# Verotoxin-Producing *Escherichia coli* in Faecal Specimens of Patients with Diarrhea in Malatya-Turkey: Scientific Letter

## Malatya Türkiye'de İshalli Hastaların Dışkı Örneklerinde Verotoksin Üreten *Escherichia coli*

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**ABSTRACT** Verotoxin producing *Escherichia coli* (VTEC) a major cause of food-born diarrhea outbreaks. *E.coli* O157H7 is the most common VTEC serotype. Its prevalence is not well known in Turkey. To date, VTEC has not been isolated from stool samples of Turkish patients with diarrhea living in Turkey; only *E.coli* O157H7 was determined in a few animal and environmental samples from our country. In consequence of few studies from Turkey have been evaluated, about this pathogen. We investigated VTEC in 940 samples and *E. coli* O157:H7 in 104 samples from diarrheic patients. *E. coli* were negative in all samples. To date, VTEC has not been isolated from stool samples of Turkish patients with diarrhea living in Turkey. This may be due to dietary habits of Turkish people or to the limited number of studies on this subject in Turkey.

**Key Words:** Verotoxin producing *Escherichia coli,* infectious diarrheal disease

**ÖZET** Verotoksin üreten *E. coli* (VTEC) besinlerle bulaşa bağlı ishal salgınlarının önemli bir nedenidir. *E. coli* O157:H7 en yaygın VTEC serotipidir. VTEC prevalansı ülkemizde iyi bilinmemektedir. Bugüne kadar Türkiye'de yaşayan ishalli Türk hastalarının dışkı örneklerinden bu bakteri izole edilememiştir. *E. coli* O157:H7, ülkemizde sadece bazı hayvanlarda ve çevresel örneklerde saptanmıştır. Türkiye'den bu patojeni değerlendiren yalnızca birkaç çalışma vardır. Biz, ishalli hastalardan 104 örnekte verotoksin üreten *E.coli* ve 940 örnekte *E. coli* O157:H7 araştırdık. Tüm örneklerde. *E. coli* O157:H7 ve verotoksin üreten *E.coli* negatif bulundu. Günümüze dek, Türkiye'de insan dışkı örneklerinden VTEC izole edilememiştir. Bu, Türk halkının beslenme alışkanlıklarına veya Türkiye'de bu konuda yapılan çalışma sayısının yetersizliğine bağlı olabilir.

**Anahtar Kelimeler:** Verotoksin üreten *Escherichia coli,* bulaşıcı ishal hastalığı

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Verotoxin producing *Escherichia coli* (VTEC) is a major cause of food-born diarrhea outbreaks and sporadic infections due to factors such as consumption of raw milk, uncooked meat, and contact with animal feces and ruminants.[1,2] VTEC infections are associated with watery or bloody diarrhea, hemolytic uremic syndrome, and hemorrhagic colitis and may be fatal, especially in the elderly.[3,4] The most common VTEC serotype is *E. coli* O157:H7. There are two major types of Verotoxin (VT1 and VT2). Current methods for the detection of VTEC require testing for VTs and strain isolation.[5,6] The prevalence of VTEC is not well-known in our

country. No VTEC has been isolated from stool samples of patients with diarrhea in Turkey,[7,8] while only one *E.coli* O157:H7 strain was isolated from food samples has been samples.[7-9] There are few studies from Turkey investigating this pathogen.

We investigated the prevalence of E. coli O157:H7 in 940 samples and VTEC in 104 samples from the stools of diarrheal patients between 2000 and 2003 in Malatya province and southeast Anatolia. In the southeastern Anatolia region of Turkey, where the study was conducted, a traditional dish known as "Çig Köfte", namely uncooked meatballs, is commonly consumed, whic makes the isolation of *E. coli* O157:H7 or VTEC likely.

All samples included the diarrheic stools. One hundred and four samples that contained at least one of the following were tested for verotoxin: blood and/or erythrocytes, mucus and/or leukocytes. Sixty patients were children (under 16 years of age) and 44 were adults (over 16 years of age). All samples were collected as part of the laboratory work. One sample from each patient was studied.

The macroscopic and microscopic characteristics of all samples were examined and recorded. *E. coli* was identified by standard biochemical tests. *E. coli* strains were confirmed by API 20E (BioMerieux, France). Sorbitol-nonfermenting colonies were tested for O157 and H7 antigens by latex agglutination.

Presence of toxins was evaluated by VTEC-RPLA detection kit (Denka Seiken, Tokyo, Japan). This is a reverse passive latex agglutination test. Study was performed according to the manufacturer's instructions.

Mucus was present in 80 of the stools, blood and/or erythrocytes in 30 samples, and leucocytes in 69 samples. Sorbitol negative *E. coli* growth was determined in the stool samples of only two patients out of 940. One of these patients was a child with bloody diarrhea and the sample contained mucus and leukocytes. The isolated strain was beta hemolytic. The other sample was of an adult with watery diarrhea. This sample did not contain any mucus, leukocyte and/or erythrocyte, and the isolated strain was non hemolytic on blood agar. They were negative for *E. coli* O157 and H7 antigens in latex agglutinations tests. No verotoxin production was found in any of the 104 samples evaluated for this pathogen. Thirty of the stools contained erythrocytes. Thirty-six *E. coli* strains hemolysed sheep red blood cells on sheep blood agar plates (17 alpha, 19 beta).

The outbreaks and sporadic infections of VTEC have been reported by different studies worldwide. It is a significant cause of food-borne infection and sporadic infections due to factors such as consumption of raw milk, uncooked meat and contact with animal feces and ruminants.[1-3,10,11] The most common VTEC serotype is *E. coli* O157:H7. Food-borne outbreaks have been reported in many countries since 1982, when it was first detected and described.[12] Non-motile strains of *E.coli* O157:NM, the mutants of *E. coli* O157:H7 that can forment sorbitol, have been recently reported in European countries.[13,14]

The prevalence of VTEC in Turkey is not well-known. In the study of Yılmaz et al, *E. coli* O157:H7 was isolated from cattle rectal swabs, cattle carcasses, and environmental samples.[15] Similarly, Aslantaş et al isolated *E.coli* O157:H7 from cattle rectal swabs at a rate of 13.6%.[16] In the study by Keskimaki et al, VTEC was reported in people returning from Turkey.[17] However, to date, there are no data from Turkey indicating that this bacterium has ever caused outbreaks among humans or is sporadically isolated. In our study, 940 stool samples from patients with diarrhea were studied for *E. coli* O157:H7. Although sorbitol negative *E. coli* was detected in two stool cultures, they were negative for *E. coli* O157:H7 by latex agglutination. None of the 940 samples had growth of sorbitol non-fermenting *E. coli* O157:H7. Among these stool samples, 104 stool samples containing at least one of the following were selected: blood and/or erythrocytes, mucus and/or leucocytes. The samples were studied for verotoxin secreting *E. coli*. None of the 104 strains isolated were secreting ve-

rotoxin. Güney et al detected 23 strains agglutinating with sorbitol non-fermenting and *E. coli* O157:H7 serums, but biochemical characteristics of these 23 isolates were compatible with *Escherichia hermannii,* not with *E. coli* (99.8%).[7] In the study of Hasçelik et al no VTEC was isolated in children under 15 years of age with acute gastroenteritis.[8] Furthermore, to the best of our knowledge, VTEC has never been investigated in patients with hemolytic uremic syndrome in Turkey. Despite the reports of strains of environmental or animal origin from Turkey, there are no studies reporting the presence of the strain or any related disease activity. Despite the dietary habits of the people in this region, where a traditional dish known as çig köfte (uncooked meatballs) is frequently consumed, no VTEC was isolated, which may be due to the low number of the samples studied. It is not surprising that VTEC was not isolated from 104 samples; however, no *E. coli* O 157: H7 was isolated from 940 diarrhea samples, which is remarkable. "Çiğ köfte" is a spicy hot food. The spices may prevent the growth of this bacterium. Except for this particular dish, Turkish people prefer to eat well-cooked meat. Even in the most classical restaurants, the waiters are often asked to serve the meat well-done. People over 25 years of age prefer ordinary restaurants. Fast food restaurants are common in metropolises and in touristic areas of the West Anatolia region. There are a few fast food restaurants in Malatya and the people of this region do not have a habit of eating fast food.

The fact that no VTEC has been isolated from human stool samples of Turkish people to date may be due to the limited number of studies evaluating this pathogen or dietary habits of Turkish people.

In addition, in the southeast of Turkey, where husbandry is widespread for livelihood, thousands of people have contact with animals. Nevertheless, to date, no Turkish studies have been conducted on human-animal contact with respect to VTEC and O157:H7 infections.

In conclusion, although, VTEC has not been isolated from the stool samples of Turkish patients with diarrhea living in Turkey up to date, further studies with larger series are recommended.

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