

## Typing of Verotoxigenic *Escherichia coli* Strains Isolated from Animal and Human Sources

Timur GÜLHAN \* ✍ Banur BOYNUKARA \* Mustafa ALIŞARLI \*\*

\* Department of Microbiology, Faculty of Veterinary Medicine, University of Yüzüncü Yıl, 65080, Van - TURKEY  
\*\* Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, University of Yüzüncü Yıl, 65080, Van - TURKEY

Yayın Kodu (Article Code): 2008/81-A

### Summary

In this study, the verotoxin types of 73 *E. coli* strains, 58 animal origin and 15 human origin, were examined by Vero cell culture technique. Typing was performed using Duopath verotoxin GLISA test tool. Of them 67 (91.8%) strains were typed; 31 (42.5%) were VT1, 20 (27.4%) were VT2 and 16 (21.9%) were VT1+VT2. 1 animal origin and 5 human origin totally 6 (8.2%) strains could not be typed. It was seen that this technique used in determining the verotoxin types of human and food origin *E. coli* strains of can also be used as practical test in determining the verotoxin types of animal origin *E. coli* strains.

**Keywords:** Verotoxigenic *Escherichia coli*, ELISA, Animal, Human

## Hayvan ve İnsanlardan İzole Edilen Verotoksijenik *Escherichia coli* Suşlarının Tiplendirilmesi

### Özet

Bu çalışmada Vero hücre kültürü tekniği ile verotoksijenik olduğu bilinen hayvan kaynaklı 58, insan kaynaklı 15, toplam 73 *E. coli* suşunun Verotoksin tipleri (VT1, VT2) araştırıldı. Tiplendirme Duopath verotoksin GLISA test kiti kullanılarak yapıldı. Bu suşların 31'i (%42.5) VT1, 20'si (%27.4) VT2, 16'sı (%21.9) VT1+VT2 olmak üzere 67 tanesi (%91.8) tiplendirildi. İnsan kaynaklı 1, hayvan kaynaklı 5, toplam 6 suş (%8.2) tiplendirilemedi. İnsan ve gıda kaynaklı *E. coli* suşlarının verotoksin tiplerinin belirlenmesinde kullanılan bu tekniğin, hayvan orijinli verotoksin tiplerinin belirlenmesinde de pratik bir test olarak kullanılabileceği görüldü.

**Anahtar sözcükler:** Verotoksijenik *Escherichia coli*, ELISA, Hayvan, İnsan

### INTRODUCTION

Verotoxin-producing *Escherichia coli* (VTEC) strains may cause severe diseases in humans, such as hemorrhagic colitis and hemolytic uremic syndrome <sup>1</sup>. These microorganisms can enter food during the processing of meat and dairy products if hygienic conditions are inadequate. Human infections from these pathogens are often caused by direct contact with domestic and wild animals or by consumption of contaminated foodstuffs like meat and unpasteurized milk <sup>2,3</sup>. Infected animals may be risk for humans so it is necessary to determine the VTEC strains particularly in healthy

animals. Therefore, rapid and reliable laboratory tests are needed to facilitate early diagnosis of these strains. VTEC strains produce mainly two types of verotoxin (VT1 and VT2). For detection of these toxins, cell culture cytotoxicity assays <sup>4</sup>, DNA hybridization <sup>5,6</sup>, ELISA <sup>7-9</sup> and PCR <sup>4,10</sup> may be used. ELISA is known to be simple to perform, easy to interpret and highly sensitive to detect toxin types of VTEC strains. The results of Duopath Verotoxin (DV) are easy to interpret and the methodology is simple. Although its assay was originally designed for use with food products, it



İletişim (Correspondence)



+90 432 2251127



tgulhan@yahoo.com

has a great potential for clinical applications as well. Test results are easy to interpret, and the methodology is simple enough for the laboratory personnel at all levels to perform the test <sup>11</sup>. The aim of this study was to detect VT1 and VT2 toxin types of *E. coli* strains found to be VTEC in Vero cells isolated from animal and human by using DV GLISA.

## MATERIAL and METHODS

In this study, of the 378 *E. coli* strains isolated from animals and human in a previously carried out study <sup>12</sup>, 73 *E. coli* strains (58 animal and 15 human origin) determined to be verotoxic by Vero cell assay <sup>13</sup> were used as main material in order to determine the verotoxin types (VT1 and VT2).

**Reference *E. coli* Strain:** Verotoxigenic *E. coli* H30 known to be verotoxigenic was used as reference strain.

### Detection of verotoxin types (VT1 and VT2):

The test was performed according to manufacturers' instruction manual. Briefly, VTEC strains were streaked onto Cefixime Tellurite-Sorbitol MacConkey (CT-SMAC, Oxoid CM0981) agar and plates incubated at 37°C for overnight. One representative colony selected from this medium was inoculated into 1 ml of Casemino Acids-yeast extract broth (CA-YE broth with Carbadox, Merck 1.00060.0100) and incubated at 37°C for 6 h. Then, 180 µl of broth culture was taken into eppendorf tube and 20 µl polymyxin B (Merck 1.09875.0001) sulfate solution was added. The mixture was incubated at 37°C for 10 min. Then, 160 µl of this mixture was transferred into test panel and incubated at room temperature for 20 min. When the red line forming on the test panel was only present at control zone, verotoxin was negative. When seen at VT1 region, verotoxin 1 was positive. Observation of red line forming at VT2 region indicates the positivity verotoxin 2. When red lines formed at both locations, verotoxin 1 and verotoxin 2 were evaluated as positive.

## RESULTS

73 of 378 *E. coli* strains isolated from animal and human were found to be verotoxigenic. Of these strains, 58 were animal origin and 15 were

human origin. In total, from 67 of 73 VTEC strains, 31 for VT1, 20 for VT2 and 16 for VT1+VT2 were typed and 6 were untyped. The distribution of VT1 and VT2 toxin types of 73 VTEC strains, by origin, are given in *Table 1*.

**Table 1.** The distribution of VT1 and VT2 toxin types of VTEC strains by their origins

**Tablo 1.** Orijinlerine göre VTEC suşlarının VT1 ve VT2 toksin tiplerinin dağılımı

Origin	Number of isolates	Toxin types			Typed	Untyped
		VT1	VT2	VT1+VT2		
Cattle	4	2	2	-	4	0
Calves	10	2	1	6	9	1
Sheep	15	8	4	2	14	1
Lamb	24	14	5	4	23	1
Gull	5	3	-	-	3	2
Human	15	2	8	4	14	1
Total (%)	73	31 (42.5)	20 (27.4)	16 (21.9)	67 (91.8)	6 (8.2)

## DISCUSSION

Cell culture, PCR, DNA hybridization and ELISA have been used for determination of verotoxin types in VTEC strains. Although sensitivities of these methods differ from each other, ELISA has widely been used for detecting and typing of VTEC strains in culture and feces <sup>4,6,7,10</sup>.

Significant numbers of studies were performed for determination of verotoxin types of *E. coli* strains from animal origins. In a study on healthy animals, it was reported that VTEC strains found to be positive were 4% VT1, 20% VT2, 14% VT1+VT2 in cattle; 9% VT1, 20% VT2, 10% VT1+VT2 in calves <sup>4</sup>. Similar results were reported in a study from Argentina Tandil food and dairy cattle <sup>14</sup>. In an another study on healthy cattle and sheep for verotoxin typing by ELISA, 8 of 52 VTEC from cattle origin were found to be VT1, 17 for VT2, 32 for VT1+VT2; 7 of 11 sheep isolates also found to be VT1, 1 for VT2 and 2 for VT1+VT2 <sup>9</sup>. Rey et al. <sup>15</sup> reported that 110 of 253 VTEC isolated from sheep were found to be positive for Stx1, 10 for Stx2 and 133 for Stx1+Stx2 by PCR. Samadpour et al. <sup>16</sup> detected 5 of 21 VTEC strains of sheep origin for VT1, 10 for VT1+VT2; 4 of 33 VTEC isolated from chicken were also detected for

VT2. In a similar study, 52 of 94 *E. coli* strains isolated from chicken and turkey were determined Stx genes by PCR and 41 of these strains were typed to be for Stx1, 3 for Stx2<sup>17</sup>. In a study performed on wild poultry, all of 13 VTEC strains isolated from gull were detected for VT1+ VT2 by phage type 4<sup>3</sup>.

The types of VTEC strains isolated from human have been revealed by several researchers. Park et al.<sup>11</sup> reported that 45 of 49 *E. coli* O157 strains were found to be Stx2 and 10 of 12 non O157 isolates were VT1 determined by DV. Researchers indicated that the sensitivity of this method is very high (100%) and may be used as a test for detecting of VTEC in a short period of time. In a similar study, 43 of 126 VTEC strains were found to be positive for VT1, 45 for VT2 and 38 for both VT1 and VT2<sup>18</sup>. Stephan and Untermann<sup>1</sup> also reported that 3 of 14 VTEC strains isolated from human fecal samples were found to be VT1, 2 for VT2 and 2 for VT1+VT2.

In our study, VT1 toxin type was found to be dominant in VTEC strains isolated from animals, while VT2 toxin type was found to be dominant among isolates from human sources. These results are similar to those of stated reports<sup>1,9,11,15,17,18</sup>. The findings of the present study indicated that both VT1 and VT2 toxin types could be detected at a high percent (91.8%) analyzed by DV. Since these toxins have been detected in *E. coli* strains isolated from samples of individuals with disease, the presence of these toxins in *E. coli* strains from healthy animals and humans must be investigated in detail.

This study showed that DV test kit, widely used for detecting verotoxin types of *E. coli* strains isolated from humans and food, is also useful for the verotoxin types of *E. coli* strains isolated from animals. It was concluded that the reason that some of the strains (8.2%) were determined by Vero cell culture analyses but could not be typed by DV GLISA test kit, might be due to the difference toxin types (VT2v). Furthermore, in this study, VTEC strains which were determined by Vero cell culture analyses but could not be typed by DV technique will be reanalyzed by PCR.

## REFERENCES

- Stephan R, Untermann F:** Virulence factors and phenotypical traits of verotoxin-producing *Escherichia coli* strains isolated from asymptomatic human carriers. *J Clin Microbiol*, 37, 1570-1572, 1999.
- Chapman PA, Siddons CA, Wright DJ, Norman P, Fox J, Crick E:** Cattle as a source of verotoxigenic *Escherichia coli* O157. *Vet Rec*, 131, 323-324, 1992.
- Wallace JS, Cheasty T, Jones K:** Isolation of vero cytotoxin-producing *Escherichia coli* O157 from wild birds. *J Appl Microbiol*, 82, 399-404, 1997.
- Blanco M, Blanco JE, Blanco J, Mora A, Cristina P, Alonso MP, Mourino M, Madrid C, Balsalobre C, Juarez A:** Distribution and characterization of fecal verotoxin-producing *Escherichia coli* (VTEC) isolated from healthy cattle. *Vet Microbiol*, 54, 309-319, 1997.
- Borie CF, Monreal Z, Martinez J, Arellano C, Prado V:** Detection and characterization of Enterohaemorrhagic *Escherichia coli* in slaughtered cattle. *J Vet Med*, 44, 273-279, 1997.
- Saridakis HO, Gared S, Vidotto MC, Guth BEC:** Virulence properties of *Escherichia coli* strains belonging to enteropathogenic (EPEC) serogroups isolated from calves with diarrhea. *Vet Microbiol*, 54, 145-153, 1997.
- Carroll KC, Adamson K, Korgenski K, Croft A, Hankemeier R, Daly J, Park CH:** Comparison of a commercial reversed passive latex agglutination assay to an enzyme immunoassay for the detection of Shiga toxin-producing *Escherichia coli*. *Eur J Clin Microbiol Infect Dis*, 22, 689-692, 2003.
- Chapman PA, Malo ATC, Siddons CA, Harkin M:** Use of commercial enzyme immunoassay and immunomagnetic separation systems for detecting *Escherichia coli* O157 in bovine fecal samples. *Appl Environ Microbiol*, 63, 2594-2553, 1997.
- Randall LP, Wray C, McLaren IM:** Studies on the development and use of a monoclonal sandwich ELISA for the detection of verotoxic *Escherichia coli* in animal feces. *Vet Rec*, 140, 112-115, 1997.
- Smith KA, Kruth S, Hammermueller J, Gyles C, Wilson JB:** A case-control study of verocytotoxigenic *Escherichia coli* infection in cats with diarrhea. *Can J Vet Res*, 62, 87-92, 1998.
- Park CH, Kim HJ, Hixon DL, Bubert A:** Evaluation of the Duopath verotoxin test for detection of Shiga toxins in cultures of human stools. *J Clin Microbiol*, 41, 2650-2653, 2003.
- Gülhan T, Boynuvara B, Alişarlı M, Dülğar M, Aksu Z:** Hayvan ve İnsan Orijinli Verotoksijenik *Escherichia coli* Suslarının VT1 ve VT2 Verotoksin Tiplerinin Duopath GLISA Tekniği ile Belirlenmesi. II. *Ulusal Veteriner Mikrobiyoloji Kongresi*. Side, Antalya (26-28 Eylül 2006). s:138-139.
- Caprioli A, Luzzi I, Rosmini F, Pasquini, P, Cirrincione, R, Gianviti A, Matteucci MC, Rizzoni G:** Hemolytic-uraemic syndrome and Vero cytotoxin-producing *Escherichia coli* infection in Italy. *J Infect Dis*, 166, 154-158, 1992.
- Parma AE, Sanz ME, Blanco JE, Blanco J, Viñas MR, Blanco M, Padola NL, Etcheverria AI:** Virulence genotypes and serotypes of verotoxigenic *Escherichia coli* isolated from

cattle and foods in Argentina. *Eur J Epidemiol*, 16, 757-762, 2000.

**15. Rey S, Blanco JE, Blanco M, Mura A, Dahbi G, Alanso JM, Hermoso M, Hermoso J, Alanso MP, Usera MA, González EA, Bernález MI, Blanco J:** Serotypes, phage types and virulence genes of Shiga-producing *Escherichia coli* isolated from sheep. *Vet Microbiol*, 94, 47-56, 2003.

**16. Samadpour M, Ongerth JE, Liston J, Tran N, Nguyen D, Whittam TS, Wilson RA, Tarr PI:** Occurrence of shiga-like toxin-producing *Escherichia coli* in retail fresh seafood,

beef, lamb, pork and poultry from grocery stores in Seattle, Washington. *Appl Environ Microbiol*, 60, 1038-1040, 1994.

**17. Parreira VR, Gyles CL:** Shiga toxin genes in avian *Escherichia coli*. *Vet Microbiol*, 87, 341-352, 2002.

**18. Blanco JE, Blanco M, Alonso MP, Mora A, Dahbi G, Blanco J:** Serotypes virulence genes, and intimin types of Shiga toxin (verotoxin)-producing *Escherichia coli* isolates from human patients: Prevalence in Luga, Spain, from 1992 through 1999. *J Clin Microbiol*, 42, 311-319, 2004.