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Prevalence of *Escherichia coli* O157 in red meat and meat products determined by VIDAS ECPT and LightCycler PCR

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Abstract: This study aimed to determine the prevalence of *Escherichia coli* O157 in retail red meat and meat products with the Vitek Immunodiagnostic Assay System, including H7 *Escherichia coli* phage technology (VIDAS ECPT), and a real-time polymerase chain reaction system (LightCycler PCR; LCPCR). A total of 106 red meat and meat product samples were analyzed with VIDAS ECPT and LCPCR. VIDAS ECPT presumptive positive samples were subjected to VIDAS Immuno Concentration *E. coli* O157, followed by culture and serology. Among the 27 out of 72 (37.50%) red meat samples and 3 out of 34 (8.82%) red meat products that tested positive by VIDAS ECPT, 5.55% and 0.00% were confirmed positive for *E. coli* O157 (but not H7), respectively. Red meat and red meat product samples were 73.61% and 20.58% positive by LCPCR. The 5.55% prevalence of *E. coli* O157 in red meats poses a significant risk for consumers and indicates insufficient hygiene management both at the farm and during the slaughtering and meat handling processes in Turkey. This is the first report on the detection of *E. coli* O157 by VIDAS ECPT and LCPCR in naturally contaminated red meat and meat products.

Key words: *E. coli* O157, VIDAS, PCR, red meat, red meat product

Escherichia coli O157'nin kırmızı etlerde ve et ürünlerinde VIDAS ECPT ve LightCycler PCR ile prevalansının belirlenmesi

Özet: Bu çalışmanın amacı, *Escherichia coli* O157 (*E. coli* O157)'yi de içeren *Escherichia coli* Vitek Ultra Performans Immunodiagnostik Assay Sistemi Faj Teknolojisi (VIDAS ECPT) ve bir gerçek zamanlı Polimeraz Zincir Reaksiyonu sistemi (LightCycler PCR- LCPCR) ile kırmızı etlerde ve et ürünlerinde *E. coli* O157'nin prevalansının belirlenmesidir. Çalışmada, 106 kırmızı et ve et ürünü örneği VIDAS ECPT ve LCPCR ile analiz edildi. VIDAS ECPT şüpheli pozitif örnekler, VIDAS Immuno-konsantrasyon *E. coli* O157 (ICE) testine tabi tutuldu, ardından kültür ve seroloji uygulandı. VIDAS ECPT ile 72 kırmızı et örneğinin 27'si (% 37,50) ve 34 et ürünü örneğinin 3'ü (% 8,82) pozitif iken, bu örneklerin sırasıyla % 5,55 ve % 0,00 oranında *E. coli* O157 (H7 hariç) pozitif olduğu doğrulandı. Kırmızı et örneklerinin % 73,61'inin, kırmızı et ürünü örneklerinin ise % 20,58'inin LCPCR ile *E. coli* O157 pozitif olduğu belirlendi. Sonuç olarak, kırmızı etlerde % 5,55 oranında *E. coli* O157 bulunması tüketici yönünden risk oluşturmaktadır. Bu durum ülkemizde çiftlik, kesimhane ve et işleme yerlerinde hijyen koşullarının yetersizliğinin bir göstergesidir. Bu çalışma, doğal kontamine kırmızı et ve et ürünlerinde *E. coli* O157'nin varlığının VIDAS ECPT ve LCPCR ile belirlendiği rapor edilen ilk çalışmadır.

Anahtar sözcükler: *E. coli* O157, VIDAS, PCR, kırmızı et, kırmızı et ürünü

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Introduction

Among all enterohemorrhagic *Escherichia coli* (EHEC), *E. coli* O157:H7 and *E. coli* O157:H– are recognized as major pathogens and a cause of foodborne disease in humans worldwide. These bacteria are mostly transmitted through undercooked minced beef and meat products, which have a relatively short shelf life; therefore, rapid detection in these particular foods is required (1-3). Statutory authorities in Turkey require testing for *E. coli* O157:H7 in red meat parts, ground meats, and heat-treated meat products by internationally recognized methods (4).

Previous studies from different countries indicated the prevalence of EHEC in meat products as follows: 0%-14% in ground meats (5-12), 0%-8% in fresh meats (12,13), and 5%-7% in red meat products (5). There are also studies from Turkey that report the prevalence of EHEC as 0.4% (14) in ground beef and 0.3%-5% in red meat products (15-17). It is impractical to compare these rates, due to the heterogeneity of the methods used in these studies. Still, the results show the persistence of the pathogen in these types of samples, and various culture-, ELISA-, and polymerase chain reaction (PCR)-based detection methods or their combinations have been used over the years for detection.

There are several validated methods incorporating culture, immunological, and molecular techniques, mainly for the detection of *E. coli* O157:H7. However, the culture in these methods is time consuming (up to 3-5 days) and not suitable for routine screening of large samples (18). The Vitek Immunodiagnostic Assay System (VIDAS; Biomerieux, Marcy L'Etoile, France), an automated enzyme-linked fluorescent assay-based system, has been used as an alternative method for the rapid detection of *E. coli* O157:H7 in food samples. VIDAS was validated by the Association Française de Normalisation and the Association of Official Analytical Chemists (AOAC). Several studies in the literature report the use of VIDAS *E. coli* O157 (VIDAS ECO) and VIDAS Immuno Concentration *E. coli* O157 (VIDAS ICE) for detecting *E. coli* O157:H7 in retail red meats (7,13) and red meat products (19). The system was recently modified to VIDAS UP *E. coli* O157 including H7 (VIDAS ECPT), which uses a novel recombinant phage ligand to increase target specificity. This novel assay was evaluated by Rozand and Feng (20) for a panel of *E. coli* O157:H7 strains, atypical variants, and other enteric bacteria and was found highly specific for this pathogen. Another study by Savoye et al. (18) noted the successful detection of *E. coli* O157:H7 with high sensitivity compared to real-time PCR and culture in artificially contaminated ground beef. Real-time PCR has been used for the rapid and reliable detection of *E. coli* O157 in retail red meats (12), as well. Perelle et al. (10) performed Shiga toxin-producing *E. coli* (STEC) serogroup specific-multiplex PCR to determine prevalence in retail minced meat samples with LightCycler (Roche Applied Science, Indianapolis, IN, USA). Today, detection of *E. coli* O157 (including H7) from foods is possible by the use of an AOAC performance-tested and NordVal-validated commercially available real-time PCR kit, which can be used by LightCycler; this is known as the LCPCR system.

To date there has been no study in the literature using the above indicated rapid detection systems, alone or in combination, for assessing the rate of *E. coli* O157 in retail red meat and meat products. In this study, we aimed to determine the prevalence of this pathogen in red meat and meat products collected randomly from local food stores by VIDAS ECPT and LCPCR.

Materials and methods

Escherichia coli O157 strains

The *E. coli* O157:H7 EDL-931 strain, obtained from the Refik Saydam National Public Health Agency, Ankara, Turkey, was used as a positive control in VIDAS ECPT, VIDAS ICE, culture, serology, biochemical, and LCPCR tests.

Samples

A total of 72 red meat (52 ground meat and 20 chopped meat samples, all veal) and 34 red meat product (20 meatball, 5 salami, and 9 sausage samples [all beef + mutton]) retail samples were purchased from local food stores in 2010 and 2011; samples were analyzed immediately after their transfer to the laboratory on ice.

Analysis of samples by VIDAS

For the VIDAS test, 25 g of the meat/product sample was aseptically placed into a sterile Stomacher bag with a filter that contained 225 mL of buffered peptone water (BPW; Biomerieux 42043). The sample was incubated at 41.5 °C for 24 h for enrichment after homogenization by hand massage from the outer surface of the bag for 2 min. Following incubation, the BPW culture was stored at 4 °C until confirmation after taking a 1-mL aliquot for LCPCR template preparation and 1.5 mL for further heat treatment at 100 °C for 5 min in a block heater (FBD02DD, Techne Corporation, Minneapolis, MN, USA). After cooling to room temperature, 500 µL of heat-treated BPW broth culture was placed into a VIDAS ECPT strip (Biomerieux 30122) and then into a miniVIDAS instrument (Biomerieux) for 50 min with a VIDAS ECPT solid phase receptacle (SPR); results were analyzed according to the manufacturer's instructions. A relative fluorescence value of ≥0.04 was considered a presumptive positive result. Each presumptive positive sample was subjected to confirmation by placing 500 µL of BPW broth culture stored at 4 °C into a VIDAS ICE strip (Biomerieux 30526), and this was placed into a miniVIDAS instrument for 40 min with the VIDAS ICE SPR. Thereafter, 10 µL from the VIDAS ICE strip was streaked onto the selective agar plate ChromID O157:H7 medium (Biomerieux 42630) and incubated at 37 °C for 24 h. Typical colonies were subjected to serological identification by anti-O157 and anti-H7 sera using the rapid latex agglutination test (Wellcolex *E. coli* O157:H7, R30959601, Remel Products, Lenexa, KS, USA). Biochemical identification was performed by the API 20E (Biomerieux 20100).

DNA isolation and LCPCR

DNA from each sample was isolated from a 1 mL-aliquot of BPW broth with the foodproof Sample Preparation Kit I (BIOTECON, Potsdam, Germany). Isolated DNA was used as a template in LCPCR, which was performed with the foodproof *E. coli* O157 Detection Kit (BIOTECON) after concentration and purity determination by a NanoDrop spectrophotometer (ND1000, Thermo Scientific, Wilmington, DE, USA). The total PCR reaction volume was 20 µL. It comprised 5 µL of template DNA added to 15 µL of PCR mix (13 µL of foodproof *E. coli* O157 Master Mix [ready-to-use primer and hybridization probe mix]), 1 µL of foodproof *E. coli* O157 Enzyme Solution (FastStart Taq DNA polymerase and uracil-DNA glycosylase, heat-labile, for prevention of carry-over contamination), and 1 µL of foodproof *E. coli* O157 Internal Control. The foodproof *E. coli* O157 Control Template DNA and DNA from *E. coli* O157:H7 EDL-931 were used as positive controls; PCR-grade water was used as a negative control in PCR. The amplification protocol included an initial denaturation step at 95 °C for 10 min, 55 cycles of denaturation at 95 °C for 0 s, annealing at 59 °C for 30 s, and 5 s of primer extension at 72 °C. Fluorescence values of the internal control and each sample were automatically measured at 705/back 530 nm (channel F3/back-F1) and at 640/back 530 (channel F2/back-F1) at the end of each annealing step. Data analysis was automatically performed by LightCycler software version 4.1.

Results

VIDAS ECPT analysis revealed that 27 out of 72 (37.50%) red meat samples (22 of 52 ground and 5 of 20 chopped meats) and 3 out of 34 (8.82%) meat products (3 of 20 meatballs) were positive for *E. coli* O157. All presumptive positive samples were tested for further confirmation by VIDAS ICE and plated onto selective ChromID O157:H7 medium. After biochemical identification by API 20E was performed on typical colonies from the selective plate, 18.05% (8 ground and 5 chopped meat; total: 13/72) and 0.00% (0/34) of red meat and meat products were found positive for *E. coli* O157, respectively. After serological identification by the rapid latex agglutination test for both O157 and H7, 4 out of 72 (5.55%) red meat samples (2 ground and 2 chopped meats) were confirmed for *E. coli* O157, whereas none of these samples were positive for H7 (Table).

In LCPCR analysis, 53 (73.61%) of the red meat (36 ground and 17 chopped meats) samples and 7 (20.58%) meatballs were determined positive for *E. coli* O157. There was no detection in the salami and sausage samples, which were also negative by VIDAS. The overall *E. coli* O157 presumptive detection rate of 28.30% (30/106) from red meat samples fell to 3.77% (4/106) after VIDAS ICE, serological, and biochemical confirmations. The *E. coli* O157 detection rate for all of the samples in LCPCR was 56.60% (60/106) (Table).

Table. Red meat and meat product VIDAS ECPT and LCPCR results.

Sample type (n)	No. positive (%)			
	VIDAS			PCR
	Presumptive	Confirmed		
		ICE + culture	Serology + biochemical identification	
Red meat				
Ground meat (52)	22 (42.30)	8 (15.38)	2 (3.85)	36 (69.23)
Chopped meat (20)	5 (25.00)	5 (25.00)	2 (10.00)	17 (85.00)
Subtotal (72)	27 (37.50)	13 (18.05)	4 (5.55)	53 (73.61)
Meat product				
Meatball (20)	3 (15.00)	0 (0.00)	-	7 (35.00)
Salami (5)	0 (0.00)	0 (0.00)	-	0 (0.00)
Sausage (9)	0 (0.00)	0 (0.00)	-	0 (0.00)
Subtotal (34)	3 (8.82)	0 (0.00)	-	7 (20.58)
Total (106)	30 (28.30)	13 (12.26)	4 (3.77)	60 (56.60)

-: not applicable

Discussion

Previous studies reported the prevalence of *E. coli* O157 in retail red meats determined by VIDAS *E. coli* O157 (VIDAS ECO) (6-8), conventional PCR (5), and simplex/multiplex real time PCR (9-12). Our study is the first to report the prevalence of *E. coli* O157 by using 2 different systems for the detection of this pathogen in retail red meat and meat products: VIDAS ECPT technology and LCPCR, a real-time PCR by a commercially available *E. coli* O157 detection kit. Although there is no one-to-one correspondence with previous prevalence studies in terms of methodologies, we compared our results to those from studies using methods similar to ours in which naturally contaminated red meat or meat product samples were tested.

In their study of VIDAS for the detection of *E. coli* O157 in ground meats, Fantelli and Stephan (6) detected 2.3% *E. coli* O157 by VIDAS ECO in minced meat in Switzerland. Another study by Tutenel et al. (8) indicated that Belgian ground beef harbored 0.18% *E. coli* O157 according to VIDAS ECO followed by immunomagnetic separation (IMS). Similarly, Vernozy-Rozand et al. (7) reported a 0.12% prevalence of *E. coli* O157:H7 in industrial French minced beef with the VIDAS ECO and VIDAS ICE systems. These prevalence rates are lower than the 3.85% prevalence rate we obtained from ground meats with VIDAS ECPT. The prevalence of *E. coli* O157 was investigated and reported as 0% by Pontello et al. (13) in various foods, including fresh meats, by a commercial EHEC screening test and VIDAS ECO. In contrast to this finding, the VIDAS ECPT detection rate for *E. coli* O157 in our meat samples was 10.00%, which is relatively high. We have not come across any previous study that used VIDAS to investigate *E. coli* O157 in naturally contaminated red meat products. In our study, we did not detect any meat products as positive for *E. coli* O157 by VIDAS ECPT, a test which was used here for the first time for this aim.

There may be many factors affecting the differences in prevalence rates among studies; these differences are mainly related to samples and sampling (type, source/location, initial bacterial load), environmental and seasonal factors, and the detection methodology used. Apart from sample-related issues, a factor that may have affected the prevalence rate by VIDAS in the current study was our decision to use the VIDAS UP *E. coli* O157 including H7 (ECPT) test for our samples. The manufacturer of this novel system indicates that it has increased target specificity because it is based on specific phage capture technology. Still, one cannot rule out the effect of the factors indicated above, which could have contributed to our slightly higher prevalence rate.

Among studies using PCR for *E. coli* O157 detection in ground meats, Alexandre et al. (5) reported a rate of 26.1% EHEC by conventional PCR, where O157 was indicated as the second most prevalent serogroup. Perelle et al. (10) determined the presence of main pathogenic *E. coli* O serogroups at 2.6% (1% O157) in

minced meat samples, first by PCR-ELISA for STEC screening and then by serogroup-specific multiplex and simplex real-time PCR. Similarly, Auvray et al. (9) reported detection of 1 of the 5 main *E. coli* O serogroups (O26, O103, O111, O145, and O157) by real-time PCR in 5.5% of retail minced beef samples. However, Stefan et al. (11) stated that neither O157 nor the other main STEC O-serogroups that represent a major public health concern, including O26, O91, O111, and O145, was detected by real-time PCR in any of the 106 mince meat samples collected in Italy. In contrast to the relatively low and zero prevalence rates indicated above by Auvray et al. (9), Perelle et al. (10), and Stefan et al. (11), Suo et al. (12) reported 14% of ground meat samples as positive for the *E. coli* O157 serotype, both by multiplex PCR and the United States Department of Agriculture/Food Safety and Inspection Service Microbiology Laboratory Guidebook modified culture method. In the same study, none of the chopped beef samples were found positive for *E. coli* O157. In Turkey, Sarimehmetoglu et al. (14) reported *E. coli* O157 prevalence in fresh ground beef at 7.6% by culture; the *E. coli* O157:H7 detection rate was 2.4% by IMS and 0.79% by multiplex PCR. Our detection rates for *E. coli* O157 from ground and chopped meat by LCPCR are higher than previously reported rates.

The presence of EHEC was investigated by Alexandre et al. (5) by conventional PCR; it was detected in 21.73% and 38.88% of sausage and hamburgers, respectively, and *E. coli* O157 was the second most prevalent serogroup in these samples. Apart from this PCR study, no other study included naturally contaminated red meat products for the detection of *E. coli* O157 by PCR. However, there are several studies that used different culture methods and determined the prevalence of this pathogen (*E. coli* O157, if not otherwise indicated) in meat products from Turkey: 2% in hamburgers and 5% in İnegöl meatballs by Sarımehmetoğlu et al. (16), 1% in sucuk and 2% in uncooked hamburgers by Noveir et al. (15), and 11.25% O157:H7 in cooked döner samples by Ulukanlı et al. (17). Our detection rate for *E. coli* O157 in red meat products by LCPCR (20.58%) was much higher than previously reported rates.

Our *E. coli* O157 detection rate in red meat and meat product samples by LCPCR, using a commercial kit specifically designed for the detection of *E. coli* O157, was very high compared to previous reports. This relatively high rate with LCPCR may be explained by the presence of *E. coli* O157 strains that show atypical biochemical profiles and therefore were not detected in that specific culture (21), or that exhibit weak agglutinations to O157 and H7 antibodies, which is likely due to insufficient expression of antigens under assay conditions (22). It may also be due to the presence of *E. coli* O157:H7 strains that are rough and do not express the O157 antigen (23), and to cells present in a viable but not culturable status and injured or dead cells, which can lead to false positives in PCR (24). In seeking other possible reasons for these results, we studied the available product information. The commercial kit was designed to amplify a highly conserved sequence specific for *E. coli* O157 antigen, and performance tests were applied including specificity (inclusivity, exclusivity) and sensitivity assays (kit insert information). The inclusion of information on sequence specificity in PCR and the possibility of encountering cross-reactivity with other *E. coli* O serogroups in the kit insert would maximize the reliability of the method. The reasons behind the high rate obtained by LCPCR in this study require further investigation; none of the above factors could have led to the unusually high detection rate obtained from these samples.

In this study, we did not detect an *E. coli* O157:H7 isolate within the O157 isolates. This may be due to the actual absence of this serotype within the isolates tested or to possible weak agglutinations to H antibodies, as indicated above. Regardless, the presence of *E. coli* O157:H− isolates in red meat samples poses a significant risk for consumers.

In conclusion, this is the first report to date to determine the *E. coli* O157 rate in naturally contaminated red meat and meat products with VIDAS ECPT and LCPCR. The presence of this pathogen in red meats indicates improper or insufficient hygiene management both at the farm and during the slaughtering and meat handling process in Turkey.

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