CONCISE COMMUNICATION

# Vero cytotoxin-producing *Escherichia coli* in a study of infectious intestinal disease in England

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An investigation of infectious intestinal disease in England included examination of feces for Vero cytotoxin-producing *Escherichia coli* (VTEC). Using DNA probe hybridization 27 VTEC strains were identified, 12 were from cases, and of these three belonged to serogroup O157. The remaining 15 strains were isolated from controls. The strains were confirmed biochemically as *E. coli*, they were serotyped and characterized according to their toxin production, the presence of sequences encoding intimin (*eae*) and enterohemolysin was determined and resistance to antimicrobial agents was determined. Six of the nine cases with non-O157 VTEC were less than 16 years old, only two of the 15 controls were under 16. Infection with more than one micro-organism was also considered.

**Keywords** Vero cytoxin-producing *E. Coli*, infectious intestinal disease, VTEC

**Accepted** 27 September 2001

*Clin Microbiol Infect* 2002; 8: 183–186

Vero cytotoxin-producing *Escherichia coli* (VTEC) are associated with human disease including mild diarrhea, severe bloody diarrhea with abdominal pain and hemolytic uremic syndrome (HUS) [1]. These organisms are also described as Shiga toxin-producing *E. coli* (STEC). The most important serogroup in the United Kingdom is O157 but more than 100 serotypes of *E. coli* have been shown to produce Vero cytotoxins. Unlike VTEC O157, few studies in the UK have investigated VTEC of other serogroups [2,3]. A major investigation was undertaken to determine the true incidence of infectious intestinal disease (IID) in England and to estimate the incidence in the community of gastrointestinal disease with a microbiological cause [4]. Between August 1993 and January 1996 fecal samples from cases and controls in two study components were examined; one was a community cohort ($n = 1316$) and the other a General Practice (GP) case–control component ($n = 5157$). Cases of IID in the GP case–control component generally had more severe symptoms than those in the community cohort since they were a selected subset from the community who had sought medical advice. The overall microbiological findings and rates of IID have been reported [5,6] but details of the properties of specific organisms were not included. The IID study included examination for six groups of pathogenic or potentially pathogenic groups of *E. coli*. The results of studies with VTEC belonging to all serogroups are reported here.

Full details of the methods have been published in the report of the IID Study [4]. Briefly, fecal suspensions were prepared for direct hybridization and a sweep of representative coliform growth was also hybridized using a mixture of fluorescein-labeled VT1 and VT2 probes [4,7]. Probe-positive colonies were identified and hybridized with individual VT1 and VT2 probes [8,9]. Isolates were confirmed biochemically as *E. coli* and serotyped as described previously [10]. Resistance to antimicrobial agents was determined by an agar dilution method [11]. The final plate concentrations of the drugs were (mg/L): ampicillin, 8; chloramphenicol, 8; gentamicin, 4; kanamycin, 16; streptomycin, 16; sulfathiazole, 64; tetracyclines, 8; trimethoprim, 2; furazolidone, 8; nalidixic acid, 16; ciprofloxacin, 0.125 and 1.0. The presence

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of sequences encoding intimin (*eae*) and enterohemolysin was determined with DNA probes [12,13]. For detection of enterohemolysin production, strains were streaked on sheep blood agar (5% sheep blood, washed three times in sterile phosphate-buffered saline added to L agar base) for single colonies and incubated at 37 °C overnight. The plates were examined for hemolysis immediately after incubation and after 4–6 h at room temperature.

A total of 27 VTEC strains was isolated during the study [4,6]. Three belonged to serogroup O157 and were isolated from cases in the GP case–control component. Fifteen non-O157 VTEC cases were also identified in this part of the study; six (0.2% of samples) were from cases and nine (0.4% of samples) from controls (Table 1). In the community cohort component of the study, the nine non-O157 VTEC comprised three that were isolated from cases (0.4% of samples) and six from controls (1.1% of samples). Six of the nine cases with non-O157 VTEC from the two components of the study were children (under 16 years) of which three were under 5 years, whereas only two of the 15 controls were children, both under 5 years. The numbers in this study were small but the results suggested that carriage of non-O157 VTEC in healthy individuals may be higher in adults than in young children ($P = 0.021$, Fisher exact test). Seven of the individuals infected with non-O157 VTEC lived in rural areas and the other 17 were from urban or suburban areas. Fifteen of the 24 non-O157 VTEC strains were detected between September and December. Eight of the 24 persons with non-O157 VTEC had mixed infections, including five of the six cases from the GP case–control component (two with *Campylobacter*, one each with *Yersinia*, rotavirus and adenovirus, respectively; one cohort-case was also positive for *Clostridium perfringens* enterotoxin and one

**Table 1** Properties of VTEC isolated in the IID study

| Serotype | Study component | Age (years) | VT1 | VT2 | *eae* | CVD 419 | Hemolysin production |
|---|---|---|---|---|---|---|---|
| O157:H7[a] | GP case | 2 | – | + | + | + | + |
| O157:H7[a] | GP case | 18 | + | + | + | + | + |
| O157:H7[a] | GP case | 23 | – | + | + | + | + |
| O52:H25 | GP case | 4 | – | + | – | – | – |
| O?:H2 | GP case | 2 | + | – | + | + | + |
| O?:H10 | GP case | 5 | – | + | – | – | – |
| O?:H10 | GP case | 62 | + | + | – | + | + |
| O?:H19 | GP case | 67 | + | – | – | – | – |
| O rough: H- | GP case | 56 | – | + | – | – | – |
| O82:H2 | GP control | 3 | + | + | – | + | + |
| O91:H- | GP control | 54 | + | – | – | + | V |
| O118:H1 | GP control | 44 | – | + | – | – | – |
| O128ab:H2 | GP control | 28 | + | + | – | + | + |
| O128ab:H2 | GP control | 30 | + | + | – | + | + |
| O146:H21 | GP control | 66 | + | + | – | + | + |
| O162:H6 | GP control | 67 | + | + | – | – | – |
| O162:H8 | GP control | 68 | + | + | – | – | V |
| O?:H- | GP control | 65 | + | + | – | – | – |
| O26:H11 | Cohort case | 8 | + | – | + | + | + |
| O91:H10 | Cohort case | 1 | – | + | – | – | – |
| O rough:H- | Cohort case | 12 | + | + | – | + | V |
| O115:H7 | Cohort control | 2 | + | – | – | – | – |
| O128 ab: H2 | Cohort control | 53 | + | + | – | + | + |
| O146:H21 | Cohort control | 34 | + | + | – | + | V |
| O?: H18 | Cohort control | 39 | + | + | – | + | V |
| O?: H21 | Cohort control | 46 | + | – | – | + | + |
| O?: H- | Cohort control | 39 | – | + | – | + | + |

[a]The phage types of the three *E. coli* O157:H7 strains were 2,14 and 32.
V = variable production.

cohort-control and one GP control each had *Yersinia* co-infection) [4].

Properties of the 27 VTEC strains are listed in Table 1. The three VTEC O157 isolates carried the VT2 and *eae* genes and were also enterohemolysin probe positive. Fourteen of the non-O157 VTEC belonged to recognized serotypes but eight were untypable using the current scheme that identifies 173 O serogroups. Serotyping was the method chosen for examining the *E. coli* isolated in the IID study. However, DNA methods have developed since that time, for example, it may be possible to study the non-serotypable isolates using pulsed field gel electrophoresis but H types can be readily used to distinguish between O? strains. Six combinations of VT toxin genes were detected and 18 of the 24 non-O157 VTEC possessed the VT1 gene. This contrasts with VTEC O157 strains isolated in England and Wales, of which 76% have the VT2 gene only [7]. Only two of the non-O157 VTEC carried the *eae* gene, both were isolated from cases (Table 1). Fourteen of the 24 non-O157 VTEC hybridized with the enterohemolysin probe CVD 419 and produced hemolysin. One strain was hemolytic but negative with the CVD 419 probe. Only two of the VTEC strains were resistant to antimicrobial agents: one, serotype O115:H7 was resistant to streptomycin and tetracyclines and the second strain, serotype O52:H25 was resistant to ampicillin, kanamycin, streptomycin, sulfathiazole, tetracylines and trimethoprim. This result contrasted with other studies of *E. coli* strains such as the enteroaggregative *E. coli* (EAggEC) isolated in the IID study. One hundred and fourteen of the 206 EAggEC strains (55%) were resistant to one or more antimicrobial agents (L.E.P., unpublished observations). In a recent study of 3429 VTEC O157 strains 20% were resistant to one agent tested but only 1.3% were resistant to four or more drugs [7].

Several studies have shown that certain VTEC strains are associated with severe human disease [1]. This subset of VTEC, known as enterohemorrhagic *E. coli* (EHEC), is linked with bloody diarrhea and HUS and includes strains of serogroup O157 and non-O157 serogroups such as O26, O1O3, O111 and O145 [14]. Almost all these EHEC strains have the *eae* gene and produce enterohemolysin. The IID study examined individuals presenting to GPs or those with intestinal disease in the community. More severe cases of VTEC infection, such as those with HUS, may not have been investigated as part of this study as persons with severe symptoms were more likely to be hospitalized. One child with VTEC O26 was identified and this strain was *eae* and enterohemolysin positive: these properties are found in most VTEC O26 [15]. In a recent report of VTEC infections in England and Wales between 1995 and 1998 11 non-O157 VTEC strains were characterized. Three of the individuals had bloody diarrhea, five had non-bloody diarrhea and one had HUS [7]. Eight of the 11 isolates were *eae* positive and seven hybridized with the CVD 419 probe for the enterohemolysin gene. Five of the isolates were O26:H11 including the one from the HUS case.

The IID study is one of the few performed in Britain to provide information on VTEC other than specifically serogroup O157. The small numbers identified means that it is difficult to draw conclusions on the statistical implications of there being a higher proportion of VTEC among controls than cases. These strains were not a significant cause of IID and were carried by several individuals, mainly adults that did not have symptoms of gastro-intestinal disease. The relative importance of VTEC of serogroup O157 compared with those of other serogroups appears to vary considerably between countries, including those with common borders such as Belgium and Germany. Studies have shown that the frequencies of VTEC infections in mainland Europe are generally lower than those seen in the UK and the USA [1,16–18]. where the vast majority of reports are of VTEC O157 infections. Piérard et al. [16] reported that in four cases of HUS seen during a study lasting over five years, three of the four were associated with VTEC O157 whilst the sample from the fourth case was polymerase chain reaction-positive without a culturable isolate. However, cases of HUS can be caused by non-O157 VTEC and therefore vigilance is required. For patients with HUS or bloody diarrhea, whose stools are negative for *E. coli* O157, *Salmonella* and *Campylobacter*, fecal specimens should be examined for non-O157 VTEC. This should allow further assessment of the role of these organisms in human disease.

## ACKNOWLEDGMENTS

We thank those who took part in this study and the Department of Health (from April 2000, Food Standards Agency) who funded the project. The contributions of laboratory colleagues and

those who analysed the data are also gratefully acknowledged.

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