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Vero cytotoxin-producing *Escherichia coli*, particularly serogroup O 157, associated with human infections in the United Kingdom: 1989–91

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SUMMARY

This survey reports the results of investigations performed by the Laboratory of Enteric Pathogens (LEP), to identify evidence of human infection with Vero cytotoxin-producing *Escherichia coli* (VTEC) in the UK during the period 1989–91. Bacterial isolates, faecal specimens and serum samples were received from patients suffering from diarrhoea, bloody diarrhoea and haemolytic uraemic syndrome. Using serotyping, Vero cytotoxin gene probing and an ELISA for serum antibodies to *E. coli* O 157, evidence of infection was detected in 232, 428 and 615 individuals in 1989, 1990 and 1991 respectively. Of these individuals, 15% were reported as having HUS. Vero cytotoxin-producing *E. coli* O 157 was the most frequently encountered serogroup, with isolations from a total of 1092 individuals over the 3-year period. The incidence of VTEC infection increased from 0·41/100000 in 1989 to 1·07/100000 in 1991. The area with the highest rate of infection in each year was Scotland, increasing from 1·37/100000 in 1989 to 3·97/100000 in 1991.

INTRODUCTION

Escherichia coli producing Vero cytotoxin (VTEC), particularly strains belonging to serogroup O 157, are an important cause of haemorrhagic colitis (HC) [1] and haemolytic uraemic syndrome (HUS) [2, 3]. Cases of HC and HUS caused by VTEC have been identified in all age groups but more frequently in infants and young children. Fatalities are more common in the elderly. Two principal Vero cytotoxins (VT1 and VT2) are produced, and occur singly or in combination [4]. VTEC were first recognized as a cause of human disease following outbreaks and sporadic cases of HC and HUS in the USA and Canada [1, 5]. The first noted outbreak of HUS in Britain occurred in 1982 in the West Midlands [6] and of HC in 1985 in East Anglia [7]. Subsequent outbreaks and sporadic cases in the UK have been reported [8–11].

Using sorbitol MacConkey agar and an O 157 specific antiserum, *E. coli* O 157 can be isolated by the clinical laboratory. Bacterial isolates may then be referred to the LEP for biochemical identification, serotyping and toxin characterisation using DNA probes specific for VT gene sequences. Strains of *E. coli* serogroup

O 157 are further subdivided by phage typing [12]. If *E. coli* O 157 or other known enteric pathogens are not isolated, faecal specimens may be sent directly to the LEP for analysis using DNA probes. Due to the short period of excretion of VTEC, isolation of the organism from the patient's faeces is not always possible, however, patients with *E. coli* O 157 infection usually produce high-titre serum antibodies to the lipopolysaccharide (LPS). An ELISA technique for serum antibodies to *E. coli* O 157 LPS may be used as an indication that infection had occurred in recent weeks or months [13].

In this study, specimens were received from the UK for investigation of VTEC infection from January 1989 to December 1991.

MATERIALS AND METHODS

Faeces, bacterial isolates and sera received over the 3-year period were examined for evidence of VTEC infection. The date recorded for each specimen was the date received by the LEP. The clinical details described were based on information accompanying the specimens.

Specimens

A total of 1312 strains of *E. coli* was examined and typed using biochemical [14] and serotyping procedures in the LEP [15]. Strains of *E. coli* serogroup O 157 were phage typed [12]. Bacteria were stored on Dorset's egg agar slopes at room temperature. In some instances, particularly when pathogens had not been isolated by the sending laboratory, faecal and serum samples were sent to the LEP.

DNA probes for VT genes

Cultures were spotted in a grid formation on nylon membranes on nutrient agar and incubated at 37 °C for 6 h. Colonies were lysed and DNA denatured using sodium hydroxide [16]. The DNA was bound to the membrane by baking, and then hybridized with DNA probes for VT1 and VT2 genes, followed by immunological detection of hybrids [17].

Faecal specimens were either spotted onto the membrane directly, or streaked first onto MacConkey agar followed by replicating onto the membrane, and then treated as above or hybridized with ^{35}S-labelled VT probes [18].

Serology

Sera were analysed by ELISA and immunoblotting, for antibodies binding to the LPS of *E. coli* O 157, as described previously [13].

RESULTS

The number of laboratories submitting specimens and isolates increased from 115 in 1989 to 160 in 1990, and declined slightly to 150 in 1991.

Faeces, bacterial isolates and sera were received from 403, 640 and 903 individuals in 1989, 1990 and 1991 respectively for the investigation of VTEC infection. A patient was defined as having a VTEC infection if either a bacterial isolate (or faecal sample) was positive with one of the VT gene probes, or if serum antibodies to *E. coli* O 157 LPS were detected. Of the 1946 individuals examined,

Table 1. *Faeces, bacterial isolates and sera examined by the LEP from patients with diarrhoea, BD and HUS: 1989–91*

	Patients		Faecal probe positive, VTEC isolated /faeces examined	Faecal probe positive, no VTEC isolated /faeces examined	VTEC* isolated/ cultures examined		
	Total	Evidence of VTEC infection			O 157	Non-O 157	Antibodies to O 157 LPS
Non-bloody diarrhoea†	664	454 (68%)	24/124 (19%)	5/124	423/522	3/522	38/91 (42%)
Bloody diarrhoea†	860	583 (68%)	41/263 (16%)	15/263	518/591	7/591	74/129 (57%)
Haemolytic uraemic syndrome‡	308	193 (63%)	17/115 (15%)	11/115	77/102	7/102	121/184 (66%)

Note. Some patients had more than one specimen type examined.
* Cultures examined includes those isolated during the faecal probe.
† Includes patients who subsequently developed HUS.
‡ Includes patients with a prodrome of non-bloody diarrhoea or BD.

Table 2. *Toxin gene type and phage type of VTEC isolations: 1989–91*

	VT producing *E. coli* O 157	Toxin gene type			Phage type				
		VT1	VT2	VT1 & VT2	1	2	4	49	Other
1989	178	2	116	60	32	55	22	45	21
1990	382	3	312	67	31	130	37	136	48
1991	532	5	430	97	50	209	38	142	100
Total	1092	10 (1%)	858 (79%)	224 (20%)	113 (10%)	394 (36%)	97 (9%)	323 (29%)	172 (16%)

evidence of VTEC infection was detected in 232, 428 and 615 for each consecutive year.

Of those examined, 1662 were recorded as having non-bloody diarrhoea, bloody diarrhoea (BD) or HUS, of which 1116 had evidence of VTEC infection. There were 284 individuals from whom clinical details were incomplete or absent, or in a few instances were asymptomatic. Of these, 159 had evidence of VTEC infection from which there were 136 isolations of *E. coli* O 157 and 7 non-O 157 VTEC. In 170 of the 308 patients with HUS, a prodromal illness of diarrhoea or BD was described.

A summary of all the tests performed and results obtained for those who had diarrhoea, BD or HUS is given in Table 1. It should be noted that for some patients, both faeces (or bacterial isolates) and sera were examined, and also a number of patients exhibited more than one of the recognized clinical symptoms.

Bacteriology

Of 1312 *E. coli* examined, 1112 hybridized with one or other or both of the VT gene probes, and 1092 (98%) of these belonged to serogroup O 157 (Table 2). Flagella antigen H7 was the most common H type (88%) of O 157 VTEC,

Table 3. *Toxin gene type of isolates from patients with diarrhoea, BD and HUS: 1989–91*

	VT producing *E. coli* O 157	VT1	VT2	VT1 and VT2
Non-bloody diarrhoea*	423	6 (1·4%)	335 (79·2%)	82 (19·4%)
Bloody diarrhoea*	518	4 (0·8%)	395 (76·2%)	119 (23·0%)
Haemolytic uraemic syndrome†	77	0	73 (94·8%)	4 (5·2%)

* Includes patients who subsequently developed HUS.
† Includes patients with a prodromal non-bloody diarrhoea or BD.

the remaining 12% being non-motile. The most frequently encountered phage types of *E. coli* O 157 were 2, 49, 1 and 4 [19]. The most common VT gene type was VT2 only (79%) (Table 2). Considering clinical symptomatology, it was shown that 76% of *E. coli* O 157 associated with BD possessed gene sequences for VT2 only, as compared with 95% of *E. coli* O 157 associated HUS (Table 3).

There were 34 *E. coli* O 157 which were negative with the VT gene probes. Two possessed the flagella antigen H7 and 6 were non-motile, and of these strains, 3 rapidly fermented sorbitol (all non-motile) and 5 did not. Other flagella types identified were; H8 (12), H19 (1), H42 (1), H45 (11) and H39 (1). All of these strains rapidly fermented sorbitol.

Twenty non-O 157 VTEC were isolated during the 3 years. The 11 strains associated with BD or HUS belonged to serotypes; O 6.H2, O 26.H11, O 128ab.H2, O 145.H25 (two), O 168.H$^-$, and 5 which did not belong to any of the 173 currently recognised O serogroups. The flagella antigens of these strains were H4, H10, and H40 and 2 were non-motile.

The remaining 9 strains belonged to serotypes; O 26.H11, O 26.H$^-$, O 100.H25, O 118.H12, O 128ab.H2 (2) and 3 not belonging to any of the 173 O serogroups, but had flagella antigens H2, H4 and H8. Of these 20 strains, 4 had gene sequences for VT1 only, 10 for VT2 only and 6 had both VT1 and VT2 genes.

Bloody diarrhoea and haemolytic uraemic syndrome

The majority of patients with VTEC associated bloody diarrhoea were under 10 years of age although all other age groups were affected to some degree. Those with HUS, however, were almost exclusively under 10 years of age. Of the total number of patients with evidence of VTEC infection, 15% developed HUS.

Epidemiology

VTEC infections occurred predominantly during summer months (Fig. 1). Using the available age and sex information of the patient, the average annual incidence of VTEC infection per 100000 of the population in defined age groups was calculated. The age groups most frequently infected with VTEC for both males and females, was 1–4 years with a mean rate of 3·3/100000. For age groups 5 years and over, incidence for both sexes decreased with the lowest incidence for males being the age group 35–44 (0·18/100000) and for females, the age group 25–34 (0·40/100000). Incidence then increased to age group 85 and over, with

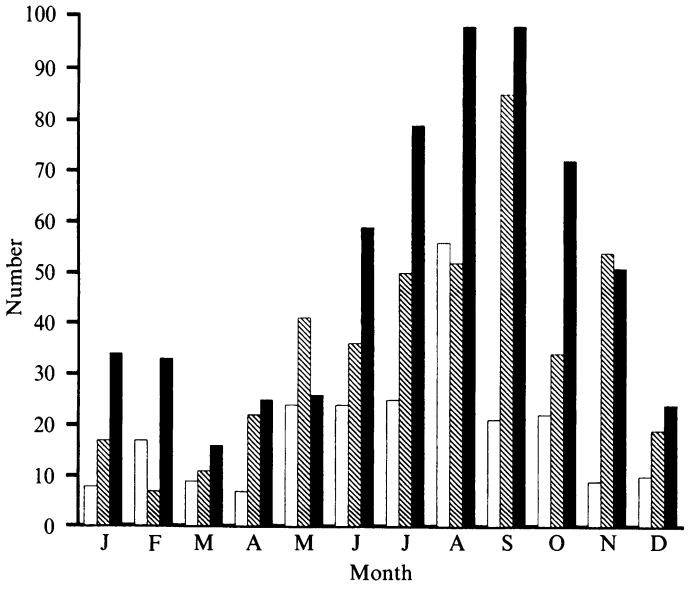

Fig. 1. Monthly incidence of VTEC infection for three years: 1989–91. □, 1989; ▨, 1990; ■, 1991.

0·63/100000 males and 1·12/100000 females infected. For individuals less than 1 year of age, the male incidence rate was slightly higher than females with 2·0/100000 males infected per year and 1·5/100000 females infected per year. There were also slightly more males than females infected in the 5–14 year age group. All other age groups, however, showed higher rates of infection for females, particularly those aged 35 years and over, where on average there were twice as many females infected/100000 as males.

The incidence of VTEC infection per 100000 of the population in each health region of the UK was calculated, and VTEC infections were shown to be most common in Scotland (Table 4). The incidence rate in Wales increased by over five times between 1989 and 1990. Certain areas in England had relatively high incidence rates, for example, N. England (1·07/100000 in 1990), W. Midlands (1·01/100000 in 1990), E. Anglia (1·27/1000000 in 1991) and N. W. England (2·10/100000 in 1991). The four lowest rates of infection were in the Thames regions N.W., N.E., S.E. and S.W., none of which had an incidence rate above 0·4/100000.

There were 17 general outbreaks during the 3-year period, involving 165 cases. Evidence of VTEC infection was detected in 143 of the patients. Nine of the outbreaks occurred in Scotland, with a further three in the north of England. Where the age of the patient was known, 33% were aged 1–14, and 28% 75 years and over. A summary is given in Table 5.

In 1989, there was only one general outbreak which was associated with an 'ox roasting'. There were eight cases of BD and one of HUS.

There were 9 outbreaks in 1990, of which 6 occurred in Scotland, and 3 in England. Four of the outbreaks (30 cases) were associated with old peoples homes and elderly people in psychiatric hospitals, where 16 were recorded as having BD, 2 as having HUS and there were 6 fatalities. A restaurant-associated outbreak [11]

Table 4. *Incidence of VTEC infections per 100000 of the population*

Health region	VTEC infection confirmed by DEP			Incidence per 100000		
	1989	1990	1991	1989	1990	1991
N. England	17	33	32	0·55	1·07	1·04
Yorkshire	10	25	32	0·27	0·69	0·88
Trent	34	27	37	0·73	0·58	0·79
E. Anglia	13	14	26	0·64	0·68	1·27
N.W. Thames	3	5	6	0·09	0·14	0·17
N.E. Thames	2	15	9	0·05	0·40	0·24
S.E. Thames	10	5	12	0·27	0·14	0·33
S.W. Thames	2	12	12	0·07	0·40	0·40
Wessex	8	6	20	0·27	0·20	0·68
Oxford	6	16	16	0·24	0·63	0·63
S.W. England	11	15	32	0·34	0·46	0·98
W. Midlands	29	53	47	0·56	1·01	0·90
Mersey	3	4	11	0·12	0·17	0·46
N.W. England	7	17	84	0·17	0·42	2·10
Wales	6	32	35	0·21	1·11	1·22
N. Ireland	1	0	2	0·06	0·00	0·13
Scotland	70	149	202	1·37	2·92	3·97
Total	232	428	615	0·41	0·75	1·07

(16 cases), which occurred in Lothian, affected mainly children and young adults, causing BD in a number of patients, and 4 cases of HUS. Other outbreaks involved a youth custody centre, a playgroup, and a family returning from a holiday in France. There was one community outbreak in Scotland.

In 1991, there were 7 outbreaks, the largest of which involved 23 cases associated with fast food outlets. While 11 of the cases had eaten beefburgers at a single branch in Preston on 19 January, the distinct properties of the organism isolated (urease positive, phage type 31) enabled the detection of contacts and also cases who had eaten at other outlets in Northampton, Glasgow, Hanley and Manchester. Those affected were predominantly young, with about 50% aged 15–24 years. There were 22 cases of BD and 3 developed HUS. Another major outbreak occurred in the Scottish borders, affecting 2 eventide homes and 3 people in the community [25]. Where the age was given, nearly all cases were over 75 years of age. Preston was the location for another outbreak later in the year affecting 17 people, where 76% were aged 1–14 years. There were 10 cases of BD and 5 of HUS. This was a community outbreak where the only epidemiological link with some of the cases was the consumption of locally made yoghurt. Other outbreaks in 1991 involved a community and a restaurant in Scotland, a Welsh nursery and school-children returning from a trip to Austria. Throughout the 3 years, VTEC were not isolated from any food or other source associated with the outbreaks, although certain foods were epidemiologically implicated. In addition to the general outbreaks, there were 45 household outbreaks involving 97 cases.

During the 3-year period, recent travel abroad was noted for 41 of the 1275 patients with evidence of VTEC infection. Countries recorded were; France (4), Belgium, Austria (6), Spain (10), Portugal (2), Ibiza (2), Majorca, Malta (2), Corfu, Cyprus, Turkey, Yugoslavia, Libya, Canary Islands, The Gambia, Kenya, China, New Zealand, USA (3).

Table 5. *General outbreaks in the UK: 1989–91*

Year	Regional health area	Location (reference)	Number of cases (HUS)	Specimens examined by LEP: Positive by bacteriology	Positive by serology only	Organism isolated
1989	Oxford	Community	8 (1)	3	—	O 157.H7, PT* 49, VT2
1990	Wales	Psychogeriatric hospital	4	4	—	O 157.H⁻, PT 14, VT1 & VT2
	Yorkshire	Youth custody centre	7	1	—	O 157.H7, PT 4, VT1 & VT2
	Oxford	Playgroup	7 (3)	5	2	O 157.H7, PT 2, VT2
	Scotland (Grampian)	Community [20]	4	2	—	O 157.H7, PT 2, VT2
	Scotland (Glasgow)	Nursing home [21]	9 (2) – 2 deaths	2	2	O 157.H7, PT 49, VT2
	Scotland (Lanarkshire)	Psychiatric hospital [22]	12 – 4 deaths	9	2	O 157.H7, PT 2, VT2
	Scotland (Lothian)	Restaurant [11]	16 (4)	12	2	O 157.H7, PT 49, VT2
	Scotland (Edinburgh)	Family	5	4	—	O 157.H7, PT 4, VT2
	Scotland (Edinburgh)	Nursing home	5	5	—	O 157.H7, PT 49, VT2
1991	N.W. England (Scotland, Oxford)	Fast-food chain [23]	23 (3)	21	1	O 157.H7, PT 31, VT2†
	Oxford	School trip to Austria	6	6	—	O 157.H7, PT 49, VT2
	N.W. England	Community [24]	17 (5)	16	1	O 157.H7, PT 49, VT2
	Wales (Carmarthen)	Nursery [24]	10	10	—	O 157.H7, PT 49, VT2
	Scotland (Borders)	Eventide homes, community [25]	21	20	1	O 157.H7, PT 1, VT1 & VT2
	Scotland (Aberdeen)	Restaurant	6	6	—	O 157.H7, PT 32, VT2
	Scotland (Lothian)	Community [26]	5 (2)	5	—	O 157.H7, PT 2, VT2

*PT, phage type. †Organism isolated was urease positive.

DISCUSSION

In this study, evidence of infection with VTEC was sought by the examination of faeces, bacterial isolates and sera. From 1989 to 1991, the number of individuals recorded as being infected increased by 165%. Part of this increase may be attributed to more laboratories screening for *E. coli* O 157. Another factor contributing to enhanced surveillance was the availability in the LEP of an ELISA technique for detecting serum antibodies to *E. coli* O 157 late in the disease. This test is particularly important in cases of HUS where diagnosis is made after prodromal diarrhoea had ceased. Of the 1275 individuals with evidence of VTEC infection, 11% were determined by serological tests alone.

DNA probes were used to confirm the presence of VT genes in isolates of *E. coli* O 157 and also to sub-divide the strains into one of three VT type groups (VT1, VT2 or VT1 and VT2). The majority of strains had gene sequences for VT2 only, followed by strains producing VT1 and VT2, and those producing VT1 only were uncommon. These probes were also used to screen faeces for VTEC, and in this respect they were not only able to detect *E. coli* O 157, but also VTEC of other serogroups.

The most common serogroup of VTEC was O 157, however, since most laboratories screen only for *E. coli* O 157, other serogroups were underestimated. As yet, gene sequences for VT have not been detected in any strains of *E. coli* O 157 other than those with flagella H7 or non-motile (H^-). Whilst the ability to produce VT is believed to be a virulence property, 2 strains of *E. coli* O 157.H7 (1 associated with diarrhoea) and 6 non-motile strains of *E. coli* O 157 (3 associated with diarrhoea, and 2 with BD) did not produce VT. There were also 26 individuals (20 with diarrhoeal disease) from whom *E. coli* O 157 with flagella antigen H19, H39, H42, H45 or H8 was isolated. Two of these strains (O 157.H8 and O 157.H45) were associated with BD, and one (O 157.H19) with HUS. Gene sequences for VT were not detected in these strains.

Of the non-O 157 VTEC serotypes recorded, some have been reported previously in the UK. Serotypes O 26.H11 (and H^-), O 118.H12, O 128.H2 and O 145.H25 have been associated with cases of BD and HUS [3, 4]. Serogroup O 100 has been detected in a UK food sample (O 100.H^-)[27].

The number of patients with VTEC related BD or HUS increased from 1989 to 1991. However, the number of HUS patients recorded as having a prodromal illness of diarrhoea or BD is thought to be underestimated. This is because the patient may no longer have diarrhoea or BD when admitted with HUS, or because clinical details accompanying specimens were incomplete.

It is not clear why some cases go on to develop HUS, and whether certain characteristics of the organism are directly responsible for this syndrome. However, most strains associated with cases of HUS produce VT2 only.

Within most age groups, VTEC were isolated more frequently from female patients than from males. There were, however, slightly more males infected than females in those aged less than 1, and 5–14 years. Analysis of results based on geographical location showed that the incidence of VTEC infection within the UK, was highest in Scotland (Table 4). Regional incidence rates were variable, and may have resulted from a number of causes. The majority of cases were apparently sporadic, however, numbers were elevated by outbreaks, as in N.W. England

(Table 5). Incidence rate in certain areas may also have increased due to the referral of patients to regional dialysis centres, for example, the Birmingham Children's Hospital. The increase in the number of hospitals submitting specimens to the LEP, and improved surveillance in a given area may have been significant. This could account for the rise in incidence in Wales, since a total population survey began in 1990 whereby stools from all cases of diarrhoea were screened for *E. coli* O 157.

In North America, foods such as raw beef and unpasteurized milk have been identified as vehicles of *E. coli* O 157 [28]. In the UK, a recent study has shown that non-O 157 VTEC are present in foods such as uncooked sausages [27] but as yet, *E. coli* O 157 has not been isolated from food in the UK. Nevertheless, in a small number of outbreaks, case control studies have implicated certain foods such as beefburgers and yoghurt [23, 24]. The isolation of VT producing *E. coli* O 157 from 5 calves in Scotland [29] and 39 cattle in an abattoir study in Sheffield [30] has provided evidence that cattle are a source of infection in the UK.

In the UK, Vero cytotoxin-producing *E. coli*, particularly those belonging to serogroup O 157, are human pathogens of importance with an appreciable mortality. In order to understand the epidemiology of VTEC infections, comprehensive follow up investigations, particularly of outbreaks, are vital. It is recommended that the search for *E. coli* O 157 should be part of routine laboratory investigations of diarrhoeal disease. If investigations are made late in the disease, particularly in cases of HUS, serum samples should be examined for antibodies to *E. coli* O 157 LPS. Recent work has demonstrated the high sensitivity of DNA probes for VT genes, particularly when examining faeces. Now that such probes are labelled non-radioactively, probe tests could be used outside the reference laboratory, to confirm the VT gene status of isolates of *E. coli* O 157 and also to allow the detection of non-O 157 VTEC.

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REFERENCES

1. Riley LW, Remis RS, Helgerson SD, et al. Haemorrhagic colitis associated with a rare *Escherichia coli* serotype. New Eng J Med 1983; **308**: 681–5.
2. Milford DV, Taylor CM, Guttridge B, Hall S, Rowe B, Kleanthous H. Haemolytic uraemic syndromes in the British Isles 1985–1988: association with Verocytotoxin producing *Escherichia coli*. Part 1: clinical and epidemiological aspects. Arch Dis Child 1990; **65**: 716–21.
3. Kleanthous H, Smith HR, Scotland SM, et al. Haemolytic uraemic syndromes in the British Isles, 1985–1988: association with Verocytotoxin producing *Escherichia coli*. Part 2: Microbiological aspects. Arch Dis Child 1990; **65**: 722–7.
4. Smith HR, Scotland SM. Vero cytotoxin-producing strains of *Escherichia coli*. J Med Microbiol 1988; **26**: 77–85.
5. Karmali MA, Steele BT, Petric M, Lim C. Sporadic cases of haemolytic-uraemic syndrome associated with faecal cytotoxin and cytotoxin producing *Escherichia coli* in stools. Lancet 1983; i: 619–20.
6. Taylor CM, White RHR, Winterborn MH, Rowe B. Haemolytic-uraemic syndrome: clinical experience of an outbreak in the West Midlands. BMJ 1986; **292**: 1513–6.

7. Morgan GM, Newman C, Palmer SR, et al. A community outbreak of haemorrhagic colitis due to verotoxin producing *Escherichia coli* O 157:H7. Epidemiol Infect 1988; **101**: 83–91.
8. Salmon RL, Farrell ID, Hutchison JGP, et al. A christening party outbreak of haemorrhagic colitis and haemolytic uraemic syndrome associated with *Escherichia coli* O 157:H7. Epidemiol Infect 1989; **103**: 249–54.
9. Scotland SM, Rowe B, Smith HR, Willshaw GA, Gross RJ. Vero cytotoxin-producing strains of *Escherichia coli* from children with haemolytic uraemic syndrome and their detection by specific DNA probes. J Med Microbiol 1989; **25**: 237–53.
10. Smith HR, Rowe B, Gross RJ, Fry NK, Scotland SM. Haemorrhagic colitis and Vero-cytotoxin producing *Escherichia coli* in England and Wales. Lancet 1987; i: 1062–5.
11. Marsh J, MacLeod AF, Hanson MF, Emmanuel FXS, Frost JA, Thomas A. A restaurant-associated outbreak of *E. coli* O 157 infection. J Publ Health Med 1992; **14**: 78–83.
12. Frost JA, Smith HR, Willshaw GA, Scotland SM, Gross RJ, Rowe B. Phage-typing of Vero-cytotoxin (VT) producing *Escherichia coli* O 157 isolated in the United Kingdom. Epidemiol Infect 1989; **103**: 73–81.
13. Chart H, Smith HR, Scotland SM, Rowe B, Milford DV, Taylor CM. Serological identification of *Escherichia coli* O 157:H7 infection in haemolytic uraemic syndrome. Lancet 1991; **337**: 138–40.
14. Cowan ST, Steel KJ. Manual for the identification of medical bacteria. Cambridge: Cambridge University Press, 1970.
15. Gross RJ, Rowe B. Serotyping of *Escherichia coli*. In: The virulence of *Escherichia coli*. Reviews and Methods, Sussman M, ed. London: Academic Press; 1985: 345–63.
16. Maniatis T, Fritsch EF, Sambrook J. Molecular cloning: A laboratory manual. New York: Cold Spring Harbour Laboratory, 1982.
17. Thomas A, Smith HR, Willshaw GA, Rowe B. Non-radioactivity labelled polynucleotide and oligonucleotide DNA probes, for selectively detecting *Escherichia coli* strains producing Vero cytotoxins VT1, VT2 and VT2 variant. Mol Cell Prob 1991; **5**: 129–35.
18. Willshaw GA, Smith HR, Scotland SM, Field AM. Rowe B. Heterogeneity of *Escherichia coli* phages encoding Vero cytotoxins: comparison of clones sequences determining VT1 and VT2 and development of specific gene probes. J Gen Microbiol 1987; **133**: 1309–17.
19. Frost JA, Cheasty T, Thomas A, Rowe B. Phage typing of Vero cytotoxin-producing *Escherichia coli* O 157 isolated in the United Kingdom: 1989–91. Epidemiol Infect 1993; **110**: 469–475.
20. Dev VJ, Main M, Gould IM. Waterborne outbreak of *Escherichia coli* O 157. Lancet 1991; **337**: 1412.
21. Anonymous. Diarrhoea outbreak in old peoples' home. Comm Dis Env Health Scot Week Rep 1990; No. 90/25: 4.
22. Anonymous. *E. coli* O 157 infection. Comm Dis Env Health Scot Week Rep 1990; No. 90/42: 5.
23. Anonymous. Haemorrhagic colitis: *Escherichia coli* O 157. PHLS Comm Dis Rep 1991; **1**, No. 6: 1.
24. Anonymous. Verotoxin producing *Escherichia coli* O 157: phage type 49. PHLS Comm Dis Rep 1991; **1**, No. 47: 1.
25. Bisset JG, Brown MI, Bimson G, et al. An outbreak of haemorrhagic colitis due to *E. coli* O 157 in 2 residential homes for the elderly in the Borders. Comm Dis Env Health Scot Week Rep 1992; 26, No. 92/19: 5–6.
26. Graham L, Ramsay C. A community outbreak of *E. coli* O 157. Comm Dis Env Health Week Rep 1991; 25, No. 91/43: 3–4.
27. Smith HR, Cheasty T, Roberts D, Thomas A, Rowe B. Examination of retain chickens and sausages in Britain for Vero cytotoxin-producing *Escherichia coli*. App Env Microbiol 1991; **57**: 2091–3.
28. Karmali MA. Infection by Verocytotoxin-producing *Escherichia coli*. Clin Microbiol Revs 1989; 2: 15–38.
29. Synge BA, Hopkins GF. Verotoxigenic *Escherichia coli* O 157 in Scottish calves. Vet Rec 1992; **130**: 583.
30. Chapman PA, Siddons CA, Wright DJ, Norman P, Fox J, Crick E. Cattle as a source of Verotoxigenic *Escherichia coli* O 157. Vet Rec 1992; **131**: 323–4.