

## Extended phage-typing scheme for *Escherichia coli* 0157:H7

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### SUMMARY

In Canada, the number of human isolates of verotoxigenic (VT +ve) *Escherichia coli* 0157:H7 from diarrhoeal cases and haemolytic uraemic syndrome and haemorrhagic colitis has increased from 25 in 1982 to 2384 in 1989. A total of 3273 VT +ve *E. coli* 0157:H7 strains (3255 strains isolated in Canada and 18 isolates from other countries) were phage typed. The phage typing scheme has been extended from 14 to 62 phage types. Of these, five types occurred exclusively in other countries (type 47 in Japan; and types 49, 50, 51 and 52 in the U.K.). Thirty-five different phage types were identified in Canada; only nine of these (1, 2, 4, 8, 14, 21, 23, 31 and 32), each accounted for more than 1% of the cases from human sources. The same nine types were the only ones observed among the isolates from non-human sources (meat and slaughter houses) suggesting a food-borne transmission in most of the human cases. Phage types 1 (30.5%); 4 (21%); 8 (13.5%); 31 (8.9%) and 14 (8%) were encountered in varying frequencies in most of the provinces; infrequently occurring phage types also showed regional variation. Thirteen different phage types were identified among 151 outbreaks representing 556 isolates of *E. coli* 0157:H7. More than one phage type were encountered in 12 outbreaks whereas in 141 outbreaks, all strains in each, had the same phage type.

### INTRODUCTION

*Escherichia coli* serotype 0157:H7 or 0157:NM (non-motile) producing verotoxin (VT) is a well recognized cause of diarrhoeal disease and has been associated with haemorrhagic colitis (HC) [1, 2] and haemolytic uraemic syndrome (HUS) [3, 4].

In Canada, the frequency of this serotype has increased from 25 isolates in 1982 to 2384 in 1989. This has created the need for differentiation of sporadic and outbreak isolates and for determination of the role that non-human sources may play in human infections. The phage typing scheme for verotoxigenic (VT) *E. coli* 0157:H7 developed in Canada [5] has been successfully used in epidemiological investigations of *E. coli* 0157:H7 infections [6, 7].

We report here an extension of this phage typing scheme from 14 phage types to 62 phage types with their geographical distribution.



Table 1. (cont.)

Phage type	Typing phages							
	9	10	11	12	13	14	15	16
1	-	-	CL	CL	-	< SCL	CL	SCL
2	OL	-	CL	CL	CL	-	CL	SCL
3	OL	OL	SCL	CL	-	-	SCL	SCL
4	OL	OL	CL	CL	-	< SCL	CL	< CL
5	OL	OL	≪ OL	< OL	CL	-	-	-
6	-	-	SCL	-	SCL	-	-	-
7	-	-	SCL	-	-	SCL	-	-
8	-	-	CL	CL	CL	< SCL	CL	< SCL
9	-	-	SCL	CL	-	-	-	CL
10	≪ OL	< OL	+++	CL	-	CL	CL	CL
11	≪ OL	-	SCL	SCL	-	SCL	SCL	SCL
12	-	-	+++	SCL	SCL	-	SCL	SCL
13	≪ OL	≪ OL	≪ OL	-	SCL	-	-	-
14	OL	OL	CL	CL	CL	≪ SCL	CL	< CL
15	-	-	SCL	SCL	SCL	-	SCL	SCL
16	< OL	< OL	-	+++	SCL	-	-	-
17	< OL	-	SCL	-	+++	-	-	-
18	-	-	≪ OL	±±	< CL	-	-	-
19	-	-	< OL	+++	-	-	-	+++
20	-	-	-	-	< SCL	-	-	-
21	< OL	< OL	P.-	-	-	-	-	-
22	< OL	< OL	-	SCL	-	-	-	-
23	-	-	CL	< CL	-	-	CL	SCL
24	OL	-	CL	CL	-	-	CL	≪ SCL
25	-	-	≪ OL	≪ OL	CL	-	-	-
26	< OL	< OL	CL	CL	-	-	CL	SCL
27	+++	+++	CL	CL	CL	-	SCL	+++
28	< OL	< OL	≪ OL°	≪ OL°	-	-	-	-
29	-	-	-	+++	-	-	+++	+++
30	-	-	≪ OL	≪ OL	+	-	++	< OL
31	-	-	P-	-	CL	-	-	-
32	OL	OL	P-	-	CL	-	-	-
33	-	-	P-	-	-	-	-	-
34	< OL	< OL	CL	CL	< CL	-	CL	CL
35	-	-	CL	CL	-	-	< CL	±±
36	< OL	< OL	CL	CL	-	-	CL	-°
37	-	-	CL	CL	-	-	CL	≪ OL
38	OL	OL	-	-	-	-	-	-
39	< OL	< OL	≪ OL	≪ OL	CL	-	≪ OL	-
40	OL	OL	CL	CL	-	-	SCL	-°
41	OL	OL	CL	CL	-	+++	CL	< CL
42	< OL	< OL	SCL	SCL	-	-	≪ SCL	-
43	-	-	≪ OL	≪ OL	CL	-	≪ OL	-
44	-	-	-	-	-	-	-	< SCL
45	OL	OL	CL	CL	-	±	SCL	+++
46	< OL	< OL	-	-	-	-	-	-
47	OL	OL	CL	CL	-	+++	CL	-
48	OL	OL	CL	CL	SCL	SCL	CL	SCL
49	OL	OL	CL	CL	CL	CL	CL	-
50	OL	-	CL	CL	CL	-	< OL	-
51	< OL	-	+	-	+++	-	-	-
52	-	-	< CL	≪ OL	-	++	≪ SCL	-
53	+++	≪ OL	≪ OL	≪ OL	≪ SCL	-	+++	+++
54	-	-	< CL	SCL	≪ SCL	-	≪ SCL	< SCL
55	-	-	CL	CL	SCL	SCL	CL	SCL
56	-	-	CL	CL	-	+++	CL	SCL
57	< OL	OL	-	-	-	-	+	SCL
58	-	-	-	-	-	-	-	SCL
59	+++	≪ OL	P-	P-	SCL	+++	-	≪ SCL
60	-	-	P-	-	-	-	-	< SCL
61	-	-	CL	CL	SCL	+++	SCL	+++
62	+++	< OL	P-	-	-	+++	< CL	-
Untypable	-	-	-	-	-	-	-	-

CL, confluent lysis; OL, opaque lysis; SCL, semi-confluent lysis; + + +, 71-100 plaques; + +, 21-70 plaques; +, 5-20 plaques; -, no reaction; °, inhibitory reaction with u plaques; p, less than 5 plaques; ± ±, a possible range of reactions between - and + +.

## MATERIALS AND METHODS

*Bacterial cultures.* A total of 3255 *E. coli* 0157:H7, or 0157:NM verotoxigenic cultures isolated in Canada were phage typed: 2675 were isolated from sporadic cases, 556 from 151 outbreaks and the remaining 24 strains were isolated from meats (17), cow faeces (5), dog faeces (1) and an environmental swab (1). In addition, 18 isolates of *E. coli* 0157:H7 were received from other countries (6 Israel, 1 Japan, 6 UK, 2 Belgium and 3 USA). These strains of 0157:H7 have been checked for verotoxin production by the Verocell assay [1]. All strains were stored on Dorset egg agar medium at room temperature.

*Phages.* Sixteen phages, their propagating strains and 14 different type strains were obtained from R. Ahmed (Ontario Ministry of Health, Ottawa, Ontario). The phages were grown in bulk by the standard agar layer technique [8], titrated, then stored at 4 °C.

*Phage typing.* *E. coli* 0157:H7 or 0157:NM strains were plated on nutrient agar and incubated for 18 h at 37 °C. A single smooth colony was selected and inoculated into 4.5 ml of Difco phage broth (pH 6.8) and incubated for 2–3 h in a shaking water bath at 37 °C. The culture was then inoculated by flooding on a Difco phage agar plate and the 16 phages at their routine test dilution (RTD) were spotted on the plate using a multiple inoculum syringe method [9]. Plates were allowed to dry until no spots were visible, then they were incubated at 37 °C for 18 h, before examination for lytic reactions.

## RESULTS

The extended phage typing scheme for *E. coli* 0157:H7 strains (Table 1) shows 62 different phage types established by using 16 typing phages. Types 1–14 were reported previously [5]. Strains showing a pattern that did not conform to any of the recognized phage types were considered atypical (AT).

If any of these AT strains gave a consistently reproducible and clearly recognizable lytic pattern and/or became epidemiologically significant (occurring with sufficient frequency during an outbreak or as sporadic cases), a phage type designation was assigned. All *E. coli* 0157:H7 strains studied reacted with the typing phages and were therefore typable.

Table 2 shows that the reported number of *E. coli* 0157:H7 strains isolated from humans has increased since 1982. Of the 6946 strains isolated during 1978–89, 3231 were submitted for phage typing.

*Phage types of E. coli 0157:H7 isolated from humans in Canada*

Thirty-five different phage types (Table 3) were identified among the 3231 *E. coli* 0157:H7 and 0157:NM strains investigated. Only two isolates gave non-characteristic patterns (AT). Between 1982 and 1989 nine phage types: 1; 2; 4; 8; 14; 21; 23; 31 and 32 occurred most frequently, representing 96.2% (3110/3231) of the human isolates. The remaining 26 phage types represented 3.8% (119/3231) of the cultures. Predominant were phage types 1 (30.5%), 4 (21%), 8 (13.5%), 31 (8.9%) and 14 (8%) (Table 3).

Table 2. *Human isolates of E. coli 0157:H7 in Canada*

1978-89	
1978-81	5
1982	25
1983	59
1984	163
1985	294
1986	750
1987	1381
1988	1885
1989	<u>2384</u>
Total	6946

Table 3. *Most common phage types of E. coli 0157:H7 from human sources in Canada*

Phage type	Human sources	Percentage
1	987	30.5
4	680	21.0
8	435	13.5
31	287	8.9
14	259	8.0
32	215	6.7
2	156	4.8
23	54	1.7
21	37	1.1
Others (26 types)	119	3.7
Atypical (AT)	2	0.1
Total	3231	100.00

Table 4. *Phage types of E. coli 0157:H7 associated with non-human sources*

Source	No.	Phage type
Beef (ground)*	14	1, 2, 4, 14, 31, 32
Veal (ground)	1	23
Pork	2	4, 21
Cow faeces*	5	1, 8, 31
Dog faeces	1	4
Environment	1	8

No. of strains examined: 24.

\* two isolates from beef and all isolates from cow faeces were from slaughter houses.

#### *Occurrence of phage types among non-human sources*

Nine phage types were associated with non-human sources, mainly isolates from meats and carcasses in slaughter houses (Table 4). All these phage types were common (more than 1% each) among human isolates (Table 3). On the other hand, the 26 infrequently occurring types have not yet been found in non-human isolates.

Table 5. *Distribution of phage types of E. coli 0157:H7 of human origin in the provinces of Canada*

Phage type	Provinces of Canada										Total	Other countries
	BC	ALTA	SASK	MAN	ONT	QUE	NB	NS	PEI	NFLD		
1	243	312	44	24	255	55	15	30	6	3	987	
2	10	38	10	18	52	3	3	5	1	16	156	2 (Israel)
4	189	195	29	21	161	48	13	12	—	12	680	1 (Belgium)
8	76	124	48	21	125	22	6	13	—	—	435	2 (Israel)
14	39	61	11	27	97	9	8	4	1	2	259	2 (Israel)
21	6	9	1	2	14	2	—	3	—	—	37	
23	8	8	4	2	22	1	—	9	—	—	54	
24	1	2	—	1	4	—	—	1	—	—	9	1 (Belgium)
31	143	62	10	5	49	6	3	8	—	1	287	3 (USA)
32	42	55	17	13	60	19	4	4	1	—	215	
33	—	2	—	1	4	3	—	—	—	—	10	
34	4	—	—	—	4	—	1	—	—	—	9	
35	1	—	—	—	1	2	—	—	—	—	4	
36	1	—	—	—	—	—	—	—	—	—	1	
37	3	2	—	—	—	—	—	—	—	—	5	
38	2	—	—	—	—	—	—	—	—	—	2	
39	3	4	2	—	12	3	—	3	—	—	27	1 (UK)
40	3	2	—	—	5	—	—	—	—	—	10	
41	—	1	—	—	—	1	—	—	—	—	2	
42	—	—	—	—	1	—	—	—	—	—	1	
43	—	—	—	2	1	—	—	—	—	2	5	1 (UK)
44	—	—	—	—	—	2	—	—	—	—	2	
45	1	1	—	—	2	1	—	1	—	—	6	
46	—	—	—	—	1	—	—	—	—	—	1	
47	—	—	—	—	—	—	—	—	—	—	—	1 (Japan)
48	—	1	6	1	3	—	—	—	—	—	11	
49	—	—	—	—	—	—	—	—	—	—	—	1 (UK)
50	—	—	—	—	—	—	—	—	—	—	—	1 (UK)
51	—	—	—	—	—	—	—	—	—	—	—	1 (UK)
52	—	—	—	—	—	—	—	—	—	—	—	1 (UK)
53	1	—	—	—	—	—	—	—	—	—	1	
54	—	1	—	—	3	—	—	—	—	—	4	
55	—	1	—	—	—	—	—	—	—	—	1	
56	—	—	—	—	1	—	—	—	—	—	1	
57	—	—	—	—	1	—	—	—	—	—	1	
58	—	1	—	—	—	—	—	—	—	—	1	
59	—	—	—	—	1	—	—	—	—	—	1	
60	—	—	—	—	—	1	—	—	—	—	1	
61	—	—	—	—	1	—	—	—	—	—	1	
62	—	1	1	—	—	—	—	—	—	—	2	
Atypical	—	1	—	1	—	—	—	—	—	—	2	
Total	776	884	183	139	880	178	53	93	9	36	3231	18

*Geographical distribution of phage types of E. coli 0157:H7 in Canada and other countries*

Five common phage types, 1, 4, 8, 14 and 31 (Table 5) representing 82% of the 3231 cultures examined were encountered in different frequencies in each province. The largest number of phage types was observed in Ontario (25 types) followed by

Table 6. Phage types of *E. coli* 0157:H7 identified in 151 outbreaks

Phage type	No. of outbreaks*	No. of cases
1	32	86
2	13	40
4	29	87
8	17	64
14	16	32
21	2	5
23	3	11
31	14	39
32	9	27
34	1	2
40	1	3
43	1	2
48	1	3
1 and 2	1	25
1 and 4	4	45
1 and 8	3	11
1 and 23	1	7
1, 4, 14 and 31	1	13
1, 4, 21 and 31	1	16
8, 23 and 31	1	38
Total	151	556

\* Settings of the outbreaks studied: nursing home outbreaks, 19; community outbreaks (day care, field trips, banquets, etc.), 12; non-specified outbreaks, 5; hospital outbreaks, 3; family outbreaks, 112.

Alberta (22) reflecting the larger sample size from these provinces. Phage type 2, more frequent in Alberta, Ontario and Newfoundland, was mainly associated with outbreaks. Phage types 31 and 32 were observed more frequently in British Columbia, Alberta and Ontario than in the other provinces. The rare phage types 36, 38, and 53 were isolated in British Columbia; types 55 and 58 in Alberta, types 42, 46, 56, 57, 59 and 61 in Ontario and types 44 and 60 in Quebec.

Among 18 strains received from other countries, type 47, isolated in Japan, and types 49, 50, 51 and 52 in UK have not been found in North America. Phage types 2, 4, 8, 14, 24, 31, 39 and 43, and others have been seen both in Canada and abroad (Table 5) [7].

#### *Phage types associated with human outbreaks of E. coli* 0157:H7

Epidemiologically-related strains from an outbreak usually showed the same phage type but occasionally were of mixed phage types. As seen in Table 6, 13 different phage types were identified among the 151 outbreaks (19 nursing homes, 12 community, 3 hospital, 112 family outbreaks and 5 non-specified) representing 556 isolates of *E. coli* 0157:H7. These cultures were associated with HC, HUS, nonbloody diarrhoea and asymptomatic infections. Multiple phage types were encountered in 12 outbreaks representing 155 isolates of *E. coli* 0157:H7; in the remaining 139 outbreaks only a single phage type was found (Table 6).

Of the 19 nursing home outbreaks, 1 outbreak of phage type 4 occurred in British Columbia; 2 outbreaks in Alberta showed mixed types 1 and 4; 1 in Saskatchewan included mixed types 8, 23 and 31; 14 outbreaks in Ontario

belonged to single phage types, 1, 4, 8, 23, 32, and 2 were mixed types (1 and 2 and 1 and 4); in New Brunswick type 4 was isolated.

Of the 12 community outbreaks, 3 occurred in British Columbia (phage type 2, mixed types 1 and 23, and 1, 4, 14 and 31); 6 in Ontario (phage types 8, 31 and 32, and mixed types 1, 4, 21 and 31); 2 in Newfoundland (phage types 2 and 4) and 1 of phage type 1 in Prince Edward Island. Three hospital outbreaks occurred in Ontario (phage types 1, 14 and mixed types 1 and 8). One hundred and twelve family outbreaks were observed across Canada.

Although meat has often been suspected as the source of infection in these outbreaks, very few incidents have been associated with specific foods. In an Alberta nursing home outbreak, left-over ground beef was positive for *E. coli* 0157:H7 phage type 1. Nine of the 10 human isolates were phage type 1 and one other, from a vegetarian, was phage type 4. In a family outbreak, two children and the associated veal cutlets consumed were positive for *E. coli* 0157:H7 phage type 31. In a community outbreak, the left-over ground beef was positive for *E. coli* 0157:H7 phage type 2. No other isolates were investigated. Raw milk was implicated as a vehicle in a community (kindergarten) outbreak of phage type 8, but the only positive non-human samples were calf faeces (phage type 8) from a farm visited by the children [10].

On the basis of epidemiological information, the highest number of outbreaks and the most sporadic cases occurred between June and September each year indicating a marked seasonal pattern of *E. coli* 0157:H7 infections.

#### DISCUSSION

A total of 6946 *E. coli* 0157:H7 were isolated in Canada between 1978 and 1989 (Table 2). There has been an exponential rise in the number of human isolates of *E. coli* 0157:H7 since it was first recognized in Canada in 1978. In 1989, 2384 isolations were made from cases of diarrhoea, HUS or HC. This made it necessary to attempt the differentiation of this serotype by phage typing. In our present study, the phage typing scheme has been extended from 14 types [5] to 62 different phage types (Table 1). All 3273 strains of *E. coli* 0157:H7 were sensitive to 2 or more of the 16 typing phages.

Phage type 1, found previously common in Canada [5] is still the predominant type in the present survey (see Table 3 and 5). The frequency of other phage types in this study was not the same as that reported previously [5] probably because of the sample size (98 strains to 3273 strains studied) and the limited geographical area as compared to the present study. Phages types 1, 2, 4, 8, and 14 seen in the previous study, were also observed in this study. However, other phage types 3, 5, 6, 7, 9, 10, 11, 12, 13 were not observed in the present study. Phage types 8 and 14 were seen as sporadic cases (3 isolates) in the previous study, whereas in the present study, these types were associated with 33 outbreaks (96 cases) across Canada.

Of the 151 outbreaks of *E. coli* 0157:H7, 32 outbreaks belonged to phage type 1 (Table 6). This phage type was also associated with other types in 11 outbreaks. The study of *E. coli* 0157:H7 phage types in the United Kingdom [7] has also shown that phage type 1 was common. Phage type 49, the third most common in



the United Kingdom [7] has not been found in Canada (Table 5). In comparing the prevalence of phage types from human and non-human sources (Tables 3 and 4), the nine most common phage types [1, 2, 4, 8, 14, 21, 23, 31 and 32] which accounted for 96.2% (3110/3231) of the human isolates were also observed among animal isolates suggesting that meat products may play a major role in the transmission of verotoxigenic *E. coli* 0157:H7 in the human population. Other studies [10–12] have also shown the prevalence of *E. coli* 0157:H7 in meats and dairy cattle. So far in Canada 26 other phage types were exclusively observed in human infections (Tables 3 and 4). These findings emphasize the necessity of examining a sufficiently large number of *E. coli* 0157:H7 strains from non-human sources.

The geographical distribution of phage types in Canada and other countries for the infrequent phage types (Table 5), shows that types 47 isolated in Japan and 49, 50, 51 and 52 in the U.K. [7] have not been observed in Canada. Similarly, within Canada, phage types 36, 38 and 53 were observed only in British Columbia and types 55 and 58 only in Alberta.

The extended phage typing scheme not only provides for strain discrimination among sporadic cases but has also been useful in the study of outbreaks (Table 6). Family outbreaks were observed across Canada indicating a widespread occurrence of these organisms. In one family outbreak, two children were positive for *E. coli* 0157:H7 phage type 31, as were the strains isolated from veal consumed. In only one family outbreak, a direct association existed between hamburger consumption in the home and *E. coli* 0157:H7 infection, suggesting that improper cooking of food prepared at home may play a role in the spread of the infection. The occurrence of more than one phage type in outbreaks of *E. coli* 0157:H7 has been reported in Canada [5] and in the UK [7]. In our present study, we have observed mixed phage types in 12 outbreaks. These outbreaks mostly occurred in institutions. Recently, Frost and colleagues [7] have demonstrated the relatedness of types 1, 4, 8 and 14 by further characterization of strains with markers other than phage typing. Similar characterization of our strains with other markers will be the subject of a further study.

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#### REFERENCES

1. Johnson WM, Lior H, Bezanson GS. Cytotoxic *Escherichia coli* 0157:H7 associated with haemorrhagic colitis in Canada. *Lancet* 1983; i: 76.
2. Smith HR, Rowe B, Gross RJ, Fry NK, Scotland SM. Haemorrhagic colitis and verocytotoxin-producing *Escherichia coli* in England and Wales. *Lancet* 1987; i: 1062–65.
3. Karmali MA, Petric M, Lim C, Fleming PC, Arbus GS, Lior H. The association between idiopathic hemolytic uremic syndrome and infection by verotoxin-producing *Escherichia coli*. *J Infect Dis* 1985; **151**: 775–82.

4. Scotland SM, Willshaw GA, Smith HR, Rowe B. Properties of strains of *Escherichia coli* belonging to serogroup 0157 with special reference to production of verocytotoxins VT1 and VT2. *Epidemiol Infect* 1987; **99**: 613-24.
5. Ahmed R, Bopp C, Borczyk A, Kasatiya S. Phage-typing scheme for *Escherichia coli* 0157:H7. *J Infect Dis* 1987; **155**: 806-9.
6. Ratnam S, March SB, Ahmed R, Bezanson GS, Kasatiya S. Characterization of *Escherichia coli* serotype 0157:H7. *J Clin Microbiol* 1988; **26**: 2006-12.
7. Frost JA, Smith HR, Willshaw GA, Scotland SM, Gross RJ, Rowe B. Phage-typing of verocytotoxin (VT) producing *Escherichia coli* 0157 isolated in the United Kingdom. *Epidemiol Infect* 1989; **103**: 73-81.
8. Adams MH. Bacteriophages. New York: Interscience Publishers Inc., 1959.
9. Farmer JJ, III, Hickman FW, Sikes JV. Automation of *Salmonella typhi* phage typing. *Lancet* 1975; ii: 787-90.
10. Borczyk AA, Karmali MA, Lior H, Duncan LMC. Bovine reservoir for verotoxin-producing *Escherichia coli* 0157:H7. *Lancet* 1987; i: 98.
11. Doyle MP, Schoeni JL. Isolation of *Escherichia coli* 0157:H7 from retail fresh meats and poultry. *Appl Environ Microbiol* 1987; **53**: 2394-96.
12. Orskov F, Orskov I, Villar JA. Cattle as reservoir of verotoxin-producing *Escherichia coli* 0157:H7. *Lancet* 1987; ii: 276.