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Verotoxin-producing *Escherichia coli* infections in Sheffield: cattle as a possible source

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SUMMARY

During 1986 and 1987, faecal samples from patients with haemorrhagic colitis (HC) or haemolytic-uraemic syndrome (HUS) were examined for evidence of infection by verotoxin-producing *Escherichia coli* (VTEC). During the 2-year period VTEC infections were found in 31 (78%) of 40 patients initially presenting with HC, and in 5 (63%) of 8 patients initially presenting with HUS. VTEC were found in only 2 (0·9%) of 229 age and sex matched control patients with acute non-bloody diarrhoea. All but one VTEC belonged to *E. coli* serogroup O 157. During 1987 this serogroup was isolated from 2 (1%) of 207 samples of faeces taken from cattle arriving at a Sheffield abattoir, indicating a possible source of these infections for man. We are unaware of previous reports of isolation of this organism from cattle in England.

INTRODUCTION

Strains of *Escherichia coli* producing a powerful cytotoxin active against cultured vero cells were first described by Konowalchuk *et al.* (1977), and have since been recognized as important human pathogens. Such verotoxigenic *E. coli* (VTEC) have been associated with outbreaks and sporadic cases of haemorrhagic colitis (HC) in North America, Europe and Japan (Riley *et al.* 1983; Pai *et al.* 1984; Itoh *et al.* 1985; Robaeys *et al.* 1987; Smith *et al.* 1987), and with sporadic cases of haemolytic-uraemic syndrome (HUS) in North America and England (Karmali *et al.* 1983, 1985; Scotland *et al.* 1988). Beef products and untreated milk have both been suggested as possible sources of VTEC infection for man (Riley *et al.* 1983; Martin *et al.* 1986; Ryan *et al.* 1986; Borczyk *et al.* 1987).

The aim of this study was to monitor the incidence of VTEC infection in patients with HC or HUS, in age/sex matched controls with non-bloody diarrhoea, and in cattle arriving at a Sheffield abattoir.

MATERIALS AND METHODS

Selection of patients

Patients selected for study were those presenting with either HC (sudden onset of grossly bloody diarrhoea, sometimes preceded by abdominal pain and cramps

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and watery non-bloody diarrhoea) or HUS (acute renal failure, thrombocytopaenia and microangiopathic haemolytic anaemia). Faecal samples were selected from those submitted by general practitioners, the Communicable Diseases Unit of Lodge Moor Hospital, Sheffield, or other hospitals in the area, on the basis of information supplied on the original request forms. For each HC or HUS patient selected for study, we also selected control patients of the same sex, whose age and date of onset of illness matched as closely as possible those of the HC or HUS patient, but who presented with acute non-bloody diarrhoea. All patients were examined for the presence of free faecal verotoxin and *E. coli* O 157.

Cattle specimens

Faeces were obtained from 207 randomly selected cattle brought to a Sheffield abattoir during 1987. These samples were examined for the presence of *E. coli* O 157, but not for free faecal verotoxin.

Detection of E. coli *O 157*

Faecal samples were inoculated onto sorbitol MacConkey agar (Oxoid CM813). After overnight incubation, apparently non-sorbitol fermenting colonies were identified using a series of standard biochemical tests. Isolates identified as *E. coli* were checked for agglutination with *E. coli* O 157 antiserum (Difco) using standard methods. Isolates identified as *E. coli* O 157 were then grown in a modified brain heart infusion broth (Chapman & Swift, 1984) and after centrifugation at 3000 g at 4 °C for 30 min and filtration of the supernatant through a 0·22 μm membrane, the filtrate was then assayed for verotoxin as described by Konowalchuk *et al.* (1978).

Detection of faecal verotoxin

Faecal samples were diluted 1 in 2 in phosphate buffered saline and centrifuged at 3000 rev./min at 4 °C for 30 min. The supernatant was then filtered through a 0·22 μm membrane filter and assayed for verotoxin as above.

Detection of VTEC serogroups other than O 157

If free faecal verotoxin was found in the absence of *E. coli* O 157, then a search for other toxigenic serogroups was made. After culture of the sample on ordinary MacConkey agar (Oxoid CM7b), colonies resembling those of *E. coli* were checked for verotoxin production as above: those proving to be VT^+ were then serogrouped.

Detection of other pathogens

HC and HUS patients and controls were examined for salmonellas, shigellas, campylobacters and *Cryptosporidium* by methods described in an earlier survey (Chapman & Mitchelmore, 1988).

RESULTS

Table 1 shows the results of the examinations of human and cattle faeces. During the 2-year period VTEC infections were found in 31 (78%) of 40 patients initially presenting with HC, and in 5 (63%) of 8 patients initially presenting with

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Table 1. *VTEC infections in Sheffield, 1986–7*

	HC	HUS	NBD	CF
Number examined	40	8	229	207
Faecal verotoxin	31	2	2	NE
Non sorbitol-fermenting *E. coli*	36	8	46	25
VT+ *E. coli* O 157	30	5	2	2
VT+ *E. coli* O 128	1	0	0	NE
Other pathogens	0	0	52	NE

HC, haemorrhagic colitis; HUS, haemolytic-uraemic syndrome; NBD, control patient with non-bloody diarrhoea; CF, cattle faeces; NE, not examined.

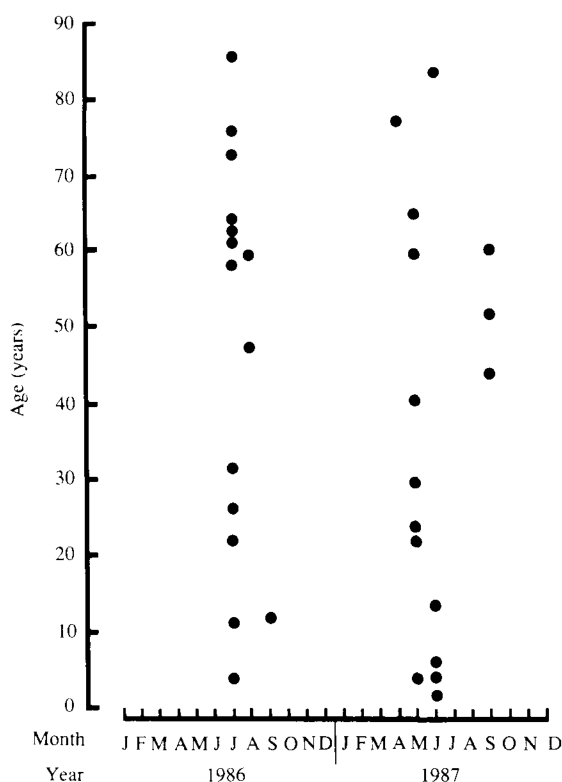

Fig. 1. Age and seasonal distribution of verotoxin-producing *E. coli* infections in Sheffield.

HUS. VTEC were found in only 2 (0·9%) of 229 age- and sex-matched control patients with acute non-bloody diarrhoea. All isolates of *E. coli* O 157 produced verotoxin and had identical biochemical test profiles (indole+, ONPG+, urea−, malonate−, PPA−, citrate−, arginine dihydrolase−, lysine decarboxylase+, ornithine decarboxylase+, sorbitol−, inositol−, adonitol−, rhamnose−, arabinose−, xylose−). Only one other VTEC serogroup, O 128, was isolated. No other pathogens were isolated from HC or HUS patients, but of those with acute non-bloody diarrhoea 52 (23%) of 229 patients had a recognized enteric pathogen.

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During 1987, *E. coli* O 157 was isolated from 2 (1%) of 207 samples of faeces taken from cattle arriving at a Sheffield abattoir. Both isolates produced verotoxin and had identical biochemical test profiles to the human isolates.

Figure 1 shows the seasonal and age distribution of VTEC associated HC cases over the 2-year period.

DISCUSSION

HUS is a serious illness previously reported mainly in young children, which may often be preceded by an attack of bloody diarrhoea and is frequently a cause of renal damage (Fong, de Chadarevian & Kaplan, 1982; Drummond, 1985; Neill, Agosti & Rosen, 1985). Many infectious agents have been reported in association with sporadic cases of HUS including shigellae (Bhuyan, Srivastava & Choudhry, 1985), campylobacters (Fumarola, Miragliotta & Jirillo, 1985; Haq, Rahman & Akbar, 1985) and *Yersinia* spp. (Davenport & Finn, 1988*a*, *b*), but few of these studies have excluded VTEC infection as a cause. A strong association between dysentery caused by *Shigella dysenteria* serotype 1 and subsequent development of HUS was observed in Indian children by Bhuyan *et al.* (1985), and workers have noted a strong homology between shigatoxin produced by *S. dysenteriae* serotype 1 and the vero cytotoxin produced by VTEC (O'Brien & Holmes, 1987). Karmali *et al.* (1983, 1985) reported an association between *E. coli* O 157 and sporadic cases of HUS. Our findings would support this, but in our study the age range for HUS patients varied from 2 to 85 years, and the disease cannot therefore be regarded as one exclusively encountered in children.

The ages of patients initially presenting with HC varied from 4 to 87 years. Of 31 patients subsequently positive for VTEC, 7 developed complications including full HUS, uraemia, haemolytic anaemia or renal failure. The seasonal distribution (Fig. 1) shows marked clusters of cases of HC in summer each year which are difficult to explain: these could perhaps be small unrecognized point-source outbreaks associated with beef products, or with a contaminated seasonal food crop as described recently by Morgan *et al.* (1988). Based on epidemiological evidence, studies in N. America and Europe have implicated cattle as possible sources of VTEC infection for man (Riley *et al.* 1983; Martin *et al.* 1986; Ryan *et al.* 1986; Borczyk *et al.* 1987), although their isolation from cattle has been reported infrequently (Martin *et al.* 1986). During 1987 we examined 207 samples of faeces from cattle arriving at a Sheffield abattoir. Of these 2 (1%) yielded heavy growths of VTEC O 157 on sorbitol MacConkey medium; we are unaware of previous reports of isolation of this organism from cattle in England. In view of the rarity of *E. coli* O 157 in relation to other enteric pathogens, this low level carriage by cattle cannot be excluded as a source of these infections in Sheffield.

The confirmation of the presence of VTEC infections can pose difficulties. Most, but not all strains, belong to serogroup O 157 and are unusual among *E. coli* strains in not fermenting sorbitol (Wells *et al.* 1983; Pai *et al.* 1984). This feature was utilized in developing a medium for screening for *E. coli* O 157 (March & Ratnam, 1986), and such a medium is now available commercially, as is antiserum to *E. coli* O 157.

Demonstration of VT production is necessary for all isolates of *E. coli* O 157

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because not all produce VT, and in the absence of *E. coli* O 157, other VTEC serogroups should be sought (Gross & Rowe, 1985). Sensitive DNA probes for VT genes have been developed (Strockbine *et al.* 1986; Willshaw *et al.* 1987) but these unfortunately hybridize with shigatoxin genes harboured by most shigellas. A probe detecting genes for fimbrial antigens of *E. coli* O 157 may not detect other serogroups of VTEC (Levine *et al.* 1987).

Demonstration of free faecal VT is a rapid and diagnostically useful procedure (Karmali *et al.* 1983). However, several other toxins that affect Vero cells may be present in faecal samples, and antitoxin to VT, which is not available commercially, is needed for specific confirmation of positive results.

Better, and more easily managed laboratory methods need to be developed to enable studies to elucidate further the epidemiology of VTEC infections.

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REFERENCES

Anon. (1987). Unravelling HUS. *Lancet* **ii**, 1437–1439.

Bhuyan, U. N., Srivastava, R. N. & Choudhry, V. P. (1985). Pathology of acute renal failure and haemolytic uraemic syndrome in acute dysentery in children. *Indian Journal of Medical Research* **81**, 402–408.

Borczyk, A. A., Karmali, M. A., Lior, H. & Duncan, L. M. C. (1987). Bovine reservoir for verotoxin-producing *Escherichia coli* O 157.H7. *Lancet* **i**, 98.

Chapman, P. A. & Mitchelmore, D. L. (1988). A two-year survey of the incidence of heat-labile enterotoxin-producing *Escherichia coli* and other enteric pathogens in travellers returning to the Sheffield area. *Epidemiology and Infection*. **101**, 239–247.

Chapman, P. A. & Swift, D. L. (1984). A simplified method for detecting the heat-labile enterotoxin of *Escherichia coli*. *Journal of Medical Microbiology* **18**, 399–403.

Davenport, A. & Finn, R. (1988*a*). Haemolytic-uraemic syndrome induced by *Yersinia pseudotuberculosis*. *Lancet* **i**, 358–359.

Davenport, A. & Finn, R. (1988*b*). Yersinia and the haemolytic-uraemic syndrome. *Lancet* **i**. 1224.

Drummond, K. N. (1985). Haemolytic-uraemic syndrome – then and now. *New England Journal of Medicine* **312**, 116–118.

Fong, J. S. C., de Chadarevian, J. P. & Kaplan, B. S. (1982). Haemolytic-uraemic syndrome: current concepts and management. *Pediatric Clinics of North America* **29**, 835–856.

Fumarola, D., Miragliotta, G. & Jirillo, E. (1985). *Campylobacter jejuni*-associated haemolytic-uraemic syndrome: a possible role for endotoxin. *Archives of Internal Medicine* **145**, 573.

Gross, R. J. & Rowe, B. (1985). *Escherichia coli* diarrhoea. *Journal of Hygiene* **95**, 531–550.

Haq, J. A., Rahman, K. M. & Akbar, M. S. (1985). Haemolytic-uraemic syndrome and campylobacter. *Medical Journal of Australia* **142**, 662–663.

Itoh, T., Kai, A., Saito, K., Yanagawa, Y., Inaba, M., Takahashi, M., Takano, I., Matsushita, S., Kudoh, Y., Terayama, T., Ohashi, M., Karaki, K., Yamashita, H., Ikenoue, S., Satoh, H., Seki, T., Katoh, K., Hirooka, Y., Yamada, M., Akiyama, H.,

https://doi.org/10.1017/S0950268800030156 Published online by Cambridge University Press

Ohzeki, T., Ohshiba, Y., Yamanouchi, J., Tsuchitani, H., Tsunakawa, T. & Tazaki, T. (1985). Epidemiological and laboratory investigation of an outbreak of acute enteritis associated with cytotoxin-producing *Escherichia coli* O 145.H⁻. *Annual Report of the Tokyo Metropolitan Research Laboratory for Public Health* **36**, 16–22.

Karmali, M. A., Petric, M., Lim, C., Fleming, P. C., Arbus, G. S. & Lior, H. (1985). The association between idiopathic haemolytic-uraemic syndrome and infection by verotoxin-producing *Escherichia coli*. *Journal of Infectious Diseases* **151**, 775–782.

Karmali, M. A., Steele, B. T., Petric, M. & Lim, C. (1983). Sporadic cases of haemolytic-uraemic syndrome associated with faecal cytotoxin and cytotoxin-producing *Escherichia coli* in stools. *Lancet* **i**, 619–620.

Konowalchuk, J., Dickie, N., Stavric, S. & Speirs, J. I. (1978). Properties of an *Escherichia coli* cytotoxin. *Infection and Immunity* **20**, 575–577.

Konowalchuk, J., Speirs, J. I. & Stavric, S. (1977). Vero response to a cytotoxin of *Escherichia coli*. *Infection and Immunity* **18**, 775–779.

Levine, M. M., Xu, J. G., Kaper, J. B., Lior, H., Prado, V., Tall, B., Nataro, J., Karch, H. & Wachsmuth, K. (1987). A DNA probe to identify enterohaemorrhagic *Escherichia coli* of O 157.H7 and other serotypes that cause haemorrhagic colitis and haemolytic-uraemic syndrome. *Journal of Infectious Diseases* **156**, 175–182.

March, S. B. & Ratnam, S. (1986). Sorbitol-MacConkey medium for detection of *Escherichi coli* O 157.H7 associated with haemorrhagic colitis. *Journal of Clinical Microbiology* **23**, 869–872.

Martin, M. L., Shipman, L. D., Wells, J. G., Potter, M. E., Hedberg, K., Wachsmuth, I. K., Tauxe, R. V., Davis, J. P., Arnoldi, J. & Tilleli, J. (1986). Isolation of *Escherichia coli* O 157.H7 from dairy cattle associated with two cases of haemolytic-uraemic syndrome. *Lancet* **ii**, 1043.

Morgan, G. M., Newman, C., Palmer, S. R., Allen, J. B., Shepherd, W., Rampling, A., Warren, R. E., Gross, R. J., Scotland, S. M. & Smith, H. R. (1988). First recognized community outbreak of haemorrhagic colitis due to verotoxin-producing *Escherichia coli* O 157.H7 in the UK. *Epidemiology and Infection* **101**, 83–91.

Neill, M. A., Agosti, J. & Rosen, H. (1985). Haemorrhagic colitis with *Escherichia coli* O 157.H7 preceding adult hemolytic-uraemic syndrome. *Archives of Internal Medicine* **145**, 2215–2217.

O'Brien, A. D. & Holmes, R. K. (1987). Shiga and shiga-like toxins. *Microbiological Reviews* **51**, 206–220.

Pai, C. H., Gordon, R., Sims, H. V. & Bryan, L. E. (1984). Sporadic cases of haemorrhagic colitis associated with *Escherichia coli* O 157.H7. *Annals of Internal Medicine* **101**, 738–742.

Riley, L. W., Remis, R. S., Helgerson, S. D. & McGee, H. B. (1983). Haemorrhagic colitis associated with a rare *Escherichia coli* serotype. *New England Journal of Medicine* **308**, 681–685.

Robaeys, G., Surmont, I., Lemmens, P., Goremans, G., Vantrappen, G. & Vandepitte, J. (1987). Haemorrhagic colitis and verotoxin-producing *Escherichia coli* O 157 in Belgium. *Lancet* **i**, 1495–1496.

Ryan, C. A., Tauxe, R. V., Hosek, G. W., Wells, J. G., Stoesz, P. A., McFadden, H. W. Jr., Smith, P. W., Wright, G. F. & Blake, P. (1986). *Escherichia coli* O 157.H7 diarrhoea in a nursing home: clinical, epidemiological and pathological findings. *Journal of Infectious Diseases* **154**, 631–638.

Scotland, S. M., Rowe, B., Smith, H. R., Willshaw, G. A. & Gross, R. J. (1988). Vero cytotoxin-producing strains of *Escherichia coli* from children with haemolytic-uraemic syndrome and their detection by specific DNA probes. *Journal of Medical Microbiology* **25**, 237–243.

Smith, H. R., Rowe, B., Gross, R. J., Fry, N. K. & Scotland, S. M. (1987). Haemorrhagic colitis and vero cytotoxin-producing *Escherichia coli* in England and Wales. *Lancet* **i**, 1062–1065.

Strockbine, N. A., Marques, L. R. M., Newland, J. W., Williams-Smith, H. & Holmes, R. K. (1986). Two toxin-converting phages from *Escherichia coli* O 157.H7 strain 933 encode antigenically distinct toxins with similar biologic activities. *Infection and Immunity* **53**, 135–140.

Wells, J. G., Davis, B. R., Wachsmuth, I. K., Riley, L. W., Remis, R. S., Sokolov, R. & Morris, G. K. (1983). Laboratory investigation of haemorrhagic colitis outbreaks associated with a rare *Escherichia coli* serotype. *Journal of Clinical Microbiology* **18**, 518–520.

https://doi.org/10.1017/S0950268800030156 Published online by Cambridge University Press

WILLSHAW, G. A., SMITH, H. R., SCOTLAND, S. M., FIELD, A. M. & ROWE, B. Heterogeneity of *Escherichia coli* phages encoding vero cytotoxins: comparison of cloned sequences determining VT1 and VT2 and development of specific gene probes. *Journal of General Microbiology* **133**, 1309–1317.

https://doi.org/10.1017/S0950268800030156 Published online by Cambridge University Press