

A Seven Month Survey for the Detection of *E. coli* O157:H7 from Ground Beef Samples in the Markets of Turkey

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Abstract: A seven month period from the beginning October to the end of April 2004, a total of 126 ground beef samples were analyzed to determine the incidence of *Escherichia coli* (*E. coli*) O157:H7. Among the sampling months, the incidence of EHEC serotypes were only observed in April. Of the 126 ground beef samples, only one ground beef sample was positive for *E. coli* O157:H7, having a prevalence of 0.79 %. Five samples were found positive for *E. coli* O157 serotype, having a prevalence of 3.96 %. The results of this study reveal that the occurrence of EHEC serotypes in ground beef in the Kars (Eastern Turkey) seem to be seasonal and the level of *E. coli* O157 was significantly higher as compared to other studies in Turkey, but it is similar to studies performed in many countries around the world. Of the tested eighteen antibiotics, resistance towards three or more antibiotics were observed among the all isolates.

Key words: *E. coli* O157:H7, ground beef, antibiotic resistance

Introduction

The need for hygienic meat production has gained importance due to awareness among consumers about health risks associated with contaminated meat (Yashoda *et al.*, 2000). The microbiological quality of either spoilage or food poisoning microorganisms depends on the meat used for mincing, sanitary conditions, practices in preparation time and temperature of storage (Duitschaever *et al.*, 1973; Khalafalla *et al.*, 1993). These factors may cause a major risk for subsequent foodborne infection in human (Huffman, 2002; Reid *et al.*, 2002). The threat posed by EHEC diseases spread via contaminated and improperly cooked meat has been well recognized and the epidemiological impact of such diseases is considerable (Huffman, 2002; Reid *et al.*, 2002). Shiga toxin-producing *Escherichia coli* (*E. coli*) (STEC) produce cytotoxins identical at the genetic and protein level to the shiga toxins produced by *Shigella dysenteriae* I. Enterohemorrhagic *E. coli* (EHEC) are a subset of STEC. *E. coli* O157:H7 can be considered as a significant prototype of the EHEC (Tutenel *et al.*, 2002). *E. coli* O157:H7 has been well recognized as cause of human diseases including diarrhea, haemorrhagic colitis (HC) and the haemolytic uremic syndrome (HUS), or thrombotic thrombocytopenic purpura (TTP) (Nataro and Kaper, 1998). Healthy cattle have been regarded as a natural reservoir of VTEC organisms for infections (Hancock *et al.*, 1994; Armstrong *et al.*, 1996). The natural reservoirs of this pathogen are also many kinds of animals especially sheep, goats, wild animals. Consumption of contaminated and improperly cooked

ground beef is often implicated in foodborne outbreaks of *E. coli* O157:H7, though a variety of foods including unpasteurized milk, apple cider, fermented sausage, mayonnaise, and water, have also been linked as routes of transmission for human illness. The contamination of this potentially pathogenic strain may be secondarily transmitted from infected animals or person to person (Mead and Griffin, 1998).

With the exception of studies that describe the presence of EHEC in raw meat and raw meat based products Turkey, there is no information available in the literatures about the true incidence of this bacterium in ground beef. The objectives of this study were (I) to determine the incidence of this bacterium in ground beef through seven months and (II) to determine the resistance towards the antibiotics used in veterinary and human therapy (III) to compare the results with other studies performed in many countries in the world.

Materials and Methods

Samples: A total of 126 ground beef samples were collected eighteen times per month from the beginning October to the end of April, 2004. For each sampling, 100 g was collected from the markets and butchers, placed in a sterile bag, and stored in a cool box for transportation to the laboratory and analyzed within 1 h.

Analyses: To detect the presence of *E. coli* O157:H7 in ground beef samples, a 25 g of sample was pre-enriched with modified novobiocin EC broth (mEC+n, Merck 14582, Berlin, Germany) at 37°C for 24 h. A swap of the enrichment broth was then spread onto selective

CT-SMAC (Cefixime-Tellurite Supplement and Sorbitol MacConkey Agar, Oxoid CM 813 and SR 172 E, Basingstoke, UK) and incubated at 42°C for 24-48 h. End of the incubation, colourless, sorbitol negative (-), suspected colonies were streaked onto Fluorocult Violet Red Bile (VRB) (Merck 1.04030, GERMANY) and these plates were incubated at 42°C for 24-48 h. aerobically. Colonies grown on VRB were checked under UV light. The suspected colonies were Gram stained and IMVIC tests were performed. The colonies were then subjected to the agglutination test to determine the serotype of the bacteria using specific antisera to *E. coli* O157 (Oxoid, 200075, UK) and Dryspot *E. coli* O157 latex agglutination test (Oxoid, UK) for *E. coli* O157 carried out in parallel. Cultures identified as *E. coli* O157 were tested with antisera H7 (Oxoid, 211057, UK) as described by the manufacturer.

Antimicrobial testing: Antibiotic resistance patterns of *E. coli* O157 and *E. coli* H7:O157 were determined by the disk diffusion method using Mueller Hinton Agar (Bauer *et al.*, 1966). Zone interpretations were based on the recommendations of the National Committee on Clinical Laboratory Standards (NCCL). The antibiotic discs used were: ampicillin (10 µg), cefoperazone (30 µg), ceftriaxone (30 µg), cephazolin (30 µg), ceftazidime (30 µg), chloramphenicol (30 µg), ciprofloxacin (10 µg), gentamicin (10 µg), kanamycin (30 µg) nalidixic acid (30 µg), norfloxacin (10 µg), oxytetracycline (30 µg), ofloxacin (5 µg), enrofloxacin (5 µg), streptomycin (10 µg), tetracycline (30 µg), trimethoprim (5 µg), trimethoprim-sulfamethoxazole (25 µg). *E. coli* ATCC 25922 was used as control organism.

Results and Discussion

In the present investigation, we used mEC+n broth and CT-SMAC method. Of the 126 ground beef samples, only 1 (0.79%) was contaminated with *E. coli* O157:H7. Five (3.96%) of the 126 ground beef samples were positive for *E. coli* O157. A seven months of ground beef survey revealed that EHEC serotypes were not detected between October and March. It has been previously noted that the occurrence of *E. coli* O157:H7 in cattle feces is often seasonal, with the warmer and increased moist conditions of summer season contributing the highest incidence (Kudva *et al.*, 1997; Johnsen *et al.*, 2001). Fecal samples harboring highest incidence of the EHEC in the warmer season may directly contaminate the ground beef samples and concordantly increased the level of detection of the pathogens in the samples analyzed in this work. No recovery of the EHEC serotypes in cooler seasons in this study may be as consequences of low infectious dose or non homogenous distribution of the organism within sample or may be the background microflora in ground beef predominate the growth of *E. coli* O157:H7. In a study of

Heuvelink *et al.* (1997), they evaluated the efficacy of selective enrichment and plating media for the isolation of O157 serogroup from minced meat and concluded that mEC+n and CT-SMAC were the most efficient media for selective enrichment and isolation, respectively. It appears from the present data, detecting level of bacteria in ground beef samples collected in the period of April seems to be augmented in warmer season rather than cooler season, using the novobiocin (mEC+n) and CT-SMAC. Hence, it can be said that more sensitive techniques such as immunomagnetic separation or else are required with the pre-enrichment procedure in cooler season.

Previously existing studies regarding the prevalence of *E. coli* O157:H7 in beef and ground beef samples were noted as a 3.7% of the 164 beef, 29.4% of the 17 beef samples in USA (Pai *et al.*, 1984; Doyle and Schoeni, 1987) and 6 % of the 50 ground beef samples in Egypt (Abdul-Raouf *et al.*, 1996). Although high prevalence was reported in these earlier studies, no recovery or false-positive *E. coli* O157:H7 serotypes resulted from the two recent studies of Silveira *et al.* (1999) and Uhtil *et al.* (2001). Silveira *et al.* (1999) investigated 886 hamburger samples from the southeast of Brazil. Uhtil *et al.* (2001) analyzed 114 beef and baby beef samples from 30 different grocery stores for the presence of *E. coli* O157:H7 in Croatia. The number of the samples examined in those studies was not low for detecting a microorganism but it is presumably due the differences and difficulties arising from the methodology.

The prevalence found in this investigation shows similarity to those reported by Vernozy-Rozand *et al.* (2002), who found the prevalence of *E. coli* O157:H7 in minced beef was 0.12% (four of 3450), but dissimilar to the more recent study of Chinen *et al.* (2001), who detected *E. coli* O157:H7 from the 6 (3.75%) of 160 ground beef samples between February and May in Argentina. In contrast, the prevalence of O157 was approximately equal to the number of the *E. coli* O157:H7 serotypes found in Chinen *et al.* (2001). Our result showed a higher prevalence when compared to the study of Heuvelink *et al.*, 1997, who detected positive *E. coli* O157 from the 1 % of the 571 minced beef in Netherlands.

Reports conducted in Turkey related to the incidence of *E. coli* O157 in raw meat and raw meat based products was found at 2% and 2.58% in hamburgers (Sarimehmetoglu *et al.*, 1998; Cebiroglu and Nazli, 1999; Noveir *et al.*, 2000), 5% in meatballs (Sarimehmetoglu *et al.*, 1998), 0.4% in minced meat, 0.99% in sodjouk (Noveir *et al.*, 2000). In a study of Gun *et al.* (2003), the presence of *E. coli* O157:H7 was investigated in the bovine carcasses and abattoir environment in Istanbul between January 2000 and April 2001. Eight (2.4%) of the 330 cattle carcasses and six positive isolates from the environments in abattoir were

shown to be positive for this serotype. Nonetheless, the incidence of this pathogen in the sampling months is not clearly mentioned. Therefore, results of the present study may be more representative of ground meat in the Kars, Eastern part of the Turkey than previous studies conducted in other parts of the country. If so, risk exposure to EHEC O157 from ground beef is higher than previous reports by Noveir *et al.* (2000). Unfortunately, the infection cases of *E. coli* O157:H7 have not been officially known in Turkey. Therefore further work is essentially needed to determine the genes encoding the virulence factors to prevent risk exposures to livings.

Food consumption is an important pathway for bacteria to enter humans, the presence of antimicrobial-resistant bacteria in foods warrants particular attention (Schroeder *et al.*, 2004). Many human *E. coli* O157:H7 infections are acquired from eating undercooked contaminated beef. Therefore, it is crucial to determine if resistant *E. coli* O157:H7 is a possible reservoir for spread of resistance factors to other microorganisms (Galland *et al.*, 2001). In the present work, both *E. coli* O157 and *E. coli* O157:H7 serotypes were resistant to three or more antibiotics. Chloramphenicol, streptomycin and trimethoprim type resistance was common among all isolates as shown in Table 1.

Table 1: Antibiotic resistance of the EHEC isolates

Isolates	Resistance pattern*
O157	C, S, W
O157	C, S, TE, W
O157	C, S, W
O157	C, S, OT, TE,W
O157	C, S, W
O157:H7	C, S, OT, TE,W

*Abbreviated symbols in the Table were C (Chloramphenicol, 30µg); S (Streptomycin, 10µg), OT (Oxytetracycline, 30µg); TE (Tetracycline, 30µg); W (Trimethoprim, 5µg).

Resistance towards at least one or more antibiotics such as ampicillin, chloramphenicol, and tetracycline has been reported for O157 and H7 serotypes by previous workers from several materials (Meng *et al.*, 1998; Radu *et al.*, 2001). In the present investigation, data concluded from antibiotic resistance pattern is in agreement as compared to the previous studies. This is possibly as a consequence of extensive usage of these antibiotics in the treatments of the cattle or in other sources e.g. a contact with feces of the animals, feeding, water sources or agriculture, which may be as a cause of the transmission of resistant genes from various vectors to food production animal. General conclusion can not be derived due to the low number of microorganism used in this preliminary test. However, three to five antibiotic resistance among the isolates reveal may be an alert for the consumption of improperly cooked meat. Molecular studies are needed to

determine the genes encoding the resistance.

Transmission vehicles are not examined in this preliminary work, it is rather to determine the true incidence of this pathogen in ground beef during the three seasons (fall, winter and spring). This harmony of seasonal rates of *E. coli* O157:H7 in cattle, retail meats and human disease is evidence not only of causation but also of the potential for farm level interventions to be transmitted to the consumer in the form of lower risk (Hancock *et al.*, 2001). Epidemiological and comprehensive studies are required to find out the incidence of this pathogen throughout the all year and major possible contamination sources including abattoir conditions, transportation of carcass, butcher's hygiene, utensils and tools used in butcher's shop and storage of ground beef should be checked routinely.

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