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Surveillance for *Listeria monocytogenes* and listeriosis, 1995–2004

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SUMMARY

Canadian cases and outbreaks of illness caused by *Listeria monocytogenes* between 1995 and 2004 were assessed. Isolates (722 total) were characterized by serotyping, and pulsed-field gel electrophoresis (PFGE) was performed to provide a means of detecting case clusters. Rates of listeriosis remained fairly consistent during the period of study, and patient characteristics were similar to those seen in studies of other populations. Most isolates were obtained from blood and cerebrospinal fluid, although during some outbreak investigations isolates were also obtained from stools. Serotype 1/2a predominated in isolates from patients in Canada, followed by serotypes 4b and 1/2b. Outbreaks caused by *L. monocytogenes* that occurred during the period of study were caused by isolates with serotypes 1/2a and 4b. A retrospective analysis of PFGE data uncovered several clusters that might have represented undetected outbreaks, suggesting that comprehensive prospective PFGE analysis coupled with prompt epidemiological investigations might lead to improved outbreak detection and control.

Key words: *Listeria monocytogenes*, listeriosis, PFGE, surveillance.

INTRODUCTION

Listeria monocytogenes is responsible for a significant proportion of deaths arising from infection with foodborne bacterial pathogens. Infection with *L. monocytogenes* was the fourth most common cause of death due to bacterial indigenous foodborne disease in England and Wales during the period 1992–2000 despite a very low incidence (0·003 cases/1000 person-years [1]). Listeriosis is responsible for about 500 fatalities annually in the USA, or about 28% of all deaths caused by known foodborne pathogens. This was second only to deaths resulting from *Salmonella* infections [2].

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Common clinical syndromes of severe (classic) listeriosis include sepsis, meningitis, encephalitis, endocarditis, and focal infections [3]. *L. monocytogenes* can also cause febrile gastroenteritis [4–6] and can be isolated from stools of healthy people [7–9]. Summaries of long-term surveillance show that severe listeriosis predominantly affects neonates and young infants, pregnant women and their unborn children, older individuals, and immunocompromised individuals [1, 8, 10–15].

Data from the U.S. Foodborne Disease Active Surveillance Network (FoodNet) showed a reduction in cases between 1996 and 2002 of 5 to 2·7 cases per million persons [13, 16]. However, between 2004 and 2005 the incidence of listeriosis increased from 2·7 to 3 cases per million persons [17, 18]. The incidence of bloodborne listeriosis in England and Wales has shown a similar recent increase [15]. In 2006, member states of the European Union reported a higher number of annual cases than had been seen in each of the last 8 years. This constituted an increasing and statistically significant trend for many of the member states during this period [19].

Canada began a laboratory surveillance programme for *L. monocytogenes* in 1987, with reporting from only four of ten provinces. However, the incidence of listeriosis in Canada from 1987 to 1994 was estimated using data from Ontario alone [8]. During this period the annual number of cases ranged from 44 to 109 nationally (1·7–4·5 cases per million). In 1996 the estimated cost of listeriosis illness and deaths in Canada was between 11·1 and 12·6 million Canadian dollars annually [8]. The only reported Canadian outbreak of listeriosis before 2000 occurred in the Maritime Provinces in 1981 [20]. There were 41 cases and 17 deaths, all caused by isolates with serotype 4b. The vehicle of infection was coleslaw made from cabbage contaminated at a farm after fertilization with raw manure from a flock of sheep. Recent outbreaks have also occurred in Québec in 2002 [21; Ministère de santé et services sociaux, http://www.msss.gouv.qc.ca/sujets/santepub/listeriose.php] and 2008, in British Columbia in 2002 (British Columbia Centre for Disease Control, 2002 Annual Summary of Reportable Disease, http://www.bccdc.org/downloads/pdf/epid/BCCDC_annual_report_2002.pdf) and across Canada in 2008 (Public Health Agency of Canada, http://www.phac-aspc.gc.ca/alert-alerte/listeria/listeria_2009-eng.php).

Serotyping has been extensively used to differentiate *L. monocytogenes* into groups that appear to be relevant in terms of human disease and clonal lineages of the organism. Three of the thirteen serotypes, 1/2a, 1/2b and 4b, predominate in human disease, with large outbreaks almost exclusively linked to serotype 4b [22].

Subtyping of *L. monocytogenes* has been accomplished using a number of methods. Pulsed-field gel electrophoresis (PFGE) has proved to be an extremely powerful tool for detecting epidemiologically relevant clusters of *L. monocytogenes*, as well as for use in trace-back investigations of implicated foods [23, 24]. It has also proved valuable for long-term surveillance [10, 11].

The National Listeriosis Reference Service (LRS) was instituted in 2001 to provide enhanced laboratory surveillance and to implement molecular subtyping in conjunction with PulseNet Canada [25]. We have retrospectively analysed by PFGE all human isolates of *L. monocytogenes* recovered in Canada from 1995 to 2004. With the inclusion of the associated case data, this analysis will provide a baseline against which the efficacy of future public health control measures can be assessed. It will also allow us to assess and improve national listeriosis surveillance data quality.

MATERIALS AND METHODS

Case definitions

From 1995 to 1999 the National Notifiable Diseases (NND) listeriosis case definition was used. A case was defined as a person exhibiting 'clinically compatible symptoms with the isolation of *L. monocytogenes* from a site which is normally sterile, including foetal gastrointestinal contents' (Canadian Diseases Weekly Report Supplement-Canadian Communicable Disease Surveillance System-Disease-Specific Case Definitions and Surveillance Methods, March 1991; volume 17S3). The case definition did not include gastrointestinal infection with *L. monocytogenes*. In addition, the definition did not specify whether abortions, stillbirths, or infected neonates were all counted separately from the infected mother. Provincial public health laboratories in some jurisdictions reported to the LRS instances of *L. monocytogenes* gastroenteritis, defined as patients with stool samples positive for *L. monocytogenes*, as well as isolates from other anatomical sites.

NND and provincial/territorial reportable data

To be collected by the NND, specimens submitted to primary microbiology laboratories must test positive

for an enteric pathogen that is reportable within the province. The laboratory must report the positive isolation to a local health authority (for instance, a health unit) directly or via a physician, who will then report the case, with associated epidemiological information, to the provincial authority (e.g. Ministry of Health). The provincial authority then reports the data to the NND. Because health falls under provincial/territorial jurisdiction, provision of data to the NND is voluntary.

From 1995 to 1999, total listeriosis case counts and epidemiological data associated with listeriosis cases (year, age, sex) were routinely collected from all provinces and territories, except Québec, through the NND system, also known as the National/Provincial Notifiable Diseases system [NPND; 'Notifiable Diseases On-Line – Listeriosis' (http://dsol-smed.hc-gc.ca/dsol-smed/ndis/diseases/list_ehtml)]. NND will be used here for simplicity. From 2000 to 2004, during which listeriosis was not nationally notifiable, listeriosis remained reportable in all provinces and territories except for Québec. Listeriosis became reportable in Québec in 2004. Total listeriosis case counts and associated epidemiological data (year, age, sex) reported to the provinces/territories from 2000 to 2004 were consequently provided directly, as noted above, by the provincial/territorial ministries of health to the NND, from which they were made available through *ad hoc* queries. For simplicity, all these data will be considered to have originated from the NND.

LRS data

Collection of data for laboratory surveillance also begins with the isolation at a primary laboratory of an enteric pathogen that is reportable in the province. Isolates or specimens may or may not then be forwarded to the provincial public health laboratories; limited patient information may or may not accompany the isolate or specimen. All *L. monocytogenes* isolates arriving at each public health laboratory were forwarded to the LRS. Information was provided, either at the time of sample submission or retrospectively, about patients' age and sex, the date of isolation or date of receipt of the isolate at the laboratory, and the province where the isolate was collected. When reporting the number of infections based on the laboratory data, individuals from whom multiple isolates were recovered were counted as a single patient.

Hospitalization data

The average annual number of hospitalizations from 1995 to 2004 due to all forms of listeriosis, excluding congenital and neonatal cases, was determined using the Canadian Institute for Health Information's Hospital Morbidity Database. Both the Ninth and Tenth Editions of the International Classification of Diseases (ICD) were in use over this time period. A hospitalized case of listeriosis was defined as an individual whose hospitalization record listed ICD-9 code 027.0 or ICD-10 code A32 in the first three diagnostic codes. The ICD-9 code 027.0 and the ICD-10 code A32 are equivalent.

Death data

Statistic Canada's Vital Statistics–Death Database code was used to determine the annual number of deaths due to listeriosis from 1995 to 2004. These were defined as deaths for which ICD-9 code 027.0 (1995–1999) and ICD-10 code A32 (2000–2004) was listed as the underlying cause of death.

Analysis

Notifiable/reportable disease surveillance data were used to describe the number and incidence of invasive listeriosis cases by year, age and sex. Census population data were obtained from Statistics Canada. Descriptive analyses of reported listeriosis cases and hospitalization and case-fatality rates were calculated using Microsoft Excel. The population of Québec was excluded from denominators for incidence rates from 1995 to 2003. Case-fatality rates were calculated using the number of deaths as the numerator and the total number of cases reported through notifiable/reportable disease surveillance as the denominator. χ^2 statistics were calculated using the SigmaStat 3.5 statistics package (Systat Software Inc., USA).

Bacterial isolates, serotyping, and PFGE

Isolates provided to the LRS were grown on brain heart infusion (BHI) agar or BHI broth and stored in skimmed milk at $-80\,^{\circ}C$. Serotyping of O- and H-antigens was done according to the methods of Seeliger & Hohne [26] using antiserum prepared, standardized, and quality assured in-house or by using commercial antiserum (Denken Seiken Co. Ltd, Japan) according to the manufacturer's instructions.

Table 1. *Rates of reported listeriosis in Canada* (*per million*) *based on National Notifiable Diseases* (*NND*) *data, by province/territory, 1995–2004*

Province/territory	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004
BC	0·8	1·0	0·8	0·5	0·7	1·5	1·5	3·4	2·9	2·4
AB	3·7	0·0	1·1	4·1	4·1	0·3	3·6	1·6	3·2	1·6
SK	2·0	0·0	7·9	7·9	3·9	1·0	5·0	3·0	2·0	0·0
MB	5·3	2·6	0·0	0·9	4·4	4·4	1·7	2·6	3·4	0·9
ON	4·0	2·3	3·3	4·5	2·7	3·2	3·0	3·5	3·5	3·4
QC	—	—	—	—	—	—	—	—	—	4·2
NS	1·1	3·2	3·2	1·1	2·1	0·0	4·3	0·0	7·5	1·1
NB	0·0	4·0	1·3	2·7	0·0	5·3	1·3	1·3	1·3	5·3
PEI	0·0	7·4	14·7	0·0	0·0	0·0	0·0	0·0	0·0	0·0
NL	3·5	0·0	0·0	1·9	3·7	0·0	5·7	1·9	1·9	1·9
NN	—	—	—	—	—	0·0	0·0	0·0	0·0	0·0
NWT	0·0	0·0	0·0	0·0	0·0	0·0	0·0	24·1	0·0	0·0
YT	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0
National total	3·1	1·8	2·5	3·4	2·6	2·3	2·9	2·9	3·3	3·0

AB, Alberta; BC, British Columbia; NB, New Brunswick; NL, Newfoundland; NN, Nunavut; NS, Nova Scotia; NWT, Northwest Territories; MB, Manitoba; ON, Ontario; PEI, Prince Edward Island; QC, Québec; SK, Saskatchewan; YT, Yukon Territory.
'–' Indicates that no data were provided to the NND from that province for that year.

All PFGE was performed by the two LRS laboratories using *Asc*I and *Apa*I restriction endonucleases according to standardized PulseNet methods [27]. PFGE was done retrospectively for the period 2001–2008 for isolates obtained for the years 1995–2003 and obtained in real-time by PulseNet Canada for the 2004. PFGE patterns were designated by a code consisting of the organism (LM), the enzyme used for restriction of genomic DNA (AA, *Apa*I; AC, *Asc*I), and a unique numerical identifier for each pattern differing by one band or more (e.g. 0·0001).

Retrospective cluster detection

The dates of isolation or dates of receipt of isolates at the LRS laboratories were used for cluster detection, as described recently for retrospective analyses of invasive *L. monocytogenes* infections in The Netherlands [14]. Clusters of cases have been defined as the occurrence of at least three listeriosis cases over a period of 14 weeks and involving the same PFGE type, or pulsovar [28]. We have adopted this definition but consider the pulsovar to be the PFGE subtype defined by the combination of the patterns obtained using two enzymes, *Asc*I and *Apa*I, which provides additional discriminatory power that is useful in outbreak investigations [29].

RESULTS

Descriptive analysis of incidence and burden of illness

A total of 670 listeriosis cases were reported to provincial/territorial and/or national notifiable disease systems between 1995 and 2004, with an average of 67·0 cases reported each year (median 68 cases, range 40–96 cases). During the same time period, infection of 738 individuals was reported through the LRS, with an average of 74 individuals identified per year (median 72, range 36–125 individuals) (see Supplementary Table 1, available online). The average annual incidence rate from 1995 to 2004 was 2·8 cases per million population based on NND data (median 2·9, range 1·8–3·4 cases per million; see Table 1). Incidence rates in the first 5-year period fluctuated widely, with highs noted in 1995 and 1998 and a 10-year low in 1996. In contrast, the incidence rates over the last 5-year period demonstrated a fairly steady increase from 2·3 cases per million in 2000 to 3·0 cases per million in 2004. Case numbers collected in the LRS database were higher in British Columbia in 2002, partly as the result of two outbreaks in the province during that year (Supplementary Table 1). Many individuals associated with these outbreaks were identified only on the basis of diarrhoeal symptoms or isolation of *L. monocytogenes* from stools,

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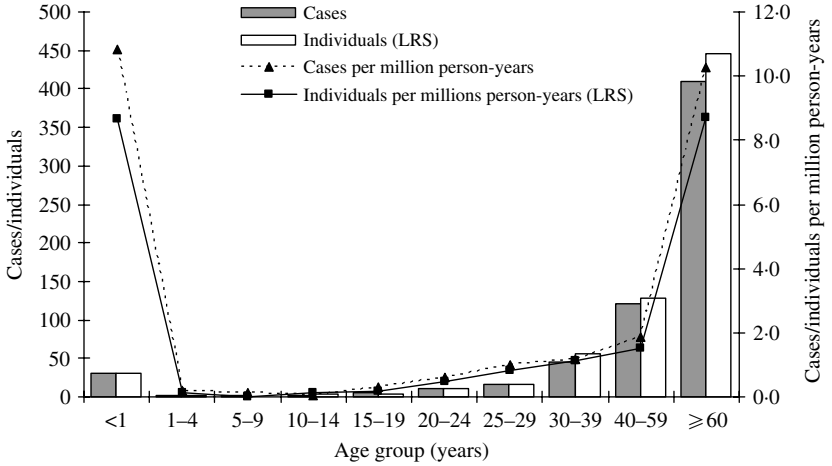

Fig. 1. Age distribution of cases and rates of infection by age group, Canada, 1995–2003.

and for this reason were not captured by the NND database.

Differences were apparent between datasets collected by the LRS and NND. Cases of listeriosis in Québec were not captured by notifiable/reportable disease surveillance until 2004 when listeriosis became provincially reportable in Québec. For most years, fewer cases were identified in the LRS data for the province of Ontario than in the NND dataset (Supplementary Table 1). During some years (e.g. 1995 and 2002) the number of cases associated with *L. monocytogenes* infection was greater in some provinces (e.g. Alberta, British Columbia) in the LRS dataset than in the NND dataset (Supplementary Table 1).

The age distributions and age-specific incidence rates for listeriosis over the 10-year study period are shown in Fig. 1. There were no significant trends over time. Although the absolute number of cases represented by the NND database was different, the age distributions and rates of infection by age group were almost indistinguishable from the LRS data (Fig. 1).

The male : female ratio of the 555 cases reported to NND for which sex data were available was about 1 : 1. However, from 1995 to 1997 the ratio ranged between 1·5–1·7 : 1, switched in 1998–1999 to 1 : 1·2–2·0, stabilized at about 1 : 1 from 2000 to 2002, and then returned to a 1·5 : 1 ratio in 2003. According to the LRS data, the male : female ratio overall was also about 1 : 1, although this varied by year. For instance, the ratio was 0·6 : 1 in 2002 and 2·7 : 1 in 1996. Data on patients' sex were available for 687 (93%) of the 738 cases for which information was submitted to the LRS.

A total of 758 *L. monocytogenes* isolates was collected from the 738 individuals. Most (84%) were isolated from blood or cerebrospinal fluid (CSF) (Table 2). Serotype 1/2a was the predominant serotype found in blood, CSF, and other body sites, while serotype 4b predominated in specimens associated with pregnancy and miscarriage. Although the numbers were extremely small, this association was statistically significant when tested using the χ^2 test (see Table 2). Serotype 4b was also found significantly more frequently in stools, but this may be more a reflection of the fact that only two outbreaks of gastrointestinal disease were investigated rather than a predilection for the gastrointestinal tract of the *L. monocytogenes* 4b themselves.

There were a total of 659 hospitalizations for which listeriosis (ICD-9 code O27.0 and ICD-10 code A32.0) was listed in the top three diagnoses from 1995 to 2004. On average, 65·9 of these hospitalizations occurred per year (median 64, range 48–84 hospitalizations per year). Listeriosis was the primary diagnosis for 455 (69·0%) of the 659 hospitalizations. The annual hospitalization rate including hospitalizations for which listeriosis was listed in the top three diagnoses averaged 2·2 hospitalizations per million population (median 2·1 hospitalizations per million population) and ranged from 1·6 to 2·8 hospitalizations per million population. The annual hospitalization rate mirrored the annual incidence of listeriosis over time with the exception of the last 2 years when it declined while the incidence rate increased. Between 1995 and 2004, there were a total of 43 deaths for which listeriosis was the underlying cause, with an average of 4·3 deaths per year (median 4·5, range 1–7 deaths). The average annual case-fatality rate for listeriosis between 1995 and 2004 was 6·5% (median 6·7%, range 1·0–10·3%).

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Table 2. *Association of serotype with specimen source for the most frequently detected serotypes*

Serotype	Blood (%)	CSF and brain tissue (%)	Specimens associated with pregnancy and miscarriage (%)*	Specimens associated with female reproductive organs (%)	Specimens associated with normally sterile sites (%)†	Specimens associated with other sites (%)	Stools (%)*	Total
1/2a	253 (45·8)	45 (52·3)	1 (12·5)	4 (50)	24 (75)	27 (61·4)	6 (21·4)	360
1/2b	82 (14·9)	11 (12·8)	0	1 (12·5)	0	3 (6·8)	0	97
1/2c	5 (0·9)	1 (1·2)	1 (12·5)	0	0	1 (2·3)	0	8
4b	160 (30·0)	22 (25·6)	6 (75·0)	3 (37·5)	7 (22)	9 (20·5)	22 (78·6)	229
Others	52 (9%)	7 (8·1)	0	0	1 (3)	4 (9·1)	0	64
Total	552	86	8	8	32	44	28	758

In some fields there were two specimen sources associated with one patient and one isolate. For that reason, the number of specimens shown in this table is greater than the number of isolates shown in some other tables.

* Statistically different from both blood and CSF and brain tissue, $P<0{\cdot}001$, power of performed test with $\alpha=0{\cdot}050$: $\geqslant 0{\cdot}966$.

† Statistically different from blood only, $P<0{\cdot}015$, power of performed test with $\alpha=0{\cdot}050$: 0·818.

Outbreaks of foodborne *L. monocytogenes* in Canada

Six foodborne listeriosis outbreaks were reported in Canada between 1995 and 2003 (Table 3). In 2000, a small outbreak of listeriosis involving two previously healthy adults occurred in Ontario [30]. Another outbreak of gastrointestinal disease caused by *L. monocytogenes* was identified in Manitoba in June, 2000. A *L. monocytogenes* strain with the outbreak PFGE patterns was also isolated from the CSF of one patient.

In the summer of 2000, an outbreak of listeriosis was associated with flat whipping cream at a Winnipeg church event. Molecular typing using PFGE and randomly amplified polymorphic DNA (RAPD) revealed indistinguishable patterns for all isolates.

An outbreak that occurred in Québec in 2002 was caused by cheese that was heat-treated but not pasteurized. Seventeen cases were identified, including two pregnant women (Table 3 [21]). Of the 17 cases, 11 required hospitalization. Isolates recovered from patients were all serotype 1/2a. In early February 2002, two cases of *L. monocytogenes* meningitis were reported in British Columbia. Following epidemiological investigations, both a recall of the implicated cheese and a 3-month period of enhanced surveillance were implemented to detect invasive listeriosis and febrile gastroenteritis associated with cheese consumption produced by a single company (Table 3). Isolates from clinical specimens, cheese, and the implicated dairy plant had indistinguishable PFGE patterns and ribotypes. A third outbreak of *L. monocytogenes* associated with cheese also occurred in September 2002 in British Columbia (Table 3). The contaminated cheese caused 86 clinical cases of febrile gastroenteritis. Only a single cheese brand from a single production date tested positive for the organism, with counts of *L. monocytogenes* ranging from 10^2 to 10^9 c.f.u./g. The probable source of this outbreak was introduction of *L. monocytogenes* through the water supply via an inadequately protected reservoir. Poor water quality rendered the disinfection system ineffective. Isolates of *L. monocytogenes* obtained in November 2002, from a pipe in the water cistern had PFGE patterns indistinguishable from those found in the implicated cheese.

Characteristics of Canadian *L. monocytogenes* isolates

Serotype 1/2a predominated over all other serotypes every year except for 2002, the year in which two large outbreaks were identified [Fig. 2, Supplementary Fig. 1 (available online)]. During the period of study, about 48% (237/490) of the isolates serotyped were serotype 1/2a, 32% (157/490) were 4b, 15% (72/490) were 1/2b, and the remaining 4–5% of isolates were distributed in serotypes 3b, 4a, 4c, 1/2c, 3a, and 4e. Most of these rare serotypes were associated with isolates from Ontario or Québec patients, although a disproportionate number (based on the relative populations of the provinces) were also from the Maritime Provinces and Newfoundland (Fig. 2). Regional and temporal variation in serotype was evident. Serotype 4b predominated in Ontario in 2000, 2002, and

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Table 3. *Outbreaks of listeriosis in Canada during the period 1995–2004*

	Year	Location	Number affected	Vehicle of infection	Specimen source for isolates	Isolate serotype	PFGE type	Reference
1	2000	Ontario	2	Imitation crab meat	Blood	1/2b	LMACI.0046	[30]
2	2000	Manitoba, British Columbia	8	n.d.	Stool (8), CSF (1)	1/2a	LMACI.0059:LMAAI.0005	Present study
3	2000	Manitoba	7	Flat whipping cream	Stool (6), blood (1), throat (1)	1/2a	LMACI.0118:LMAAI.0122	Present study
4	2002	Québec	17	Heat-treated cheese	Blood (11), CSF (5), placenta (1)	1/2a	LMACI.0044:LMAAI.0074 (16) LMACI.0044:LMAAI.0080 (1)	[21]
5	2002	British Columbia	47	Cheese	Stool (8), blood (3), CSF (3)	4b	LMACI.0023:LMAAI.0140	Present study
6	2002	British Columbia	86	Pasteurized milk cheese	Stool (14)	4b	LMACI.0082:LMAAI.0017	Present study

n.d., Not determined.

2003, in Alberta during 2001, and in British Columbia in 2002 (Fig. 2). Serotype 1/2b predominated in Ontario in 1997, and was found in approximately equal numbers with serotype 1/2a in that province in 2001.

PFGE was used as the primary subtyping method to characterize *L. monocytogenes* isolates from across Canada. When both the *Asc*I and *Apa*I PFGE patterns were used to define the bacterial subtype, 232 (72%) of the 324 combined subtypes were found in only a single isolate, and 243 (75%) combined subtypes were found only in a single year. PFGE subtyping data for the remaining isolates demonstrated different temporal patterns (Table 4). Some subtypes, including subtype LMACI.0001:LMAAI.0001, were found in most years with little evidence for a trend to increasing or decreasing isolation rates. Other subtypes persisted for a shorter, well-defined period. For instance, isolates with combined subtype LMACI.0009:LMAAI.0234 were not seen before 2002, but have been detected in each subsequent year (Table 4). Several clusters, defined as $\geqslant 3$ isolates with indistinguishable PFGE *Asc*I and *Apa*I subtypes occurring within a 14-week period [28], were detected in the retrospective analysis (Table 5). Clusters were clearly visible within the PFGE data, and the PFGE patterns and combined subtypes associated with isolates from the three outbreaks of diarrhoeal disease were in the types most frequently found (see Table 5).

DISCUSSION

The annual national rates of listeriosis ranged between 1·8 and 3·4 per million population throughout the study period. The distribution of patient ages was similar to those reported previously [22], with the majority of *L. monocytogenes* infections occurring in elderly people, neonates and women of child-bearing age. Similarly, the number of people hospitalized with listeriosis (as the most responsible diagnosis) in Canada has remained fairly constant ranging between 1·6 and 2·8 per million population between 1995 and 2004. Listeriosis case-fatality rates for Canada ranged from 1·0 to 10·3% between 1995 and 2004. According to Lee & Middleton, the incidence of listeriosis in Ontario between 1997 and 2001 was $<0{\cdot}5$ cases/100 000, but the case-fatality rate was 23·8% [31]. This was higher than for Canada as a whole. Recent case-fatality rates for other jurisdictions are: Denmark 21% [32, 33], England 44% [15], France 20% [28], Germany 9% [34], Italy 20% [35], The

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Table 4. *The most frequently detected combined* Asc*I and* Apa*I PFGE patterns in Canada, 1995–2004*

PFGE pattern	Year										Total number of isolates (%)	Rank
	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004		
LMACI.0001 LMAAI.0001	1	0	5	11	5	8	12	5	7	3	56 (7·9)	1
LMACI.0001 LMAAI.0003	1	0	5	2	9	2	4	0	0	0	23 (3·2)	2
LMACI.0004 LMAAI.0013	1	0	0	0	1	2	1	7	6	2	20 (2·8)	3
LMACI.0015 LMAAI.0024	2	2	3	3	1	1	0	1	4	2	19 (2·7)	4
LMACI.0044 LMAAI.0074	0	0	0	0	1	1	0	16	0	0	18 (2·5)	5
LMACI.0007 LMAAI.0014	3	2	0	3	5	1	0	0	0	1	15 (2·1)	6
LMACI.0023 LMAAI.0140	0	0	0	0	0	0	1	14	0	0	15 (2·1)	6
LMACI.0082 LMAAI.0017	0	0	0	0	0	0	0	14	0	0	14 (2·0)	7
LMACI.0009 LMAAI.0234	0	0	0	0	0	0	0	1	5	4	10 (1·4)	8
LMACI.0059 LMAAI.0005	0	0	0	0	0	10	0	0	0	0	10 (1·4)	8
LMACI.0002 LMAAI.0001	0	1	2	0	1	1	2	0	1	1	9 (1·3)	9
LMACI.0028 LMAAI.0023	0	0	3	5	0	0	0	0	0	0	8 (1·1)	10

The total number of isolates fully typed by PFGE with both enzymes was 710.

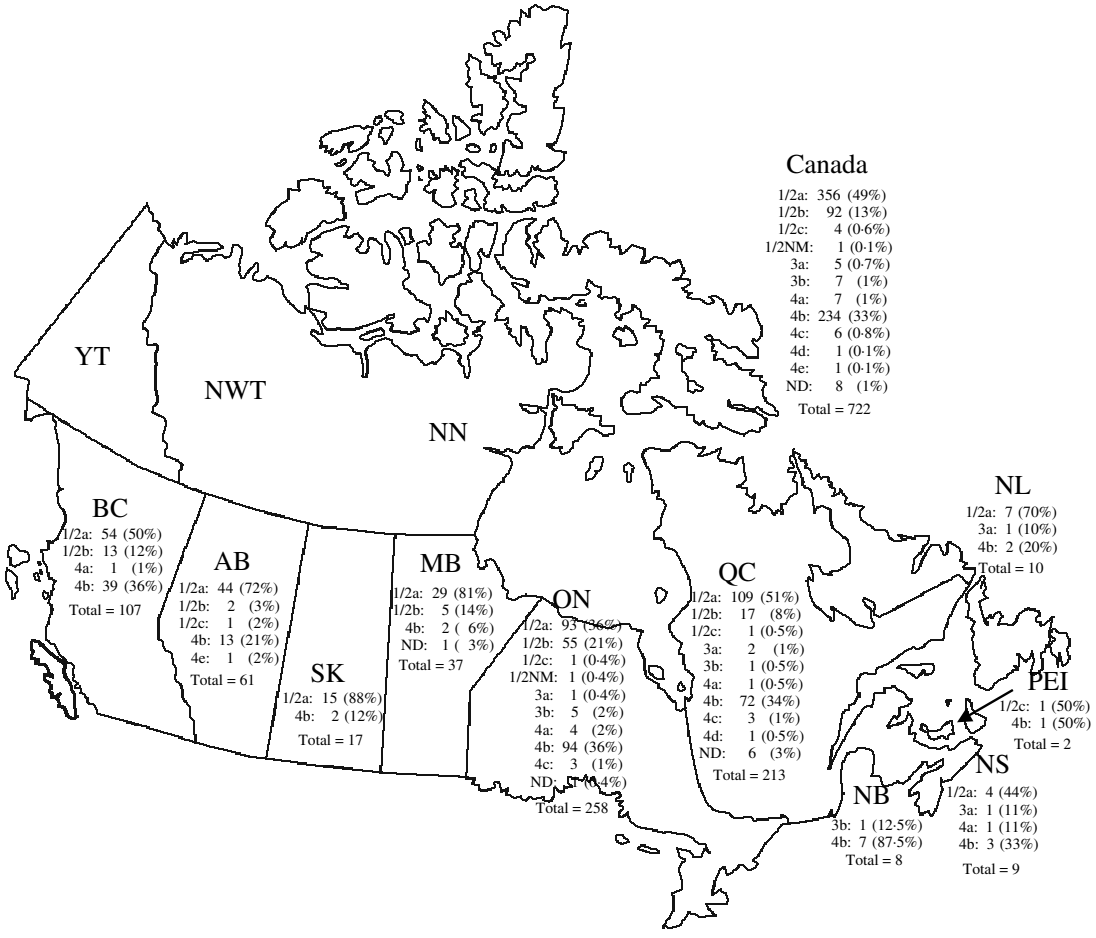

Fig. 2. Map of Canada showing the distribution of *L. monocytogenes* serotypes within each province and in Canada as a whole. ND, Not determined; one 1/2a isolate not shown, province of origin not known; total isolates ($n = 722$). For abbreviations see Table 1.

Netherlands 18% [14], and Norway 41–45% [33]. However, as noted by several groups [33, 35] low case-fatality rates can result from the collection of incomplete or imprecise information. The case-fatality rate reported here is probably an underestimate. It is based on Vital Statistics data while estimates from

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Table 5. *Clusters of cases revealed by PFGE typing. A cluster was defined as three or more isolates with indistinguishable PFGE patterns isolated or arriving at the laboratory within a 14-week period*

Year	No. of cases	Province(s)	Date range specimens isolated/received	Serotype	PFGE LMACI	PFGE LMAAI	PFGE pattern frequency: rank	Additional comments
1997	3	QC	21 June–11 Sept.	1/2a	0·0001	0·0001	1	
1997	5	AB, SK	16 Nov.–12 Dec.	1/2a	0·0001	0·0003	2	
1997	3	ON	9 June–8 Aug.	1/2b	0·0028	0·0023	10	
1998	4	BC, QC	18–25 June	1/2a	0·0001	0·0001	1	One mother–baby pair
1998	4	SK, ON	7 Aug.–9 Oct.	1/2a	0·0001	0·0001	1	Part of BC, QC cluster?
1998	3	ON	22 Sept.–20 Nov.	1/2a, 1/2b	0·0028	0·0023	10	
1999	7	AB, MB, ON	10 Oct.–20 Dec.	1/2a	0·0001	0·0003	2	
1999	5	BC, AB, ON	17 July–12 Oct.	1/2a	0·0007	0·0014	6	
2000	4	AB, MB, QC, NL	28 Feb.–19 May	1/2a	0·0001	0·0001	1	
2000	3	BC, ON, QC	14 July–15 Aug.	1/2a	0·0001	0·0001	1	
2000	8	BC, MB	12–20 June	1/2a	0·0059	0·0005	8	6 isolates from stools; outbreak 2, Table 3
2001	6	AB, ON	1 Mar.–12 Apr.	1/2a	0·0001	0·0001	1	
2001	6	BC, AB, MB, ON	21 June–27 Sept.	1/2a, 1/2b, 1/2NM	0·0001	0·0001	1	
2001	3	MB, ON	1 June–3 Aug.	1/2a	0·0001	0·0003	2	
2001	3	BC, AB, SK	9 Mar.–26 Apr.	1/2a	0·0001	0·0081	15	
2001	3	QC	25 Sept.–5 Oct.	1/2a	0·0033	0·0122	15	
2002	3	MB, ON	13 June–19 Sept.	1/2a	0·0001	0·0001	1	
2002	3	BC, QC	15 Feb.–16 May	1/2a	0·0004	0·0013	3	
2002	4	ON, QC	19 Sept.–24 Oct.	1/2a	0·0004	0·0013	3	
2002	13	BC, ON	22 Feb.–9 May	4b	0·0023	0·0140	6	Outbreak 5, Table 3; 8 isolates from stools
2002	3	QC	7 April–1 July	1/2a	0·0044	0·0074	5	Outbreak 4, Table 3; see reference [21]
2002	12	QC	30 July–24 Oct.	1/2a	0·0044	0·0074	5	Outbreak 4, Table 3; 3 newborns involved
2002	14	BC	26 Sept.–10 Oct.	4b	0·0082	0·0017	7	Outbreak 6, Table 3; all isolates were from stools
2003	4	ON, NS, NL	14 Aug.–10 Nov.	1/2a	0·0001	0·0001	1	
2003	4	ON	27 Feb.–28 May	1/2a	0·0004	0·0013	3	
2003	4	QC	8 April–27 June	1/2b, 4b	0·0009	0·0234	8	
2003	3	BC, AB, QC	15 May–13 Aug.	1/2a, 3a	0·0015	0·0024	4	
2004	3	ON, QC	24 Aug.–22 Sept.	1/2a	0·0001	0·0001	1	
2004	4	ON, QC, NL	22 Sept.–21 Dec.	4b	0·0009	0·0234	8	
2004	3	ON, QC	7 Sept.–18 Nov.	1/2a	0·0149	0·0325	15	

For abbreviations see Table 1.

other countries are based on direct follow-up of listeriosis cases or hospitalization data. In addition, listeriosis predominantly affects individuals with underlying medical conditions such that determining the cause of death is not always clear. Although listeriosis may have contributed to the individual's death, the disease may not be listed as the underlying cause. Further differences could arise from differences in case definitions – for example, whether miscarriages were included in case definitions of listeriosis – and in methods and data used to calculate case-fatality rates.

The numbers of *L. monocytogenes* cases were somewhat different from each of the data sources used. Since listeriosis was not reportable in Québec until 2004 no cases were reported through notifiable/reportable disease surveillance between 1995 and 2003 (Supplementary Table 1), although an outbreak of severe invasive *L. monocytogenes* occurred in that province in 2002 [21]. The NND data identified more cases in most other provinces than the LRS. It is possible that information and isolates were not forwarded to provincial public health laboratories. The LRS depends on the forwarding of isolates and associated information provided by provincial public health laboratories for the capture of this information in national laboratory-based surveillance systems. If some data were not supplied to provincial laboratories, this could subsequently affect the ability of the national surveillance system to detect case clusters that may be outbreaks. In 2002 the LRS captured more listeriosis cases in British Columbia. The main reason for this may be the inclusion within the LRS database of cases of diarrhoea and/or cases identified only by isolation of *L. monocytogenes*, which would not be reportable to the NND. There would be clear advantages to ensuring that surveillance for *L. monocytogenes* in Canada identified all cases of listeriosis through creation of a complete, standardized dataset in which the results of epidemiological and laboratory investigations would be available for each case. One possibility would be to implement national case-based surveillance and to integrate the results with LRS data.

Most cases (73%, 552/758) of severe listeriosis manifested as septicaemia, while only 11% of cases (86/758) were associated with CSF or brain tissue. Data from England and Wales was similar, with bacteraemia in 70% of non-pregnancy associated listeriosis cases and CNS infections in 24% of cases [15]. However, more cases in The Netherlands presented as meningitis (expressed as an incidence of 0·9–1·0 per million) compared with septicaemia (0·08–1·0 per million) [14].

The public health significance of diarrhoea caused by *L. monocytogenes* is not well understood. Capturing enteric listeriosis cases, in addition to the current case definition which captures invasive listeriosis cases only, could provide important surveillance information about *L. monocytogenes* circulating in the human population. However, systemic or comprehensive surveillance for gastrointestinal listeriosis may be difficult and impractical to achieve. A clear case definition would be needed, perhaps as simple as diarrhoea with isolation of *L. monocytogenes* in the absence of other known diarrhoeal pathogens. In a recent outbreak investigation in the USA associated with ready-to-eat turkey delicatessen meat a case of 'listeriosis was defined as illness in a person from whom *L. monocytogenes* was isolated (from any clinical specimen)' [36]. It would be very difficult to distinguish carriage of the organism from illness caused by the organism. If a decision were made to collect surveillance data on enteric infections caused by *L. monocytogenes*, any information collected on diarrhoeal listeriosis should be categorized separately from cases of severe listeriosis. The data provided to the LRS in Canada on the isolation of *L. monocytogenes* from stools indicates that this type of information is already being generated and that some consensus on how the information should be used is sorely needed.

Several previous reports have recommended that PFGE on *L. monocytogenes* isolates should always be done using two or more restriction enzymes, *Asc*I and *Apa*I [23, 37]. It has been noted that the use of *Apa*I PFGE patterns can complicate the assignment of isolates as outbreak clones, and that the use of *Asc*I PFGE patterns alone may be optimal for identifying clusters that may be outbreaks [38]. We found that the 10 highest ranked PFGE *Asc*I and *Apa*I combined subtypes comprised only 30% (217/717) of the total isolates represented in the database (see Table 4). The use of two enzymes provided additional discriminatory power and resulted in the clear definition of known outbreaks against a background of sporadic cases. However, Sauders and colleagues [24] have suggested that PFGE may be too discriminatory for outbreak detection, and suggested instead that a combination of PFGE and ribotyping be used. However, the use of ribotyping (automated) with *Eco*RI alone would not have been sufficient to identify

an outbreak that occurred in 1999 in Finland; PFGE was necessary [39]. It should be noted that since April 2006 the CDC has discontinued routine ribotyping of all *L. monocytogenes* samples submitted.

Additional clusters of listeriosis have been detected in retrospective analyses using PFGE [10, 27, 29, 38], including a putative outbreak in Norway [33, 39]. Clusters of cases have been defined as the occurrence of at least three listeriosis cases over a period of 14 weeks and involving the same PFGE type, or pulsovar [28]. A 14-week window for defining possible case clusters should be considered as extremely stringent. For example, dates of illness onset ranged over a period of 13 months in the 1998–1999 hot-dog-associated *L. monocytogenes* outbreak in the USA [6]. In the current work PFGE performed retrospectively with two enzymes proved to be useful for detecting case clusters that may have been outbreaks. Clusters, other than those identified as outbreaks by other means, were generally small. It is not clear that epidemiological investigations would have been productive if they had been undertaken. Other groups use data from PFGE with one restriction enzyme, usually *Asc*I [14, 35]. More clusters, or larger clusters, may have been apparent in our data if only one enzyme had been used.

Prompt analysis of isolates by PFGE is critical for public health response [6, 29]. Implementation of a nationwide system for molecular subtyping of *L. moncytogenes* was considered necessary for outbreak detection in Germany [34]. In Canada most PFGE is currently done at an LRS laboratory, but isolates are often batched before submission to this laboratory. We recommend that PFGE should be done immediately at the appropriate provincial public health laboratory or that *L. monocytogenes* isolates should be sent as soon as possible to the LRS. PFGE can then be done within a reasonable time and any apparent clusters can be investigated by the appropriate jurisdiction. It is also recommended that food isolates of *L. monocytogenes* obtained during routine surveillance of ready-to-eat foods, which for the most part is done by the Canadian Food Inspection Agency, be typed using PFGE and other molecular methods as soon as received, and that their patterns be compared against both the LRS/PulseNet Canada and PulseNet USA databases.

PFGE typing may also be useful for studying the interaction of the organism with the human population. For instance, a recent report showed that an outbreak isolate of *L. monocytogenes* was grouped closely by PFGE with food and environmental isolates, as well as more recent clinical isolates from North Carolina, suggesting that a particular strain type continued to circulate in food [40]. In our study it was possible to detect the time at which some patterns were no longer found in isolates associated with severe human disease, as well as to detect the introduction of new types. If the appropriate epidemiological information could be assembled for similar situations in the future it may be possible to determine what source(s) were associated with these types of changes. PFGE had been used in Norway to successfully trace the source of human infections to vacuum-packed cold cuts produced in a specific packing plant [41].

The most predominant serotypes found in Canada between 1995 and 2004 were 1/2a, 4b, and 1/2b. While serotypes 1/2a, 1/2b, and 1/2c are most commonly found from food or the food processing environment, serotypes 1/2a, and 4b cause most human disease [22]. It is thought that serotype 4b strains may be more virulent. When comparing serotypes of the infecting *L. monocytogenes* bacterium with the source of infection, we found a clear preponderance of serotype 4b isolates associated with pregnancy and miscarriage, but not with any other source except stools. Although these associations found were statistically significant when tested using the χ^2 test (see Table 2), the results should be interpreted with great caution as many of the cells in the contingency table constructed for the test contained zeroes or values <5; the theoretical χ^2 distribution would therefore not be expected to accurately describe the actual distribution of the χ^2 test statistic and the resulting P values may not be accurate. The observation suggests that virulence studies comparing 4b with other serotypes could include studies on the tropism of that serotype for tissues associated with pregnancy and childbirth. Further data on the prevalence of different serotypes in stools would be required to establish whether further research on this topic would be fruitful.

It has been strongly recommended that standardized food histories be taken from all listeriosis patients [6, 24, 37] and this recommendation was formally made in 2003 by the Council of State and Territorial epidemiologists in the USA [36]. Currently the timeliness and depth of case investigations routinely conducted by public health authorities varies by province/territory across Canada. Outbreaks of listeriosis typically involve a small proportion of particularly susceptible people in the total population

exposed and cases may be temporally dispersed. This in conjunction with a long incubation period – up to 91 days [20] – for listeriosis means that case patients may not recall their food histories by the time a cluster is identified. Prospective collection of standardized case data will increase the likelihood that a common source will be identified when one exists and will shorten the time period between cluster detection and recall of implicated products. In the USA and several European countries, these case data are routinely reviewed in conjunction with *L. monocytogenes* PFGE subtyping data at the national level to facilitate both outbreak and source identification. We recommend that these surveillance methods be reviewed and discussed with stakeholders at all levels across Canada to determine their value here.

A review of listeriosis in Canada published in 1984 [42] made similar recommendations, including: typing of all isolates, routine administration of questionnaires to cases and controls, thorough epidemiological investigations of outbreaks, and closer collaboration between federal and provincial authorities. The process of meeting these recommendations was begun with the creation of the LRS and has been supported by the continued development of PulseNet Canada and through the collaborations essential for the development and writing of this paper. We expect that the next retrospective analysis will show the fruits of this labour and that the financial and human costs of disease caused by *L. monocytogenes* in Canada will decrease.

APPENDIX. Canadian Public Health Laboratory Network (CPHLN)

Dr J. L. Isaac-Renton, British Columbia Centre for Disease Control Laboratory Services; Dr J. K. Preiksaitis, ProvLab Alberta; Dr G. Horsman, Saskatchewan Provincial Laboratory; Dr Paul van Caeseele, Cadham (Manitoba) Provincial Laboratory; Dr F. Jamieson, Dr D. Pillai, Central Public Health Laboratory, Ontario Ministry of Health and Long-Term Care; Dr A.-M. Bourgault, Laboratoire de santé publique du Québec; Dr Lewis Abbot, Queen Elizabeth Hospital, Prince Edward Island; Dr G. J. Hardy, Microbiology Division, St. John Regional Hospital, St. John, New Brunswick; Dr D. Haldane, Division of Microbiology, QE II Health Sciences Centre, Halifax, Nova Scotia; Dr S. Ratnam Newfoundland Public Health Laboratory.

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NOTE

Supplementary material accompanies this paper on the Journal's website (http://journals.cambridge.org/hyg).

DECLARATION OF INTEREST

None.

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