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Determination of *Escherichia coli* O157:H7 in Chicken Meats Sold in Sanliurfa Region

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Abstract: *Escherichia coli O157:H7 has been an important problem of public health in most countries of the world since 1982. This study was therefore aimed to investigate the presence of E. coli O157:H7 in chicken meat samples collected from various markets in Sanliurfa region which was located in Southern Turkey. For this purpose, 155 chicken meat samples were analyzed between September 2005 and February 2006. The samples were plated onto Cefixime Tellurite Supplement and Sorbitol Mac Conkey Agar after enrichment process. Suspected colonies were then analyzed for identification of E. coli O157:H7 as given in materials and methods section. E. coli O157 and E. coli O157:H7 were found in 9 (5.81%) and 3 (1.94%) of the total of 155 samples, respectively. The results showed that control measures should be developed to prevent contamination with this pathogen in chicken meats in this region. To our knowledge, this is the first report of isolation of E. coli O157:H7 from chicken meat samples in Southern Turkey.*

Keywords: *Chicken meat, cultural method, E. coli O157:H7, prevalence, public health*

Şanlıurfa Bölgesinde Satılan Tavuk Etlerinde *Escherichia coli* O157:H7'nin Tespiti

Öz: *Escherichia coli O157:H71982'den beri dünyanın birçok ülkesinde önemli bir halk sağlığı problemi olmuştur. Bu nedenle, bu çalışma Türkiye'nin güneyinde yer alan Şanlıurfa bölgesindeki çeşitli marketlerden toplanan tavuk eti örneklerinde E. coli O157:H7'nin varlığını araştırmak amacıyla yapıldı. Bu amaçla, 2005 Eylül ile 2006 Şubat döneminde toplanan 155 adet tavuk eti örneği analiz edildi. Zenginleştirme işleminden sonra örnekler Cefixime Tellurite Supplement içeren Sorbitol Mac Conkey Agara aktarıldı. Daha sonra şüpheli koloniler E. coli O157:H7'nin identifikasyonu için materyal ve metot kısmında belirtildiği gibi analiz edildi. Toplam 155 örneğin 9'unda (%5.81) E. coli O157 ve 3'ünde (%1.94) E. coli O157:H7 tespit edildi. Bu sonuçlar, bu bölgedeki tavuk etlerinin bu patojenle kontaminasyonunu önlemek için kontrol önlemlerinin artırılması gerektiğini gösterdi. Bildiğimiz kadarıyla, elde ettiğimiz sonuçlar Türkiye'nin güney bölgesindeki tavuk eti örneklerinden E. coli O157:H7'nin izole edildiği ilk rapordur.*

Anahtar Kelimeler: *Tavuk eti, kültürel metot, E. coli O157:H7, prevalans, halk sağlığı*

INTRODUCTION

E. coli O157:H7 is one of the well-known and the most serious bacterial agent among the food borne pathogens [1]. This was first recognized as a cause of illness in 1982 when it caused two major outbreaks of hemorrhagic colitis traced to the consumption of hamburgers in the USA [2]. Undercooked hamburgers from the same fast food restaurant chain were identified as the vehicle, and *E. coli* O157:H7 was isolated from patients and a frozen ground beef patty [3]. Such outbreaks increased dramatically and became widespread in the following years and this bacteria has become one of the most important foodborne pathogens. *E. coli* O157:H7 infections have continued to occur in large outbreaks and sporadic cases, although outbreaks were decreased after 1999 [4]. It has been estimated that *E. coli* O157:H7 causes 73,000 illnesses and 250 deaths annually in the United States [5].

This pathogenic microorganism causes hemolytic uremic syndrome (HUS), hemorrhagic colitis (HC) and trombotic trombositopenic purpura (TTC). The pathogen is likely to be responsible for 85-95 % of hemolytic uremic syndrome cases [6, 7]. The natural reservoirs of pathogen are many kind of animals especially cattle, sheep, goats, and wild animals. Consumption of undercooked or contaminated foods of animal origin is often implicated in foodborne outbreaks of *E. coli* O157:H7. However, fecal contaminations of other food products or direct contact with infected animals have also been linked as routes of transmission for human illness [5, 8].

Ground beef is the main source of these infections. Microbial contamination of raw and ready-to-eat (RTE) meat products with human pathogens is a consequence of a wide array of pre-harvest, harvest, and postharvest processes. *E. coli* O157:H7 can colonize the intestinal tract of cattle and other animals [9]. This microorganism was also detected in chicken caeca and feces and it has also been noted that chicken meat is a vehicle for transmission of *E. coli* O157:H7 to humans [10].

It is highly probable that foods produced, stored and/or marketed under unhygienic conditions be contaminated with *E. coli* O157:H7. Since the foods sold in the market in the Southeastern Anatolia region are often produced by ignoring the basic hygienic rules, these foods are likely available for *E. coli* O157:H7 contamination. This eventually threats the health of the people living in this region.

This study was carried out to determine the *E. coli* O157:H7 contamination level of chicken meats sold in the local butchers and the supermarkets, if any.

MATERIALS AND METHODS

Collection of Samples

During the six month period, 155 chicken meat samples were collected from the markets in Sanliurfa region located in Southern part of Turkey. The samples were taken between September 2005 and February 2006. After purchase, the samples in their original packaging were placed directly into cool boxes and transported to the laboratory within 2 h on the day of collection. Samples were stored at 4 °C in the laboratory prior to processing on the day following receipt. The samples were analyzed to determine if any *E. coli* O157:H7 serotype exist, according to the procedure proposed by Food and Drug Administration [11].

Enrichment and Isolation

Modified novobiocine EC Broth (mEC+n, Merck 14582, Berlin, GERMANY) was used as enrichment medium. Enrichment cultures for each sample were carried out by combining 25 g of each sample with 225 ml of EC Broth supplemented with 20 mg/l novobiocin (Novobiocin/ N1628, Sigma, GERMANY)

sterile bag, homogenized for at least 2 min into a stomacher and incubated at 37 °C for 24 h. CT-SMAC (Cefixime-Tellurite Supplement and Sorbitol MacConkey Agar, Oxoid CM 813 and SR172 E, Basingstoke, UK) were used for as selective solid medium. A swap of the enrichment broth was then spread onto selective CT-SMAC and incubated at 42 °C for 24-48 h. At the end of the incubation, colorless, sorbitol negative (-), suspected colonies were streaked onto Fluorocult Violet Red Bile (VRB) Agar (Merck 1.04030, GERMANY) and these plates were incubated at 42 °C for 24-48 h aerobically.

Identification and Serological Confirmation

Colonies grown on VRB were checked under UV light. Gram stain and IMVIC tests were performed on suspected colonies. 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.

RESULTS

The results showed that 9 (5.81%) of 155 chicken meat samples were contaminated with *E. coli* O157 serotype. *E. coli* O157:H7 was detected in 3 (1.94%) of total samples. Distribution of those serotypes detected in chicken meats is shown in Table 1. A six months of chicken meat survey showed that *E.coli* O157:H7 serotype was not detected between November and February. Between the same months, *E. coli* O157 was detected only in December. The highest prevalence of *E. coli* O157 and *E. coli* O157:H7 were detected in September and October. The climate is warm from June to November in Sanliurfa region. Pathogen incidence is the highest level, contributing high warm weather at this season.

Table 1. Distribution of *E. coli* O157:H7 and *E. coli* O157 serotypes isolated in chicken meats according to months.

Sampled Months	Sample Numbers	Positive Sample Numbers for *E. coli* O157:H7	Positive Sample Numbers for *E. coli* O157
September	29	2 (6.90%)	5 (17.24%)
October	27	1 (3.70%)	3 (11.11%)
November	28	-	-
December	24	-	1 (4.17%)
January	25	-	-
February	22	-	-
TOTAL	155	3 (1.93%)	9 (5.81%)

DISCUSSION

Infection with *E. coli* O157:H7 has become emerging foodborne disease in developed countries. *E. coli* O157:H7 has been isolated from dairy cattle, calves, chickens, swine and even sheep and from their meat. However, its incidence and prevalence shows intensely instability because of different reasons [12, 13]. In these, chicken has been considered as vehicles of transmission of *E. coli* O157:H7, since chicks can be colonized by small populations of this pathogen and continue to be long-term shedders [14].

There are a lot of studies to show importance of *E. coli* O157:H7 for public health from different countries concerning the incidence of *E. coli* O157:H7 on a variety of foods. Some of them show similarity to the prevalence found in our investigation while some researchers reported dissimilar results.

One of these was carried out by Samedpour and Liston [15], 4 (12%) of the 33 chicken samples obtained from local grocery stores in the Seattle area were positive for *E. coli* O157:H7. In the other study [16] this pathogen was isolated from 3 (2%) of the 150 chicken giblets purchased from markets in Costa Rica. Researchers in this article pointed out cross contamination possibility.

Contrasting Reuben et al. [16], Miri [17] reported *E. coli* O157:H7 wasn't isolated using microbiological culture and PCR from the 70 chicken nugget samples collected in Isfahan, Iran. Similarly, this bacteria wasn't isolated in 80 chicken samples examined by Soriano et *al.* [18]. Griffin and Tauxe [19] did not isolate this bacterium from raw chicken. Jo et al. [20] detected no *E.coli* O157 in 2843 different meat samples including 52 chickens by cultural method. Tutenel et al. [21] reported same prevalence for *E.coli* O157 in 241 chicken samples.

El-Safey [22] detected no *E. coli* O157:H7 in 100 Austrian food samples, including 20 chicken by cultural and immunomagnetic separation (IMS) methods. In a study [23] on some Egyptian foods, 5 (21.7%) of 23 chicken samples were positive for *E.coli* O157:H7. These results are meaningful to show in countries having different hygienic conditions for this pathogen.

Diseases occurring with *E. coli* O157:H7 aren't problem only in underdeveloped countries. This pathogen was detected from various meats and meat products in different countries both developed and underdeveloped [24, 25, 26, 27, 28, 29, 30].

In our country, some other studies also reported the presence of this pathogen on different meat and meat products. Aksu et al. [31] examined 500 food samples for this bacteria in a study carried out to investigate the presence of *E. coli* O157:H7 in various foods of animal origin. Among the group of meat products, they determined *E. coli* O157:H7 in 3 (6%) out of 50 ground beef samples, 1 out of 25 ground lamb samples and 1 (2%) out of 50 meatball samples. Abay et al. [32] reported none of 400 chicken meat samples collected from various markets in Kayseri between March 2010 and February 2011 was positive for *E. coli* O157 via cultural method and PCR. *E. coli* O157:H7 and *E. coli* O157 were investigated from 330 meat samples (120 beef, 105 chicken's meat and 105 turkey's meats) sold in butchers and markets using cultural methods by Unsal [33] who found in 1 (0.9%) and 3 (2.7%) of 105 chicken samples respectively. Baran and Gulmez [34] examined 100 samples of animal origin (50 ground beefs and 50 chicken hams) for *E. coli* O157: H7 in a study carried out in Kars Province. Consequently, they isolated bacteria from 3 ground beef samples (6%), but they didn't isolate any bacteria in chicken samples.

Akkaya et al. [35] determined that 2 (1.05%) out of 190 chicken meat samples were contaminated with *E. coli* O157:H7. In Afyonkarahisar, Akkaya et al. [36] took samples using swaps from four different points of 250 beef carcasses each. Prevalence of *E. coli* O157 and *E. coli* O157:H7 in the taken samples was found at 3.2% and 0.8%, respectively.

Using the cultural method, Mercanoglu and Aytac [37] detected *E. coli* O157 in 1 (1.8) of 57 chicken samples collected in Ankara during 8 month period. In their study, Alisarli and Akman [38] investigated 300 ground meat samples (150 ground beef and 150 ground lamb) sold in butcher shops and markets in Van. They determined *E. coli* O157 serotype in 7 ground beef samples (4.66%) and 3 ground lamb samples (2%), and concluded that ground meat had high risks of this bacteria.

Cebiroglu and Nazli [39] examined 115 hamburger and meatball samples sold in Istanbul for *E. coli* O157:H7 and isolated bacteria from 4 samples (3.47%). In the conclusion of the study, they determined that these foods had high risks for public health, and hygiene rules should be followed in every stage of production and consumption.

Obtained data indicated that the chicken meats marketed under uncontrolled conditions have threatening effects on public health with regard to *E. coli* O157:H7 serotype. One should bear in mind that meat is always considered intrinsically unsafe regarding *E. coli* O157:H7 contamination. Therefore, adequate standards of hygiene must be observed and controlled to avoid the presence of this pathogen in meat. To our knowledge, this is the first report of isolation of these bacteria from chicken meat samples in Southern part of Turkey.

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