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 uraemic 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 O157 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 O157 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 33 (35%). In contrast, in patients with HUS serological antibodies to O157 O-antigen were found in only 3 (4%) of 85 family contacts.

**Key words** Haemolytic uraemic syndrome · Verocytotoxin-producing *Escherichia coli* · Epidemiology

**Abbreviations**

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<th>Abbreviation</th>
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<tr>
<td>HUS</td>
<td>Haemolytic uraemic syndrome</td>
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<td>VT</td>
<td>Verocytotoxin</td>
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<td>VTEC</td>
<td>Verocytotoxin-producing <em>Escherichia coli</em></td>
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**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 1, 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 sera 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). The control group consisted of 65 children (28 fathers, 28 mothers, 35 siblings and two other family contacts) and serum specimens were also obtained from 95 family contacts of all the 28 patients with HUS referred to the paediatric department of the University Hospital Nijmegen for examination of the presence of VTEC infection. Specimens of patients with HUS referred to the paediatric department of the University Hospital Nijmegen and to three hospitals in the Netherlands between September 1990 and September 1993. Of the controls, 11 patients (18%) had bloody diarrhoea. On admission, stools were collected from patients and controls as soon as possible. Blood samples were taken on admission, and when possible, after 2 to 3 weeks (convalescent phase). After centrifugation sera samples were frozen at −20°C and transported on dry ice to the medical microbiology department, where they were kept frozen at −70°C until the assays were performed.

**Methods**

**Stool samples**

All stool samples examined for VTEC were plated on sorbitol MacConkey (Oxoid Sorbitol MacConkey agar containing 1% sorbitol) and blood agar. After 24 h incubation, non-sorbitol fermenting, colourless colonies were tested for agglutination with anti-O157 O-antigen serum (Difco, Detroit, Michigan, USA) and tested for VT activity in the Verocell assay. VTEC O157 strains were serotyped by Dr. W. Jansen, RIVM, Bilthoven, the Netherlands. Furthermore, faecal samples were tested by a procedure for VT detection in polymyxin B extracts of colony sweeps and for free faecal VT as described by Karmali et al. [8]. The stools of all patients with HUS and patients with acute gastro-enteritis were also tested for the most common enteric pathogens.

**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 Verocell assay [8]. Paired samples were always tested in the same Verocell 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 Verocell 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 Verocell 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-