

A longitudinal study of Vero cytotoxin producing *Escherichia coli* in cattle calves in Sri Lanka

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SUMMARY

Two cohorts of 10 and 16 calves were followed at weekly or fortnightly intervals from 4–28 and 1–9 weeks respectively to determine whether natural infection by Vero cytotoxin (VT) producing *Escherichia coli* (VTEC) occurred. Ninety-one of 171 (53%) faecal specimens were VTEC positive and 20–80% of animals at any given time excreted VTEC. Of 104 VTEC strains studied further, 6 different serogroups (O 22.H16; O 25.H5; O 49.H–; O 86.H26; O 88.H25; O 153.H12) and an untypable strain (O ? .H21) were identified. All strains belonging to the same serotype had identical profiles of reactivity with DNA probes to toxins VT1 or 2, LTI or II and a probe (CVD419) derived from a plasmid carried by enterohaemorrhagic *Escherichia coli* O 157.H7. Four of these serotypes were found in the faecal flora of the calves, taken as a group, throughout the 4-month study period. Sixty percent of the strains hybridized with the probe for VT1, 4% with the probe for VT2, and 36% with both probes. Faecal VTEC were significantly associated with overt diarrhoeal illness in animals < 10 weeks of age, but no characteristic profile of markers (serotype or hybridization pattern) in *E. coli* isolates was associated with diarrhoea. A serological response to VT1 was detected in some animals, but faecal VT1 VTEC excretion persisted in spite of seroconversion. VT1 seroconversion was not associated with diarrhoea. A serological response to VT2 was not detected even in those animals excreting VT2 VTEC in the faeces.

INTRODUCTION

The detection of Vero cytotoxin (VT), a toxin of *Escherichia coli*, was first reported by Konowalchuk and colleagues [1]. Subsequent studies have shown that VT is antigenically and biochemically related to Shiga toxin of *Shigella dysenteriae* [2] and has also been called Shiga-like toxin (SLT). The VTs of *E. coli* are heterogeneous, two major groups being recognized, namely VT1 (SLTI) and VT2 (SLTII) [3].

Vero cytotoxin producing strains of *E. coli* (VTEC) are a cause of haemorrhagic

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colitis [2, 4, 5] and the haemolytic uraemic syndrome in man [6, 7], and also of pig oedema disease [8]. More tentative associations have been made between VTEC and diarrhoea in calves [9], piglets [10] and cats [11].

The epidemiology of VTEC in cattle is relevant not only from a veterinary point of view, but also because VTEC associated outbreaks of human disease have often been food borne [4, 12]. Many VTEC outbreaks have been attributed to the serotype O 157.H7, and therefore many epidemiological studies have been focused exclusively on this serotype. However, human illness caused by other serotypes has been well documented [13]. The study reported here was initiated with the aim of detailing the epizootiology of natural infection by VTEC, of any serotype, in cohorts of cattle in a single farm over time, and to study the serological response to VT toxins following natural infection.

MATERIALS AND METHODS

Animals studied

Two cohorts of Holstein cattle calves from a dairy in the Kandy district were studied between September 1986 and February 1987. The first cohort of 10 animals was monitored from the age of 4 weeks, with faecal samples collected at 4–10, 12–14, 16, 18, 20, 24 and 28 weeks of age. The second cohort of 16 animals was followed from the age of 1 week with faecal samples at 1–3, 5, 7 and 9 weeks of age. Blood samples were collected at fortnightly intervals during the period of follow up from 24 of the 26 animals. At each visit to the dairy, the clinical status of the animals was noted, with particular reference to diarrhoea.

Assays for VT and heat labile (LT) and heat stable (ST) toxins

Faecal specimens were cultured on MacConkey agar. Colonies morphologically typical of *E. coli* on the primary culture plate were screened for VT toxin production at the University of Peradeniya laboratory as follows. Three colonies were inoculated into Syncase broth and, after overnight incubation, the culture supernatants were tested for VT activity by a microtitre adaptation [9] of the method described by Konowalchuk and others [1]. All supernatants giving VT-like cytopathic effects were tested for lability to heat at 100 °C for 10 min.

Preliminary studies were done on 22 faecal specimens where 10 colonies of *E. coli* from each primary culture plate were tested for VT. Of 11 faecal specimens yielding one or more VTEC colonies, the number of positive colonies was in the range 1–10 per 10 colonies tested. Nine of 11 positive specimens had > 2 positive colonies per 10 colonies tested. On the basis of this distribution of VTEC positive colonies, the testing of three colonies per specimen would detect 92% of specimens detected by the testing of 10 separate colonies. For logistical reasons, the number of colonies tested from each faecal specimen in the subsequent study was therefore limited to three, unless otherwise specified.

A random selection of *E. coli* isolates (134 strains from 13 diarrhoeal and 44 non-diarrhoeal stools) found to be VTEC positive in the University of Peradeniya laboratory and stored either frozen in 15% glycerol or on sugar free agar slopes at 4° for 12–18 months, were further studied at the Division of Enteric Pathogens (DEP), Central Public Health Laboratory, Colindale, London. In general, a single VTEC positive strain from each stool was studied. A limited number of stools were

investigated more intensively by studying multiple colonies of VTEC to determine the diversity of VTEC strains in a single faecal specimen. At the DEP the bacterial strains were grown in Trypticase Soy Broth (BBL), and the filtered supernatants were retested for VT in a Vero cell test, and further tested for LT, in a Y1 adrenal cell test, and for heat stable toxin (ST) in the infant mouse test [14]. Rounding of Y1 cells indicated the presence of LT and this was tested for neutralization by antisera specific for LTI or LTII as described by Holmes and colleagues [15]. The antiserum against LTII was kindly provided by R. K. Holmes, Uniformed Services University for the Health Sciences, Maryland, USA.

The strains were serotyped, and hybridization with gene probes for VT1, VT2, LTI, LTII and the CVD419 plasmid was carried out (see below).

Detection of serum antibodies to VT1 and VT2

Fifty μ l of a 1/10 dilution of test serum was mixed with an equal volume of culture supernatants of VT1-producing *E. coli* H19 or VT2-producing *E. coli* E32511 diluted to give four toxin units. One toxin unit was defined as the highest dilution of supernatant producing a cytotoxic effect on > 25% of the cell monolayer. The mixture was incubated for 2 h (37 °C in 5% CO₂), and tested for residual toxin activity as described above. A serum was considered to have antibody to the respective toxin if the cytotoxic effect on the cell sheet was completely abolished. A toxin 'back titration' was included in each experiment. Selected positive sera were titrated to determine the antibody titre defined as the reciprocal of the highest dilution that neutralized four toxin units.

DNA hybridization tests

Strains were tested by colony hybridization [16]. The VT1 probe was a 0.75 kb *Hinc* II fragment of NTP705 that was derived from a VT1-encoding phage in *E. coli* H19 [17]. The VT2 probe was a 0.85 kb *Sma* I-*Pst* I (equivalent to *Ava* I-*Pst* I) fragment of NTP707 derived from the VT2-encoding phage of *E. coli* E32511 [3]. Strains were tested for the presence of LTI sequences with a 1.2 kb *Hinc* II fragment of plasmid EWD299 [18] and for LTII genes with a probe comprising a 0.8 kb *Hind* III-*Pst* I fragment of plasmid pCP2725 [19]. The LTII producing strains were also tested with a probe developed by Dr C. Clayton. This probe comprised a 1.1 kb *Pst* I-*Mlu* fragment of the LTII recombinant plasmid pCLC158 derived from strain 357900-Z [20, 21]. Strains were also tested with a 3.4 kb *Hind* III fragment, the CVD419 probe, which was derived from the plasmid present in strains of *E. coli* O 157.H7 [22]. All probes were radioactively labelled by the random primer method [23]. Hybridization and washing were at high stringency conditions [16].

Serotyping

The O and H antigens of *E. coli* were determined using antisera for O groups O 1-O 170 and for flagellar antigens H1-H56 [24].

RESULTS

One hundred and seventy-one faecal specimens were studied, of which 91 (53%) contained VTEC. At any given visit to the dairy, the prevalence of VTEC in the study population was in the range 20-80% without a particular age predominance.

Table 1. *Prevalence of VTEC in a cohort of 26 calves studied for a period of 2-6 months*

| Age of calves at sampling (weeks) | No. of animals sampled | Diarrhoeal | | Healthy | |
|---|------------------------------|------------|-----------|---------|-----------|
| | | Total | VTEC + ve | Total | VTEC + ve |
| 1 | 16 | 0 | 0 | 16 | 7 |
| 2 | 16 | 1 | 1 | 15 | 7 |
| 4 | 26 | 10 | 7 | 16 | 8 |
| 6 | 26 | 10 | 8 | 16 | 4 |
| 8 | 26 | 1 | 1 | 25 | 12 |
| 10 | 10 | 1 | 1 | 9 | 1 |
| 12 | 10 | 1 | 0 | 9 | 8 |
| 14 | 10 | 1 | 0 | 9 | 6 |
| 16 | 10 | 0 | 0 | 10 | 8 |
| 18 | 10 | 0 | 0 | 10 | 7 |
| > 20 | 10 | 0 | 0 | 10 | 5 |
| Total | | 25 | 18 | 145 | 73 |

The overall isolation of VTEC from the animals in relation to diarrhoeal status and age is shown in Table 1. The peak age for diarrhoeal illness was at 4-6 weeks of age. Of the 25 specimens obtained from animals with diarrhoea, 18 (72%) were VTEC positive, whilst 73 of 146 (50%) of the samples from animals without diarrhoea at the time of sampling were VTEC positive. The association of VTEC with diarrhoeal symptoms in the study population overall was not statistically significant ($\chi^2 = 3.3$; $P = 0.069$). However, when animals < 10 weeks of age are considered, 72% (18/25) of diarrhoeal animals excreted VTEC compared with 40% (39/97) non-diarrhoeal animals. In this age group, the association of VTEC with diarrhoea of calves was significant ($\chi^2 = 6.9$; $P = 0.009$).

Of the 134 strains studied at the DEP, 104 were still positive for VT by Vero cell assay and DNA probes (Table 2). It is possible that the remaining 30 strains had lost VT activity during prolonged storage and subculture (see below). It is likely that the initial designation of the relevant stool sample as containing VTEC was correct, because on testing at Peradeniya soon after the original isolation, the culture supernatants produced a characteristic cytopathic effect on Vero cells that was heat labile. Further, three of the strains found to have 'lost' VT activity were among 23 randomly selected VTEC culture supernatants tested for neutralization by Shiga antitoxin (kindly provided by Dr A. D. O'Brien, Maryland, USA). All three supernatants were neutralized by the Shiga antitoxin, supporting the view that the initial designation of these three faecal specimens at least, as VTEC positive was correct. In any event, of the strains giving discrepant results between the two laboratories, half were from faecal specimens in which other *E. coli* isolates were confirmed to be VTEC at both laboratories. The association of faecal VTEC isolation and diarrhoea in animals < 10 weeks of age still remains statistically significant even if the discrepant isolates are regarded as VTEC negative.

Table 2 shows the numbers of isolates, serotypes and DNA probe results on the 104 strains confirmed as VT positive at DEP. All strains of a particular serotype gave identical results in hybridization and toxin tests. All strains hybridizing with the VT1 and/or VT2 probes were positive in the Vero cell test and vice versa. Sixty-three strains (60%) hybridized with the VT1 DNA probe, 4 (4%) were VT2

Table 2. Virulence markers of confirmed VTEC strains

| No. of strains | Serotype | Hybridization with DNA probe for | | | Production of LTII |
|----------------|------------|----------------------------------|-----|--------|--------------------|
| | | VT1 | VT2 | CVD419 | |
| 19 | O 22. H16 | + | - | + | - |
| 1 | O 25. H5 | - | + | - | + |
| 3 | O 49. H- | - | + | + | - |
| 38 | O 86. H26 | + | - | - | - |
| 1 | O 88. H25 | + | + | + | - |
| 36 | O 153. H12 | + | + | - | - |
| 6 | O ? . H21 | + | - | + | - |

Table 3. Serotypes and DNA probe results on selected faecal specimens where multiple isolates were studied

| Animal | Specimen date | No. of strains | Serotype | | Probe results |
|--------|---------------|----------------|----------|---------|---------------|
| | | | O | H | |
| 3222 | 15 Dec. 86 | 1 | 153 | 12 | VT1/VT2 |
| | | 1 | 22 | 16 | VT1/CVD419 |
| | 1 Jan. 87 | 3 | 22 | 16 | VT1/CVD419 |
| 3236 | 15 Jan. 87 | 2 | 86 | 26 | VT1 |
| | | 3 | 153 | 12 | VT1/VT2 |
| | 30 Nov. 86 | 4 | 86 | 26 | VT1 |
| | | 3 | 153 | 12 | VT1/VT2 |
| | 15 Dec. 86 | 4 | 86 | 26 | VT1 |
| 4 | | 153 | 12 | VT1/VT2 | |
| 5278 | 30 Nov. 86 | 4 | 86 | 26 | VT1 |
| | | 1 | 153 | 12 | VT1/VT2 |
| | 15 Dec. 86 | 7 | 86 | 26 | VT1 |
| 7 | | 153 | 12 | VT1/VT2 | |
| 5279 | 1 Jan. 87 | 1 | 86 | 26 | VT1 |
| | | 3 | 153 | 12 | VT1/VT2 |
| 5280 | 30 Nov. 86 | 3 | 86 | 25 | VT1 |
| | | 2 | 153 | 12 | VT1/VT2 |

positive, and 37 (36%) were positive for both VT1 and VT2 (Table 2). Four of the 8 confirmed VTEC serotypes (29 strains) hybridized with the CVD419 probe. None of the strains produced STa. One serotype (O 25. H5) was also positive in the Y1 adrenal cell test for LT. Of the 30 strains that had lost VT activity, 6 were positive by the CVD419 probe, and 7 were positive for LT toxin.

All eight strains positive in the Y1 adrenal cell test had this activity neutralized by antiserum against LTII but not by antiserum against LTI. One of these strains still retained its VT genes. The strains did not hybridize with the LTI probe. Only 3 of the 8 strains showing Y1 cell activity hybridized with the 0.8 kb *Hind* III-*Pst* I LTII probe but all 8 strains hybridized with the 1.1 kb *Pst* I-*Mlu* probe.

Where several VTEC isolates from a single faecal specimen were tested, simultaneous excretion of different serotypes of VTEC could sometimes be demonstrated (e.g. calf 3236, calf 5278, Table 3). Some animals (e.g. calf 3205; Table 4) shed a single VTEC serotype through 24 weeks follow up with apparently

Table 4. *Isolation of VTEC, serotype, DNA probe results and VT1/2 serostatus*

| | Age (wks) | | | | | | | | | |
|-----------------|-----------|----|-----|----|-----|-----|----|----|----|----|
| | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
| Calf no. 3205 | | | | | | | | | | |
| VTEC isolated | + | - | - | + | - | - | - | - | - | + |
| VT DNA probe | 1 | | | nd | | | | | nd | |
| O group | 22 | | | nd | | | | | | nd |
| H type | 16 | | | nd | | | | | | nd |
| Antibody to VT1 | + | | + | | + | | + | | + | |
| Antibody to VT2 | - | | - | | - | | - | | - | |
| Diarrhoea | + | - | - | - | - | - | + | | - | - |
| Calf no. 3208 | | | | | | | | | | |
| VTEC isolated | - | - | + | + | - | - | - | | - | |
| VT DNA probe | | | 1+2 | nd | | | | | | |
| O group | | | 153 | nd | | | | | | |
| H type | | | 12 | nd | | | | | | |
| Antibody to VT1 | - | | + | | + | | + | | + | |
| Antibody to VT2 | - | | - | | - | | - | | - | |
| Diarrhoea | - | + | + | + | - | - | - | | - | |
| | Age (wks) | | | | | | | | | |
| | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 24 | 28 | |
| Calf no. 3205 | | | | | | | | | | |
| VTEC isolated | + | | + | | + | | - | - | - | |
| VT DNA probe | nd | | 1 | | 1 | | | | | |
| O group | nd | | 22 | | 22 | | | | | |
| H type | nd | | 16 | | 16 | | | | | |
| Antibody to VT1 | + | | + | | | | | ± | | |
| Antibody to VT2 | - | | - | | | | | - | | |
| Diarrhoea | - | | - | | - | | - | - | - | |
| Calf no. 3208 | | | | | | | | | | |
| VTEC isolated | | | + | + | | + | + | - | + | |
| VT DNA probe | | | nd | 1 | 1+2 | 1+2 | nd | | nd | |
| O group | | | nd | 22 | 153 | 88 | nd | | nd | |
| H type | | | nd | 16 | 12 | 25 | nd | | nd | |
| Antibody to VT1 | + | | + | | | | | | + | |
| Antibody to VT2 | - | | - | | | | | | - | |
| Diarrhoea | - | | - | - | | - | - | - | - | |

VTEC negative cultures in between. On the other hand, many animals excreted different VTEC genotypes as well as serotypes during the period of follow up. For example calf 3208 shed three VTEC serotypes (O 153.H12 VT1 and 2; O 22.H16 VT1 and O 88.H25 VT1 and 2) within a 15 week period (Table 4).

The VTEC serotypes (confirmed at DEP) circulating in the dairy during the study period are shown in Table 5. A number of VTEC serotypes (O 22.H16; O 86.H26, O 153.H12) were found right through the 5 month period of the study.

Of the 24 calves monitored serologically at fortnightly intervals, 12 had antibodies to VT1 in the first sample tested. Of these, 6 animals remained seropositive throughout the study period, while the other 6 calves became seronegative by the age of 2-23 weeks, presumably due to a loss of maternal antibody. Of the latter, 3 animals remained seronegative, whilst the other calves seroconverted subsequently. Of the total of 12 calves seronegative at the

Table 5. VTEC serotypes isolated from calves in relation to time

| Serotype | Date of specimen collection | | | | | | | |
|------------|-----------------------------|------------|------------|------------|------------|------------|-----------|------------|
| | 1986 | | | | | 1987 | | |
| | 1 Sep. | 18 Sep. | 15 Nov. | 23 Nov. | 30 Nov. | 15 Dec. | 1 Jan. | 15 Jan. |
| O 22. H16 | | 1 | | | | 6 | 6 | 1 |
| O 25. H5 | | | | | | 1 | | |
| O 49. H- | | | | | | | 2 | 1 |
| O 86. H26 | 1 | 1 | | | 3 | 2 | 5 | |
| O 88. H25 | | | | | | | 1 | |
| O 153. H12 | 1 | 1 | 4 | 1 | 3 | 6 | 3 | |
| O ? . H21 | | | 1 | | 1 | | 1 | 2 |

commencement of the study, 9 seroconverted during the course of this study, while 3 animals remained seronegative throughout the study. At the end of the study, 18 calves of the total 24 tested, had antibodies to VT1. Neutralizing antibody titres to VT1 ranged from 80–640. While in some instances, seroconversion to VT1 could be associated with VT1 VTEC in the faeces (calf 3208) (Table 4), in other instances the animal remained seronegative in spite of prolonged excretion of VT1 VTEC. Conversely, VTEC excretion occurred in the face of serum antibody to VT1 (e.g. calf 3205) (Table 4). There was no correlation between seroconversion and diarrhoea in the calves studied.

None of the calves studied at any stage of the study showed antibodies to VT2, although many animals (e.g. calf 3208) (Table 4), shed VT2 producing *E. coli* in the faeces.

DISCUSSION

Previous studies on the prevalence of VTEC in cattle have found 4–19% of animals in temperate countries such as the United States of America and Germany to be infected at any given time [25, 26]. In tropical countries, such as Sri Lanka and Thailand, VTEC have been found in 11–84% of faecal samples from cattle [9, 27]. However, no longitudinal studies on VTEC infections in cattle have previously been reported. The data obtained in our study showed that VTEC are shed very frequently in the faeces of calves; at any given time 20–80% of the calves excreted VTEC.

Of the 134 VTEC strains studied further at the DEP, London, 104 were still VTEC positive, but 30 strains were VT negative. It is likely that these strains lost VT activity due to prolonged storage and subculture (more than one year) between initial isolation and their dispatch to the DEP. This view is supported by the following. Firstly, six of these strains were still positive for the CVD419 marker which is associated with VTEC, though not invariably so [22]. The CVD419 marker is rare in non-VT producing *E. coli*. Secondly, 3 strains were among 30 strains tested soon after primary isolation for toxin neutralization with Shiga antitoxin. The Vero toxic effects of all three were neutralized by Shiga antitoxin confirming their initial designation as VTEC. Finally, frequent loss of VT genes in clinical isolates of VTEC when grown in laboratory culture has recently been documented. Karch and colleagues [28] reported that 15 of 45

isolates from human faecal specimens lost VT activity and genes following a single subculture. The proportion of isolates (30 of 134) that lost VT activity in the present study is comparable.

A given VTEC serotype always exhibited the same profile when tested by DNA probes for VT1, VT2, CVD419 and LTII (Table 2). For example, 38 isolates of O 86.H26 obtained from 12 faecal specimens collected at different times from 7 animals were all VT1 probe positive, but negative for VT2, LTI/II and CVD419, while 36 isolates of O 153.H12 from 19 faecal samples collected from 8 calves were all exclusively VT1 and VT2 positive. In contrast, of 37 strains of VTEC serotype O 26.H11 isolated from humans and cattle in the UK between 1977 and 1989, there was diversity in relation to the VT1/2 and CVD419 profile [29].

When multiple isolations of the same serotype from one faecal specimen were excluded, over half of the isolates were VT1-producing strains, while most of the remainder had DNA encoding for both VT1 and VT2. Only 4/55 (7%) of strains hybridized exclusively with the VT2 gene probe. Wells and colleagues [25] also found all three combinations of VTEC (VT1, VT2, VT1/VT2) in dairy cattle in the USA.

In the present study, 4 confirmed VTEC serotypes (Table 2) were CVD419 positive. In addition, 5 other serotypes/untypable strains (O 2.H25, O 108.H25, O 145.H-, O ?.H11, O ?.H12) which were VT positive at primary isolation but VT negative subsequently, were CVD419 positive. It has been suggested that the CVD419 probe may be a marker of VTEC that are pathogenic to man [30], but this requires further confirmation.

One confirmed VTEC strain and 7 other strains that had lost VT activity produced LTII toxin. Two probes for LTII genes were used in this study because the *Hind* III-*Pst* I probe used by Pickett and colleagues [19] detected only 3 of 8 LTII producing strains. Tests with the *Pst* I-*Mlu* probe developed by Clayton and co-workers [20] detected all 8 LTII strains suggesting that the latter probe may be more useful in the detection of LTII *E. coli*.

In a previous study where cattle and buffalo farms in different areas of Sri Lanka were sampled, a total of 27 O serogroups of VTEC were documented [31]. While four of these serogroups were found in the dairy studied here, only one was an identical serotype (O 86.H26). Wells and others [25] also found a diversity of VTEC serotypes in 22 farms in Wisconsin and Washington, USA. In our present study, seven confirmed VTEC serotypes were found in one farm during a 5-month period (Table 5). At least four of these (O 22.H16, O 86.H26, O 153.H12 and O ?.H21) were repeatedly isolated right through the period studied, and appeared to be part of a stable resident gut flora in the calves. This is in marked contrast to the high turnover of *E. coli* strains found to occur in the calf intestinal flora by Hinton and colleagues [32]. In a longitudinal study they found 416 distinct strains over a 21 day period, 75% of these being only detected once, pointing to a continual turnover of *E. coli* strains in the calf gut.

Each VTEC serotype gave an identical profile of reactivity with the DNA probes. It is possible that all isolates of a VTEC serotype were clonally derived. It is noteworthy that of two Sri Lankan goat and one porcine VTEC strains similarly studied at DEP (unpublished data), one strain was serotype O 25.H5 and had an identical profile of reactivity in the DNA hybridization test (VT2/LT2) to that

found in cattle in the present study (Table 2). The strain was isolated from a goat in a farm over 50 miles away. However, the farm in question is a breeding and distribution site for goat husbandry in Sri Lanka, and there were small numbers of goats in the vicinity of the dairy we have studied herein. The possibility of transmission of a VTEC 'clone' by the movement of animals remains possible.

While VT1 neutralizing antibody response has been documented in human serum [33, 34] little or no evidence of VT2 neutralizing antibody was found. In one study [34] there was evidence of low titre non-specific VT2 neutralizing activity which was not immunoglobulin mediated. There have been no studies on serological responses to VTEC infections in cattle. In the present study, VTEC infection of calves by VT1 producing strains appears to evoke a serum antibody response to VT1, though this is by no means invariable. Further, faecal shedding of VT1 producing *E. coli* can persist in spite of detectable serum antibody to VT (Table 4). While *E. coli* carrying VT2 genes appear to evoke little or no serum antibody response to VT2 (of strain E32511) (Table 4), there was no evidence of 'non-specific' neutralizing activity noticed in this study. The reasons for a lack of an antibody response to VT2 could be many. Possibilities include VT2 being less immunogenic than VT1; VT2 VTEC producing less tissue invasion than VT1 VTEC; or that the VT2 produced by E32511 (used in the toxin neutralization test in this study) is antigenically different from the VT2 infecting cattle. Schmitt and colleagues [35] have recently found that the strain E32511 produces two genetically distinct Verotoxins designated SLT II and SLT IIc. The two toxins had differences in the nucleotide sequences of their B sub unit genes, and were antigenically related, but distinct.

A previous study in Sri Lanka had noted an association between VTEC and calf diarrhoea [9]. Hall and co-workers [36] reported dysentery in calves associated with *E. coli* (strain S102-9) found to be serogroup O 5.H- and to produce VT1. There have been no other published epidemiological studies on an association of VTEC and calf diarrhoea. In experiments with gnotobiotic calves [36], *E. coli* strain S102-9 attached to the intestinal mucosa and effaced microvilli, and reproduced a bloody diarrhoea. The present study was an attempt to clarify an association between VTEC and calf diarrhoea through a longitudinal study, and is the only such study reported to date. Faecal VTEC excretion was not inevitably correlated with disease in the animals, though a possible correlation with diarrhoea in animals under 10 weeks old was demonstrated. However, given that VTEC appear to be ubiquitous in the gut of calves, both healthy and diarrhoeal, it becomes difficult to assign a disease association with confidence. In the limited number of specimens from which multiple isolates were processed for VTEC, it is clear that a VTEC (of one or more serotypes) can dominate the flora of the gut. In this study, there was no obvious association of clinical diarrhoea with particular VTEC serotypes, the presence of the CVD419 marker, or seroconversion to VT1. With one exception, diarrhoea in calves was not blood associated. Enteric pathogens such as VTEC, salmonella or viruses may be found in apparently healthy animals, and whether they cause disease depends on a number of other factors such as colostrum deprivation and 'stress'. This could well be the reason why a clear association between the presence of VTEC and clinical illness is difficult to establish in a 'field' setting.

It is well established that haemorrhagic colitis in humans can be transmitted by food [4, 37, 38]. The best recognized serotype of human VTEC disease (O 157. H7) was not seen in the course of the present study or in a previous study of VTEC serotypes in Sri Lanka [31], although it has been found at low prevalence in cattle faecal specimens [25] and retail meat products from North America [39]. Of VTEC O serogroups previously found in Sri Lanka [31], O 86, O 128, O 84, O 103, O 111, and O 153 have been associated with human diseases [7, 40–2]. However, the H types of the strains isolated from Sri Lankan cattle were different from those associated with human disease in the temperate world [41]. There is little information on the role of VTEC in human disease in Sri Lanka. However, in a hospital-based study of childhood diarrhoea, 702 *E. coli* isolated from 209 diarrhoeal children were examined for VTEC with negative results (Peiris, unpublished).

The data presented here and that from previous studies confirm that cattle are a reservoir of VTEC in the environment both in temperate [25, 43] and tropical [9, 27] regions. While non-O 157. H7 VTEC have been associated with human disease [13], the pathogenic potential to humans of the other VTEC serotypes isolated in this study remains to be fully evaluated.

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