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Phage typing of Vero cytotoxin-producing *Escherichia coli* O 157 isolated in the United Kingdom: 1989–91

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SUMMARY

Between 1989 and 1991 a total of 1092 Vero cytotoxin-producing *Escherichia coli* O 157 isolated in the United Kingdom were phage typed in the Laboratory of Enteric Pathogens (LEP). Twenty-three phage types was identified, the most frequent being types 2 (36·1%), 49 (29·6%), 1 (10·3%) and 4 (8·9%). Although isolations of O 157 VTEC have increased each year from 1 in 1982 to 532 in 1991, the predominant phage types have remained unchanged although the proportion of strains belonging to types 2 and 49 has increased. O 157 VTEC from 17 outbreaks were phage typed during this period with phage type 49 predominating (7 of 17 outbreaks).

INTRODUCTION

Vero cytotoxin-producing *Escherichia coli* (VTEC) [1] have been isolated from patients with symptoms ranging from mild, non-bloody diarrhoea to haemorrhagic colitis (HC) [2, 3]. Furthermore haemolytic uraemic syndrome (HUS) has often been associated with a prodrome of diarrhoeal disease caused by VTEC [4, 5]. The serotype most frequently associated with VT production is *E. coli* O 157.H7 although some non-motile strains do occur (for review see Karmali, 1989 [6]). Outbreaks and sporadic cases have been reported in several countries particularly the USA and Canada where beef products and unpasteurized cows' milk have been cited as vehicles of infection.

The first outbreaks reported in Great Britain were of HUS in the West Midlands in 1982 and 1983 [7]. Between 1989 and 1991 *E. coli* O 157 from a total of 17 outbreaks were received by the Laboratory of Enteric Pathogens (LEP) [8]. Total *E. coli* O 157 isolations referred to LEP have increased from 1 in 1982 to 532 in 1991 [9]. This increase has been paralleled by a significant increase in cases of HUS in children [10].

In a survey of VTEC infection in the United Kingdom between 1989 and 1991 [8] specimens from 1275 patients yielded serological or bacteriological evidence of infection. *E. coli* O 157 was isolated from 1092 (85·6%) of these patients.

All *E. coli* O 157 strains referred to LEP since 1982 have been tested for the presence of genes encoding Vero cytotoxins VT1 and VT2 using DNA probes and

it has been shown that there is excellent correlation between a positive reaction with the probes and production of VT as detected using Vero cells grown in tissue culture [11].

E. coli O 157 VTEC have also been phage typed using a scheme developed in Canada [12, 13]. Phage typing of *E. coli* O 157 isolated in the UK between 1982 and 1988 [14] demonstrated 12 of the 62 phage types described by Khakhria and others [13]; the predominant phage types were type 2, type 1 and type 49. This paper describes the phage types and the distribution of VT types of *E. coli* O 157 strains isolated in the UK between 1989 and 1991 [8] and compares the results with those described for previous years.

MATERIALS AND METHODS

Bacterial strains

E. coli O 157 isolated in the UK and referred to LEP between 1989 and 1991 were included in the study.

Serotyping, phage typing and probe tests

All isolates were serotyped using rabbit antisera for *E. coli* somatic (O) antigens O 1–O 173 and flagella (H) antigens H1–H56 [15]. *E. coli* O 157 were phage typed [14] and tested with DNA probes specific for VT1 and VT2 which have been described previously [11].

RESULTS

Between 1989 and 1991 LEP identified VT probe-positive *E. coli* O 157 isolated from 1092 individuals in the UK (Table 1). Of these, 518 patients were recorded as having bloody diarrhoea and 77 had HUS. The clinical features associated with these isolations have been discussed fully elsewhere [8].

Of the 1092 *E. coli* O 157, 961 (88%) were H7, the remainder being non-motile. Ten strains (1%) were VT1 probe positive and 858 (79%) were VT2 positive; 224 (21%) were positive with both probes. Twenty-three phage types were identified of which nine were represented by a single isolate. Six isolates reacted with the phages but did not conform to a published type [13]. Phage type 2 was the most common (36·1% of strains), followed by types 49 (29·6%), 1 (10·3%) and 4 (8·9%). These four phage types accounted for 84·9% of the strains examined.

The distribution of VT genes among the different phage types was similar to that seen in previous years [14] (Table 1). Three hundred and twenty-one of 323 phage type 49 strains (99·4%) and 390 of 394 phage type 2 strains (99%) carried the VT2 gene only. Of the 4 phage type 2 strains which hybridized with both VT1 and VT2 probes, 1 was isolated in 1990 and 3 in 1991. Strains of phage type 1 carried both VT1 and VT2 genes and produced segregant colonies of phage types 4, 8 or 14 as has been described previously [14]. One strain of phage type 1 hybridized with the VT2 gene probe only. Phage types 4, 8, 14, 21, 31, 32 and 34 were also heterogenous with respect to VT production; each included strains which hybridized with both probes and those which hybridized with either the VT1 or VT2 probe only (Table 1). Analysis of sporadic isolations showed that.

Table 1. *VT+* Escherichia coli *O 157 isolated in Great Britain 1989–91*

Phage type	VT1	VT2	Number	Total	Percent
1	+	+	112	113	10·3
	−	+	1		
2	−	+	390	394	36·1
	+	+	4		
4	+	+	49	97	8·9
	−	+	43		
	+	−	5		
8	+	+	23	26	2·4
	+	−	2		
	−	+	1		
14	+	+	25	47	4·3
	−	+	22		
21	−	+	10	11	1·0
	+	+	1		
31	+	+	22	23	2·1
	−	+	1		
32	+	+	4	25	2·3
	−	+	19		
	+	−	2		
34	+	+	1	3	0·3
	−	+	2		
39	−	+	2	2	0·2
40	−	+	6	6	0·5
49	−	+	321	323	29·6
	+	+	2		
50	−	+	2	2	0·2
66	−	+	5	5	0·5
9	−	+	9	9	0·8
types*					
NC†	−	+	4	6	0·5
	+	−	1		
	−	+	1		
Total				1092	100·0

* 9 phage types (23, 24, 27, 43, 47, 51, 54, 63, 65) were each represented by only one isolate.
† 6 isolates reacted with the phages but did not conform to a recognized type.

although isolation rates varied from region to region, the distribution of phage types is consistent throughout the United Kingdom. There was no obvious association among sporadic isolates between phage type and geographical area.

Outbreaks

Between 1989 and 1991, LEP received strains from 17 VTEC outbreaks [8] all of which involved *E. coli* O 157 (Table 2).

Only one outbreak of *E. coli* O 157 (phage type 49) was recorded during 1989 despite the increase in isolations. There were nine outbreaks in 1990, two of which occurred in Lothian during September [16]. One of these outbreaks was associated with a restaurant and all strains belonged to phage type 49. The second outbreak occurred in a family recently returned from a holiday in France who were infected with *E. coli* O 157 phage type 4. The remaining 7 outbreaks in 1990 involved phage type 49 (2 outbreaks), phage type 2 (3), phage type 4 (1) and phage type 14 (1).

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Table 2. E. coli *O 157 outbreaks 1989–91*

Year	Region	Total cases (HUS)	*E. coli* O 157 Phage type	*E. coli* O 157 VT type
1989	Oxford	8 (1)	49	VT2
1990	Wales	4	14*	VT1, VT2
	Yorkshire	7	4	VT1, VT2
	Oxford	7 (3)	2	VT2
	Grampian	4	2	VT2
	Strathclyde	9 (2)	49	VT2
	Strathclyde	12	2	VT2
	Lothian	16 (4)	49	VT2
	Lothian (France)	5	4	VT2
	Lothian	5	49	VT2
1991	N.W. England	23 (3)	31†	VT2
	Oxford (Austria)	6	49	VT2
	N.W. England	16 (5)	49	VT2
	Wales	9	49*	VT2
	Borders	20	1	VT1, VT2
	Grampian	6	32	VT2
	Lothian	5 (2)	2	VT2

* All *E. coli* O 157 were H7 except isolates from these two outbreaks which were non-motile.
† All isolates were urease positive.

Three of the 1991 outbreaks involved phage type 49: one was epidemiologically-associated with the consumption of yoghurt [17] one affected children attending a nursery [17] and one followed the return of a school trip to Austria. Epidemiological investigations following an outbreak of *E. coli* O 157 phage type 31 indicated an association with the consumption of hamburgers at several fast-food restaurants [18]. The phage type 31 strain involved was unusual in that all isolates were urease positive. The remaining outbreaks were in Scotland and involved phage types 1, 32 and 2.

Microbiological confirmation of food source was not obtained in any of the above outbreaks even where a food vehicle was indicated by case control studies, as in the hamburger- and yoghurt-associated outbreaks.

During the period 1989–91 there have also been at least 45 incidents in which the same phage type of *E. coli* O 157 has been isolated from children and their parents or siblings. As with sporadic infections, in these family incidents, phage types 2 and 49 predominated.

Incidence of VT+ E. coli *O 157 1982–91*

The number of strains referred to LEP each year has increased from 1 in 1982 to 532 in 1991 (Table 3). The predominant phage types have been present throughout this period although the proportion of strains belonging to phage types 2 and 49 has increased at the expense of phage type 1 and its related types 4, 8 and 14. The increase in numbers of strains referred has been paralleled by an increasing range of phage types identified with 20 different phage types isolated in 1990.

Table 3. *VT+* E. coli *O 157. Variation in phage type over time*

Phage type	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991	Total
1	—	2	2	27	12	11	11	32	31	50	178
2	—	3	5	7	32	56	31	55	130	209	528
4	—	—	—	7	11	8	3	22	37	38	126
8	—	—	1	4	—	1	—	7	8	11	32
14	—	—	—	2	7	3	5	8	16	23	64
21	—	—	—	—	—	1	1	2	2	7	13
23	—	—	—	—	—	—	—	—	1	—	1
24	—	—	—	2	—	7	—	—	—	1	10
27	—	—	—	—	—	—	—	1	—	—	1
31	—	—	—	—	—	—	—	1	1	21	23
32	1	—	1	1	1	1	5	3	1	21	35
34	—	—	—	—	—	—	—	—	1	2	3
39	—	—	—	—	—	—	—	—	2	—	2
40	—	—	—	—	—	—	2	1	5	—	8
43	—	—	—	—	2	—	—	—	1	—	3
47	—	—	—	—	—	1	—	1	—	—	2
49	—	1	—	10	7	7	27	45	136	142	375
50	—	—	—	2	—	—	—	—	1	1	4
51	—	1	—	—	—	—	—	—	1	—	2
52	—	—	—	—	—	—	1	—	—	—	1
54	—	—	—	—	—	—	—	—	1	—	1
63	—	—	—	—	—	—	—	—	1	—	1
65	—	—	—	—	—	—	—	—	1	—	1
66	—	—	—	—	—	—	—	—	5	—	5
NC	—	—	—	—	—	—	—	—	—	6	6
Total	1	7	9	62	72	96	86	178	382	532	1425
No. of phage types	1	4	4	9	7	10	9	12	20	13	24 (+NC)

DISCUSSION

The number of VT+ *E. coli* O 157 isolated in the UK has increased steadily throughout the decade (Table 3) although some of the increase may be due to increased awareness of the importance of VTEC, particularly *E. coli* O 157, or to improved methods of isolation. The majority of strains are isolated during the summer months and most of the outbreaks have occurred at this time. Although the annual incidence of *E. coli* O 157 is still small the possibility of chronic kidney damage following HUS infection is a cause for concern [10].

Most *E. coli* O 157 phage types have a characteristic VT profile, thus phage type 1 strains usually have both VT1 and VT2 genes whereas phage types 2 and 49 usually have only VT2. Isolates which carry only the VT1 gene are rare among *E. coli* O 157 but are more frequently found in other VTEC serogroups [19]. The 1989–91 study [8] and an earlier study of HUS infections between 1985 and 1988 [20] both showed a clear predominance of isolates producing VT2 only in patients with HUS.

Strains from 4 outbreaks were included in the first report of phage typing in the UK [14] and a further 17 outbreaks have been investigated in the present survey. These have involved the phage types which also predominate among sporadic

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infections. The coincident outbreaks of phage types 49 and 4 in the Edinburgh area [16] demonstrated the value of phage typing as a first line epidemiological tool. The outbreak involving phage type 31 was of particular interest as 21 of the total 23 UK strains belonging to this phage type were found to be associated epidemiologically with the outbreak. This outbreak strain also had a distinctive plasmid profile and was urease positive [18].

The proportion of *E. coli* O 157 strains belonging to phage type 1 has declined in recent years relative to an increase in frequency of phage type 49 (Table 3). The latter phage type was first identified in the UK in 1983 and since 1988 has been the second most common type after phage type 2 accounting for 29·5% of all *E. coli* O 157 isolates. *E. coli* O 157 VTEC phage type 49 have not been reported from North America [13].

The number of phage types identified in the UK, has increased from 12 to 24 since the original UK survey [14] and a number of these were first identified in the UK. However, the predominant phage types have remained unchanged.

E. coli O 157 has been recognized to be a significant public health problem in Canada [21]. Despite a continuing increase in isolations, it is likely that the incidence of *E. coli* O 157 in the UK may still be underestimated as there are indications that not all UK laboratories routinely screen faeces for *E. coli* O 157. The combination of phage-typing with the use of VT probes has proved to be a rapid and discriminatory typing system for the investigation of *E. coli* O 157 epidemiology.

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