Properties of Vero cytotoxin-producing *Escherichia coli* of human and animal origin belonging to serotypes other than O157:H7

C. R. DORN*, S. M. SCOTLAND, H. R. SMITH, G. A. WILLSHAW AND B. ROWE

Division of Enteric Pathogens, Central Public Health Laboratory, Colindale Avenue, London NW9 5HT

(Accepted 14 March 1989)

SUMMARY

Eight non-O157:H7 Vero cytotoxin (VT)-producing *Escherichia coli* (VTEC) strains isolated from ill persons and nine bovine and lamb strains of serogroups matching the human strains, were characterized for various properties known to be associated with *E. coli* virulence. Five different serogroups were represented: O5, O55, O103, O111 and O153. The bovine and lamb strains produced VT1, while 3 human strains produced VT1, 3 produced VT2 and 2 were positive for both VT1 and VT2. The strains were non-haemolytic on horse blood agar, did not produce either heat stable toxin A (ST A) or heat labile toxin (LT), and were non-invasive. The CVD419 probe which has been proposed to identify enterohaemorrhagic *E. coli* (EHEC) hybridized with all of the O5 and O103 strains, none of the O55 and O153 strains, and 3 of the 4 O111 strains. The strains carried several different sized plasmids and hybridization of Southern blots with the CVD419 probe identified plasmids ranging in size from $42 \times 10^6$ to $90 \times 10^6$. The strains did not hybridize with the enteroadherence factor (EAF) probe derived from an enteropathogenic strain and associated with the ability to give localized adherence to HEp-2 cells. Nevertheless five of the strains adhered in a localized pattern to HEp-2 cells and Intestine 407 cells. Adhesion to either HEp-2 or Intestine 407 cells did not correlate with hybridization with the CVD419 probe or haemagglutinating properties.

INTRODUCTION

Vero cytotoxin-producing *Escherichia coli* (VTEC) were first recognized when Konowalchuk, Speirs & Stavric (1977) reported that some strains from cases of human and animal disease produced cytotoxin acting on Vero cells. VTEC produce one or both of two characterized cytotoxins, VT1 and VT2. VT1 is neutralized by anti-Shiga toxin (O'Brien *et al.* 1983b), whereas VT2 is not neutralized by either anti-VT1 or anti-Shiga toxin (Scotland, Smith & Rowe, 1985; Scotland *et al.* 1987). VT1 and VT2 have also been termed SLT I and SLT II (Strockbine *et al.* 1986).

Much attention has been focused on VTEC due to their association with outbreaks of haemorrhagic colitis (HC) in the United States (Riley *et al.* 1983).

* Current address: Department of Veterinary Preventive Medicine, The Ohio State University, 1900 Coffey Road, Columbus, Ohio 43210, USA
Canada (Pai et al. 1984) and Britain (Smith et al. 1987). VTEC were also responsible for cases of haemolytic uraemic syndrome (HUS) in Canada (Karmali et al. 1985), United States (Spika et al. 1986) and Britain (Scotland et al. 1988). The most frequently isolated VTEC are of serotype 0157:H7, but the following serogroups have also been isolated from cases of diarrhoea, HC and HUS: 01, 02, 04, 05, 06, 026, 038, 045, 050, 055, 091, 0103, 0104, 0105, 0111, 0113, 0114, 0115, 0118, 0121, 0125, 0128ab, 0145, 0153, 0163, and 0165 (Levine et al. 1987; Smith & Scotland, 1988). Levine (1987) has proposed that such strains constitute a class of enteric pathogens, termed enterohaemorrhagic E. coli (EHEC), based on their ability to cause HC, VT production, inability to produce heat stable toxin ($ST_A$) or heat labile toxin (LT) and lack of invasiveness. However, symptoms of infection by VTEC do not always include bloody diarrhoea. Serotype 0157:H7 was the prototype of this EHEC class and the source of a probe (CVD419). Probe CVD419 hybridized with all but one of 107 strains of 0157:H7, and with 55 of 70 VTEC belonging to other serogroups (Levine et al. 1987). The probe consists of plasmid sequences of unknown function on a plasmid reported to be responsible for expression of a new fimbrial antigen and for adhesion to epithelial cells (Karch et al. 1987).

Serotype 0157:H7 has been isolated from cattle on farms where persons drank raw milk and subsequently developed HC (Borczyk et al. 1987) and HUS (Martin et al. 1986; Borczyk et al. 1987). In these outbreaks serotype 0157:H7 was isolated from ill persons. Because of these reports and earlier reports of epidemiologic association of HC and HUS with ingestion of ground beef (Riley et al. 1983; Wells et al. 1983; Ryan et al. 1986), zoonotic transmission of the pathogenic strains from animals to man is thought to occur. Person-to-person transmission is possible; however, this route has infrequently been reported (Spika et al. 1986; Carter et al. 1987; Karmali et al. 1988). Serotype 0157:H7 is not a common isolate from animals and there is only one report of an isolation from a sick animal, a calf (Orskov, Orskov & Villar, 1987).

In contrast, VTEC belonging to serogroups other than 0157 have been isolated frequently from cattle with diarrhoea (Sherwood, Snodgrass & O’Brien, 1985; Mohammad et al. 1985; Mohammad, Peiris & Wijewanta, 1986; Orskov, Orskov & Villar, 1987) and from weaned pigs with diarrhoea and oedema disease (Kashiwazaki et al. 1980, 1981; Blanco et al. 1983; Dobrescu, 1983; Smith, Green & Parsell, 1983; Gonzalez & Blanco, 1985; Hampson et al. 1986; Smith et al. 1988). The pig oedema disease strains produce extra-intestinal endothelial lesions in the central nervous system and other tissues similar to those observed in some HUS patients (Dobrescu, 1983; Fong, de Chadarevina & Kaplan, 1982) and have been investigated to learn if they possess microbiological characteristics and virulence factors common to human VTEC. Smith et al. (1988) found that all six of the oedema disease strains studied produced only VT2 and their titres were low. There have been no reports of human VTEC serogroups which match the following serogroups isolated from post-weaning pigs with diarrhoea or oedema disease: 045, 0138, 0139 and 0141. Furthermore, VT1 and VT2 production by the human VTEC studied thus far are encoded by distinct bacteriophages (Willshaw et al. 1987), but Smith, Green & Parsell (1983) could not transfer the ability to produce VT from a VT-producing pig oedema strain by phage lysogenization of E. coli K12.
as achieved for the human strains. Genes for SLT II production have more recently been identified on the chromosome of a porcine (oedema disease) strain (Weinstein et al. 1988).

Phage-encoded VT production has been reported in two bovine strains (Rietra et al. 1989) and bovine strains do produce VT at similar titres to those reported for human strains (Smith et al. 1988). Some of the non-O157:H7 VTEC strains from cattle have serotypes in common with human VTEC. Because of these similarities to human VTEC there is need for more detailed study of non-O157:H7 VT-producing strains from cattle and other ruminants. In the present study a series of VTEC isolated from diarrhoeal or HUS patients was compared to a series of serogroup-matched animal VTEC. The strains were examined for shared characteristics, in addition to serogroup and VT production, to relate their properties to those of established classes of pathogenic E. coli and identify virulence factors for further study.

MATERIALS AND METHODS

Bacterial strains. The E. coli collection of the Division of Enteric Pathogens (DEP) was searched for VTEC strains from ill persons belonging to serogroups in which VTEC of bovine or ovine origin had also been isolated. Seventeen strains were found (Table 1). All the organisms were serotyped by the methods of Gross & Rowe (1985). Of the eight human strains, one diarrhoeal strain (S22-1) was from Canada and the other (E42724) was from England. The six HUS isolates were submitted to DEP from England and Canada; strain E31695 has been described previously (Scotland et al. 1988).

Seven strains were of bovine origin: two (E37446 and E37450) were from calves in Sri Lanka, one (S102-9) was from a calf with haemorrhagic diarrhoea (Hall et al. 1985; Chanter et al. 1986), one (E40867) was from a calf with diarrhoea (Sherwood, Snodgrass & O’Brien, 1985). Strains S102-9, E37446, E40867 and E40875 had been included in an earlier study of animal VTEC (Smith et al. 1988).

Resistance (R) typing and colicin production. For R-typing, overnight nutrient broth cultures were incubated at 37 °C and tested by the method of Anderson & Threlfall (1974). The following antibiotics were used: amikacin, ampicillin, cefazidine, cephalexin, cephaloridine, chloramphenicol, colistin, furazolidone, gentamicin, kanamycin, mecillinam, naladixic acid, netilmicin, spectinomycin, streptomycin, sulphathiazole, tetracycline, tobramycin and trimethoprim. Colicin production was determined using a K12 strain (14R519) in a soft agar overlay procedure described by Fredericq (1957).

Toxin assays. The bacterial strains were grown in Trypticase Soy Broth (BBL). After filtration, supernatants were tested for VT in a Vero cell test, for LT in a Yl adrenal cell test, and for STa in the infant mouse test as described by Scotland, Gross & Rowe (1985). To determine VT titres, fivefold dilutions of the cytotoxin were tested on Vero cell monolayers and these were examined after 4 days for cytotoxin effects. Neutralization tests were performed using antisera raised against partially purified VT1 and VT2 in rabbits as previously described (Scotland et al. 1988).

DNA probes and hybridization experiments. The VT1 probe was a HindII fragment of 0.75 kb specific for VT1 sequences obtained by cloning from a VT1
encoding phage carried by H19, *E. coli* O26:H11 (Willshaw et al. 1985). The VT2 probe was a 0.85 kb *Aam* I-*Pst* I fragment specific for VT2 sequences obtained by cloning from a VT2-encoding phage carried by *E. coli* strain E32511 of serotype O157:H1 (Willshaw et al. 1987). The CVD419 probe was a *Hind* III fragment of 3.4 kb cloned into pBR325 (Levine et al. 1987). The EAF probe was a *Bam* H1-*Sal* I fragment of 1.0 kb derived from pMAR22 which is a cloned derivative of the EAF plasmid pMAR2 (Baldini, Nataro & Kaper, 1986; Nataro et al. 1985). The probe fragments were cut from low-gelling temperature agarose gel and labelled by the random primer method (Feinberg & Vogelstein, 1984) using deoxyadenosine 5'-α-[35S]thiotriphosphate (Amersham International, Amersham, UK). Hybridization with the 35S-labelled probes was performed according to Maniatis, Fritsch & Sambrook (1982).

Non-radioactive alkaline phosphatase-labelled synthetic oligonucleotide probes were used for detection of ST A and LT genes (SNAP hybridization system, DuPont). The ST probe consisted of a mixed probe containing ST A1 and ST A2 sequences kindly provided by the manufacturer at our request. Hybridization and detection of probe positive colonies was carried out according to the protocols supplied with the SNAP hybridization kits NEP-010 and NEP-009 (DuPont).

**Plasmid DNA preparation, agarose gel electrophoresis and Southern hybridization.** Plasmid DNA was prepared by the alkaline extraction procedure of Birnboim & Doly (1979). The plasmid DNA extract was run on vertical 0.6% (w/v) agarose gels in TB buffer containing 89 mM-Tris base, 89 mM-boric acid and 2.5 mM-EDTA. Each strain was run at least two times. Using seven molecular weight (MW) standards, each observed plasmid band was assigned a corresponding MW and a mean MW was calculated for each plasmid. After treatment of gels with 0.25 M-HCl, plasmid DNA was transferred to Hybond N membrane by the method of Southern (1975) and hybridized with the CVD419 probe as described above.

**HEp-2 cell and Intestine 407 cell adhesion.** Each strain was tested for ability to adhere to HEp-2 cells and Intestine 407 cells in the presence of D-mannose by the method of Scotland, Gross & Rowe (1985) using a 6 h incubation period. Adherence of bacteria to these cells was classified as localized, diffuse or aggregative (Scaletsky, Silva & Trabulsi, 1984; Nataro et al. 1987). Localized adherence was observed and it was recorded as the percentage of HEp-2 or Intestine 407 cells with 10 or more adherent bacteria. Strains which adhered to less than 10% of the HEp-2 or Intestine 407 cells were considered non-adhesive for that cell line.

**HEp-2 cell test of invasiveness.** The test for ability of each strain to invade HEp-2 cells was as described by Scotland, Gross & Rowe (1985) using a 2 h infection period and a 3 h intracellular growth period. At least 300 healthy cells were examined and each cell containing more than one bacterium was counted as positive. Tests with more than 1% positive cells were considered positive. Tests with less than 1% positive cells were considered negative.

**Haemolysis test.** Each strain was streaked onto horse blood agar plates to obtain well separated individual colonies. After 18 h incubation at 37 °C, each plate was observed for a zone of haemolysis. If no zone was observed, the strain was considered non-haemolytic for horse blood.
**Human and animal VTEC other than O157: H7**

*Haemagglutination test.* The strains were grown overnight at 37 °C on CFA agar slopes and in Hedley–Wright broth. Each strain was tested in the presence and absence of mannose for haemagglutination of calf, human and guinea-pig erythrocytes. When strains were negative for haemagglutination on the first test, subcultures were made and retested through six passages to allow expression of haemaggutination.

**RESULTS**

*General properties of the strains.* The search of the DEP collection provided 8 VTEC strains from ill persons and 9 bovine and lamb VTEC strains with matching serogroups (Table 1). These strains were in serogroups O5, O55, O103, O111 and O153. None of the strains produced STₐ or LT, nor were they invasive. Three of the 8 human strains and 6 of the 9 bovine/lamb strains were sensitive to all of the antibiotics tested. All of the serogroup O103 strains were sensitive. The specific antibiotic resistances of each of the resistant strains are shown in Table 1. All strains of O5, O111 and O153 and 3 of the 4 serogroup O103 strains produced colicin.

The strains produced VT at titres ranging from 250 to 156250 (Table 1). Two strains of serotypes O5; H⁻ and O111ac:H⁻ from humans produced both VT1 and VT2. Of the other human strains, 3 produced VT1 and 3 produced VT2. All 9 of the bovine and lamb strains produced VT1. The VT1 antiserum completely neutralized the toxin of 4 of the VT1-producing strains. Incomplete neutralization of the VT1 antiserum was observed with toxin preparations from the remaining 8 VT1-producing strains: in 3 cases the VT titre was reduced by one dilution, in 4 cases by 2 dilutions and by 3 dilutions in the eighth preparation. The VT2 antiserum completely neutralized the filtrates containing VT2.

*Hybridization with DNA probes.* The hybridization of all 17 human and animal strains with either the VT1 probe, VT2 probe or both probes exactly matched the neutralization test results (Table 1). These results confirm the heterogeneity of VT production in the O5, O55, O111 and O153 serogroups. In contrast, hybridization with the CVD419 probe was very consistent within the serogroups (Table 2); all O5 and O103 strains were positive and all O55 and O153 strains were negative. The exception was serogroup O111 in which one strain was negative and the others were positive. None of the strains hybridized with the EAF probe.

The number and size of plasmids carried by the strains varied widely within and between serogroups (Table 2). All of the strains had one or more large plasmids. Plasmid DNA from the CVD419 probe positive strains was run in a single agarose gel and a Southern blot of this gel was hybridized with the CVD419 probe (Fig. 1). The plasmids hybridizing with the CVD419 probe were identified from the autoradiograph and their molecular weights, ranging from 42 x 10⁶ to 90 x 10⁶, are shown in Table 2.

*Adhesion to cells grown in tissue culture.* Four of the eight human strains and one of the nine animal strains adhered in a localized manner to more than 10% of HEp-2 and intestine 407 cells (Table 2). Strain E37450 caused detachment of tissue culture cells after 6 h, and so the adhesion test was ended after the 3 h attachment period. One of the strains, E31695, showed diffuse adhesion.
<table>
<thead>
<tr>
<th>Strain no.</th>
<th>Serotype</th>
<th>Hybridization with probes to VT</th>
<th>VT titres†</th>
<th>Source*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>VT1 VT2</td>
<td>Without antiserum With anti-VT1 With anti-VT2 With anti-VT1 and anti-VT2</td>
<td></td>
</tr>
<tr>
<td>E41787</td>
<td>O5:H7</td>
<td>+ +</td>
<td>156250</td>
<td>6250</td>
</tr>
<tr>
<td>S102-9</td>
<td>O5:H7</td>
<td>+ -</td>
<td>6250</td>
<td>250</td>
</tr>
<tr>
<td>E47411</td>
<td>O5:H7</td>
<td>+ -</td>
<td>6250</td>
<td>1250</td>
</tr>
<tr>
<td>E47412</td>
<td>O5:H7</td>
<td>+ -</td>
<td>1250</td>
<td>50</td>
</tr>
<tr>
<td>E40230</td>
<td>O55:H7</td>
<td>- +</td>
<td>1250</td>
<td>1250</td>
</tr>
<tr>
<td>E43938</td>
<td>O55:H10</td>
<td>- +</td>
<td>1250</td>
<td>1250</td>
</tr>
<tr>
<td>E37446</td>
<td>O55:H17</td>
<td>+ -</td>
<td>1250</td>
<td>250</td>
</tr>
<tr>
<td>S22-1</td>
<td>O103:H2</td>
<td>+ -</td>
<td>6250</td>
<td>250</td>
</tr>
<tr>
<td>E42724</td>
<td>O103:H2</td>
<td>+ -</td>
<td>1250</td>
<td>250</td>
</tr>
<tr>
<td>E47406</td>
<td>O103:H2</td>
<td>Calf, Healthy</td>
<td>1250</td>
<td>0</td>
</tr>
<tr>
<td>E47409</td>
<td>O103:H2</td>
<td>Calf, Healthy</td>
<td>1250</td>
<td>0</td>
</tr>
<tr>
<td>E45035</td>
<td>O111ac:H-</td>
<td>Human, HUS</td>
<td>6250</td>
<td>50</td>
</tr>
<tr>
<td>E48973</td>
<td>O111ac:H-</td>
<td>Human, HUS</td>
<td>6250</td>
<td>50</td>
</tr>
<tr>
<td>E40867</td>
<td>O111ac:H-</td>
<td>Calf, D</td>
<td>6250</td>
<td>250</td>
</tr>
<tr>
<td>E40875</td>
<td>O111ac:H-</td>
<td>Calf, D</td>
<td>6250</td>
<td>0</td>
</tr>
<tr>
<td>E31695</td>
<td>O155:H25</td>
<td>Human, HUS</td>
<td>1250</td>
<td>1250</td>
</tr>
<tr>
<td>E37450</td>
<td>O153:H12</td>
<td>Calf, D</td>
<td>250</td>
<td>0</td>
</tr>
</tbody>
</table>

* HUS, haemolytic uraemic syndrome; HD, haemorrhagic diarrhoea; D, diarrhoea.
† Toxin titrations were done at least twice.
Table 2. Properties of human and animal VTEC strains, including plasmid profiles and adhesiveness

<table>
<thead>
<tr>
<th>Serogroup and no.*</th>
<th>Plasmid MW (x 10^6)†</th>
<th>Drug resistance‡</th>
<th>Colicin production</th>
<th>Hybridization with CVD419</th>
<th>Adhesion tests§</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>HEp-2</td>
<td>Intestine 407</td>
</tr>
<tr>
<td>O5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E41787</td>
<td>H 65, 42, 23, 2 ≤ 4 (x 2)</td>
<td>Ap, Me, Sm, Su</td>
<td>+</td>
<td>+</td>
<td>61 44</td>
</tr>
<tr>
<td>S102-9</td>
<td>C 59, 53, 46, 35, 4-4, ≤ 4</td>
<td>Sm, Su</td>
<td>+</td>
<td>+</td>
<td>41 18</td>
</tr>
<tr>
<td>E47411</td>
<td>L 89, 4-9, ≤ 4 (x 2)</td>
<td>None</td>
<td>+</td>
<td>+</td>
<td>0 0</td>
</tr>
<tr>
<td>E47412</td>
<td>L 90, 51, ≤ 4</td>
<td>None</td>
<td>+</td>
<td>+</td>
<td>2 0</td>
</tr>
<tr>
<td>O55</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E40230</td>
<td>H 60, 42, 39, 6-4, ≤ 4 (x 2)</td>
<td>Ap, Ce, Me, Sm, Su</td>
<td>-</td>
<td>-</td>
<td>40 49</td>
</tr>
<tr>
<td>E43938</td>
<td>H 70, 60, ≤ 4</td>
<td>Ap, Sm, Su</td>
<td>-</td>
<td>-</td>
<td>4 0</td>
</tr>
<tr>
<td>E37446</td>
<td>C 72</td>
<td>None</td>
<td>-</td>
<td>-</td>
<td>2 3</td>
</tr>
<tr>
<td>O103</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S22-1</td>
<td>H 51, 44</td>
<td>None</td>
<td>-</td>
<td>+</td>
<td>22 25</td>
</tr>
<tr>
<td>E42724</td>
<td>H 59, 37, 4-5</td>
<td>None</td>
<td>+</td>
<td>+</td>
<td>8 3</td>
</tr>
<tr>
<td>E47406</td>
<td>C 58, 4-3</td>
<td>None</td>
<td>+</td>
<td>+</td>
<td>5 0</td>
</tr>
<tr>
<td>E47409</td>
<td>C 59, 4-2</td>
<td>None</td>
<td>+</td>
<td>+</td>
<td>5 2</td>
</tr>
<tr>
<td>O111</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E45035</td>
<td>H 48, 4-8</td>
<td>None</td>
<td>+</td>
<td>+</td>
<td>70 41</td>
</tr>
<tr>
<td>E48973</td>
<td>H 59, 24, 4-3</td>
<td>Sm</td>
<td>+</td>
<td>+</td>
<td>0 1</td>
</tr>
<tr>
<td>E40867</td>
<td>C 67, 55, ≤ 4 (x 2)</td>
<td>Cm, Sp, Sm, Su</td>
<td>+</td>
<td>+</td>
<td>2 1</td>
</tr>
<tr>
<td>E40875</td>
<td>C 67, 60, 48, ≤ 4</td>
<td>Sp, Sm, Su, Tc</td>
<td>+</td>
<td>+</td>
<td>0 0</td>
</tr>
<tr>
<td>O153</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E31965</td>
<td>H 63, 55, 24, 6-5</td>
<td>Ce, Sm, Su</td>
<td>+</td>
<td>-</td>
<td>Diffuse</td>
</tr>
<tr>
<td>E37450</td>
<td>C &gt; 100, 63, 31, 4-4</td>
<td>None</td>
<td>+</td>
<td>-</td>
<td>Diffuse</td>
</tr>
</tbody>
</table>

* Listed in same order as Table 1; H, Human; C, Calf; L, Lamb.
† Italicized molecular weights represent plasmids hybridizing with the CVD419 probe.
‡ Bacterial strains were resistant to the listed antibiotics: Ap, ampicillin; Ce, cephaloridine; Me, meccillin; Sp, spectinomycin; Sm, streptomycin, Su, sulfathiazole; Tc, tetracycline.
§ Number of cells with 10 or more locally adherent bacteria per 100 cells examined; 6 h test except for strain E37450 (see text); all tests performed at least twice.
Haemolysis and haemagglutination. None of the strains was haemolytic on horse blood agar after 24 h at 37 °C. Thirteen strains grown on either CFA slopes or Hedley–Wright broth, haemagglutinated guinea-pig erythrocytes only in the absence of mannose indicating production of type 1 fimbriae. Two strains (E47411 and E47412) grown on CFA slopes haemagglutinated calf erythrocytes in the presence of mannose (MRHA). Strains E31695 and E37450 (both 0153) grown either on CFA slopes or in Hedley-Wright broth, haemagglutinated human erythrocytes in the presence and absence of mannose. Strain E40875 grown in Hedley-Wright broth demonstrated MRHA of human erythrocytes only after six passages. MSHA and MRHA did not correlate with adherence to tissue culture cells or with hybridization with the CVD419 probe.

DISCUSSION

This study documents properties of some of the diverse serogroups of VTEC infecting humans and animals. Selection for study of only VTEC from ill persons which had one or more matching bovine or lamb VTEC in the DEP collection, limited this series to five serogroups. These serogroups were quite diverse in that they included two serogroups, O55 and O111, to which enteropathogenic E. coli may belong and one serogroup, O153, to which enterotoxigenic E. coli may belong. Within some of these serogroups only one common serotype was found for both the human and animal isolates (i.e. O5:H−, O103:H2 and O111ac:H−). Although the numbers are small, this observation of common serotypes of the human and animal isolates is similar to the finding of the common serotype O157:H7 isolated from humans and cattle during HC outbreak investigations (Martin et al. 1986; Borczyk et al. 1987; Wells et al. 1983). In addition, enzyme typing indicated phenotypic similarity of strains of the serotype O157:H7 isolates from humans and animals in North America indicating that they probably represent a single clone (Whittam et al. 1988). The presence of a single 60 MDa plasmid in most
serotype O157:H7 strains and hybridization with the CVD419 probe (Karch et al. 1987; Scotland et al. 1987) further support the clone theory for this serotype. Nevertheless, O157:H7 strains differ in types of VT produced, plasmid profiles and colicin production (Scotland et al. 1987) and numerous phage types have been recognized (Ahmed et al. 1987). In most respects the properties of the strains studied in the present communication indicated that all strains of the same serogroup were not closely related in origin. A large number of plasmids of different molecular weight were found within a single serogroup and, in particular, the plasmid hybridizing in the Southern blot with the CVD419 probe differed in size also within a single serogroup. For example in serogroup 05 the hybridizing plasmids in human and calf strains had molecular weights of c. 44 x 10^6 whereas in strains from lambs the plasmids were c. 90 x 10^6. The different dates that the strains were received in the DEP, and assumed different dates of isolation, may explain some of this genetic diversity.

The VT2 antiserum completely neutralized the three filtrates containing only VT2, but the VT1 antiserum did not completely neutralize 8 of the 12 filtrates containing only VT1. Further experiments are needed to determine whether the incomplete neutralization indicates differences in the properties of VT1 produced by different strains as noted for VT2 of human and porcine origin (Marques et al. 1987).

In experiments with gnotobiotic calves, strain S102-9 (O5:H~) attached to the intestinal mucosa and effaced microvilli so that closely adherent bacteria were associated with cup-like depressions or pedestals (Hall et al. 1985). Strains of E. coli O157:H7 have also been shown to attach to and efface the intestinal mucosa in piglets (Tzipori et al. 1986). The CVD419 probe was derived from a 60 x 10^6 plasmid carried by a strain of serotype O157:H7 and was reported to be associated with fimbriae production and adherence of bacteria in small numbers to Intestine 407 cells (Karch et al. 1987). However, two O157 VTEC strains lacking the 60 x 10^6 plasmid still showed attachment and effacement (Tzipori et al. 1987). Thus, the role of this plasmid in the virulence of O157 strains remains to be demonstrated. Strains of serogroup O111 were reported by Levine et al. (1987) to hybridize with the CVD419 probe. In the present study, 3 of the 4 strains of serotype O111ac:H~ hybridized and one strain did not hybridize with the CVD419 probe. Strains of serogroups O5 and O103 also hybridized with the CVD419 probe. Five strains attached to cells grown in tissue culture in a localized manner. There was no correlation between localized adhesion and hybridization with the CVD419 probe as, although 4 of the 5 strains giving localized adhesion hybridized with the CVD419 probe, so did 7 non-adhering strains. For enteropathogenic E. coli, localized attachment is associated with hybridization with the EAF probe (Nataro et al. 1985) but none of the VTEC gave such hybridization. It remains to be determined whether the adhesive ability of the VTEC demonstrated by attachment to tissue culture cells has any role in virulence. Clearly more basic information is needed about virulence properties of VTEC, other than VT production, and whether these properties are shared by human and animal pathogens.

The non-O157:H7 VTEC strains described in this paper showed considerable diversity in their properties. Within a serogroup there were differences between
strains of human and animal origin with respect to VT production, plasmid profiles and adhesive properties as judged by attachment to tissue culture cells or haemagglutination. These observations give no strong support to the hypothesis that non-O157:H7 VTEC strains of the same clonal origin are responsible for disease of man and animals, although the number of strains examined was small and they were not isolated from humans and animals during related episodes of disease.

ACKNOWLEDGEMENTS

The assistance of Dr H. Chart and Ms B. Said in the adhesion tests, Dr M. McConnell in the haemagglutination tests and Mr R. Gross in the serotyping is greatly appreciated. This investigation was supported in part by a Fogarty Senior International Fellowship awarded to C. R. Dorn by the National Institutes of Health, Bethesda, Maryland.

REFERENCES


Hampson, D. J., Bettleheim, K. A., Winter, P. J., Marshall, R. B. & Wilson, M. W.
Human and animal VTEC other than O157:H7


Human and animal VTEC other than O157 : H7


