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Vero cytotoxin-producing *Escherichia coli*, particularly serogroup O157, associated with human infections in England and Wales: 1992–4

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SUMMARY

Investigations were performed by the Laboratory of Enteric Pathogens on Vero cytotoxin-producing *Escherichia coli* (VTEC) in England and Wales from 1992–4. Bacterial isolates, faeces and sera obtained from patients with diarrhoea, bloody diarrhoea and haemolytic uraemic syndrome were examined. Using serotyping, Vero cytotoxin gene probing and serodiagnostic tests for *E. coli* O157, evidence of infection was detected in 543, 434 and 491 individuals in 1992, 1993 and 1994 respectively; VTEC of serogroup O157 were isolated from 470, 385 and 411 cases. The O157 VTEC strains belonged to at least 19 different phage types (PT) although 84% belonged to PT2, PT49, PT8, PT1 or PT4. Antibodies to *E. coli* O157 lipopolysaccharide were detected in 13% of the cases. The average annual rate of infection with O157 VTEC was 0·83/100000 and 12% of the 1458 individuals with evidence of infection with VTEC or *E. coli* O157 developed haemolytic uraemic syndrome. There were at least 18 general outbreaks and many family outbreaks.

INTRODUCTION

Vero cytotoxin-producing *Escherichia coli* (VTEC), particularly strains belonging to serogroup O157, are an important cause of diarrhoea, haemorrhagic colitis (HC) [1] and haemolytic uraemic syndrome (HUS) [2, 3]. In the United Kingdom, cases of diarrhoea, HC and HUS caused by VTEC have been identified in all age groups but most frequently in infants and young children. Fatalities are more common in the elderly [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 reported in 1983 [1, 5]. In the largest outbreak of O157 VTEC infection reported by the end of 1995 the consumption of undercooked hamburgers from a fast food chain in the western United States was linked with over 700 cases [6]. The first confirmed outbreak of O157 VTEC infection in Britain occurred in 1983 in the West Midlands [7]. Further outbreaks and sporadic cases have occurred with increasing frequency and in 1991 there was a total of 615 individuals in the UK with evidence of VTEC infection [4]. Details of general outbreaks caused by O157 VTEC in England and Wales from 1992–4 have been reported recently [8].

This report describes the continued surveillance of VTEC infections in England and Wales by the Laboratory of Enteric Pathogens (LEP), Central Public Health Laboratory, between 1992 and 1994.

METHODS

A case was defined as having had a VTEC infection if either a bacterial isolate carried or faecal sample had present, gene sequences encoding VT production or there was evidence of infection with *E. coli* O157 as demonstrated by detecting serum antibodies to *E. coli* O157 lipopolysaccharide (LPS). As described by Wall and colleagues [8] some cases in outbreak investiga-

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Table 1. *Evidence of infection with VTEC or* E. coli *O157 in patients in England and Wales*

Year	Evidence of infection	O157 VTEC isolated	Non-O157 VTEC isolated	Faeces positive for VT genes using DNA probes*	Antibodies to O157 LPS detected† (No. tested)
1992	543	470	4‡	3	67 (122)
1993	434	385	5§	0	45 (129)
1994	491	411	4	0	76‖ (166)
Totals	1468	1266	13‡§	3	188

* No VTEC isolated.
† No O157 VTEC isolated from faeces.
‡ VTEC of serogroups O157 and O173 were isolated from the same patient.
§ Non-O157 VTEC belonging to two different serogroups were isolated from one patient.
‖ This total includes 11 sera from one outbreak (see section on Outbreaks).

tions were included based on epidemiological evidence and clinical symptoms alone. Methods for epidemiological investigation of outbreaks have been described previously [8]. A general outbreak is defined as an outbreak affecting members of more than one private residence, or residents of an institution.

Bacterial isolates, faeces and sera were referred to the LEP from laboratories in England and Wales. The samples had been isolated from patients with diarrhoea, bloody diarrhoea and HUS. Cultures and faecal specimens were examined for evidence of VTEC infection using digoxigenin or fluorescein labelled DNA probes specific for VT genes [9–11] and sera were tested for evidence of infection with *E. coli* O157 by detection of antibodies to O157 LPS [12]. LPS was also prepared from a strain of *Shigella dysenteriae* 1. Well separated single colonies grown from faecal specimens spread onto MacConkey agar plates were replicated onto a nylon membrane supported on a nutrient agar plate. VT gene sequences were detected with fluorescein labelled VT gene probes using the enhanced chemiluminescence system (Amersham International, UK). Hybridizing colonies were isolated, identified and serotyped. A number of faecal specimens was also screened directly for organisms possessing VT genes using polymerase chain reaction (PCR) gene amplification [13], for *E. coli* O157 using immunomagnetic separation (IMS) [14] and for the *eae*A (*E. coli* *a*ttaching and *e*ffacing) gene derived from O157 VTEC [15].

Bacterial isolates were identified biochemically and serotyped by standard procedures [16], and strains belonging to serogroup O157 were differentiated by phage typing [17]; the scheme now recognizes at least 80 types [H. Lior, unpublished communication]. All isolates were screened for resistance to the following antimicrobial agents using an agar incorporation method [18] at the levels quoted: ampicillin (A) 8 and 128 mg/l, chloramphenicol (C) 8 mg/l, ciprofloxacin (Cp) 0·125 and 1 mg/l, furazolidone (Fu) 8 and 32 mg/l, gentamicin (G) 4 and 32 mg/l, kanamycin (K) 16 mg/l, nalidixic acid (Nx) 16 mg/l, streptomycin (S) 16 and 128 mg/l, sulphathiazole (Su) 64 mg/l, tetracycline (T) 8 and 128 mg/l and trimethoprim (Tm) 2 mg/l.

RESULTS

For the years 1992, 1993 and 1994, evidence of VTEC or *E. coli* O157 infection was detected in 543, 434 and 491 individuals respectively (Table 1). VTEC belonging to eight serogroups were detected, although, 99% belonged to serogroup O157. It was not possible to determine accurately the prevalence of particular clinical symptoms associated with infection since the information accompanying specimens for examination was often incomplete. It is likely, therefore, that the number of cases of HUS and bloody diarrhoea was underestimated.

Haemolytic uraemic syndrome

Haemolytic uraemic syndrome is characterized by microangiopathic haemolytic anaemia, thrombocytopenia and acute renal failure [2, 3, 5]. The incidence of HUS was based on the information included on the forms completed by the laboratories submitting samples to LEP. For the 3-year period 177

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Table 2. *Haemolytic uraemic syndrome: 1992–4*

	1992	1993	1994
Number of patients with HUS	103	83	83
Cases of HUS with evidence of infection with VTEC or *E. coli* O157*	80 (78%)	47 (57%)	50 (60%)
Cases of HUS with O157 VTEC isolated	29†	12	18
Cases of HUS positive for *E. coli* O157 by serodiagnosis, no VTEC isolated	51	34	30
Cases of HUS with only non-O157 VTEC isolated (serotypes)	0	1 (09ab:H-, O101:H-)‡	2 (O134:H25, O128:H7)

* Evidence of infection by isolation of VTEC, DNA probing for VT genes in faeces or *E. coli* O157 serodiagnosis.
† VTEC of serotypes O157:H7 and O173:H2 were isolated from one patient.
‡ Both strains isolated from one patient.

(12%) of the 1458 individuals with evidence of infection with VTEC or *E. coli* O157 developed HUS (Table 2); 88% of these cases were children under 15 years. A high proportion of HUS cases was identified by ELISA for antibodies to *E. coli* O157 LPS, and where necessary with confirmation by immunoblotting. The majority of bacterial isolates associated with HUS belonged to serogroup O157, although VTEC belonging to serogroups O9, O101, O128, O134 and O173 were also isolated.

E. coli belonging to serogroup O157

Strains carrying VT gene sequences

There were 1266 *E. coli* strains of serogroup O157 isolated which hybridized with at least one of the VT gene probes; the highest number of isolations was in 1992 (Table 1, Fig. 1*a*). The most common VT gene type continued to be VT2 only (75%); 24% were VT1 plus VT2 and less than 1% were VT1 only (Table 3). The O157 VTEC strains possessed the H7 flagellar antigen or were non-motile (H-). The percentages of H- strains were 14, 18 and 20 in 1992, 1993 and 1994 respectively.

Phage typing

The most frequently encountered phage types (PT) of O157 VTEC were PT2 (46%), PT49 (17%), PT8 (8%), PT1 (7%), PT4 (6%) and PT14 (4%) (Table 3). Phage types could be subdivided further by distinguishing the VT gene sequences carried although

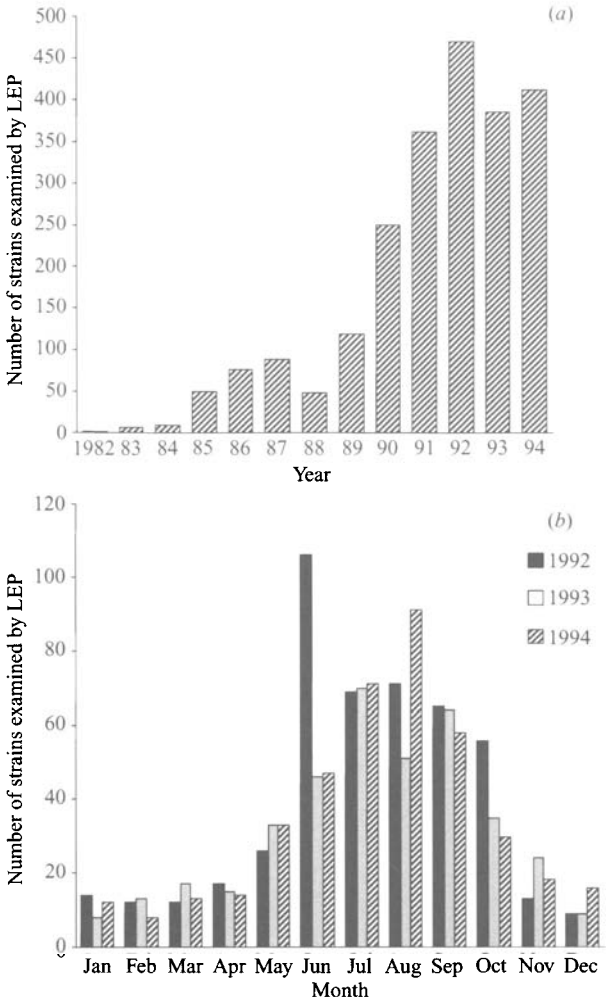

Fig. 1. (*a*) Annual incidence of VT producing *E. coli* O157 in England and Wales: 1982–94. (*b*) Monthly incidence of VT producing *E. coli* O157 in England and Wales: 1992–4.

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Table 3. *Phage types and VT types of O157 VTEC: 1992 to 1994*

Phage type	VT1	VT2	VT1 and VT2	Total (%)
1	—	7	85	92 (7·3)
2	—	556	25	581 (45·9)
4	1	34	43	78 (6·2)
8	3	5	87	95 (7·5)
14	—	23	26	49 (3·9)
21	—	20	3	23 (1·8)
23	—	—	2	2 (0·2)
24	—	4	—	4 (0·3)
27	—	1	—	1 (0·1)
28	—	7	—	7 (0·6)
31	1	5	—	6 (0·5)
32	1	25	19	45 (3·6)
34	—	8	4	12 (0·9)
43	—	1	2	3 (0·2)
49	—	214	1	215 (17·0)
50	—	4	—	4 (0·3)
51	—	6	2	8 (0·6)
54	—	6	1	7 (0·6)
RDNC*	—	29	5	34 (2·7)
Total	6 (0·5)	955 (75·4)	305 (24·1)	1266

* RDNC, Reacts does not conform (reacts with typing phages but does not conform to a designated type).

Table 4. *Drug resistance of O157 VTEC*

	1992	1993	1994
Number examined	470	385	411
Number with R type (percentage)			
SSuT	9 (19)	17 (31)	41 (49)
SSu	11 (23)	8 (14)	8 (9)
T	3 (6)	3 (5)	6 (7)
CSSuTTm	—	3 (5)	9 (11)
Other R types	25 (52)	25 (45)	20 (24)
Total no. drug resistant (percentage no. examined)	48 (10·2)	56 (14·5)	84 (20·4)
Total no. of R types	21	22	17

certain combinations predominated, such as, PT2/VT2 (44%) and PT49/VT2 (17%). The proportion of isolates belonging to PT2 has increased as the number of PT49 has decreased with the ratio of PT2:PT49 being 1·3:1 in 1989 [4] and 2·9:1 in 1994.

Resistance to antimicrobial agents

The percentage of O157 VTEC demonstrating resistance to at least one antimicrobial agent increased from 10% in 1992 to 20% in 1994 (Table 4). The distribution of resistance was sometimes related to phage type; for example, of the strains with resistance type (R type) SSu 85% belonged to PT8 and over 84% of strains of R type SSuT were PT2 VT2. Between 1992 and 1994 there was a 4·6-fold increase in the number of strains with the R type SSuT so that by 1994 20% of the PT2 VT2 strains had this R type.

E. coli *O157 not producing VT*

Forty-four *E. coli* O157 strains were negative with the VT gene probes. Fifteen were non-motile, one of which belonged to phage type 4 and did not ferment sorbitol. Other flagellar types identified were H2 (1), H6 (1), H8 (3), H16 (1), H18 (1), H20 (1) and H45

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Table 5. *The detection of antibodies to* E. coli *O157 LPS: 1992 to 1994*

	1992	1993	1994
Number of patients with sera examined	122	129	166
Number with antibodies to O157 LPS detected	83 (68%)	59 (46%)	83 (50%)
Number with antibodies to O157 LPS detected, O157 VTEC isolated	16	14	7
Number with antibodies to O157 LPS detected, O157 VTEC not isolated	67	45	76*

* Includes 11 from one outbreak (see Outbreaks section).

(19). The two strains possessing the H7 antigen did not ferment sorbitol promptly and belonged to phage types 14 and 34.

Non-O157 VTEC

Thirteen non-O157 VTEC strains were isolated; five strains were associated with HUS (serotypes O9:H-, O101:H-, O128:H7, O134:H25 and O173:H2) and four with bloody diarrhoea (serotypes O26:H11 (2), O103:H2 and O146:H21). One patient with HUS was infected with VTEC of serotypes O173:H2 and O157:H7. From another patient with HUS, two VTEC strains of serotypes O9:H- and O101:H- were isolated and were shown to carry gene sequences for the VT2 variant VT2e, normally associated with isolates from pigs [19].

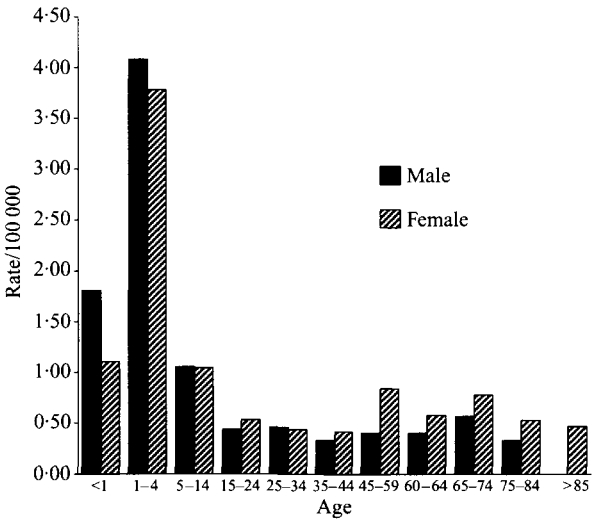

Fig. 2. Incidence of VT producing *E. coli* O157 by age and sex: England and Wales, 1992–4.

Serum antibodies to *E. coli* O157 LPS

Sera from patients, most of whom had bloody diarrhoea or HUS, were examined for antibodies to O157 LPS; antibodies were detected in 54% of the serum samples (Table 5). The figures for 1994 included 11 antibody-positive sera from one outbreak identified predominantly by serodiagnosis (see Outbreaks section).

Serological cross reactions between antibodies to *E. coli* O157 LPS and that of other organisms have been reported [20]. In 1994, two children presented with bloody diarrhoea and suspected HUS. Faecal specimens were examined for *E. coli* O157 using IMS [14] and for *eae* O157 [15] and VT genes [13] using PCR. *E. coli* strains belonging to serogroup O157 were not detected by IMS, despite antibodies to *E. coli* O157 LPS having been detected by ELISA. Gene sequences coding for VT1 were detected by PCR, although *eae* O157 sequences were not found. The children had been in contact with another child with a *Shigella dysenteriae* 1 infection and serum antibodies against the LPS of *Sh. dysenteriae* 1 were detected in both children and it was concluded that the most likely cause of infection was *Sh. dysenteriae* 1.

Epidemiology

Infection with VTEC was more prevalent during the summer months (Fig. 1*b*). Males and females of all ages were affected, although the peak incidence was in children aged 1–4 years (Fig. 2). Table 6 shows the rates of infection with O157 VTEC per 100000 of the population in each Health Region for 1992–4. The increased rates for certain areas in particular years could be attributed to outbreaks, for example, in the Oxford Health Region in 1992 (see Outbreaks section). The lowest rates of infection for each of the 3 years were in the NW, NE and SE Thames Health Regions. Information on the screening policies for *E. coli* O157 in the Health Regions is not available but differences will provide bias in the incidence rates.

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Table 6. *Regional incidence of O157 VTEC infections in England and Wales: 1992–4*

	1992*		1993*		1994*	
Health region	Number	Rate†	Number	Rate	Number	Rate
Northern	47	1·52	31	1·00	54	1·74
Yorkshire	30	0·81	31	0·84	21	0·57
Trent	64	1·35	43	0·91	41	0·86
E Anglian	27	1·29	17	0·81	30	1·44
NW Thames	10	0·29	12	0·34	14	0·40
NE Thames	11	0·29	13	0·34	14	0·37
SE Thames	15	0·40	12	0·32	13	0·35
SW Thames	19	0·64	28	0·94	10	0·33
Wessex	20	0·64	16	0·51	32	1·02
Oxford	50	1·94	21	0·81	25	0·97
S Western	29	0·87	29	0·87	35	1·06
W Midlands	44	0·83	56	1·06	46	0·87
Mersey	8	0·33	10	0·41	14	0·58
N Western	64	1·59	35	0·87	36	0·90
Wales	32	1·10	31	1·07	26	0·90

* Data calculated using 1992 OPCS figures.
† Rate expressed per 100000.

Outbreaks of O157 VTEC infection

Outbreaks of O157 VTEC infection for the years 1992–4 are summarized in Table 7; this shows 18 general outbreaks involving 176 individuals. Three further outbreaks were described by Wall and colleagues [8]; these were outbreaks 7 and 13 (1993) and outbreak 16 (1994). However, because of lack of availability of samples the microbiological studies by LEP were very incomplete and these outbreaks have not been included in Table 7.

Isolation of O157 VTEC or serological evidence of *E. coli* O157 infection was detected in 121 individuals. Most isolates associated with the outbreaks were PT2/VT2 strains although in one hospital outbreak in 1992 the causative organism was PT2, VT1 and VT2 [21]. The mortality rate in these outbreaks was 7/176 (4%); where known the ages of the patients were between 27 and 48 years and 2 were over 80 years old. In the largest outbreak recorded by December 1994 in England and Wales, there were 37 cases, 5 with HUS, associated with a nursery and also the community in the Northampton area [22]. In 1994, in the first reported UK outbreak associated with visiting a community farm there were 7 cases, 4 with HUS, and *E. coli* O157 PT2/VT2 was isolated from 4 cases [25]. O157 VTEC strains of the same type were isolated from cattle and goats on the farm. An outbreak of cases associated with travel to the same area of Majorca was identified following the isolation of O157 VTEC strains which reacted with the typing phages in the same pattern, which did not confirm to a recognizable phage type. The strains were also resistant to chloramphenicol, streptomycin, sulphathiazole, tetracycline and trimethoprim. Towards the end of 1994 an outbreak of diarrhoea with two cases of HUS was associated with a hospital nursery in Warrington and 11 cases were identified by the use of serodiagnosis for antibodies to *E. coli* O157 LPS. Only one faecal isolate was referred to the LEP and this was *E. coli* O157, PT49/VT2.

DISCUSSION

The results of the surveillance of VTEC infections in England and Wales for the years 1992–4 have shown that these organisms continue to cause public health problems. The highest number of infections with O157 VTEC occurred in 1992 and in June of that year there were 106 cases which was considerably higher for that month than observed previously and in the two subsequent years in which the highest number was 45 (Fig. 1). Part of this rise was due to a large outbreak in the Northampton area; the source of infection was not identified although person-to-person spread probably occurred [22]. The use of serodiagnosis has continued to make a significant contribution to the identification of evidence of *E. coli* O157 infection and in particular to the confirmation of an outbreak

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Table 7. *Outbreaks of O157 VTEC infections in England and Wales: 1992–4*

Year	Month	Regional health area (Town)	Location [reference]	Number of clinical cases (HUS)	Cultures and sera examined by LEP		
					Positive by culture	Positive by serodiagnosis	PT type, VT type of *E. coli* O157
1992	Jan	NW England (Manchester)	Psychiatric hospital [21]	14 – 3 deaths	4	1	PT2, VT1 and 2
	Mar	Trent (Doncaster)	Nursing home	5 – 2 deaths	5	—	PT2, VT2
	May	Trent (Retford)	Public house	19 – 1 death	3	—	PT2, VT2
	Jun	Oxford (Northampton)	Nursery and community [22]	37 (5)	28	2	PT2, VT2
	Aug	Wessex (Dorchester)	Scout camp	3	3	—	PT2, VT2
	Aug	Northern (Barrow)	Community	4	4	—	PT1, VT1 and 2
1993	May	Trent (Sheffield)	Community [23]	7 (3)	6	—	PT2, VT2†
	Jun	W Midlands (Hereford)	Communal meal	9 (2)	6	1	PT49, VT2
	Jun	Various*	Community	4	4	—	PT28, VT2
	Jun	SW Thames	Community [24]	7 (3) – 1 death	6	1	PT2, VT2
	Aug	W Midlands (Hereford)	Community (Barbecue)	4 (1)	3	1	PT49, VT2
	Aug	Wales (Gwent)	Community [9]	17 (1)	7	—	PT49, VT2‡
	Sep	Yorkshire (Leeds)	Community	17 (3)	9	—	PT2, VT2
1994	Apr	Wales	Farm	2	1	—	PT4, VT2§
	May	Various	Travellers from Majorca	5	5	—	RDNC, VT2
	Jun	Trent (Leicester)	Community farm [25]	7 (4)	4	2	PT2, VT2‖
	Oct	W Midlands (Shrewsbury)	Community	3	3	—	PT49, VT2
	Oct	Mersey (Warrington)	Nursery	12 (2)	1	11	PT49, VT2

* O157:H7, PT28/VT2 isolated in Harrogate, Leeds, Portsmouth and Guildford.
† O157:H7, PT2/VT2 also isolated from milk and cattle [23].
‡ O157:H7, PT49/VT2 also isolated from raw beefburger [9].
§ This *E. coli* O157 strain was non-motile, all the other outbreak strains were O157:H7.
‖ O157:H7, PT2/VT2 also isolated from cattle and goats on the farm [25].

in Mersey in 1994. Of the 1468 individuals with evidence of VTEC or *E. coli* O157 infection, 13% were determined by serological tests alone which is slightly higher than during the period 1989–91 [4]. The polymerase chain reaction has been evaluated as a method for screening faecal specimens for VT genes and has been shown to be more sensitive than DNA probe hybridization [13]. Current methods include the use of multiplex PCR with primers for both the *eae* O157 gene and VT gene sequences (LEP, unpublished data).

In common with North America [26] ground (minced) beef and milk have now been shown to be vehicles of O157 VTEC infection in the UK [9, 23, 27]. In 1993, O157 VTEC strains were isolated for the first time in Britain from raw milk and raw beefburgers. *E. coli* O157 strains with the same properties were isolated from dairy cows as well as the milk following an outbreak in Sheffield in which three children suffered from HUS after drinking unpasteurized milk [23]. *E. coli* O157, PT49, VT2 was isolated from beefburgers following a community outbreak in Gwent [9]. In 1994 an outbreak of O157 VTEC infection affecting more than 100 people was associated with the consumption of pasteurized milk in Scotland; the organism was identified as *E. coli* O157, PT2, VT2, and was also isolated from samples taken from a pipe carrying milk from the pasteurization apparatus to the bottling machine and from a bottling machine rubber [27]. Since the isolation of O157 VTEC from unpasteurized milk and raw beefburgers, further isolations have been made from raw minced beef, beefburgers, sausages and frying steak (PHLS unpublished data). The bovine reservoir for O157 VTEC strains has been demonstrated by isolation from healthy cattle as part of outbreak investigations and in abattoir studies [23, 28].

Phage typing and toxin gene typing of the O157 VTEC strains have aided epidemiological investigations but a small number of types account for the majority of strains. Toxin gene sub-typing by oligonucleotide hybridization and polymerase chain reaction gene amplification has allowed further differentiation of these common types such as PT2 [19, 29]. Other methods, such as restriction fragment length polymorphism analysis [9] and pulsed field gel electrophoresis [30], also provide additional discrimination of O157 VTEC strains and have been applied to investigation of strains from outbreaks. Enhanced strain discrimination for O157 VTEC strains may be achieved from the combination of phage typing with a range of DNA based techniques and this needs to be evaluated epidemiologically. Resistance of O157 VTEC strains to antimicrobial agents doubled between 1992 and 1994 with a five-fold increase in strains with the drug resistance pattern SSuT from 1992 to 1994 and over 80% of these strains were PT2. In combination with phage type and toxin gene type, the patterns of resistance may serve as useful epidemiological markers as seen with the outbreak associated with visiting Majorca. An increase in resistance of O157 VTEC to a similar range of antimicrobial agents has also been reported in Washington State in the USA [31].

The incidence of O157 VTEC infection in England and Wales continues to be lower than the rates observed in Scotland and parts of Canada [4, 26, 32, 33]. A recent report by the Advisory Committee on the Microbiological Safety of Food has recommended that surveillance for O157 VTEC should be extended to include non-bloody diarrhoea as well as cases of bloody diarrhoea and haemolytic uraemic syndrome [34]. This should increase the detection of cases and improve our epidemiological understanding of these infections. The importance of infections due to VTEC of serogroups other than O157 is still unclear, although each year such strains are isolated from cases of HUS and bloody diarrhoea. Further studies are required to evaluate their importance in human disease and to establish the sources and vehicles of infection.

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