu/g. The ratio of *E. coli* among the total of coliform bacteria is rather high (67.1%). For meatballs and the ready-to-eat foods investigated, the ratios were considerably lower, 28.3% and 23.1% respectively. Another study carried out in Turkey reported high coliform and *E. coli* counts in white cheese [35], which failed to meet the *E. coli* condition. Tawfek *et al* [33] found coliform bacteria in all of the 100 Kariesh cheese samples with average coliform counts of $18.9x10^3$/g. Reibnitz *et al*. [29] found, in a study carried out in Brasil that 79% of the examined cheese samples did not conform to legal requirements while 20% exceeded the limits for *E. coli*. Aleksieva [2] reported that 3.1% of the analysed white pickled cheese samples contained between 10-100 cfu/g, a finding which is far below the results obtained in this study. The high contamination with coliform and *E. coli* of the examined cheese samples may be an indicator that most products have been produced from raw milk and had not matured sufficiently.

In the 96 samples comprising thirteen different food varieties of ready-to-eat products, the average coliform count was $2.2x10^4$ cfu/g and the average *E. coli* count $5.1x10^3$ cfu/g (Tables 2 and 4). Coliform and *E. coli* growth were not determined at a countable level in 22.9% and 28.1% of the samples, respectively. The *E. coli* count in 12 samples (12.5%) was in the range of 10-100 cfu/g, in 41 of the samples (42.7%) between 100 and 10.000 cfu/g, and in 16 samples (16.7%) it exceeded 10.000 cfu/g. The highest average coliform count, $8.8x10^4$ cfu/g, was detected in kýsýr samples - a cold dish made with cracked wheat, salad vegetables, and olive oil – while the lowest count, $3.5x10^2$ cfu/g, was found in tarator samples – an appetizer made with yoghurt, garlic, mashed corn, chopped carrot, sesame oil, mashed chickpea and mashed eggplant. With respect to *E. coli*, the highest count with $1.4x10^4$ cfu/g was detected in paste with hot pepper samples - and the lowest count with $2.9x10^2$ cfu/g in tarator samples.

Phls [24] classified ready-to-eat foods with respect to their *E. coli* count in four categories: <10 cfu/g – suitable; <100 cfu/g – acceptable; 100-10 000 cfu/g – insufficient; (>10 000 cfu/g) – not acceptable. On the basis of that classification 28.1% of the ready-to-eat foods investigated in this study conform to the quality of „suitable", 12.5% are „acceptable", 42.7% „insufficient" and 16.7% „not acceptable". The values and ratios reported in other studies on ready-to-eat foods are lower than in this work. Gillespie *et al* [11] examined the microbiological quality of 2354 samples of ready-to-eat foods. They reported that 157 samples (6%) were of insufficient quality and only 2 samples (<1%) with *E. coli* counts exceeding 10^4 cfu/g were not acceptable. Mosupye and Holy [21], who analysed 51 ready-to-eat food samples in South Africa, reported an average *Enterobacteriaceae* count of 2.0 log cfu/g. Only in 2% of the samples inspected could *E. coli* be isolated. Kaneko *et al.* [16] found in their study of ready-to-eat foods in Tokyo a coliform count range of 0.1-2.3 log cfu/g.

Table 2. *Coliform counts of the ready-to-eat food samples (cfu/g)*
Tabela 2. Koliformna merenja uzoraka gotove hrane (cfu/g)

Sample* / *Uzorak*	n / *n*	The lowest / *Najniže*	The highest / *Najviše*	Average / *Prosečno*
Paste with hot pepper *(acýlý ezme)* / *Namaz sa feferonama (acili ezme)*	11	<10 (2)	$2.1x10^5$	$3.9x10^4$
American salad / *Američka salata*	10	<10 (3)	$2.3x10^5$	$4.6x10^4$
Cold white beans vinaigrette *(barbunya pilaki)* / *Hladna salata od belog pasulja (barbunya pilaki)*	8	<10 (2)	$1.2x10^5$	$4.1x10^4$
Kýsýr / *Kisir*	10	$1.2x10^3$	$2.1x10^5$	$8.8x10^4$
Fried eggplant / *Prženi plavi patlidžan*	7	<10 (3)	$3.0x10^4$	$4.3x10^3$
Fava / *Fava*	6	<10 (1)	$4.6x10^4$	$1.9x10^4$
Haydari / *Haydari*	8	<10 (2)	$6.0x10^3$	$1.2x10^3$
Humus / *Humus*	6	<10 (1)	$3.5x10^4$	$1.4x10^4$
Sarma / *Sarma*	8	<10 (3)	$2.5x10^4$	$5.0x10^3$
Mushroom salad / *Salata od pečurki*	6	<10 (1)	$2.5x10^4$	$1.1x10^4$
Pasa mezesi / *Pasa mezesi*	5	<10 (1)	$3.6x10^4$	$1.2x10^4$
Vegetable salad with yogurt / *Salata od povrća sa jogurtom*	5	<10 (1)	$1.5x10^4$	$5.9x10^3$
Tarator / *Tarator*	6	<10 (2)	$1.3x10^3$	$3.5x10^2$
Total / *Ukupno*	96	<10 (22)	$2.3x10^5$	$2.2x10^4$

* Turkish names of traditional cold dishes were written as italic.
* *Turski nazivi tradicionalnih hladnih jela ispisani su kurzivom*

Table 3. *E. coli* counts of the cheese and ready-to-cook meatball samples (cfu/g) /
Tabela 3. Merenja E. coli u uzorcima sira i polupripremljenih mesnih ćufti (cfu/g)

Sample / *Uzorak*	n / *n*	The lowest / *Najniže*	The highest / *Najviše*	Average / *Prosečno*
Meatball / *Mesna ćufta*	30	$1.0x10^1$	$1.2x10^5$	$3.4x10^4$
White pickled cheese / *Beli začinjeni sir*	30	<10 (3)	$3.0x10^5$	$5.3x10^4$

Despite the fact that high counts of coliform and *E. coli* have been detected in the meatball, white cheese and ready-to-eat food samples investigated in this study, no verotoxins and consequently no verotoxinogenic *E. coli* could be found with the *ELISA* technique. Meat and meat products, which may be easily contaminated with verotoxin producing *E. coli* strains during processing, are ideal medium supporting the bacterial growth. Abdul-Raouf *et al* [1] investigated the presence of verotoxin 1 produced by *E. coli* O157:H7 in roasted beef. They incu-

bated beef (pH 5.9) at different temperatures and found that samples incubated at 37°C had a 15-24% higher verotoxin content than samples incubated at 21°C. A decrease of the pH to 5.4 (with the addition of acetic acid and citric acid) reduced the amount of toxins considerably. Weeratna and Doyle [37] reported the highest concentrations of verotoxin 1 in milk and ground meat samples incubated at 37°C for 48 hrs. Samples incubated at 25°C and 30°C were found to produce much less toxin. In this study no toxin was detected despite the fact that all samples were incubated in an enriched medium at 37°C. It can, therefore, be concluded that non of the examined samples contained verotoxinogenic *E. coli*. All studies on the incidence rate of *E. coli* O157:H7 in risk foods reported only very few cases. Coia *et al*. [8] who analysed 1190 raw meat products in Scotland, found VTEC O157 in only 2 beef burgers. Chapman *et al.* [7], found *E. coli* O157:H7 in 21 out of 1500 cattle carcasses (1.4%), in 10 out of 1500 lamb carcasses (0.7%) an in 22 out of 4983 raw meat samples (0.44%). Heuveling *et al*. [13] tested, in 1996 and 1997 in The Netherlands, 2,941 meat products for the presence of verotoxin producing *E. coli*. They isolated O157 VTEC in 6 out of 571 samples of ground beef (1.3%), in 2 out of 402 samples of raw beef and ground pork (0.5%), in 1 out of 76 samples of raw ground pork (1.3%) and in 1 out of 393 other raw pork products (0.3%). The results for all other raw beef products as well as all poultry, lamb and game products were negative. Little and Louvois (1998) who analysed 2330 samples of raw meat in Great Britain reported O157 VTEC in 3 samples while in 2192 cooked samples no traces of the organism could be detected. However low the incidence rate of verotoxinogenic *E. coli* in meat products may be, the agent survives in meat and meat products for extended periods of time. Ansay *et al.* [5] reported that *E. coli* O157:H7 remained active for a long time in contaminated ground beef kept at different storage temperatures.

Due to their low pH, cheeses are not an ideal medium for the development and support of *E. coli*. However, *E. coli* remains present in cheese produced from contaminated raw milk which is not sufficiently matured. Maher *et al*. [18] investigated the behaviour of *E. coli* O157:H7 in cheese prepared from contaminated raw milk. They found that a count of 1.52 log cfu/g in milk increased in one day old cheese to 3.4 log cfu/g while rapidly decreasing during maturing; after 21 days the count fell below 10 cfu/g. However, even on the 90th day of maturing the bacteria could still be detected in an enriched medium. Quinto and Capeda [26] investigated the presence of *E. coli* in 221 samples of soft cheese made from raw milk and in 75 samples of soft cheese made from pasteurised milk. They reported that 3 samples of the raw milk cheese (1.4%) were contaminated with toxigenic *E. coli,* one of which contained verotoxin. Coia *et al*. [8] examined 500 samples of raw milk and 739 samples of cheese made from raw milk. They reported no presence of the bacteria in any of the samples.

In the present study *E. coli* O157:H7 was not directly determined in the foods with high *E. coli* count. And even after enrichment according to procedures no verotoxin could be detected. This means that the samples did not contain vero-

toxinogenic *E. coli*. Ansay and Kaspar [5a] also found contamination with *E. coli* in 58% of the cheese samples tested, however, they did not find *E. coli* O157:H7 in any of the samples.

Table 4. *E. coli counts of the ready-to-eat food samples (cfu/g)*
Tabela 4. Merenja E. Coli u uzorcima gotove hrane (cfu/g)

Sample * / *Uzorak*	n / *n*	The lowest / *Najniže*	The highest / *Najviše*	Average / *Prosečno*
Paste with hot pepper *(acýlý ezme)* / *Namaz sa feferonama (acili ezme)*	11	<10 (3)	$6.1x10^4$	$1.4x10^4$
American salad / *Američka salata*	10	<10 (3)	$6.0x10^3$	$1.5x10^3$
Cold white beans vinaigrette *(barbunya pilaki)* / *Hladna salata od belog pasulja (barbunya pilaki)*	8	<10 (2)	$5.0x10^4$	$8.6x10^3$
Kýsýr / *Kisir*	10	$5.6x10^2$	$1.8x10^4$	$3.4x10^3$
Fried eggplant / *Prženi plavi patlidžan*	7	<10 (5)	$2.2x10^4$	$3.1x10^3$
Fava / *Fava*	6	<10 (1)	$1.6x10^4$	$8.1x10^3$
Haydari / *Haydari*	8	<10 (3)	$5.0x10^3$	$1.0x10^3$
Humus / *Humus*	6	<10 (1)	$2.0x10^4$	$6.6x10^3$
Sarma / *Sarma*	8	<10 (4)	$4.0x10^3$	$6.8x10^2$
Mushroom salad / *Salata od pečurki*	6	<10 (1)	$1.3x10^4$	$7.2x10^3$
Pasa mezesi / *Pasa mezesi*	5	<10 (1)	$2.4x10^4$	$9.8x10^3$
Vegetable salad with yogurt / *Salata od povrća sa jogurtom*	5	<10 (1)	$4.0x10^3$	$1.6x10^3$
Tarator / *Tarator*	6	<10 (2)	$3.5x10^2$	$2.9x10^2$
Total / *Ukupno*	96	<10 (27)	$6.1x10^4$	$5.1x10^3$

* Turkish names of traditional cold dishes were written as italic /
* *Turski nazivi tradicionalnih hladnih jela ispisani su kurzivom*

Conclusion / *Zaključak*

The findings of this study indicate that the analysed meatball, cheese and ready-to-eat samples have a low microbiological quality, but that samples, however, were reliable from the point of view of verotoxinogenic *E. coli*.

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PRISUSTVO VEROTOKSINOGENE *E.COLI* U NEKIM NAMIRNICAMA

K. Bostan, O. Cetin, O. Ergun

U ovom radu, 30 uzoraka polupripremljenih mesnih ćufti i belog sira, kao i 96 uzoraka raznih gotovih namirnica, uzetih sa različitih prodajnih objekata u Istanbulu, analizirano je na prisustvo verotoksina (stoga, verotoksigene *E.coli) E. coli* koristeći tehniku enzim imunoeseja. Osim toga, ustanovljen je ukupan broj koliformnih i hromogenih *E. coli* koristeći metode kulture kod svih uzoraka namirnica. *E. coli* je ustanovljena kod svih polupripremljenih ćufti (100%), kod 27 uzoraka belog sira (90%) i kod 69 od ostalih uzoraka gotovih namirnica (71,9%). Međutim, ELIZA tehnika nije dala rezultate za verotoksine ni u jednom od ispitivanih uzoraka. Nalazi ovih istraživanja pokazuju nizak mikrobiološki kvalitet analiziranih uzoraka mesnih ćufti, belog sira i gotove hrane; priličan deo njih nije odgovarao zakonskim standardima. Međutim, u okviru granica osetljivosti primenjene metode, nije bilo moguće da se ustanovi verotoksinogena *E. coli*.

Ključne reči: Verotoksin, *Escherichia coli*, mesna ćufta, sir, gotova hrana

РУССКИЙ

ПРИСУТСТВИЕ ВЕРОТОКСИНОГЕННОЙ *E. COLI* В НЕКОТОРЫХ ПИЩЕВЫХ ПРОДУКТАХ

K. Bostan, O. Cetin, O. Ergun

В этой работе, 30 образчиков полуподготовленных мясных тефтелей и белого сыра, словно и 96 образчиков раных готовых пищевых продуктов, взятых с различных продажных объектов в Стамбуле, анализировано на присутствие веротоксина (оттого, веротоксигенные *E. coli*) *E. coli*, пользуя технику энзим иммуноэсе. Кроме того, установлено совокупное число колиформных и хромогенных *E. coli*, пользуя методы культуры у всех образчиков пищевых продуктов. *E. coli* установлена у всех полуподготовленных тефтелей (100%), у 27 образчиков белого сыра (90%), и у 69 из остальных образчиков готовых пищевых продуктов (71,9%). Между тем, *ELIZA* техника не дала результаты для веротоксинов ни в одном из испытыванных образчиков. Результаты этих исследований показывают низкое микробиологическое качество анализированных образчиков мясных тефтеле, белого сыра и готовой пищи; изрядная часть их не отвечала законным стандартам. Между тем, в рамках границ чувствительности применённого метода,не было возможно установить веротоксигенную *E. coli*.

Ключевые слова: Веротоксин, *Escherichia coli*, мясная тефтеля, сыр, готовая пища