大韓獸醫學會誌(2000) 第40卷 第3號
Korean J Vet Res(2000) 40(3) : 525~531

Characteristics of verotoxin non-producing *Escherichia coli* O157 and verotoxin-producing *E coli* isolated from healthy cattle

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(Accepted by Jul 27, 2000)

Abstract : Verotoxin non-producing *E coli* O157 strains have been isolated from cattle feces and compared in particular regard to biochemical properties and genotypes with verotoxin-producing *E coli* (VTEC). *E coli* O157 : nonH7 strains had different phenotypes in sorbitol fermentation and β-glucuronidase activity from *E coli* O157 : H7. Regardless of verotoxin production ability of *E coli* O157 : H7, *uidA* gene was uniquely detected from sorbitol and β-glucuronidase negative *E coli* O157 : H7.

Forty five fecal samples from 6 dairy farms were obtained and VTEC was detected as 15.6% (7 strains) of the samples. Most VTEC isolates were positive for sorbitol fermentation and β-glucuronidase activity but negative for *eaeA* gene. This study suggested that cattle could be a reservior for VTEC. However, absence of *eaeA* gene in VTEC isolates from most of healthy cattle suggested that they might be less virulent than *eaeA*-positive *E coli* against human health.

Key words : *E coli* O157 : H7, *E coli* O157 : nonH7, Verotoxin, *uidA*, *eaeA* .

Introduction

Strains of verotoxin (VT ; shiga-like toxin)-producing *Escherichia coli* (VTEC) O157 have been associated with hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS) in human[1]. Bovine food products, particularly ground beef, have been inplicated in the majority of food-borne outbreak, but diversity of foods, as well as water and person-to-person transmission, have also been linked with outbreak[2,3].

Most biochemical properties of *E coli* O157 : H7 isolates were typical of *E coli*, with the exception of sorbitol fer-

This work was supported by the Korea Agricultural Research and Development Promotion Center(grant No. 298014-3).
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mentation and β-glucuronidase activity, About 80.3% of *E coli* isolates fermented sorbitol within 24 h, but *E coli* O157 : H7 did not. Additionally, 97% of *E coli* possessed the enzyme β-glucuronidase that was not phenotypically expressed in *E coli* O157 : H7[4]. However, some *E coli* O157 : H7 isolates fermented sorbitol and exhibited β-glucuronidase activity[5,6]. For example, Gunzer *et al*[5] found that 17 strains fermented sorbitol and were positive for β-glucuronidase activity among 44 VT II-producing *E coli* O157 isolated from patients with diarrhea or HUS. There was increasing evidence suggesting that phenotypic variations existed among *E coli* O157 : H7. Therefore, it was necessary to compare biochemical properties and genotypes of VTEC O157 and VT non-producing *E coli* O157.

Isolation and serotyping of VTEC from animal feces or environmental samples were important to understand the epidemiology of human VTEC infection and to establish devise strategies for its control. Although VTEC from different sources and geographical areas belonged to many different O serotypes[7], many VTEC serotypes isolated from cattle have not been isolated from humans[8] and most documented outbreaks of HUS and HC were attributed to only a few serotypes (eg. O26 : H11, O111 : NM and O157 : H7 or NM) which have been designated enterohemorrhagic *E coli* (EHEC). The relatively low incidence of non-EHEC VTEC infection in human suggested that VT production alone might not be sufficient to cause human infection. One of the factors that might affect virulence of VTEC was *eaeA* gene present in most human EHEC strains. The *eaeA* gene was essential for adherence to intestinal epithelial cells and for effacing to microvilli[9]. Blanco *et al*[10] reported that only 9.6% of VTEC isolated from healthy cattle were positive for *eaeA* gene. The Center for Disease Control and Prevention in USA reported that *E coli* O157 : NM (nonmotile) isolates had increased from 6% of the total VT positive isolates in the early 1990s to 47% in 1996[11]. Accordingly, it was required that *E coli* isolates should be checked for their virulence factors as well as O : H serotypes in public health standpoint.

In this paper, we reported the first isolation of VT non-producing *E coli* O157 : nonH7 in Korea, In addition, we described the prevalence of VTEC infection in healthy cattle that was useful for the correlation with human VTEC infection.

Materials and Methods

Specimen collections and bacterial strains : Since 1998, *E coli* O157 strains have been isolated from cattle feces by using O157 enzyme immunoassay (EIA) kit[12]. In brief, O157 positive enrichment broth in EIA was spread simultaneously onto sorbitol MacConkey agar (Difco, USA) added cefixime (0.05 μg/ml), potassium tellurite (2.0 μg/ml) and 4-methylumbelliferyl-β-D-glucuronide (100 μg/ml) (CTM-SMAC) and MacConkey agar (Difco), respectively. Sorbitol-negative colonies from CTM-SMAC were tested according to the previous report to isolate *E coli* O157 : H7[13]. Lactose-positive colonies on MacConkey agar were tested with O157 antiserum for rapid presumptive isolation of *E coli* O157. FH9749 strain was kindly provided by Choong H Park, Fairfax hospital, VA, USA, and A2, 5306-56, Stroke W strains were received from Statens Serum Institute, Denmark.

Forty five fecal samples of 6 dairy farms were obtained to isolate VTEC between October and November of 1998. Fecal samples were taken to the laboratory for immediate processing. They were inoculated on MacConkey agar and then approximately twenty *E coli* suspected colonies were chosen from each sample and examined for their VT production ability by using Verocell assay.

The isolates were confirmed as *E coli* by using VITEK system (bioMérieux, France) and Easy 24E Plus kit (KOMED, Korea). Sorbitol fermentation and β-glucuronidase activity were examined by sorbitol MacConkey agar and Fluorocult Brila broth (Merck, Germany).

Serotyping and genotyping : Serological analyses were performed by slide and tube agglutination test with 51 O and 22 H antisera, according to the procedure of the manufacturer (Denka seiken, Japan). The presence of VT I, II, *eaeA* and *uidA* genes in the isolates was investigated by multiplex PCR as described previously[14].

Verotoxin assay : The culture supernatants of isolates to tested for VT were filtered through 0.45 μm pore size cellulose membrane filters (Sartorius, Germany). In brief, 10 μl of the

filtrates were injected into the fresh monolayer of Vero cells. The plates were incubated at 37℃ in 5% CO_2 atmosphere and examined daily for cytotoxic effect. Positive reaction of VT production was determined as the filtrate to kill 50% of the monolayer after 48 h of incubation.

Results

We examined biochemical properties, serotypes and genotypes of *E coli* O157 isolated from cattle feces. *E coli* O157 : nonH7 (it was motile but flagella antigen wasn't H7) strains had different phenotypes of sorbitol fermentation and β-glucuronidase activity from *E coli* O157 : H7. Although strain 98520 was serotyped as O157 : H7 and had sorbitol and β-glucuronidase negative phenotypes, it did not produce VT. Regardless of VT production ability of *E coli* O157 : H7, *uidA* gene was uniquely detected from *E coli* O157 : H7 that was negative for sorbitol fermentation and β-glucuronidase activity (Table 1).

VTEC were isolated 7 strains (15.6%) from 45 fecal sam-

Table 1. Characteristics of verotoxin non-producing *E coli* O157 isolates

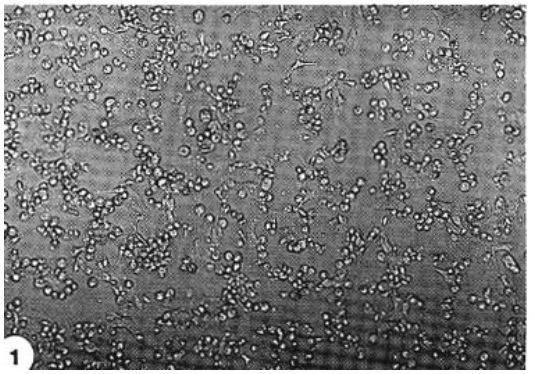
Strains	Serotype		Sorbitol fermentation	β-glucuronidase activity	PCR with primers for				Verotoxin assay
	O	H			*uidA*	VT I	VT II	*eaeA*	
96251	157	NM*	-	+	-	-	-	-	-
96291	157	12	+	-	-	-	-	-	-
9871-1	157	NM	-	+	-	-	-	-	-
98520	157	7	-	-	+	-	-	+	-
FH9749	157	NM	+	-	-	-	-	-	-
ATCC43894	157	7	-	-	+	+	+	+	+
A2	157	19	+	+	-	-	-	-	-

* nonmotile.

Table 2. Characteristics of verotoxin-producing *E coli* isolates

Strains	Serotype		Sorbitol fermentation	β-glucuronidase activity	PCR with primers for				Verotoxin assay
	O	H			*uidA*	VT I	VT II	*eaeA*	
99412	26	NM*	+	+	-	+	-	-	+
99530	NT**	NM	+	+	-	+	-	-	+
99612	125	19	+	+	-	-	-	-	+
99612-2	153	NT	+	+	-	+	+	-	+
99720	NT	2	+	+	-	+	+	-	+
99842	NT	21	+	+	-	-	+	-	+
991212	NT	NM	+	+	-	-	+	-	+
5306-56	26	46	+	+	-	+	-	-	+
Stroke W	111	NT	+	+	-	+	+	-	+

* nonmotile, ** nontypable.



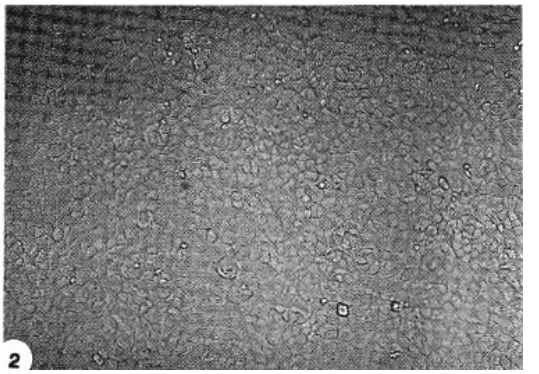


Fig 1. Phase-contrast photomicrographs of Vero cells in monolayer culture in the absence or presence of the verotoxin. The left(①) panel showed verotoxin-treated cells and toxin-untreated cells appeared in right(②) panel.

ples. Most VTEC isolated from healthy cattle showed sorbitol fermentation and β-glucuronidase activity and did not possess *uidA* and *eaeA* genes. Strains 99612 did not carry VT I, II genes but produced VT. Strains 99612 and 99612-2 were isolated from one fecal samples, but serotypes were O125 : H19 and O153 : NT, respectively (Table 2).

Discussion

Many outbreaks of VTEC O157 : H7 infections in human were associated with ingestion of contaminated food of cattle origin[1]. The isolation of VTEC from cattle has identified that cattle was a principal reservior of *E coli* O157 : H7 and other VTEC[15,16].

Unlike *E coli* O157 : H7, *E coli* O157 : nonH7 isolates were not shown sorbitol and β-glucuronidase negative phenotypes (Table 1). These results were similar to the reports that both *E coli* O157 : H7 and VTEC O157 : NM were negative for sorbitol fermentation and β-glucuronidase activity, whereas most of the VT non-producing *E coli* O157 : nonH7 ; NM were positive for sorbitol fermentation and β-glucuronidase activity[17].

Regardless of VT production ability, sorbitol and β-glucuronidase negative *E coli* O157 : H7 strains possessed *uidA* gene that appeared to be unique to *E coli* O157 : H7. Although strain 98520 was serotyped as *E coli* O157 : H7, it didn't produce VT. These were similar to our previous reports that some of *E coli* O157 : H7 did not possess VT I, II genes[18]. Accordingly, serotyping alone, whether O157 and H7 or not, was not more important than VT assay in public health standpoint.

Most VTEC, expect for *E coli* O157 : H7, had no consistent features other than VT to differentiate them from other non-pathogenic *E coli*. Consequently, VTEC isolates were obtained by testing numerous individual colonies for VT. VT positive isolates could then be identified by biochemical characterization and serotyping. The higher cost and time consumed in these methods might be the main reasons which did not apply to isolation non-O157 : H7 VTEC more often. Nevertheless, it has been the only approach at present for detection and isolation of VTEC with any serotype.

We found VTEC from 15.6% of healthy dairy cattle in Korea. Montenegro *et al*[15] in Germany identified VTEC in 17% of cows and 9% of bulls. These results indicated that VTEC were widespread among healthy cattle. According to PCR of 7 VTEC isolates, 2 isolates (28.6%) carried VT I gene only (Table 2). These data were similar to other authors' reports that there were similar distributions in VTEC from healthy cattle[15,19]. But Blanco *et al*[11] reported that 52% VTEC isolates from cattle possessed VT II gene only while 72% were VT I positive in previous study[20]. Interestingly, strain 99612 (O125 : H19) did not show VT I, II genes in PCR but produced VT, which were the evidence that several antigenic types of VT might further expand[21,22] from previous described VT[1].

The *eaeA* gene-positive VTEC were more commonly

found among calves or cattle with diarrhea than among adult healthy cattle, confirming that calves or cattle with diarrhea were important reserviors of pathogenic VTEC strains[11]. The *eaeA* gene-positive VTEC from feces of healthy dairy cattle was not isolated in this study (Table 2).

VTEC isolated from animals had more than 100 O : H serotypes[15,19,23,24]. However, many serotypes of animal VTEC isolates were not shown in VTEC serotypes of human illness[8,25]. It was probable that the majority of bovine VTEC strains isolated from healthy cattle would not be pathogenic for human. This result indicated, in part, why different serotypes of VTEC were isolated from stools of diarrhoeic patients in spite of being so prevalent VTEC serotypes present in cattle.

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大韓獸醫學會誌(2000) 第40卷 第3號
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정상 소 분변에서 분리한 verotoxin을 산생하지 않는 *Escherichia coli* O157과 verotoxin을 산생하는 *E coli*의 특성 조사

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(2000년 7월 27일 게재승인)

국문초록 : Verotoxin을 산생하지 않은 *Escherichia coli* O157과 verotoxin을 산생하는 *E coli* (VTEC)를 건강한 소의 분변에서 분리하여 생화학적 및 유전적인 특성에 대해서 비교하였다. *E coli* O157 : nonH7(운동성은 있으나 H혈청형이 7이 아님)의 sorbitol 분해능과 β-glucuronidase 활성은 *E coli* O157 : H7이 나타내는 것과는 차이가 있었다. 그리고 *uidA* 유전자는 verotoxin 산생능과 상관없이 sorbitol과 β-glucuronidase 음성인 *E coli* O157 : H7에서 특이적으로 검출되었다.

한편 6개 목장에서 수거한 소 분변 45예에서 VTEC를 분리한 결과, 7주(15.6%)가 분리되었으며 이들은 모두 sorbitol을 분해하였으며 β-glucuronidase 활성이 있었으나 장벽 부착인자를 지배하는 *eaeA* 유전자가 없었다.

비록 소가 VTEC의 보균원으로 추정되나, 정상 소에서 분리한 VTEC는 *eaeA* 유전자가 결여된 균주가 많으므로 공중위생학상 *eaeA* 유전자를 보유한 *E coli* 보다 위해성이 낮으며, 이러한 결과는 왜 사람에서 유행하는 VTEC 혈청형과 소에서 유행하는 것과 차이가 있는지를 일부 설명해준다.

Key words : *E coli* O157 : H7, *E coli* O157 : nonH7, Verotoxin, *uidA*, *eaeA* .