Epidemiol. Infect. (2012), **140**, 1757–1772. © Cambridge University Press 2011
doi:10.1017/S0950268811002548

Integrated surveillance and potential sources of *Salmonella* Enteritidis in human cases in Canada from 2003 to 2009

A. NESBITT[1]*, A. RAVEL[2], R. MURRAY[1], R. McCORMICK[1], C. SAVELLI[1], R. FINLEY[1], J. PARMLEY[3], A. AGUNOS[3], S. E. MAJOWICZ[4], M. GILMOUR[5], and the Canadian Integrated Program for Antimicrobial Resistance Surveillance Public Health Partnership[1,5,6] and the Canadian Public Health Laboratory Network[6]

[1] *Centre for Food-borne, Environmental, and Zoonotic Infectious Diseases, Public Health Agency of Canada, Guelph, Ontario, Canada*
[2] *Laboratory for Foodborne Zoonoses, Public Health Agency of Canada, St-Hyacinthe, Québec, Canada*
[3] *Laboratory for Foodborne Zoonoses, Public Health Agency of Canada, Guelph, Ontario, Canada*
[4] *Department of Population Medicine, University of Guelph, Guelph, Ontario, Canada*
[5] *National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Manitoba, Canada*
[6] *The Canadian Public Health Laboratory Network, Winnipeg, Manitoba, Canada*

(*Accepted 7 November 2011; first published online 14 December 2011*)

SUMMARY

Salmonella Enteritidis has emerged as the most prevalent cause of human salmonellosis in Canada. Recent trends of *S.* Enteritidis subtypes and their potential sources were described by integrating *Salmonella* data from several Canadian surveillance and monitoring programmes. A threefold increase in *S.* Enteritidis cases from 2003 to 2009 was identified to be primarily associated with phage types 13, 8 and 13a. Other common phage types (4, 1, 6a) showed winter seasonality and were more likely to be associated with cases linked to international travel. Conversely, phage types 13, 8 and 13a had summer seasonal peaks and were associated with cases of domestically acquired infections. During agri-food surveillance, *S.* Enteritidis was detected in various commodities, most frequently in chicken (with PT13, PT8 and PT13a predominating). Antimicrobial resistance was low in human and non-human isolates. Continued integrated surveillance and collaborative prevention and control efforts are required to mitigate future illness.

Key words: Antimicrobial susceptibility, exposure sources, human, phage type, poultry, *Salmonella* Enteritidis, temporal trend.

INTRODUCTION

In Canada, human salmonellosis is notifiable, with about 5000 cases reported annually, second only to campylobacteriosis in causing bacterial gastroenteritis [1]. The epidemiology of human non-typhoidal salmonellosis is complex involving various animal reservoirs and several transmission pathways, the most important of which is foodborne. Several serovars also cause disease in animal species. Of note, two reportable host-specific serovars (*S.* Gallinarum and *S.* Pullorum) cause severe disease in poultry [2]. Additionally, the detection of antimicrobial resistance in *Salmonella* increases both human and animal health concerns. Because of the burden of human

* Author for correspondence: A. Nesbitt, Centre for Food-borne, Environmental, and Zoonotic Infectious Diseases, Public Health Agency of Canada, 120-255 Woodlawn Road, West, Guelph, Ontario, Canada, N1H 8J1.
(Email: Andrea.Nesbitt@phac-aspc.gc.ca)

salmonellosis, its zoonotic and foodborne epidemiology, and the impact of *Salmonella* infections on the health of animals and the sustainability of animal livestock industries, Canada has long-standing surveillance systems, with specific objectives to monitor *Salmonella* in humans, food animals, and food.

Salmonella serovar Enteritidis emerged as a prevalent cause of human salmonellosis in Canada and globally in the 1980s [3]. *S.* Enteritidis has been ranked in the top three non-typhoidal serovars, ranging from 12% to 27% of all *Salmonella* infections reported in Canada from 1999 to 2006 [4]. Large outbreaks since 2005 (see Results section), and increasing rates of illness overall [4], have highlighted *S.* Enteritidis as an emerging pathogen in Canada causing a significant burden of illness. This paper provides a comprehensive picture of *S.* Enteritidis in Canada from 2003 to 2009 utilizing available data on *Salmonella* from national and targeted animal and human surveillance programmes. The specific objectives were to describe the trends of *S.* Enteritidis in humans and the agri-food sector and to explore the potential driving causes of national trends.

METHODS

Data sources

National surveillance data

The National Enteric Surveillance Program (NESP) provides weekly aggregate reports of laboratory-confirmed enteric disease cases reported by the provincial public health authorities. The purpose of NESP is to detect outbreaks and report national trends (see http://www.nml-lnm.gc.ca/NESP-PNSME/index-eng.htm for details).

Laboratory-confirmed human salmonellosis isolates are submitted to the National Microbiology Laboratory (NML) as part of reference requests, active and passive surveillance programmes, surveys or outbreak and cluster investigations. Provincial laboratories perform pulsed-field gel electrophoresis (PFGE) using standardized PulseNet Canada protocols [5] and submit the resulting data to the NML for PFGE pattern designation. Submitted isolates are phage-typed at the NML using the international *S.* Enteritidis phage-typing scheme [6], and for select provincial microbiology laboratories, PFGE and serotyping are also performed.

Targeted surveillance data

The Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) monitors antimicrobial use and antimicrobial resistance in selected species of enteric bacteria, including *Salmonella*, from humans, animals and animal-derived food sources across Canada (see http://www.phac-aspc.gc.ca/cipars-picra/surv-eng.php for details). Human *Salmonella* isolates are submitted by provincial public health laboratories to NML for further subtyping and antimicrobial susceptibility testing. Data on *Salmonella* from the agri-food sectors are collected for various commodities: pigs on farm, caecal samples from slaughtered chickens and pigs, and retail chicken and pork meats. All *Salmonella* isolates recovered from animals and food are forwarded to the *Salmonella* Typing Laboratory (STL) of the Laboratory for Food-borne Zoonoses, Public Health Agency of Canada (PHAC), to have serotype, phage type, and antimicrobial susceptibility determined. Some *Salmonella* isolates from diagnostic animal samples submitted by veterinarians and from various government monitoring programmes, such as the testing of fluff from approved hatchery supplier flocks [7] undergo the same testing as the CIPARS isolates described above. However, isolates from government monitoring programmes do not routinely undergo antimicrobial susceptibility testing.

C-EnterNet is an integrated sentinel site surveillance programme that systematically samples and tests human enteric pathogens from three exposure sources [retail food (chicken, pork, beef), on-farm (broiler chicken, swine, dairy, beef), and untreated surface water] in parallel with human enteric pathogen laboratory-based surveillance and enhanced case follow-up on exposures (see http://www.phac-aspc.gc.ca/c-enternet/index-eng.php for details). The programme was implemented in June 2005 at the pilot sentinel site (Waterloo Region, Ontario): a population of about 500 000 residents. The enhanced follow-up of all human cases enables classification of cases as international travel-related, outbreak-related or endemic sporadic cases.

Outbreak data

Outbreak data are not captured in a standardized or systematic manner across the country. *S.* Enteritidis outbreaks in Canada during the study period were retrieved through (1) a peer-reviewed literature search limited to Canadian outbreaks from 2003–2009

https://doi.org/10.1017/S0950268811002548 Published online by Cambridge University Press

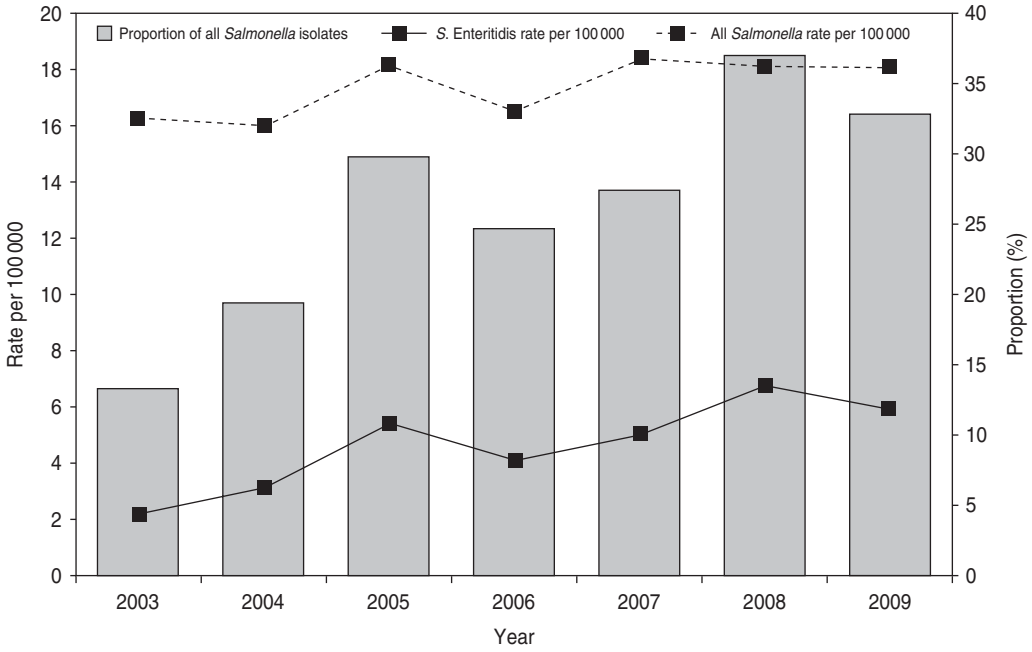

Fig. 1. Laboratory-confirmed *Salmonella* Enteritidis case incidence rate (per 100 000) and proportion among all non-typhoidal *Salmonella* isolates, by year, in Canada, 2003–2009 (*source*: National Enteric Surveillance Program).

conducted in PubMed using the key words: *Salmonella*, Enteritidis, and outbreak; (2) a review of investigation records documented by the Outbreak Management Division at the Centre for Food-borne, Environmental and Zoonotic Infectious Diseases, PHAC; and (3) provincial outbreak investigation records requested from provincial and territorial partners.

Data analysis

Data collected from national and targeted animal and human surveillance programmes from January 2003 to December 2009 were analysed. Annual incidence rates for human salmonellosis were computed using the national and sentinel site population estimates [8]. Temporal trends (annual and seasonal) were analysed based on monthly counts using a negative binominal regression model for all cases and for the six most frequent phage types separately. Cases were described by age, sex, phage type, PFGE patterns and antimicrobial susceptibility. *S.* Enteritidis isolated from non-human samples were described by phage type, antimicrobial susceptibility, PFGE pattern and trends by food commodity and data source. To test statistical differences, when appropriate, logistic regression analysis was conducted using SAS version 9.1 (SAS Institute Inc., USA) and the χ^2 test and critical ratio (z) test using EpiCalc2000 version 1.02 [9]. All statistical significant differences were identified by using a P value threshold of 0·05.

RESULTS

S. Enteritidis in humans

Overall incidence

A total of 10 616 laboratory-confirmed *S.* Enteritidis human cases were reported in Canada from 2003 to 2009 via NESP. The national annual incidence rate increased from 2·16/100 000 person-years in 2003 to 5·79/100 000 in 2009 (63 % increase), while the incidence rate for all non-typhoidal *Salmonella* infections increased from 16·29/100 000 person-years in 2003 to 18·03/100 000 in 2009 (Fig. 1) (10 % increase). Of all reported *Salmonella*, the proportion of *S.* Enteritidis isolates rose from 12·7 % in 2003 to 32·1 % in 2009 (Fig. 1). The increase of *S.* Enteritidis cases was observed in each of the 10 provinces and three territories, with varying incidence rates (Fig. 2) and distribution of genetic subtypes (see Microbial features section below).

Endemic sporadic cases

Nationally, it was difficult to distinguish between outbreak-related cases, international travel-related cases, and endemic sporadic, domestically acquired

https://doi.org/10.1017/S0950268811002548 Published online by Cambridge University Press

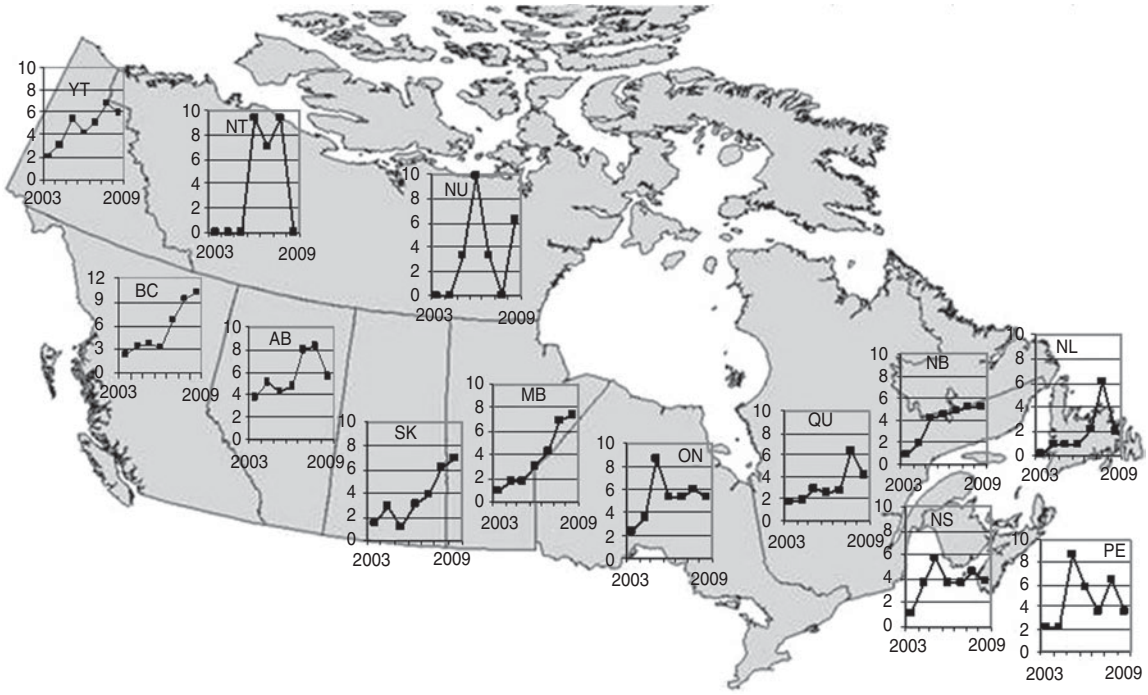

Fig. 2. Laboratory-confirmed *Salmonella* Enteritidis case incidence rate (per 100 000), by year and province/territory, in Canada, 2003–2009 (*source*: National Enteric Surveillance Program). YT, Yukon Territory; NT, Northwest Territory; NU, Nunavut; BC, British Columbia; AB, Alberta; SK, Saskatchewan; MB, Manitoba; ON, Ontario; QU, Québec; NB, New Brunswick; NS, Nova Scotia; PEI, Prince Edward Island; NL, Newfoundland.

cases because of limited epidemiological data accompanying the isolates. At the sentinel site level (C-EnterNet) the incidence rate for endemic sporadic *S*. Enteritidis cases increased from 0·41/100 000 person-years in 2005 to 3·66/100 000 in 2009.

Outbreak-related cases

Six *S*. Enteritidis outbreaks with well documented investigations were identified from 2003 to 2009. *S*. Enteritidis outbreaks were largely attributed to PT8 and PT13 (Fig. 3). In 2004, a cluster of 10 cases of *S*. Enteritidis PT8 linked to a single restaurant in Alberta was identified. Although a food source was not identified, the resulting investigation implicated a food handler as the probable source (V. Mah, Alberta Health and Wellness, personal communication, February 2009). In 2005, a large *S*. Enteritidis PT13 outbreak occurred in Ontario, resulting in 552 laboratory-confirmed cases of illness, and the investigation identified mung bean sprouts as the source of infection. This outbreak was described in the peer-reviewed literature [10]. In 2006, an increase in *S*. Enteritidis PT13 occurred in Ontario resulting in an investigation that identified either chicken or eggs as a potential source of infection, although this could not be confirmed (Ontario Ministry of Health and Long-Term Care, personal communication, February 2009). In 2007, a notable increase in *S*. Enteritidis PT13 occurred across three provinces (Ontario, British Columbia, New Brunswick). The subsequent investigations, undertaken provincially and nationally, indicated several potential sources of infection including chicken and eggs; however, no definitive source was confirmed (Outbreak Management Division, CFEZID, personal communication, February 2009). In August 2008, an outbreak in the province of Québec involving more than 150 cases of *S*. Enteritidis PT13 was attributed to a pasteurized cheese made in this province (C. Gaulin, Québec Ministère de la Santé et des Services sociaux du Québec, personal communication, February 2009). British Columbia identified an increase in *S*. Enteritidis PT8 in June 2008. The resulting investigation identified eggs as the most likely source of infection. The increase and accompanying investigation have continued into 2011 [11].

International travel-related cases

At C-EnterNet's sentinel site, 36% (69/190) of *S*. Enteritidis cases reported between mid-2005 and

https://doi.org/10.1017/S0950268811002548 Published online by Cambridge University Press

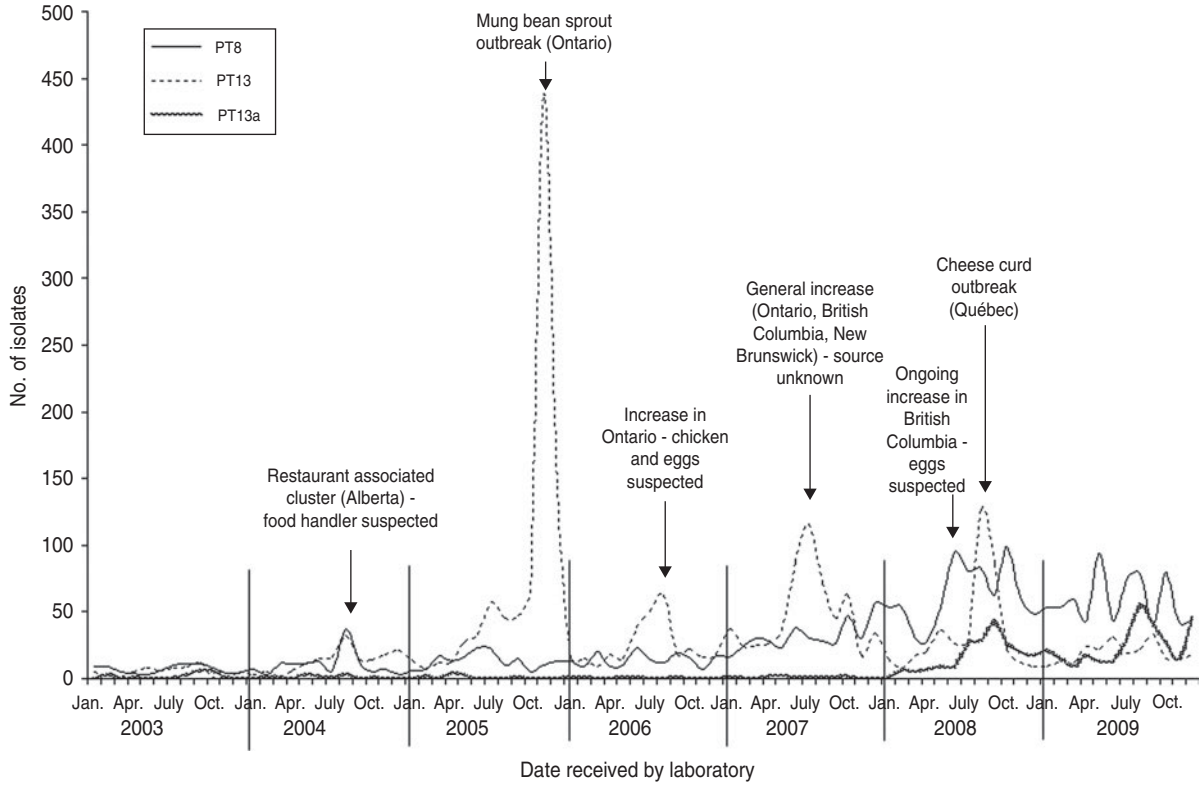

Fig. 3. *Salmonella* Enteritidis PT8, PT13a and PT13 isolates by month, Canada 2003–2009 (*source*: National Microbiology Laboratory and outbreak identifications, Outbreak Management Division, Centre for Food-borne, Environmental, and Zoonotic Infectious Diseases, Public Health Agency of Canada and Provincial Public Health Authorities).

2009 were linked to travel outside Canada. Overall, the most frequent travel destination reported by these travel-related cases was to the Caribbean.

Age and gender distribution of cases

There was no significant difference in the gender distribution across all surveillance systems (Table 1). Nationally, CIPARS data showed that the *S.* Enteritidis cases were older than all *Salmonella* cases, with fewer cases in children aged <10 years and more cases in adults aged between 30 and 59 years (Table 1). The age distribution of the *S.* Enteritidis cases at the sentinel site level was similar to the national estimate, except it was higher for adults aged 20–24 years (15% and 8%, respectively) (Table 1).

Microbial features

From 2003 to 2009, 10555 human *S.* Enteritidis isolates were phage-typed by the NML. The most common phage types were PT13 (25%), PT8 (22%), PT4 (13%), and PT1 (8%) (Table 2). Prior to 2004, PT13, PT8 and PT13a represented less than 25% of all phage types. Combinations of these three phage types are presently found in most provinces, representing 62% nationally in 2009. The phage-type distribution was comparable to CIPARS data, which is a subset of 6923 isolates of the national database (Table 2). The temporal trends for the six most common phage types showed various annual and seasonal patterns (Figs 4 and 5). Overall, compared to December, significantly more *S.* Enteritidis cases were reported in August and September and fewer in November. Phage types 13, 13a and 8 had summer seasonal peaks, while PT1, PT4 and PT6a showed winter seasonal peaks. PT13 was associated with four large outbreaks between 2003 and 2009 (Fig. 4). With removal of the Ontario outbreak related to mung bean sprouts in 2005, PT13 significantly increased in 2004, 2005 and 2007 compared to the preceding year and decreased in 2008 and 2009. PT13 showed an increase in Ontario in 2005, 2006 and 2007; a marked increase was observed in British Columbia in 2007; and Québec had a large outbreak in 2008 associated with cheese curds. PT13 showed a seasonal peak in summer (July–September) and a drop in winter (January to March). PT8 showed an upward trend from 2003 to 2009 with a statistically significant increase from 2006 to 2007 and from 2007 to 2008.

https://doi.org/10.1017/S0950268811002548 Published online by Cambridge University Press

Table 1. *Age and gender distribution (%) of non-typhoidal* Salmonella *and* S. *Enteritidis cases, in Canada, 2003–2009 (sources:* C-*EnterNet and Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS)*

	C-EnterNet Sentinel site		CIPARS	
	Salmonella ($n=587$)*	*S.* Enteritidis ($n=190$)*	*Salmonella*	*S.* Enteritidis
Sex	($n=586$)		($n=14\,868$)	($n=4128$)
Male	48·5	50·0	49·3	50·5
Female	51·5	50·0	50·7	49·5
Age (years)			($n=17\,992$)	($n=4799$)
<5	15·2	9·5	13·1	8·4†
5–9	7·0	4·2	7·9	6·9†
10–14	6·1	6·3	5·4	5·7
15–19	6·1	5·8	5·7	6·2
20–24	12·4	14·7‡	7·8	8·1‡
25–29	9·4	10·5	7·7	8·1
30–39	12·6	12·1	11·8	13·3†
40–59	20·4	24·7	24·7	27·9†
⩾60	10·7	12·1	15·9	15·4

* Includes endemic, travel- and outbreak-related cases.
† Significantly different proportion of CIPARS *S.* Enteritidis cases than all CIPARS *Salmonella* serovars ($P<0·05$).
‡ Significantly different proportion of *S.*Enteritidis cases than other surveillance programme ($P<0·05$).

No statistically significant monthly differences were found. Between 2005 and 2006, regional differences were observed, with PT8 as the predominant phage type in the Eastern and Western provinces. Notable increases in PT8 were observed in almost all provinces since 2007; British Columbia showed a dramatic increase in 2008. PT13a increased significantly from one year to another starting in 2008 in almost all provinces and without any obvious seasonality (Fig. 4). PT4 peaked in 2006, followed by a significant decrease from 2006 to 2007 and from 2008 to 2009 with two seasonal peaks; one from January to April and another in late summer (August and September; Fig. 5). PT1 showed no obvious annual trend, but peaked seasonally from January to April (Fig. 5). Finally, PT6a significantly increased from 2003 to 2004 and from 2007 to 2008 before significantly decreasing in 2009. There was a significant seasonal drop in cases between June and August (Fig. 5).

In C-EnterNet's sentinel site, PT13 was the most common phage type (Table 2). All PT13 isolates were endemic, with the majority (40/57) associated with an outbreak in 2005. The majority of PT8 isolates (21/24) were also endemic. All PT1 isolates were travel-related and 77% of PT4 isolates were also travel-related.

In general, the predominant *Xba*I PFGE patterns identified by C-EnterNet with their corresponding phage types were as follows: SENXAI.0001 with PT4, SENXAI.0003 with PT8, SENXAI.0006 with PT13a and SENXAI.0038 with PT13 (Table 3). Considering isolates from both human cases and the chicken commodity, 10 (83%) of 12 PT4 isolates were SENXAI.0001, 92 (89%) of 103 PT8 isolates were SENXAI.0003, 35 (69%) of 51 PT13a isolates were SENXAI.0006 and 48 (96%) of 50 PT13 isolates were SENXAI.0038.

Through CIPARS, 5691 human *S.* Enteritidis isolates were tested for antimicrobial susceptibility during the study period. Eighty-two percent (4683/5691) of isolates were susceptible to all antimicrobials tested, while resistance to ⩾1 antimicrobials was observed in 17% of isolates, and resistance to ⩾5 antimicrobials was observed in 1%. Resistance to one or more antimicrobials was most prevalent in PT1 strains (38%), followed by PT4 (19%), PT29a (4%), and PT6a (6%). Resistance was observed most often to nalidixic acid (14%), tetracycline (3%), ampicillin

https://doi.org/10.1017/S0950268811002548 Published online by Cambridge University Press

Table 2. *Top five* S. *Enteritidis phage types in human and non-human isolates by surveillance data sources, in Canada, 2003–2009* (*sources: National Microbiology Laboratory* (*NML*), *Canadian Integrated Program for Antimicrobial Resistance Surveillance* (*CIPARS*) *and C-EnterNet*)

		NML (2003–2009)	CIPARS (2003–2009)	C-EnterNet (2005–2009)
Human		PT13 (2614, 25%) PT8 (2306, 22%) PT4 (1346, 13%) PT1 (903, 9%) PT13a (556, 5%) PT6a (340, 3%)	PT8 (1769, 26%) PT13 (1459, 21%) PT 4 (814, 12%) PT 1 (603, 9%) PT6a (215, 3%)	PT13 (57, 38%) PT8 (24, 16%) PT13a (15, 10%) PT4* (13, 9%) PT1* (8, 5%)
Chicken	Farm	No data	No data	PT13 (3, 23%) PT8 (2, 15%) PT8a (1, 8%) Atypical PT (1, 8%) Other (3, 23%)
	Abattoir (slaughtered chicken)	No data	PT8 (51, 37%) PT13 (24, 17%) PT13a (20, 14%) Atypical PT (20, 14%) PT14b (9, 6%) PT51 (9, 6%)	No data
	Retail	No data	PT8 (79, 42%) PT13a (31, 16%) Atypical PT (26, 14%) PT13 (20, 11%) PT51 (18, 10%)	PT8 (11, 46%) PT13 (8, 33%) PT13a (3, 13%) Other (2, 8%)
	Diagnostic cases	No data	PT8 (260, 55%) PT23 (39, 8%) PT13a (38, 8%) PT13 (35, 7%) Atypical PT (32, 7%)	No data
Porcine	Farm	No data	PT8 (1, 33%) Atypical PT (1, 33%)	PT13 (1, 100%)
	Abattoir (slaughtered)	No data	PT8 (6, 40%) PT13 (4, 27%) PT11b (3, 20%) PT13a (1, 7%) PT2 (1, 7%)	No data
	Retail	No data	PT8 (1, 100%)	No *S.* Enteritidis isolates
	Diagnostic cases	No data	PT8 (4, 33%) PT20 (3, 25%) Untypable (2, 17%) PT13 (1, 8%) PT13a (1, 8%) Atypical PT (1, 8%)	No data
Bovine	Farm	No data	No data	PT8 (1, 50%)
	Retail	No data	No data	PT13 (1, 50%)
	Diagnostic cases	No data	PT13 (4, 33%) PT8 (2, 17%) PT33 (2, 17%) PT51 (1, 8%) Atypical PT (13, 14%)	No data
Other animal species	Diagnostic cases	No data	PT8 (26, 29%) PT13 (17, 19%) Atypical PT (13, 14%) PT9b (12, 13%) PT23 (9, 10%)	No data

* Data from C-EnterNet indicate that these phage types are primarily associated with cases linked to international travel.

† Other animal species include: unspecified bird species (55), ducks (12), dogs (6), cats (4), rodents (3), aquatic mammals (2), geese (2), reptiles (2), turkeys (2), amphibians (1), horses (1), and mink (1).

https://doi.org/10.1017/S0950268811002548 Published online by Cambridge University Press

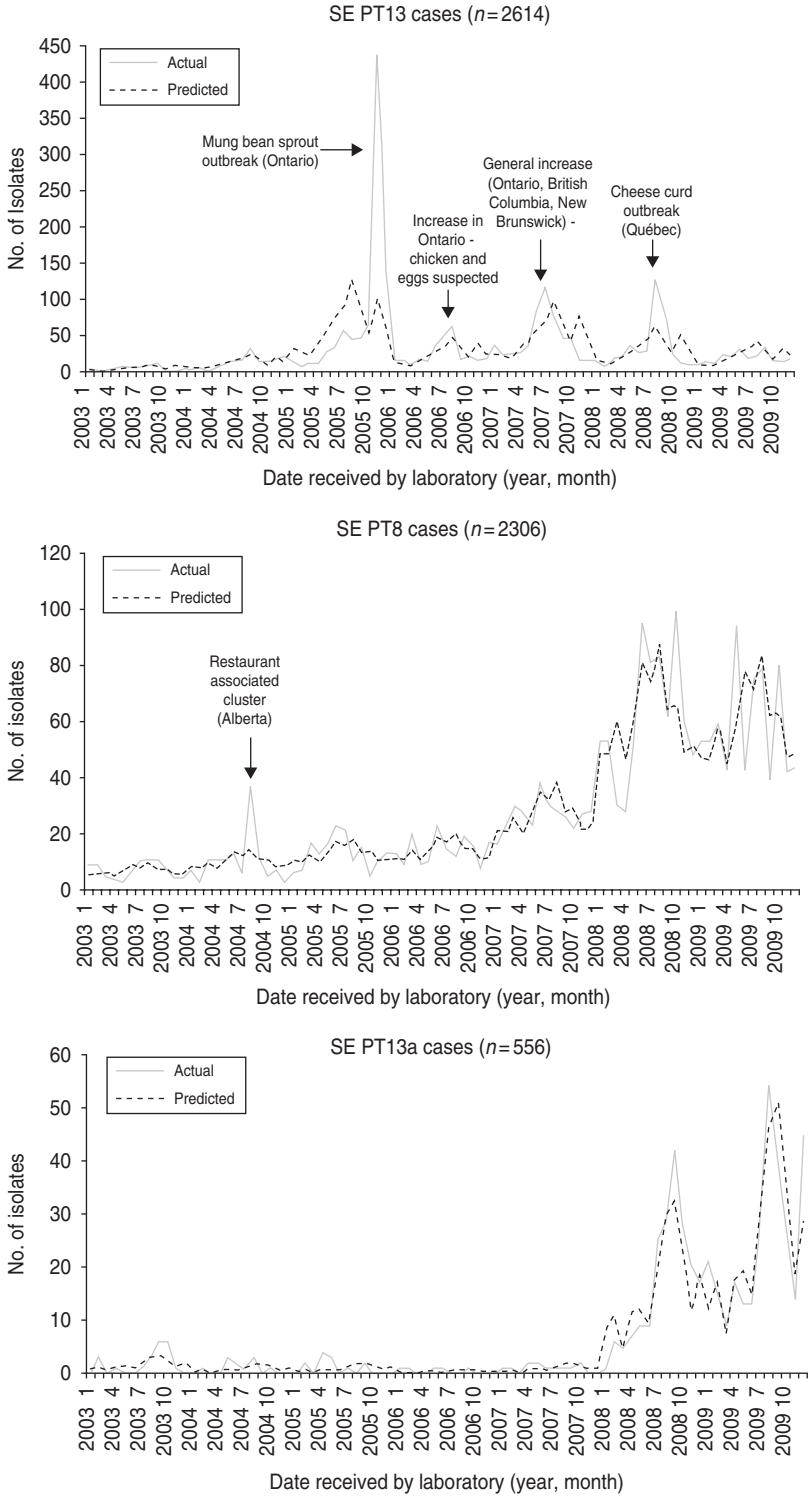

Fig. 4. Monthly actual and predicted numbers of *S*. Enteritidis cases for phage types 13, 8 and 13a, in Canada, 2003–2009 (*source*: National Microbiology Laboratory). The predicted values are the result of modelling the data using a negative binomial regression and assist in predicting seasonality.

https://doi.org/10.1017/S0950268811002548 Published online by Cambridge University Press

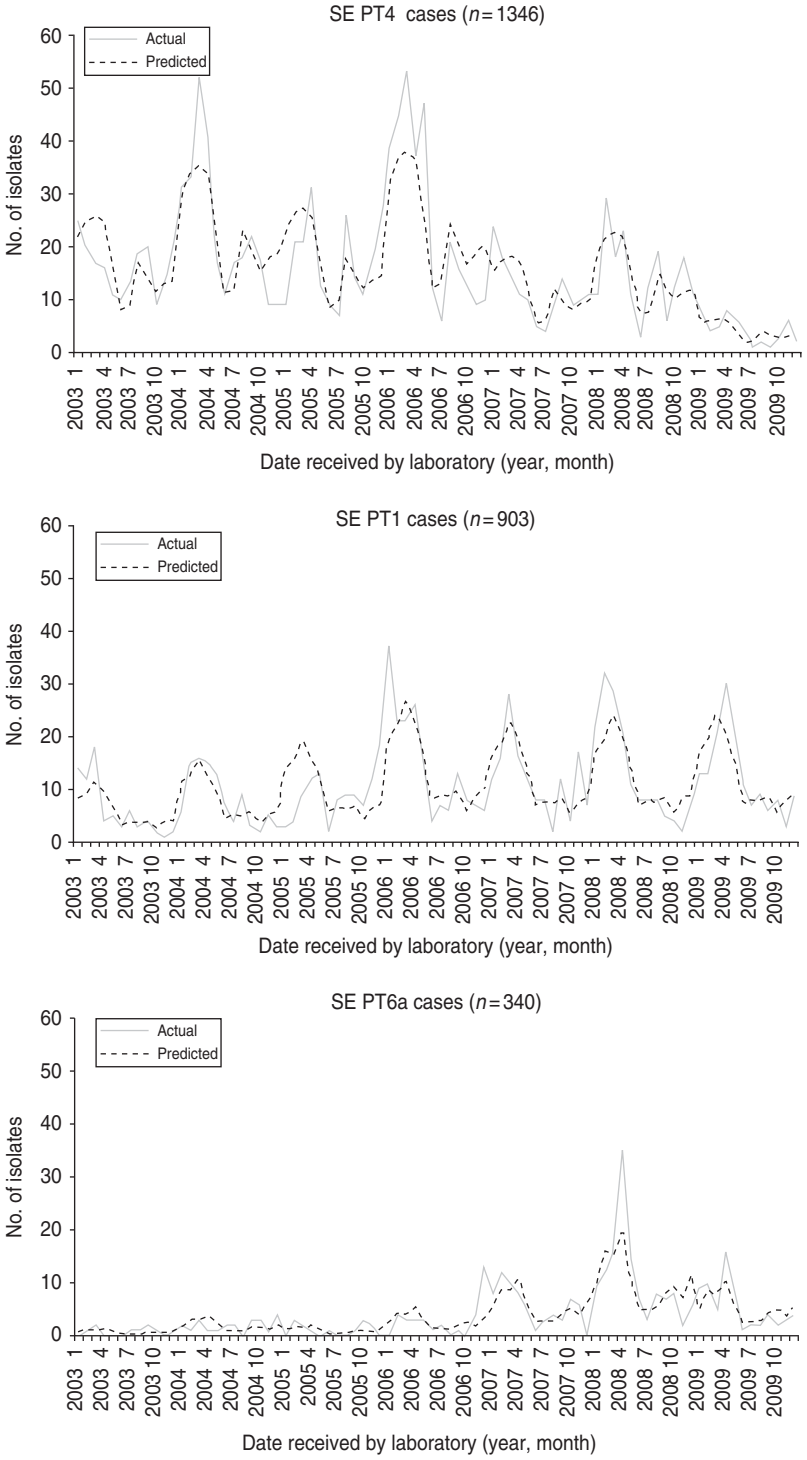

Fig. 5. Monthly actual and predicted numbers of *S*. Enteritidis cases for phage types 1, 4 and 6a, in Canada, 2003–2009 (*source*: National Microbiology Laboratory). The predicted values are the result of modelling the data using a negative binomial regression and assist in predicting seasonality.

(2%), and sulfisoxazole (2%). All strains were susceptible to amikacin, two strains were resistant to ceftriaxone and one strain was resistant to ciprofloxacin. The resistance to tetracycline in all *S*. Enteritidis isolates increased significantly in 2007 compared to 2003.

https://doi.org/10.1017/S0950268811002548 Published online by Cambridge University Press

Table 3. *Phage type and PFGE pattern for* S. *Enteritidis isolated from human cases, chickens, and retail chicken meat in Canada, 2005–2009*

		PT4	PT8	PT13	PT13a	Other PT	Atypical PT
Human endemic cases*	SENXAI.0001	3				2	1
	SENXAI.0002					1	
	SENXAI.0003		19	1	3		
	SENXAI.0004					1	1
	SENXAI.0006				8	1	
	SENXAI.0007		2		1		
	SENXAI.0008					1	
	SENXAI.0009					1	
	SENXAI.0038			23		1	
Human travel-related cases*	SENXAI.0001	7				12	
	SENXAI.0002	1					
	SENXAI.0003		3		2	1	
	SENXAI.0004					4	
	SENXAI.0006				1		
	SENXAI.0008					9	
	SENXAI.0017	1					
	SENXAI.0093					1	
	SENXAI.0123						2
	SENXAI.0123					1	
Chicken farm*	SENXAI.0003		3		1	4	1
	SENXAI.0007				1		
	SENXAI.0038			3			
Abattoir chicken†	SENXAI.0003		25			5	6
	SENXAI.0006				10	1	
	SENXAI.0007		1				
	SENXAI.0038			8	3	1	3
	SENXAI.0062			1			
Retail chicken*†	SENXAI.0003		42			25	8
	SENXAI.0006				16	2	1
	SENXAI.0007		5				2
	SENXAI.0038			14	5	2	2
	SENXAI.0068				1	1	
	SENXAI.0104		1				
	SENXAI.0168		1				
	SENXAI.0183		1				

* C-EnterNet data (2005–2009).
† CIPARS non-human data (2008 and partial 2009).

S. Enteritidis in agri-food sources

S. *Enteritidis in broiler chicken*

Through CIPARS and C-EnterNet, *S.* Enteritidis was isolated from chicken on farms, slaughtered chicken, and retail chicken (Table 4). There are no results for chicken eggs since they are not actively monitored by these programmes. CIPARS data show that the relative proportion of *S.* Enteritidis in all *Salmonella* isolates recovered from slaughtered chicken increased from 0% (0/126) to 19% (44/230) over the study period (Table 4). The proportion of *S.* Enteritidis in CIPARS retail chicken increased from 0% (0/54) to 20% (94/473) and this upward trend was observed across all participating provinces. PT8, PT13a, and PT13 were the most common phage types recovered from the chicken commodity (Fig. 6, Table 2). All *S.* Enteritidis isolates from retail and abattoir chicken were fully susceptible to the antimicrobials tested except for two isolates from slaughtered chicken: one resistant to tetracycline and one resistant to streptomycin. Of all chicken *S.* Enteritidis isolates tested by PFGE, the most common pattern was SENXAI.0003, followed by SENXAI.0038 and SENXAI.0006 (Table 3). Pattern SENXAI.0003 was most closely associated with PT8.

https://doi.org/10.1017/S0950268811002548 Published online by Cambridge University Press

Table 4. *Number of* Salmonella *and* S. *Enteritidis recovered from non-human samples by active surveillance systems in Canada, 2003–2009* (*source: Canadian Integrated Program for Antimicrobial Resistance Surveillance* (*CIPARS*) *and C-EnterNet*)

	2003			2004			2005			2006			2007			2008			2009			Total (2003–2009)				
	Tested	Salm+	SE+	Tested	Salm+	SE+	Tested	Salm+	SE+	Tested	Salm+	SE+	Tested	Salm+	SE+	Tested	Salm+	SE+	Tested	Salm+	SE+	Tested	Salm+	SE+	% SE+ in tested samples	% Salm+ in tested samples
CIPARS																										
Retail chicken	338	54	0	636	107	3	756	73	3	755	94	10	828	346	17	960	382	62	1090	473	94	5363	1529	189	3·5	28·5
Retail pork	125	1	0	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	621	4	0	841	13	0	967	17	1	1102	18	0	3656	53	1	0·03	1·4
Slaughtered chicken	803	126	0	893	142	9	1103	199	7	831	187	14	902	206	20	854	234	45	851	230	44	6237	1324	139	2·2	21·2
Slaughtered pigs	1393	391	5	703	269	1	486	212	3	352	145	1	284	105	0	340	151	1	327	147	4	3885	1420	15	0·4	36·6
Pig farm	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	349	72	0	533	110	1	486	61	1	698	124	1	2066	367	3	0·2	17·8
C-EnterNet																										
Retail chicken	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	81	17	0	145	43	5	318	101	5	185	60	7	200	57	9	929	278	26	2·8	29·9
Retail pork	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	81	2	0	140	4	0	187	6	0	178	1	0	200	3	0	785	16	0	0	2·0
Retail beef	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	81	0	0	139	1	0	187	1	0	180	1	1	200	1	0	787	4	1	0·1	0·5
Untreated surface water	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	42	2	0	140	28	0	134	13	0	100	34	2	112	28	1	588	105	3	0·5	17·9
Chicken farms	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	36	26	4	100	62	3	120	37	6	256	109	13	5·1	42·6
Pig farms	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	115	36	0	120	33	0	120	40	1	113	33	0	120	41	0	588	183	1	0·2	31·1
Dairy farms	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	179	20	0	112	14	0	112	9	0	120	22	0	523	65	0	0	12·4
Beef farms	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	80	8	0	112	7	1	120	15	0	312	30	1	0·3	9·6

n.t., Samples not tested.

Salm+, *Salmonella*-positive isolates; SE+, *Salmonella* Enteritidis-positive isolates.

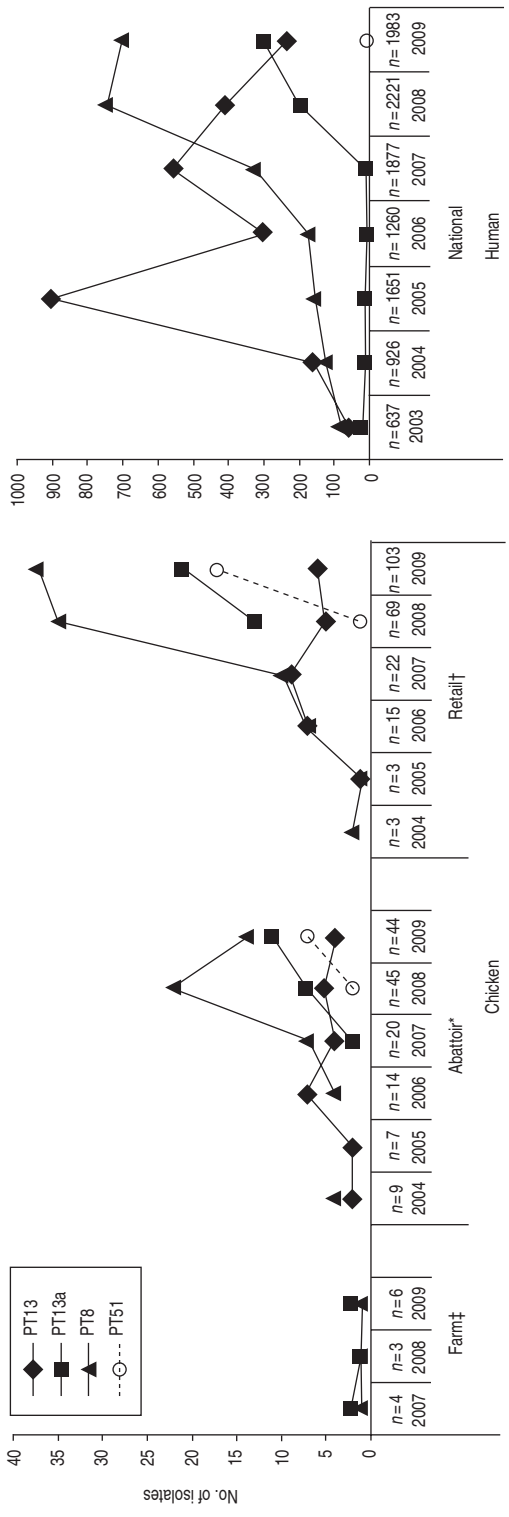

Fig. 6. Common *S*. Enteritidis phage types recovered in humans and non-humans, by year, in Canada, 2003–2009 [*sources*: National Microbiology Laboratory, Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) and C-EnterNet]. * CIPARS data only. † CIPARS and C-EnterNet data combined. ‡ C-EnterNet data only.

S. *Enteritidis in other food animal commodities and the environment*

S. Enteritidis was detected in pigs on farm, slaughtered pigs (caecal contents), and retail pork far less frequently than in the chicken commodity (Table 4). *S*. Enteritidis represented less than 1% (20/2039) of all *Salmonella* isolated from pigs on farm, at slaughter and at retail. Overall, PT8 was the most common phage type recovered from the pig commodity (Table 2). All pig isolates tested were fully susceptible to the antimicrobials tested. Two *S*. Enteritidis isolates were recovered by C-EnterNet from the beef commodity, one in retail ground beef and another in beef farm manure. Both were PT8 (Tables 2 and 4). Two *S*. Enteritidis isolates were detected in untreated surface water samples (Table 4), both were PT13.

S. *Enteritidis in animal clinical isolates*

Between 2003 and 2009, *S*. Enteritidis represented 11% (586/5261) of all *Salmonella* isolates recovered from all animal diagnostic submissions sent to STL. *S*. Enteritidis represented 0·66% (4/608) and 22% (191/861) of all diagnostic *Salmonella* isolates in 2003 and 2009, respectively. *S*. Enteritidis was isolated most often from chicken (471/586, 80%) and PT8 was the most common phage type (260/449 isolates with phage-type data, 58%) in chicken diagnostic isolates (Table 2). PT8 and PT13 were most commonly detected in diagnostic isolates from pigs, cattle and other animals (Table 2). Ten diagnostic *S*. Enteritidis isolates demonstrated antimicrobial resistance: three from chicken, two from cattle, two from pigs, and three from unspecified bird species. Four isolates showed resistance to $\geqslant 5$ antimicrobials; these included one pig and three chicken isolates. All chicken isolates showed resistance to antimicrobials of very high importance to human medicine: three were resistant to ceftriaxone and ceftiofur and two were resistant to amoxicillin-clavulanic acid.

S. *Enteritidis from government monitoring programmes*

S. Enteritidis was isolated from 15% (1188/7852) of all *Salmonella* isolates from government monitoring programmes submitted to the STL. Of these isolates, 93% (1107/1188) originated from chickens (e.g. broilers, broiler breeders, layers, layer breeders), ducks, turkeys or other avian species. The most common phage types across all government monitoring isolates were PT8 (43%, 516/1188), PT13 (15%, 174/1188), and PT13a (9%, 102/1188).

https://doi.org/10.1017/S0950268811002548 Published online by Cambridge University Press

DISCUSSION

Through the integration of data provided by Canadian surveillance programmes, this study confirms and documents an increasing trend in *Salmonella* Enteritidis in the Canadian population and several exposure sources. *S*. Enteritidis has become the most common serotype of human salmonellosis in Canada, surpassing *S*. Typhimurium and representing about 33% of all human *Salmonella* isolates reported in 2009. The rates of *S*. Enteritidis infections in humans have increased threefold from 2003 to 2009, with most provinces and territories affected. The increase has been primarily associated with three main phage types, 8, 13 and 13a. These phage types had summer seasonal peaks and were associated with cases of domestically acquired infections. Similar phage types have been reported in the USA [12, 13]. In contrast, European countries have reported PT4, PT1, PT8, PT14B and PT21 as the most commonly identified phage types [14].

The various agri-food data examined in this study showed that the broiler chicken commodity was more often contaminated with *S*. Enteritidis than other livestock commodities that were regularly sampled. PT8, PT13 and PT13a were the most common strains in broiler samples, similar to human cases. The data showed an overall increase in recovery of *S*. Enteriditis from broiler chicken between 2005 and 2009, which paralleled that observed in humans. For example, a concurrent increase in PT8 was observed early on in the reporting period in slaughtered chicken, retail chicken, and humans, while an increase in PT13a was observed in slaughtered chicken, retail chicken, and humans in 2008 and 2009. Outbreak investigations involving PT8 and PT13 have implicated chicken and eggs as the source of human infection. These results are supported by the fact that broiler meat has recently been identified as a risk factor for *S*. Enteritidis in North America [13, 15] and that *S*. Enteritidis has been increasingly recovered from retail chicken meat [16–18] and eggs [19, 20]. In the USA, data (2001–2005) showed an increase in the proportion of PT8 isolates from chicken meat and a slight drop in the proportion of PT13 [21]. It is understood that gradual changes in predominant strains occur naturally over time; however, the exchange of broiler hatching eggs, chicks, and chicken meat products both inter-provincially [22] and internationally [23], may also contribute to the distribution and emergence of certain *S*. Enteritidis phage-type strains in Canada. Further investigation is needed to establish if changes in predominant phage-type strains occurring in both Canada and the USA occurred independently. Although monitoring of *S*. Enteritidis in this commodity is covered both by CIPARS and C-EnterNet, there is no current programme monitoring *S*. Enteritidis in imported poultry products to explain the increasing trend of *S*. Enteritidis and emergence of certain phage types in broiler chickens.

The egg commodity has also been recognized as a major *S*. Enteritidis exposure source for humans [12, 24–28]. *S*. Enteritidis in this commodity is not monitored by either CIPARS or C-EnterNet. However, monitoring and intervention programmes are conducted by the egg industry and the Canadian Food Inspection Agency (CFIA) to ensure egg safety [29–31]. While some of these data are captured by government monitoring described above, the data available for this study were insufficient to determine which isolates were recovered from the egg industry *vs*. other poultry industries such as the layer industry. Currently, Health Canada is finalizing a policy on *S*. Enteritidis in Canadian shell eggs, which is informed by the risk assessment of Canadian Grade A shell eggs internally contaminated with *S*. Enteritidis [32]. This policy is intended to harmonize current inter-provincial differences in sampling and *S*. Enteritidis laboratory methods. This policy will enhance safety of eggs reaching the consumer by implementing early risk mitigation approaches in *S*. Enteritidis-positive laying flocks.

Beyond the egg and broiler commodities, *S*. Enteritidis was detected in other food animals (e.g. beef cattle, pigs), pets, wild animals, food (e.g. pork, beef, cheese, produce), and surface water, highlighting each as a potential source of human infection. However, few *S*. Enteritidis isolates have been recovered from these sources and the available data were insufficient to explore their contribution to the level and trend of *S*. Enteritidis infections in humans.

Antimicrobial resistance levels in *S*. Enteritidis sourced from healthy animals on farms and at slaughter, and from retail meats remained low over the study period. This is consistent with data from other surveillance programmes [16, 33]. In human isolates, resistance to nalidixic acid, tetracycline or ampicillin was observed in phage types that were not identified in food animal isolates (PT1, PT4, PT6a, PT29a). PT1 has repeatedly demonstrated the highest level of resistance to nalidixic acid and reduced susceptibility to ciprofloxacin [34–36]. PT6a has also

shown resistance to nalidixic acid [36]. Currently, antimicrobial resistance in *S*. Enteritidis remains low. However, changes in resistance profiles should be monitored, especially for drugs of high importance to human medicine [37] such as fluoroquinolones and newer generation cephalosporins.

This study provides further insight into the epidemiology of *S*. Enteritidis infections in the Canadian population and the role of international travel. At the sentinel site level, international travel represents an important source of *S*. Enteritidis infection. Common phage types in travel-related cases included PT4, PT1 and PT6a. These findings corroborate with other studies, which identified PT4 [12, 13] and PT1 [38] as being associated with international travel. PT1, PT4 and PT6a are different from those contributing to the increase in endemic cases and are absent from all non-human sources in this study and from previous reports [39–41]. Of the CIPARS data, these phage types exhibited higher levels of resistance to nalidixic acid compared to all other phage types. In addition, these phage types showed a different seasonal pattern than the phage types associated with endemic cases, with larger peaks in the winter. While, *S*. Enteritidis infections acquired abroad appear to be different from infections acquired domestically, they do contribute to the overall burden of *S*. Enteritidis cases in Canada. This warrants enhanced surveillance and prevention efforts.

This study synthesized the best available information provided by many surveillance and monitoring programmes. Although each system has its own limitations, these synthesized findings provide a comprehensive picture of the epidemiology of *S*. Enteritidis in Canada. These results demonstrate the importance of ongoing national surveillance at different points in food production and the ability of integrated surveillance to identify human and animal health issues. Considering the increasing trend of human *S*. Enteritidis incidence in Canada and the difficulties in establishing epidemiological linkages between contaminated source and human disease, surveillance of this pathogen in humans, animals and the environment should continue and be conducted in a coordinated and collaborative manner. Future research should also be undertaken to elucidate the role of various potential sources in order to target public health actions to reduce exposure and to better understand the exposures and routes of transmission for human cases. Finally, enhanced collaboration between public health, animal health and the poultry industry is needed to implement effective prevention and control measures and mitigate the burden of illness caused by *S*. Enteritidis.

ACKNOWLEDGEMENTS

The authors acknowledge provincial and territorial epidemiologists and laboratory directors and programme stakeholders for their valued input on the manuscript. We also acknowledge provincial epidemiologists from the provinces of Ontario, British Columbia, Québec, and Alberta for providing additional outbreak information. The authors thank Andrea Ellis and Diane MacDonald for their advice and early discussions on the scope of the paper, Pia Muchaal and Lisa Scott for their assistance in the data analysis, Rebecca Irwin, Rafiq Ahmed, Frank Pollari, and Lisa Landry for their critical review of the manuscript. The authors especially thank the contribution provided by all the Provincial Public Health Laboratories for submitting isolates for further characterization and antimicrobial susceptibility testing. The Canadian Integrated Program for Antimicrobial Resistance Surveillance Public Health Partnership and the Canadian Public Health Laboratory Network including the provincial public health laboratories or enteric reference centres of British Columbia, Alberta, Saskatchewan, Manitoba, Ontario, Québec, New Brunswick, Nova Scotia, Prince Edward Island and Newfoundland and Labrador; the Laboratory for Foodborne Zoonoses, Guelph, Ontario and Saint-Hyacinthe, Québec; the National Microbiology Laboratory, Winnipeg, Manitoba; and the Centre for Food-borne, Environmental and Zoonotic Infectious Diseases, Guelph, Ontario.

DECLARATION OF INTEREST

None.

REFERENCES

1. **Government of Canada.** Laboratory surveillance data for enteric pathogens in Canada: annual summary 2006. National Microbiology Laboratory, 2007.
2. **Canadian Food Inspection Agency.** Federally reportable diseases in Canada – 2011 (http://www.inspection.gc.ca/english/anima/disemala/rep/repe.shtml). Accessed 7 October 2011.
3. **Rodriguez DC, Tauxe RV, Rowe B.** International increase in *Salmonella* Enteritidis: a new pandemic? *Epidemiology and Infection* 1990; **105**: 21–27.

4. **Government of Canada.** Laboratory surveillance data for enteric pathogens in Canada: annual summary 1999–2005. National Microbiology Laboratory, 2007.
5. **Ribot EM, *et al.*** Standardization of pulsed-field gel electrophoresis protocols for the subtyping of *Escherichia coli* O157:H7, *Salmonella*, and *Shigella* for PulseNet. *Foodborne Pathogens and Disease*. 2006; **3**: 59–67.
6. **Ward L, De Sa RJ, Rowe B.** A phage-typing scheme for *Salmonella* Enteritidis. *Epidemiology and Infection* 1987; **99**: 291–294.
7. **Canadian Food Inspection Agency.** Hatchery Regulations (http://laws-lois.justice.gc.ca/eng/H-3.3/C.R.C.-c. 1023). Accessed 5 October 2009.
8. **Statistics Canada.** Demographic estimates section, July population estimates, 2009 final intercensal estimate. Demography Division, 2010.
9. **Gilman J, Myatt M.** EpiCalc2000 version 1.02. Computer Software, Brixton Books, 1998.
10. **Olson AB, *et al.*** Limited genetic diversity in *Salmonella* enterica serovar Enteritidis PT13. *BMC Microbiology* 2007; **7**: 87.
11. **Thomas W, Wilson W.** Canadian *Salmonella* Enteritidis control symposium and workshop. *Proceedings of the Canadian Salmonella Enteritidis Control Symposium and Workshop*, Vancouver, British Columbia: Intersol Group, 2011.
12. **Marcus R, *et al.*** Re-assessment of risk factors for sporadic *Salmonella* serotype Enteritidis infections: a case-control study in five FoodNet sites, 2002–2003. *Epidemiology and Infection* 2007; **135**: 84–92.
13. **Voetsch AC, *et al.*** Analysis of the FoodNet case-control study of sporadic *Salmonella* serotype Enteritidis infections using persons infected with other *Salmonella* serotypes as the comparison group. *Epidemiology and Infection* 2009; **137**: 408–416.
14. **European Centre for Disease Prevention and Control.** Annual epidemiological report on communicable diseases in Europe, 2010.
15. **Kimura AC, *et al.*** Chicken consumption is a newly identified risk factor for sporadic *Salmonella* enterica serotype Enteritidis infections in the United States: a case-control study in FoodNet sites. *Clinical Infectious Diseases* 2004; **38** (Suppl. 3): 244–252.
16. **United States Food and Drug Administration.** National antimicrobial resistance monitoring system: retail meat annual report, 2007 (http://www.fda.gov/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/NationalAntimicrobialResistanceMonitoringSystem/ucm164662.htm). Accessed 6 April 2010.
17. **Little CL, *et al.*** Prevalence, characterisation and antimicrobial resistance of *Campylobacter* and *Salmonella* in raw poultry meat in the UK, 2003–2005. *International Journal of Environmental Research and Public Health* 2008; **18**: 403–414.
18. **Ribeiro AR, *et al.*** *Salmonella* spp. in raw broiler parts: occurrence, antimicrobial resistance profile and phage typing of the *Salmonella* Enteritidis isolates. *Brazilian Journal of Microbiology* 2007; **38**: 296–299.
19. **Suresh T, *et al.*** Prevalence and antimicrobial resistance of *Salmonella* Enteritidis and other *Salmonellas* in the eggs and egg-storing trays from retail markets of Coimbatore, South India. *Food Microbiology* 2006; **23**: 294–299.
20. **Centers for Disease Control and Prevention.** Outbreaks of *Salmonella* serotype Enteritidis infection associated with eating shell eggs – United States 1999–2001. *Morbidity and Mortality Weekly Report* 2003; **51**: 1149–1152.
21. **Altekruse SF, *et al.*** *Salmonella* Enteritidis in broiler chickens, United States, 2000–2005. *Emerging Infectious Diseases* 2006; **12**: 1848–1852.
22. **Agriculture and Agri-Food Canada.** Profile of the Canadian Chicken Industry: Interprovincial trade in chickens (http://www.agr.gc.ca/poultry-volaille/prindc6_eng.htm#sec63). Accessed 20 September 2010.
23. **Agriculture and Agri-Food Canada.** Profile of the Canadian Chicken Industry: Canadian imports of chicken meat and meat products (http://www.agr.gc.ca/poultry-volaille/prindc6_eng.htm#sec64). Accessed 20 September 2010.
24. **Doorduyn Y, *et al.*** Risk factors for *Salmonella* Enteritidis and Typhimurium (DT104 and non-DT104) infections in The Netherlands: predominant roles for raw eggs in Enteritidis and sandboxes in Typhimurium infections. *Epidemiology and Infection* 2006; **134**: 617–626.
25. **Patrick ME, *et al.*** *Salmonella* Enteritidis infections, United States, 1985–1999. *Emerging Infectious Diseases* 2004; **10**: 1–7.
26. **St Louis ME, *et al.*** The emergence of grade A eggs as a major source of *Salmonella* Enteritidis infectious. *Journal of the American Medical Association* 1988; **259**: 2103–2107.
27. **European Food Safety Authority.** The community summary report of zoonoses, zoonotic agents, antimicrobial resistance and foodborne outbreaks in the European Union in 2005. *European Food Safety Authority Journal* 2007; **130**: 34–117.
28. **Braden CR.** *Salmonella* enterica serotype Enteritidis and eggs: a national epidemic in the United States. *Clinical Infectious Diseases* 2006; **43**: 512–517.
29. **Egg Farmers of Canada.** Canadian Egg Marketing Agency – egg producer testing program (http://www.eggs.ca/IndustryHealthPro/Media_Centre.aspx). Accessed 6 April 2010.
30. **Frenette D.** *Salmonella* Enteritidis detection and eradication program. The Quebec egg board food safety program. In: *Proceedings of the Ontario Association of Poultry Practitioners Symposium: Salmonellosis, antimicrobial use, and antimicrobial resistance*. Guelph, Ontario, 2009.
31. **Canadian Food Inspection Agency.** Egg and egg products (http://www.inspection.gc.ca/english/fssa/eggoeu/eggoeue.shtml). Accessed 16 September 2010.
32. **DeWinter LM, *et al.*** Risk assessment of shell eggs internally contaminated with *Salmonella* Enteritidis. *International Food Risk Analysis Journal* 2011; **1**: 40–81.

33. **DANMAP.** DANMAP 2008 – Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, foods and humans in Denmark (http://www.danmap.org/pdfFiles/Danmap_2008.pdf). Accessed 6 April 2010.
34. **Browning LM, *et al.*** Antimicrobial resistance of *Salmonella* in Scotland, 2004 (excluding Typhi and Paratyphi). *Health Protection Scotland Weekly Report* 2005; **39**: 268–272.
35. **Threlfall EJ, *et al.*** Assessment of factors contributing to changes in the incidence of antimicrobial drug resistance in *Salmonella* enterica serotypes Enteritidis and Typhimurium from humans in England and Wales in 2000, 2002 and 2004. *International Journal of Antimicrobial Agents* 2006; **28**: 389–395.
36. **Carrique-Mas JJ, *et al.*** Trends in phage types and antimicrobial resistance of *Salmonella* enterica serovar Enteritidis isolated from animals in Great Britain from 1990 to 2005. *The Veterinary Record* 2008; **162**: 541–546.
37. **Health Canada Veterinary Drug Directorate.** Categorization of antimicrobial drugs based on importance in human medicine (http://www.hc-sc.gc.ca/dhp-mps/consultation/vet/consultations/amr_ram_hum-med-rev-eng.php). Accessed 17 September 2010.
38. **Molbak K, Neimann J.** Risk factors for sporadic infection with *Salmonella* Enteritidis, Denmark, 1997–1999. *American Journal of Epidemiology* 2002; **156**: 654–661.
39. **Poppe C, *et al.*** The prevalence of *Salmonella* Enteritidis and other *Salmonella* sp. among Canadian registered commercial chicken broiler flocks. *Epidemiology and Infection* 1991; **107**: 201–211.
40. **Renwick SA, *et al.*** Epidemiological associations between characteristics of registered broiler chicken flocks in Canada and the *Salmonella* culture status of floor litter and drinking water. *Canadian Veterinary Journal* 1992; **33**: 449–458.
41. **Poppe C, *et al.*** *Salmonella* Enteritidis and other *Salmonella* in laying hens and eggs from flocks with *Salmonella* in their environment. *Canadian Journal of Veterinary Research* 1992; **56**: 226–232.