

Distribution of *Salmonella enteritidis* phage types in Canada

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SUMMARY

The distribution of *Salmonella enteritidis* phage types in Canada is described; 606 of 674 strains examined were of human origin. Typable strains of all sources, constituted 99.6% (671/674) of all strains examined, and were representative of 15 different phage types. Five phage types (8, 13, 4, 13a and 1) accounted for 92.4% of the total. Phage type 8 consistently showed the highest incidence in human (69.96%) and non-human (72.05%) sources and appeared to be the most common in North America. Phage type 4, the most prevalent in the UK, is infrequent in Canada (38/674). The distribution of phage types showed regional variation among infrequent phage types, whereas the common type, 8, was observed in different frequencies in all provinces. Examination of 29 outbreaks of *S. enteritidis* representing 254 isolates for humans revealed 5 different phage types, the highest number of outbreaks (11) were type 8. A study of these outbreaks and the animal-host-associations of the common phage types, 8 and 13, indicated that contaminated poultry appeared to be the most common source of human infection in Canada.

INTRODUCTION

In Canada, through a nationwide surveillance system of the National Reference Laboratory for Enteric Pathogens, Laboratory Centre for Disease Control, *Salmonella typhimurium* ranked first among human and non-human isolates [1-3]. During 1976-89, 38.7% (46195) of the 119354 human isolates belonged to serotype, *S. typhimurium*. This was followed by *S. heidelberg* 6.8% (8055); *S. enteritidis* 6.7% (8047); *S. hadar* 6.4% (7628) and *S. thompson* 3.0% (3591). Overall, these five common serotypes comprised 61.6% (73516) of the total salmonella isolated in Canada during this period. Although the order of frequency of these common serotypes varied from year to year, *S. enteritidis* has remained one of the five most frequent serotypes among human isolates during these years [1-3]. However recently, *S. enteritidis* has been implicated in outbreaks in Canada [3], the USA [4, 5], the UK [6, 7], and in western European countries (Finland, France, West Germany, Denmark, Spain and Portugal) [8, 9]. Poultry or poultry products have been the main source of human infections [6, 10]; and recently, eggs have also been incriminated in human infections in the UK, Spain and northeastern US [5, 9, 11].

For the discrimination of *Salmonella enteritidis*, phage-typing schemes have been described [12-15]. In order to determine the possible role that *S. enteritidis*

isolated from non-human sources plays in human infections of *S. enteritidis*, we have applied a phage-typing scheme developed in the UK [13] by which we investigated isolates received from both human and non-human sources.

MATERIALS AND METHODS

Bacterial strains

We have investigated 674 *S. enteritidis* strains by phage-typing. These representative cultures, isolated from human and non-human sources between 1977 and 1989, included 254 strains from 29 outbreaks, 352 cultures from sporadic cases and 68 from non-human sources (poultry, milk, water, etc.).

Phage-typing

The phage-typing scheme for *S. enteritidis*, developed in the UK [13], together with its phages and type strains were obtained from Dr B. Rowe, Director of Division of Enteric Pathogens, Central Public Health Laboratories, Colindale, UK. The standard phage-typing technique described by Anderson and Williams [14] was employed throughout this investigation. Strains showing a pattern that did not conform to any recognized phage type were considered atypical (AT). Strains which did not react with any of the typing phages were considered untypable (UT).

RESULTS

Isolations of S. enteritidis in Canada (1976-89)

Table 1 shows the number of *S. enteritidis* isolated in Canada compared with the total salmonella isolated between 1976 and 1989. Although the percentage frequency of *S. enteritidis* varied from year to year, there has been no major increase of *S. enteritidis* reported from human sources. However, *S. enteritidis* ranked among the five common serotypes during this period.

Frequency of phage types of S. enteritidis in human and non-human sources

The frequency of *S. enteritidis* phage types from human and non-human sources is presented in Table 2. Fifteen different phage types were identified from all sources and represented 96.3% (649/674) of the strains; 3.3% (22/674) were designated AT, whereas only 0.4% (3/674) of the strains were found to be UT.

Thirteen different phage types were identified among 606 strains of *S. enteritidis* isolated from human sources; the most frequent, phage type 8, represented approximately 70% of the isolations. Among the 68 *S. enteritidis* isolates from non-human sources, 9 phage types were encountered; phage type 8, represented approximately 72% of the isolations.

Phage type 8 appears to be the predominant type among human (70%) and non-human (72%) isolates. Although the frequency of other phage types varied in both groups, two phage types, 14a and 23, were exclusively identified among non-human isolates while six phage types were encountered only among human isolates (Table 2).

Seven different phage types were identified among poultry isolates (Table 3); the majority of poultry isolates (33/49) belonged to phage type 8. Five isolations

Table 1. *Salmonella enteritidis* isolations in Canada (1976–89)

Year	Number of isolates in human		yearly rank	(%)
	Salmonella total	<i>S. enteritidis</i> total		
1976	4310	375	2	(8.7)
1977	5471	454	2	(8.3)
1978	8471	359	4	(4.2)
1979	8704	405	4	(4.7)
1980	8749	667	3	(7.6)
1981	9457	717	3	(7.6)
1982	9452	351	4	(3.7)
1983	8094	307	5	(3.8)
1984*	10303	283	4	(2.8)
1985	7128	602	3	(8.5)
1986	9861	927	3	(9.4)
1987	10644	888	3	(8.3)
1988	9957	914	4	(9.2)
1989	8762	798	3	(8.9)

* The totals for 1984–9 include only partial reports provided by the Province of Quebec Public Health Laboratory.

Table 2. *Phage types of S. enteritidis in Canada*

Phage type	Human isolates	Non-human isolates	Total	Rank
1	13	2	15	5
2	4	—	4	
4	36	2	38	3
6	3	—	3	
6a	2	—	2	
8	424	49	473	1
9	1	—	1	
9b	4	2	6	
13	59	3	62	2
13a	31	4	35	4
14	2	—	2	
14a	—	2	2	
19	3	1	4	
23	—	1	1	
26	1	—	1	
Atypical (AT)	21	1	22	
Untypable (UT)	2	1	3	
Total	606	68	674	

of phage type 8 and one of phage type 13 were from eggs and egg products. *S. enteritidis* phage type 4 was isolated from turkey litter in Canada in 1980 and recently from imported finches (country unspecified).

Geographical distribution of phage types for S. enteritidis in Canada

The geographical distribution of *S. enteritidis* phage types from human infections is presented in Table 4. The greatest variety of phage types was observed in British Columbia (10 phage types), followed by Ontario (6 types). The

Table 3. *Phage types of S. enteritidis from non-human sources in Canada*

Phage type	Non-human sources	No. of isolates	Total isolates
1	Poultry (chicken litter)	2	2
4	Imported Finch	1	2
	Litter (Turkey)	1	
8	Poultry	33	49
	Eggs and egg products	5	
	Buttered bread crumbs and flour	2	
	Porcine and ham	2	
	Milk (bovine)	1	
	Water	1	
	Avian	3	
	Animals (fox and mink)	2	
9b	Poultry	1	2
	Feed supplement (poultry)	1	
13	Avian	1	3
	Poultry	1	
	Egg (cake)	1	
13a	Poultry	2	4
	Chicken nugget	1	
	Cheese curd	1	
14a	Water	2	2
19	Poultry	1	1
23	Poultry	1	1
AT (Atypical)	Avian	1	1
UT (Untypable)	Buttered bread crumbs	1	1
Total		68	68

predominant phage type, 8, was encountered in different frequencies in all provinces. We found regional differences among the 606 *S. enteritidis* studied. Phage types 9 and 26 occurred only in British Columbia, type 2 only in Alberta and Saskatchewan, and type 14 only in Newfoundland. Phage type 13, the second most common type was only seen in British Columbia (55 isolates) and in Ontario (4 isolates).

The phage-typing of *S. enteritidis* isolates from human outbreaks are shown in Table 5.

Five different phage types were associated with 29 food-poisoning outbreaks – 10 restaurants, 5 institutional (1 nursing home, 3 hospitals and 1 church supper), 1 airline caterer and 13 family outbreaks. The highest number of outbreaks (17/29) were due to phage type 8, followed by 5 outbreaks belonging to phage type 13. Fourteen of the outbreaks (11 of type 8, 1 of type 13 and 2 of 13a) were observed in Ontario, and 7 outbreaks (4 of type 13 and 1 each of type 4, 8 and AT) in British Columbia.

Four outbreaks have been associated with specific foods. A large food poisoning outbreak of *S. enteritidis* phage type 8 due to contaminated cake occurred in Ontario; this phage type was identified from the cake and one of the cases examined from the total of 104 laboratory confirmed cases of *S. enteritidis*. In a hospital outbreak, 30 cases as well as food isolates (bread crumbs, breaded chicken and pork, and flour) belonged to *S. enteritidis* phage type 8. The same phage type

Table 4. Frequency of phage types for *S. enteritidis* in Canada from human sources

Phage type	BC*	Alta	Sask	Man	Ont	PQ	NB	NS	PEI	Nfld	Total	(%)
1	3	-	7	-	2	-	-	1	-	-	13	(2.1)
2	-	3	1	-	-	-	-	-	-	-	4	(0.7)
4	17	-	2	3	11	-	-	2	1	-	36	(5.9)
6	2	-	-	-	-	-	1	-	-	-	2	(0.5)
6a	1	-	-	-	-	-	1	-	-	-	2	(0.3)
8	111	10	10	2	159	1	83	24	12	12	424	(70.0)
9	1	-	-	-	-	-	-	-	-	-	1	(0.1)
9b	2	-	2	-	-	-	-	-	-	-	4	(0.7)
13	55	-	-	-	4	-	-	-	-	-	59	(9.7)
13a	6	-	-	-	22	-	-	3	-	-	31	(5.1)
14	-	-	-	-	-	-	-	-	-	2	2	(0.3)
19	-	-	-	-	2	-	-	1	-	-	3	(0.5)
26	1	-	-	-	-	-	-	-	-	-	1	(0.2)
Atypical (AT)	3	2	1	-	3	9	-	3	-	-	21	(3.5)
Untypable (UT)	1	-	-	-	1	-	-	-	-	-	2	(0.3)
Total	203	15	23	5	204	10	85	34	13	14	606	

* BC, British Columbia; Alta, Alberta; Sask, Saskatchewan; Man, Manitoba; Ont, Ontario; PQ, Quebec; NB, New Brunswick; PEI, Prince Edward Island; Nfld, Newfoundland.

Table 5. *Phage types of S. enteritidis identified from outbreaks*

Phage type	Province	No. of outbreaks	No. of cases studied
1	Sask	2	4
4	BC	1	2
8	BC	1	2
	Alta	1	2
	Ont	11*	107
	NB	1	72
	NS	1	3
	PEI	1	7
	Nfld	1	2
13	BC	4†	30
	Ont	1	2
13a	Ont	2	10
	BC	1	2
Atypical	PQ	1	9
Total		29	254

* Three outbreaks were associated with bakery cake, breaded chicken and egg salad – ham.

† One outbreak was associated with cream-filled cake.

was isolated from 10 cases and from the egg salad and ham in a restaurant outbreak. Phage type 13 was identified in a large outbreak (36 laboratory confirmed cases) in British Columbia involving cream-filled cakes sold by two bakeries.

DISCUSSION

During 1976–89, salmonella isolates from human sources in Canada varied from year to year (Table 1). In each of these years, increasing isolations of a few serotypes (*S. typhimurium*, *S. hadar*, *S. heidelberg*), resulted in increased cases in an annual total of reported cases of salmonellosis [1–3, 17] and *S. typhimurium* always ranked first although with variable frequency during this period. However, it is interesting to note that overall, *S. enteritidis* remained fairly stable, with variable frequencies from year to year among human isolates (Table 1). In 1987, *S. enteritidis* was not reported among the 15 most common salmonella serotypes from non-human sources in Canada [3]. In 1988, it ranked twelfth (63/3208) of the total salmonella from non-human sources; 42 of the 63 isolates were reported from poultry and 2 from eggs.

In other countries (USA, UK and western European countries) recently, there has been a dramatic increase of *S. enteritidis* infection [2, 4, 5, 9] among human isolates. Evidence has suggested that this *S. enteritidis* epidemic is due to consumption of contaminated eggs and poultry.

Our phage-typing study identified 15 different phage types (Table 2), and each of these 15 accounted for more than 1% of the cases of human or non-human origin. Phage types 8 (70.2%) and 13 (9.2%) were most common.

We studied a limited number of isolates (130) from the USA and found the prevalence of phage types 4, 6, 8, 9b, 13, 13a, 19, 23 and 25 (data not shown). It has been reported that in the USA, *S. enteritidis* isolates from human outbreaks belonged to phage types 8 and 13a [9].

In the UK and from western European countries [7, 9, 13], *S. enteritidis* phage type 4 has been the most frequent type followed by type 8.

In Canada, *S. enteritidis* type 4 was infrequent (38/674) (Table 2). The first human isolate of type 4 was identified in 1979 in the province of Nova Scotia. Only one family outbreak (1989) was associated with phage type 4 (Table 5) and the remaining cases were sporadic. Among the sporadic cases of phage type 4, there were at least 19 cases (8 in British Columbia, 3 in Alberta and 8 in Ontario) that acquired the infection after returning from abroad.

On comparing the frequency of less common phage types it was observed in the UK that phage type 24 has increased from 24 isolations in 1987 to 341 isolations during the first 6 months of 1989 [17]. To date, type 24 has not been isolated in Canada. Phage type 22 was only recently identified in Canada in a food poisoning outbreak. This type was isolated from six cases and from tapioca, lemon and chocolate pudding. In the UK phage type 22 has been a rare type [13] and in 1989 there was only one isolate of this type (B. Rowe and L. Ward, personal communication). Overall, phage-typing showed the predominance of two phage types, 4 and 8, among *S. enteritidis* isolates, suggesting the need for a further subdivision of these types by plasmid profiles [19].

Our plasmid analysis (data not shown) of 185 *S. enteritidis* strains of different phage types identified six different plasmid patterns. Five plasmid profile patterns were observed in 30 strains of phage type 4 and the most common pattern was characterized by the presence of a 38 MDa plasmid (23/30 of strains). This pattern was also most common among phage type 4 strains in the UK [19]. In Canada, as well as in the UK [18], this method has been used as an adjunct to phage-typing in support of epidemiological investigations of *S. enteritidis*.

The strains examined in our study reflect the trends of distribution patterns of the phage types of *S. enteritidis* in Canada based on a sample of the available cultures in our collection.

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