Verocytotoxin-producing *Escherichia coli* infection in hemolytic uremic syndrome in part of Western Europe

**Abstract** From September 1989 until September 1993, stool specimens and sera from 113 children with diarrhoea-associated haemolytic uremic syndrome (HUS) from the Netherlands, two university hospitals in Belgium and one university hospital in Germany were examined for the presence of verocytotoxin-producing *Escherichia coli* (VTEC) infection. Evidence for VTEC infection was observed in 88 (78%) patients with HUS compared to 2 (3%) of the 65 children with acute gastro-enteritis. Serotype 0157 was the causative agent in 76 (86%) of these 88 patients with VTEC-associated HUS and verocytotoxin-2 (VT-2) was the most frequent toxin produced. Serological testing for antibodies to 0157 O-antigen yielded the highest number of positive results compared to the other test methods. Antibodies to O157 were found in sera of 71 (65%) of 110 patients with HUS and one control serum. Stool and sera examination for VTEC in 95 family contacts of 28 patients with HUS demonstrated an evidence for VTEC infection in 33 (35%). In contrast, in patients with HUS serological antibodies to O157 O-antigen were found in only 3 (4%) of 85 family contacts.

**Conclusion** In this part of Western Europe, VT2-producing *Escherichia coli*, mainly those belonging to serogroup O157, are the major cause of HUS in childhood.
Introduction

Haemolytic uraemic syndrome (HUS), preceded by an acute, often bloody gastro-enteritis, is mostly seen in children and is a major cause of acute renal failure in childhood [9]. Since the first report by Karmali and coworkers [8], verocytotoxin-producing *Escherichia coli* (VTEC) infections are recognized as an important cause of diarrhoea-associated HUS in the United States, Canada, and United Kingdom [3]. VTEC strains may belong to different serogroups, but the most commonly isolated VTEC is the serotype O157:H7. A family of at least three verocytotoxins (VT) has been identified: VT1 or Shiga-like toxin I, VT2 or Shiga-like toxin II and VT-2 variants. Not all those infected with VTEC will develop HUS. Infection with VTEC can be asymptomatic, can lead to a mild diarrhoea, bloody diarrhoea, haemorrhagic colitis or HUS [3]. In this study, we report the results of a 4-year prospective study in which we examined the presence of VTEC infection in patients with diarrhoea-associated HUS in the Netherlands, two adjacent university hospitals in Belgium and one in Germany at a distance of approximately 150 km from the University of Nijmegen, The Netherlands.

Patients and methods

Patients

Between September 1989 and September 1993, stool and serum specimens from 113 patients with diarrhoea-associated HUS (58 female, 55 male; mean age ± SD: 46 ± 35 months; range 9–162 months) were received by the Department of Medical Microbiology of the University Hospital Nijmegen for examination of the presence of VTEC infection. Specimens of patients with HUS were obtained from 77 Dutch patients admitted to paediatric nephrology departments of the academic hospitals in the Netherlands, 21 patients admitted to the paediatric nephrology department of the Children's Hospital, Cologne in Germany and 15 patients admitted to the University Hospitals of Leuven and Antwerp in Belgium. HUS was determined by a sudden onset of illness with a prodromal phase of acute gastro-enteritis and by laboratory evidence of microangiopathic haemolytic anaemia, thrombocytopenia and disturbed renal function [6]. All patients with HUS included in this study had a prodromal phase with acute gastro-enteritis; in 83 (73%) of the HUS patients the gastro-enteritis was reported to be bloody. Stool and serum specimens were also obtained from 95 family contacts (27 fathers, 28 mothers, 35 siblings and two other family contacts) of all the 28 patients with HUS referred to the paediatric department of the University Hospital, Nijmegen. The family members included all family contacts who lived with the HUS index case in the same house. The control group consisted of 65 children aged 1 year in the stool. The observed cytotoxicity in the filtrates of two HUS patients was also found in three patients

Serum samples

Paired serum samples, collected on admission and after 14 days were used to detect neutralizing ability to VT1 and VT2 or VT variants with the Vero cell assay [8]. Paired samples were always tested in the same Vero cell microtitre plate. A fourfold or more rising titre to VT1, VT2 and/or VT variants in the sera was regarded as positive for recent VTEC infection. Serum antibodies to the lipopolysaccharide of *E. coli* O157 were analysed by ELISA and immunoblotting, as described previously [5]. A case was defined positive for VTEC infection when one or more of the above described detection methods were positive.

Statistical analysis

The significance of differences between the groups was determined by using the Fisher’s exact test (two tailed).

Results

The results are shown in Table 1. Evidence for VTEC infection was found in 88 (78%) out of 113 patients with HUS. Only two patients (3%) from the control group of 65 children with acute gastro-enteritis demonstrated a VTEC infection. All 19 isolated strains demonstrated cytotoxicity in the Vero cell assay which could be neutralized by polyclonal antibodies to VT2 alone. Infection with *Shigella dysenteriae* I occurred in one patient with HUS. No *Campylobacter*, *Salmonella* or *Yersinia* species were isolated in the stool of the HUS group. Evidence for *Clostridium difficile* infection was found in three patients with HUS. One HUS patient had both *E. coli* O157:H7 and *Clostridium difficile* in the stool. The observed cytotoxicity observed in the Vero cell assays for both culture and fecal filtrate was in all, except two, cases neutralizable with antibodies to VT2. Antibodies to VT1 neutralized the cytotoxic effect in the filtrates of two HUS patients.

*E. coli* O157:H– was isolated in two control cases. One strain *E. coli* O157:H– was isolated from the non-
Table 1. VTEC infection in patients with HUS and controls with acute gastro-enteritis.

<table>
<thead>
<tr>
<th>Methods</th>
<th>No. of patients with HUS positive for VTEC (n = 113)</th>
<th>No. of controls positive for VTEC (n = 65)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faeces</td>
<td></td>
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<tr>
<td>Isolation of VTEC O157</td>
<td>19/90 (21%)</td>
<td>1/57 (2%)</td>
</tr>
<tr>
<td>O157 : H-</td>
<td>18/19 (95%)</td>
<td>0/57 (0%)</td>
</tr>
<tr>
<td>O157 : H- VT/PECS</td>
<td>1/19 (5%)</td>
<td>1/57 (2%) *</td>
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<tr>
<td>Free faecal VT</td>
<td>24/90 (27%)</td>
<td>1/57 (2%)</td>
</tr>
<tr>
<td></td>
<td>49/90 (54%)</td>
<td>1/56 (2%)</td>
</tr>
<tr>
<td>Serology VT-neutralizing ability</td>
<td>4/76 (5%)</td>
<td>0/19 (0%)</td>
</tr>
<tr>
<td>Antibodies to O157-antigen</td>
<td>71/110 (65%)</td>
<td>1/53 (2%)</td>
</tr>
<tr>
<td>Total VTEC infection</td>
<td>88/113 (78%)</td>
<td>2/65 (3%)</td>
</tr>
</tbody>
</table>

*The other isolated E. coli O157 : H- strain in the control group did not produce VT and is not included. All methods, except the assay for VT-neutralizing ability, had P < 0.001 compared with controls. VT/PECS VT detection in polymyxin B extracts of colony sweeps. The Verocell assay for neutralizing ability to VT was positive in paired sera samples from 4 (5%) of the 76 patients with HUS (Table 1). All detected neutralizing ability to VT were against VT1 not to VT2 or VT variants. Antibodies to O157 O-antigen were present in 71 (65%) of the 110 tested HUS patients and in 1 (2%) of the 53 control cases. Although no further investigation was done to identify the VTEC strains with serotypes other than serogroup O157, combining the results of stool and sera examinations, VTEC infection due to serotype O157 was present in 76 (86%) of the 88 cases.

Table 2. In 17 (61%) families one or more members were found to have a VTEC infection at the time the patient was admitted to the hospital. Thirty-three (35%) family members had evidence of VTEC infection; 50% of these family members had diarrhoea. Evidence for VTEC infection was most prominent in the siblings of the patient (49%). All the 16 isolated VTEC O157 strains of family members produced VT2. Only 3 of the 85 family members, children of 1, 4, and 6 years of age, had serum antibodies to the O157 O-antigen. All three children had diarrhoea. Bloody diarrhoea was a more prominent feature of VTEC infection in patients with HUS than in the family members and correlated significantly with the presence of antibodies to O157 antigen (P < 0.05).

Discussion

Combined microbiological and serological procedures provided evidence for VTEC infection in 78% of the patients with HUS compared with 3% in the control group of children with acute gastro-enteritis. Serotype O157 was the predominant cause of VTEC-associated HUS in our patients. All isolated VTEC O157 strains were VT2 producers only. These results are comparable with other European epidemiological studies [1, 4, 12]. VT2-producing...
strains have been associated with a higher frequency of systemic complications in human diseases [2, 12]; however, a recently published Canadian study reported that 22 out of the 26 VTEC O157: H7 isolated from HUS patients were both VT1 and VT2 producers [10].

In this study serological examination for antibodies to O157 was the most successful method compared to the stool examination. Serological testing for antibodies to O157 O-antigen can provide evidence of VTEC infection for several weeks after the onset of diarrhoea [5]. The lower detection percentage of VTEC from stool, is probably due to the fact that the recovery rate for VTEC from stool depends on the interval of days between the onset of symptoms and the collection of stool. Tarr et al. [11] reported that this rate decreased from 100% to 33% if the stool was cultured within 2 days or 1 week after the diarrhoea began. In our study, stool was sampled 7 ± 3 (SD) days after the onset of diarrhoea.

Combined faecal and serological tests demonstrated a VTEC infection in 33 (35%) of the 95 family members of HUS patients. It is remarkable that although VTEC infection could often be demonstrated in the stool of family members, serum antibodies to O157 O-antigen were significantly lower in the family members as compared to the patients. The three family members who were positive for serum antibodies O157 LPS, were children. VTEC O157 isolates presented in this study have recently been characterized by phase typing, polymerase chain reaction for VT genes, E. coli attaching and effacing (eae) gene and random amplified polymorphic DNA fingerprinting. No differences were found in the strains isolated from different persons within one family, demonstrating that VTEC infection in one family was due to the same strain of E. coli O157 [7]. These family studies might indicate that other, yet unknown, host factors in the intestine are involved in making it more easier for VTEC lipopolysaccharides to cross the mucosal surface. A difference in adherence of VTEC to the intestinal epithelium of adults and children might be an explanation for the occurrence of HUS in mostly children as compared to adults.

Several outbreaks of VTEC infection by strains of E. coli of serotype O157: H7 in the United States and Canada have revealed that the major route of acquisition of VTEC seems to be the consumption of contaminated meat, unpasteurized milk, exposure to contaminated water, and through person-to-person transmission [3]. Although there is some indication that in our family study VTEC transmission through person-to-person contact might be very important, this study was not designed to detect the source of VTEC.

We conclude that in part of Western Europe, VT2-producing Escherichia coli, mainly those belonging to serogroup O157, are the major cause of HUS in childhood. Further studies to establish the source of these strains are currently in progress.

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References