POLYMERASE CHAIN REACTION FOR DETECTION OF SHIGA LIKE TOXIN PRODUCING ESCHERICHIA COLI

HEUVELINK A.E.*, VAN DE KAR N. **, DE BOER E.***3, WERNARS K.****, HUIS IN 'T VELD J.* and MONNENS L.**

* Department of the Science of Food of Animal Origin, Faculty of Veterinary Medicine, University of Utrecht, Utrecht, The Netherlands, ** Department of Pediatrics, Academic Hospital Nijmegen, Nijmegen, *** Inspectorate for Health Protection, Food Inspection Service, Zutphen, **** National Institute of Public Health and Environmental Protection, Bilthoven.

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SUMMARY

In this study a Polymerase Chain Reaction (PCR) assay was used for the detection of Shiga like toxin-producing *E. coli* (SLTEC) in retail raw meats (beef). In addition, the presence of the *E. coli* attaching and effacing (*eae)* gene in these strains was investigated. Fifty-three human fecal *E. coli* isolates of serotype O157, representing isolates from patients with (HUS) and family contacts collected between 1989 and 1993, and 6 *E. coli* O157 isolates previously isolated from raw meats were also included. A total of 29 (16%) of 180 beef samples tested showed a positive reaction: 1 for the SLT I gene only, 17 for the SLT II gene only, and 11 for both the SLT I and SLT II gen. From 10 SLT positive enrichment cultures a total of 46 SLTEC strains were isolated. Serotyping revealed that the isolates belonged to 9 different serotypes: 27 (59%) to serotype O22:K-, 7 (15%) to O113:K-, 1 (2%) to O2:K?, O8:K?, O71:K-, O75:K1, O88:K-, O101:K(A)?, and O123:K? each. The remaining 5 (≈11%) isolates could not be serotyped. All 46 isolates showed a negative PCR for the *eae* gen. In 1 of the 10 examined enrichment cultures *eae* positive *E. coli*'s belonging to serotype O26 were found, which were negative for the SLT genes. Of the 53 *E. coli* O157 isolates from patients with HUS or from family contacts, 48 (91%) were positive for only SLT II and 5 (9%) were positive for both SLT I and SLT II. No isolate was positive for only SLT I. All strains contained *eae* gene sequences.

This study clearly showed that *E. coli* O157:H7 strains isolated from HUS-patients contained the *eae* gene, in addition to SLT I and SLT II, or only SLT II gene sequences. None of the SLTEC isolated from the retail beef samples harboured the *eae* gene.

Introduction

Enterohaemorrhagic *Escherichia coli* (EHEC) are implicated in the pathogenesis of the diarrhea-associated (D+) form of haemolytic uraemic syndrome (HUS), which is the most common cause of acute renal failure in childhood (Kaplan, 1990). EHEC strains produce two immunologically distinct verocytotoxins (VT1 and VT2), either single or in combination. Therefore, EHEC strains belong to verocytotoxin-producing *E. coli* (VTEC). The VTs are encoded by bacteriophages. Since these VTs are closely related to the Shiga toxin produced by *Shigella dysenteriae*, they are often referred to as Shiga-like toxins (SLT I and SLT II) (Strockbine et al., 1986). In the past few years, several variants of VT2 (VT2c or SLT IIc) have been identified (Thomas et al., 1993). The exact mechanisms of EHEC attachment to the human intestinal mucosa are poorly understood. However, based on in vitro and animal studies the following three-stage model has been proposed: (1) initial attachment mediated by plasmid-encoded fimbrial adhesins (Karch et al., 1987), followed by (2) more intimate adhesion with (3) localized effacement of the microvilli and disruption of the cellular cytoskeleton at the site of attachment mediated by the chromosomally encoded *E. coli* attaching and effacing (*eae*) gene product (Donnenberg et al., 1993) has been proposed. Additionally, other bacterial factors may contribute to the attachment of the EHEC. The damage of the epithelium would allow the passage of bacterial products which are normally excluded from the circulation, such as SLTs. It is assumed that HUS results from the systemic action of SLT on vascular endothelial cells (Kaplan et al., 1990). *E. coli* serotype O157 is the most

common and most studied member of the EHEC group. However, at least 50 other human SLTEC serotypes are known (Karmali, 1989).

E. coli O157:H7 has been isolated from cattle, foods of animal origin and contaminated water (Karmali, 1989; Swerdlow et al., 1992). Person-to-person transmission is also believed to have an important role in the spread of this pathogen (Rowe et al., 1993). This study was part of an epidemiological survey with the aim to obtain knowledge about the source of SLTEC infection in The Netherlands. One-hundred and eighty retail raw meats were examined for the presence of SLT and *eae* gene sequences by the Polymerase Chain Reaction (PCR). The isolated SLTEC were characterized by biochemical tests and serotyped. The results were compared with those obtained for SLTEC strains isolated from patients with HUS and their family contacts.

Materials and Methods

E. coli O157 isolates. Fifty-three human fecal *E. coli* isolates belonging to serotype O157 were studied, including 31 isolates from 20 children with D+ HUS, 1 isolate from an adult with HUS, 20 isolates from 9 family contacts and 1 isolate from patients with acute, bloody gastro-enteritis. The isolates had been collected in the period from 1989 to 1993 (Kar van de, 1994). Patients were from The Netherlands, Belgium and Germany. In addition, 6 *E. coli* O157:non-H7 strains previously isolated from raw meats were tested (Boer de et al., 1992). As controls, *E. coli* O157:H7 reference strain EDL931 and 3 non-*E. coli* strains were included. The bacterial cells were kept frozen at -70°C in glycerol-containing (10%) medium. Performing a PCR assay, the cells were grown overnight in Brain Heart Infusion (BHI) broth and subsequentially 1:10 diluted in pepton-water.

Preparation of raw meat products. The raw meat products were taken from retail outlets, and kept frozen at -20°C until examination. Twenty g of raw meat was mixed with 180 mL modified Trypticase Soy Broth with acriflavin (10 µg/mL) (mTSB+A) and incubated at 37°C with aeration (100 rpm) for 20-24 h. Then, 0.1 mL culture was subcultured in 10 mL BHI and incubated for another 6 h at 37°C (100 rpm). Following dilution in pepton-water (1:10), 5 µL was used in the PCR assay.

Polymerase Chain Reaction. PCR reaction mixtures (25 µL) contained 5 µL sample, 50 m*M* TRIS-HCl (pH 8.5), 50 mM NaCl, 1.5 m*M* $MgCl_2$, 2 m*M* DTT, 0.2 m*M* dNTPs each, 0.5 U of Thermus aquaticus (Taq) DNA polymerase (Perkin Elmer Cetus), and 0.3 µM (each) of the primers SLT I-1 and SLT I-2, or SLT II-1 and SLT II-2, or *eae*-1 and *eae*-2. The oligonucleotide primer pairs targeted conserved sequences found in the genes encoding SLT I, SLT II and types or variants of the SLT II family, and the *eae* gene product (Wernars et al., 1993). Negative (no template DNA) and positive (overnight culture of reference strain EDL931, 1:10 diluted) controls were included in each experiment. Amplification was performed in a DNA thermal cycler (Perkin-Elmer Cetus). The thermal profile involved an initial denaturation at 94°C for 3 min followed by 40 cycles of denaturation at 94°C for 1 min, primer annealing at 54°C for 3 min, and primer extension at 72°C for 3 min. The final step was a 10 min incubation at 72°C. After the PCR, 20 µL aliquots were analyzed by electrophoresis in 1,6% agarose gels containing ethidium bromide (0.4 µg/mL), visualized by UV transullimination and photographed. Molecular mass markers (PUC19 DNA digested with *Rsa*1) were electrophoresed simultaneously, showing DNA fragments of 240, 676 and 1764 bp.

Isolation and serotyping of SLT and/or *eae* positive *E. coli*'s. From 10 SLT-positive meat products several SLTEC were isolated. The positive enrichment cultures were plated onto Sorbitol-MacConkey agar (SMAC), and sorbitol-negative cfu were tested in the PCR. The isolates were serotyped at the National Institute of Public Health and Environmental Protection (Bilthoven, The Netherlands).

Results and Discussion

Of the 53 human fecal *E. coli* isolates of serotype O157, 48 (91%) were positive for only the SLT II gene, and 5 for both SLT I and SLT II gene sequences (Table 1). In previously performed verocel cytotoxicity tests, culture filtrates of all these strains were neutralized by only SLT II antibodies. Possibly, the SLT I gene does not become expressed in the 5 strains yielding a positive PCR for the SLT I gene. The higher prevalence of infection among HUS-patients with SLT II-producing *E. coli* strains, rather than with SLT I- and SLT I+ SLT II-producing *E. coli* strains was also observed in other studies carried out in Western Europe and North-America (Karmali, 1989; Kleanthous et al., 1990). All 53 SLTEC strains contained the chromosomally-located *eae* gene (Table 1), which is assumed to be a better virulence marker than the SLT genes, since spontaneous loss of bacteriophages carrying the SLT genes has been documented (Karch et al., 1992).

The 6 *E. coli* O157 isolates from raw meats were negative for all 3 gene sequences (Table 1) as were the 3 non-*E. coli* strains.

Twenty-nine of the 180 beef products tested showed a positive PCR: 1 for only the SLT I gene, 17 for only the SLT II gene, and 11 for both SLT I and SLT II (Table 2). A total of 46 SLTEC were isolated from 10 SLT-positive enrichment cultures: 27 (59%) belonging to serotype O22:K-, 7 (15%) to O113:K-, 1 (2%) to O2:K?, O8:K?, O71:K-, O75:K1, O88:K-, O101:K(A)?, and O123:K? each. The remaining 5 (11%) isolates could not be serotyped. In 1 of the 10 cultures, *eae*-positive *E. coli* strains belonging to serotype O26 were found, which were negative for the SLT genes. Possibly, these isolates are members of the Enteropathogenic *E. coli* (EHEC) group.

Conclusions

Ninety-one % of the *E. coli* O157 strains isolated from patients with HUS and their family contacts were positive for only the SLT II gene. The remaining human fecal isolates contained both SLT I and SLT II gene sequences. All strains carried the *eae* gene. Sixteen % of the retail raw meat products examined in this study contained SLTEC. The isolated SLTEC belonged to 9 different serotypes and were all negative for the *eae* gene. Possibly, the *eae* gene is a good virulence marker for human pathogenic SLTEC.

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