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Characterization of *Salmonella* isolates from beef cattle, broiler chickens and human sources on Prince Edward Island

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Abstract

Non-typhoid *Salmonella* serovars remain a potential threat to human health, and beef cattle and broiler chickens are possible sources of these organisms on Prince Edward Island (PEI). In this study, the ceca of beef cattle belonging to fasted and non-fasted groups, and broiler chickens were examined for *Salmonella* at the time of slaughter. The characteristics of the isolates, including antimicrobial resistance patterns and virulence genes, were studied along with the isolates obtained from cases of human salmonellosis on PEI during the study period (1996–97). The prevalence of *Salmonella* in beef cattle was 4.6% (11/240). The rate was significantly higher in fasted cattle (7.46%), than in non-fasted cattle (0.94%). The prevalence rate in chickens was 32.5% (39/120). In beef cattle, *Salmonella typhimurium* phage type (PT) or definitive type (DT) 104 which was resistant to ampicillin, chloramphenicol, streptomycin, sulfisoxazole and tetracycline, was the most predominant type (64%). In chickens, *S. heidelberg*, with resistance to gentamicin, streptomycin and sulfisoxazole, predominated. Of 26 isolates from humans, the most common serovar was *S. typhimurium*, including a multidrug-resistant strain of DT104. Examination by PCR revealed presence of the virulence gene *invA* in all serovars, and the *spvC* gene in all *S. typhimurium* isolates, of both beef cattle and human origin. Among the other serovars the latter gene was found in 7 human isolates, but in none of the chicken or beef isolates. All but 3 of the *spvC*-positive isolates possessed a 90 kilobasepair (kbp) plasmid suggesting that the 3 isolates had the *spvC* gene on their chromosome. These findings were confirmed by

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plasmid DNA isolation using 3 different protocols and by sequence analysis of the *spvC*-PCR product. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Salmonella; Beef cattle; Chickens; Humans; Characteristics

Résumé

Les sérovars de salmonelles de type non-typhoïdes constituent une menace potentielle à la santé humaine. À l'Ile du Prince Édouard (IPE) les bovins et les poulets sont des sources possibles de ces organismes. La présence de salmonelles au moment de l'abattage a été vérifiée au niveau du caecum de bovins soumis ou non à un jeûne, de même que chez des poulets. Les caractéristiques des isolats, incluant les supports de résistance antimicrobienne et les gènes de virulence, ont été étudiés à partir d'isolats obtenus des cas de salmonellose humaine sur l'IPE pendant la période d'étude (1996–97). La prévalence de salmonellose chez les bovins était de 4,6% (11/240). Ce taux était sensiblement plus élevé dans le groupe d'animaux soumis à un jeûne. Le taux de prévalence chez les poulets était de 32,5% (39/120). Pour les bovins, *Salmonella typhimurium* DT104 résistant à l'ampicilline, au chloramphénicol, à la streptomycine, au sulfisoxazole et à la tétracycline était le type prédominant (64%). Chez les poulets, *S. heidelberg* résistant à la gentamicine, à la streptomycine et au sulfisoxazole, prenait le premier rang. Des 26 isolats humains, le sérovar le plus commun était *S. typhimurium*, incluant une souche multi-résistante de type DT104. L'examen par la réaction d'amplification en chaîne par la polymérase (PCR) a indiqué la présence du gène de virulence *invA* dans tous les sérovars, et le gène *spvC* dans tous les isolats de *S. typhimurium* d'origine bovine et humaine. Parmi les autres sérovars, ce dernier gène a été trouvé dans 7 isolats humains, mais dans aucun des isolats de poulet ou de bovin. Tous les isolats positifs pour *spvC*, sauf 3, possédaient un plasmide de 90 kbp indiquant que les 3 isolats étaient porteurs du gène *spvC* sur leur chromosome. Ces résultats ont été confirmés par l'isolement d'ADN plasmidique en utilisant 3 protocoles différents et par séquençage du produit *spvC*-PCR. © 2000 Elsevier Science Ltd. All rights reserved.

Mots-clé: Salmonelles; Bovins; Poulets; Humaines; Caractéristiques

1. Introduction

Non-typhoid *Salmonella* represent an important public health problem in many parts of the world including Canada. Human infection with *Salmonella* often is associated with consumption of foods of animal origin [1]. *Salmonella* may be present as a part of the indigenous flora of the intestinal tract of carrier animals. Coincidental isolation of the same *Salmonella* serovars from human and animal sources underlines the importance of a zoonotic relationship [2]. Monitoring the occurrence and frequency distribution of *Salmonella* serovars from human and animal sources is important to detect possible outbreaks, to identify possible sources of infection and to target prevention and control measures [3]. A study during 1984–85 indicated that the incidence of *Salmonella* in Canadian beef

carcasses was 3.3% [4]. The results of a recent study on PEI showed slaughter hogs on the island are not reservoirs of *Salmonella* serovars commonly associated with human disease in Canada [5]. However, there has been no information whether beef cattle and broiler chickens at the time of slaughter harbor *Salmonella* similar to those associated with human disease on PEI. At the abattoir, the initial source of contamination are the carrier animals with *Salmonella* in their digestive tracts and on the hides. It has been suggested that stress associated with transportation, overcrowding and feed withdrawal experienced by animals before slaughter increases shedding of *Salmonella* [4,6]. However, there is little published information on the effect of feed withdrawal in cattle before slaughter. Cattle held for more time in the abattoir have been found more likely to be positive for *Salmonella*, than cattle held for shorter periods [7]. The emergence of multidrug-resistant strains such as *S. typhimurium* DT104 in other geographic areas also created a need to study the characteristics of food animal-associated *Salmonella* on Prince Edward Island (PEI).

The objectives of the study described here, were to compare presence of *Salmonella* in the ceca of fasted and non-fasted cattle at slaughter, as well as to determine the carriage rates, serovars, and phenotypic and genotypic characteristics including the presence of virulence genes *invA* and *spvC*, of the isolates from beef cattle and chickens at abattoirs on PEI. Human clinical isolates of *Salmonella* obtained during the study period from the main hospital on PEI were compared with the animal isolates.

2. Materials and methods

2.1. Sample collection from beef cattle

During June–August 1996, a total of 240 samples from beef cattle originating from a number of different farms were collected from a federally-inspected plant on PEI. On the beef slaughter days of every week, 20 animals (35% of the slaughter volume) were randomly selected prior to slaughter by computer generated random numbers (Epi Info, Version 6 database and statistical program). Animals for sampling were selected randomly under two categories. Animals detained in the lairage for 18–24 hours were categorized as fasted and those slaughtered immediately after arrival, as non-fasted. Information on last feeding was not available; however, all animals were from PEI, and travelled less than 3 h to reach the abattoir. Cecal sampling method was chosen over rectal sampling because the amount of cecal contents taken was more consistent. Samples were placed in sterile plastic bags and transported to the laboratory on ice for processing the same day.

2.2. Sample collection from broiler chickens

A total of 120 cecal samples were collected from broilers at the only poultry

processing plant in PEI during April–May 1997. Sampling was done in a random systematic manner with every fourth or fifth bird in the processing line being sampled. The birds originated from different farms in PEI, however, individual farms were not identified.

2.3. Culture of samples

Samples were cultured for *Salmonella* using selective enrichment and selective plating [8,9]. Twenty-five grams of cecal sample from beef cattle or 1 g of cecal content from each broiler chicken were inoculated into 225 ml or 9 ml, respectively, of tetrathionate broth (Oxoid, Nepean, Ontario), and incubated at 37°C for 24 h. Subcultures were done on modified semisolid Rappaport–Vassiliadis medium (MSRV) as per the methods described for *Salmonella* isolation from fecal samples, by Dusch and Altwegg [9]. Pure cultures were obtained by streaking on blood agar and MacConkey agar plates. Slide agglutination test using polyvalent *Salmonella* antiserum (Difco, Detroit, MI) was used for preliminary identification of isolates.

2.4. Human isolates

Twenty-six *Salmonella* strains from human sources were supplied in pure culture by the Queen Elizabeth Hospital, PEI. These comprised of all isolates recovered from unrelated clinical cases of salmonellosis during 1996–97 on PEI.

2.5. Serotyping and phage typing

All isolates were confirmed as *Salmonella*, and then serotyped [2] and phagetyped [2] at the Health Canada, OIÉ Reference Laboratory for Salmonellosis, Guelph, Ontario, Canada.

2.6. Determination of antimicrobial drug resistance

Antibiotic-susceptibility tests were carried out by the agar dilution method employing a replicator, as described by Poppe et al. earlier [10]. The drugs and the concentrations used were: amikacin (AK), ampicillin (A), ceftriaxone (Ctr), cephalothin (Clt), streptomycin (S) and chloramphenicol (C) at 8, 16 and 32 μg/ml; ciprofloxacin (Cp) at 1, 2 and 4 μg/ml; cotrimoxazole (trimethoprim/sulfamethoxazole) (Cot) at 10, 40 and 80 μg/ml; gentamicin (G), neomycin (N), tetracycline (T) and tobramycin (Tob) at 4, 8 and 16 μg/ml; kanamycin (K) at 16, 32 and 64 μg/ml; nalidixic acid (Nal) at 32 μg/ml; nitrofurantoin (F) at 64 μg/ml; polymyxin B (Pol) at 2, 8 and 16 μg/ml; and sulfisoxazole (Su) at 256 μg/ml. The concentrations of antimicrobial agents and interpretations of susceptibility, intermediate susceptibility and resistance were those suggested by the National Committee for Clinical Laboratory Standards (NCCLS) [11,12].

To examine for low-level resistance, the minimal inhibitory concentrations of

ciprofloxacin for *S. typhimurium* strains were determined using E-test strips (AB Biodisk, Solna, Sweden) with concentrations ranging from 0.002 to 32 μg/ml. The test was done according to manufacturer's instructions. *Escherichia coli* ATCC strain 25922 was used for quality control of the test as suggested by the National Committee for Clinical Laboratory Standards (NCCLS) [12].

2.7. Plasmid characterization

Plasmid DNA of all *Salmonella* isolates were prepared by the method of Kado and Liu [13], except that the isolates were grown on blood agar plates and colonies were scraped off the surface of the agar with a disposable loop and suspended in lysis buffer. Plasmids were resolved by gel electrophoresis on a 0.7% agarose gel, and visualized by staining with ethidium bromide, and viewing under ultraviolet light. For estimation of molecular sizes, reference plasmids of the following bacterial strains were used: *S. typhimurium* LT2 containing a single plasmid (pSLT2) [14] of 90 kbp, and *E. coli* V517 carrying 8 plasmids (53.7, 7.2, 5.6, 5.1, 3.9, 3.0, 2.7, and 2.1 kbp) [15]. Supercoiled DNA ladder ranging from 16.0 to 2.0 kbp (Gibco-BRL, Burlington, Ontario) was used to estimate the molecular size of small plasmids (<16 kbp).

2.8. Examination for invA and spvC virulence genes

All *Salmonella* isolates collected in this study, except one untypeable chicken strain, were tested for the virulence genes. Whole cell DNA for polymerase chain reaction (PCR) was prepared as described by Rahn et al. [16]. Plasmid DNA was prepared by three different protocols: Kado and Liu [13], Birnboim and Doly [17], and Olsen [18]. Plasmid DNA was characterized by gel electrophoresis on a 0.7% agarose gel, as described by Kado and Liu [13].

For PCR amplification, two pairs of oligonucleotide primers specific for *Salmonella spvC* and *invA* genes previously designed by Swamy et al. [19] according to the published DNA sequences of *invA* and *spvC* genes (GenBank accession numbers M64295 and M90846, respectively), were used. The *spvC* sense primer sequence was 5′-CGG AAA TAC CAT CAA ATA-3′, and the *spvC* antisense primer sequence was 5′-CCC AAA CCC ATA CTT ACT CTG-3′; this primer pair was predicted to yield a 669-bp product. The *invA* sense primer sequence was 5′-TTG TTA CGG CTA TTT TGA CCA-3′, and the *invA* antisense primer sequence was 5′-CTG ACT GCT ACC TTG CTG ATG-3′; this primer pair was predicted to yield a 521-bp product. PCR amplifications were carried out in a DNA Thermal Cycler Model 480 (Perkin–Elmer Cetus, Dartmouth, Nova Scotia). The PCR mixtures were subjected to the following temperature parameters: an initial denaturation step for 1 min at 93°C followed by 30 cycles of denaturation at 93°C for 1 min, primer annealing at 48°C for 1 min and extension at 72°C for 2 min. Following the amplification, the PCR products were analyzed immediately on 1.5% agarose gels or were stored at −20°C prior to analysis. The PCR products of *spvC* gene were purified by extraction from low melting

Table 1
The frequency of *Salmonella* isolation from cecal contents of fasted and non-fasted beef cattle

Condition of the animals	Sample size[a]	Number of positive[b]	% Positive
Fasted	134	10	7.46%[c]
Non-fasted	106	1	0.94%
Total	240	11	4.6%

[a] One sample from each of the randomly selected animal.
[b] Confirmed by serological identification.
[c] Level of significance (Chi-square = 4.358, *P*-value = 0.037).

temperature agarose gels [20]. The PCR product was then subcloned using a TOPO TA cloning Kit™, version D (Invitrogen, San Diego, CA, USA). Selected recombinant plasmids containing the insert DNA were sequenced by a modification of the Sanger dideoxynucleotide chain termination procedure described by Chen and Seeburg [21] with either the M13/pUC universal or the reverse primer M13pUC (Boehringer Mannheim, Laval, Quebec) and [α^{35}S] dATP (sp. act. > 600 Ci/mmol; Amersham, Oakville, Ontario). The nucleotide sequences on the autoradiograph were read manually and the data were analyzed using the FASTA program [22].

3. Results

3.1. Isolation and subtyping

Of the 240 cecal samples from freshly slaughtered beef cattle, salmonellae were isolated from 4.6% (n = 11). The rate of isolation for fasted animals was 10/134 (7.46%) while for non-fasted animals it was only 1/106 (0.94%) (Table 1). Salmonellae were isolated in significantly higher frequency from fasted animals than from non-fasted ones (Chi-square = 4.358, P = 0.037). The data analysis was done by using Epi Info, Version 6. The most common type (63.6% of all isolates) was *S. typhimurium* DT104 (Table 2).

Table 2
Serovars and phage types of *Salmonella* identified in beef cattle

Serovars	Phage type	No. isolated (n = 11)	Percentage
S. typhimurium	104	7	63.6%
S. typhimurium	108	1	9.1%
S. agona	ND[a]	2	18.2%
S. infantis	ND	1	9.1%

[a] Not done.

Table 3
Salmonella serovars identified in broiler chickens

Serovar	No. isolated ($n = 39$)	Percentage
S. heidelberg	17	43.6%
S. schwarzengrund	11	28.2%
S. infantis	10	25.6%
I:4,5:i:-[a]	1	2.6%

[a] Untypeable (subspecies I, serogroup B).

Thirty-nine (32.5%) of 120 cecal samples of broiler chickens (Table 3) were positive for *Salmonella*, the most common serovar being *S. heidelberg* (43.6%).

The most common serovar found among 26 *Salmonella* isolates from human salmonellosis was *S. typhimurium* (24%) phage types 104, 108, 195 and one undetermined type (Table 4).

3.2. Drug resistance

The drug resistance patterns and the presence and sizes of plasmids of 47 of the 76 *Salmonella* isolates are shown in Table 5. Resistance to one or more drugs was seen in 47 (61.8%) of isolates. The proportion of drug resistant *Salmonella* was as follows: 10/11 (91%) from beef, 28/39 (71.8%) from chickens, and 9/26 (34.6%) from humans. Eight of the 11 beef isolates, including all six *S. typhimurium*

Table 4
Serovars and phage types of 26 Salmonella isolates from cases of human salmonellosis

Serovar	Phage type	No. isolated	Percentage
S. typhimurium	104	1	3.84%
S. typhimurium	108	1	3.84%
S. typhimurium	195	3	11.5%
S. typhimurium	Atypical	1	3.84%
S. hadar	2	4	15.4%
S. enteritidis	29	2	6.9%
S. enteritidis	4	1	3.84%
S. enteritidis	8	1	3.84%
S. heidelberg	8	2	6.6%
S. heidelberg	10	1	3.84%
S. infantis	ND[a]	3	11.5%
S. agona	ND	1	3.84%
S. bareilly	ND	1	3.84%
S. kiambu	ND	3	11.5%
S. brandenburg	ND	1	3.84%

[a] Not done.

Table 5
Drug resistance and plasmids of 76 *Salmonella* isolates

Serovar and phage type	Source	No. of isolates	R-type
S. typhimurium 104	Human[a]	1	ACSSuTKN
S. typhimurium 104	Beef cattle[b]	6	ACSSuT
S. typhimurium 108	Beef cattle	1	ACSSuT
S. typhimurium 104	Beef cattle	1	SSuT
S. agona	Beef cattle	1	ACSSuT
S. heidelberg AT[c]	Chicken[d]	16	SSuTG
S. heidelberg 6	Chicken	1	SSuG
S. heidelberg 8	Human	1	AST
S. agona	Human	1	SSuT
S. agona	Beef cattle	1	SSuT
S. enteritidis 29	Human	2	ASTK
S. hadar 2	Human	4	ST
S. schwarzengrund	Chicken	11	F

[a] Information on specimens is not available.
[b] Beef cattle: cecal contents.
[c] AT = Atypical phage type.
[d] Chicken: cecal contents of broilers.

DT104 isolates showed simultaneous resistance to ampicillin, chloramphenicol, streptomycin, sulfisoxazole, and tetracycline (R-type ACSSuT). The single human isolate of *S. typhimurium* DT104 was resistant to kanamycin and neomycin in addition to the above drugs (R-typeACSSuTKN). Forty-one percent of the *Salmonella* isolates from chickens were *S. heidelberg* with R-type SSuTG (G = gentamicin). The MIC value for ciprofloxacin against all *S. typhimurium* isolates both from beef cattle or human sources, was identical, and the reading on the E-test strip 0.012 μg/ml.

3.3. Plasmids

Of the total 76 isolates examined for the presence of plasmids, 38 (50%) were positive. All multi-drug resistant *S. typhimurium* DTs 104 and108 from beef cattle possessed a single 90 kbp plasmid DNA. In contrast, the *S. typhimurium* DT104 isolate of human origin had the 90 kbp plasmid and an additional plasmid of 3.6 kbp. Sixteen of 17 *S. heidelberg* from chickens possessed 90 and 45 kbp plasmids and two small plasmids of molecular sizes 7.2 and 2.1 kbp. All 8 human isolates of *S. heidelberg* had only the 90 kbp plasmid. Other plasmid positive isolates were mainly of human origin, and these included both drug resistant and drug susceptible isolates of serovars other than *S. typhimurium* and *S. heidelberg* (Table 5).

3.4. Occurrence of invA gene and spvC

The oligonucleotide primers generated PCR products of the predicted sizes. The *invA* and *spvC* products were approximately 521 bp and 669 bp, respectively, as determined by agarose gel electrophoresis. The controls, *S. typhimurium* SR 11 and *E. coli* HB101 were positive and negative, respectively.

All but one of the 76 isolates were tested for virulence factors. All 75 were positive for *invA* gene sequence (Table 6) as indicated by the size of the PCR product in agarose gels (approximately 521 bp) (Fig. 1). Twenty-one (28%) of the isolates had *spvC* gene sequence as determined by the size of the PCR product (approximately 669 bp) (Fig. 2). Sequence analysis of the *spvC* PCR product showed 99% identity with the published *spvC* sequence, thus establishing the specificity of the product. Isolates that were positive for *spvC* gene included all *S. typhimurium*, and originated from humans or beef cattle, but not from chickens. Analysis of the plasmid DNA revealed that 18 of the 21 *spvC*-positive isolates had large molecular size plasmids, but 16 *spvC*-negative isolates did not (Table 6). All three plasmid-negative, *spvC*-positive isolates were serovars of human origin.

Table 6
PCR results for *invA* and *spvC* virulence genes in 75 *Salmonella* isolates

Salmonella serovar	No. tested	Source	Plasmid profile (in kbp)	PCR result	
				invA	*spvC*
S.typhimurium	1	Human	90, 3.6	+	+
S.typhimurium	4	Human	90	+	+
S. typhimurium	1	Human	None[a]	+	+
S. enteritidis	2	Human	72,54	+	+
S. enteritidis	2	Human	54	+	+
S. hadar	4	Human	5.1, 3 and 2.1	+	–
S. infantis	2	Human	None	+	–
S. infantis	1	Human	None[a]	+	+
S. heidelberg	1	Human	90	+	+
S. heidelberg	1	Human	None[a]	+	+
S. heidelberg	1	Human	None	+	–
S. agona	1	Human	None	+	–
S. kiambu	3	Human	None	+	–
S. bareilly	1	Human	None	+	–
S. brandenburg	1	Human	None	+	–
S. typhimurium	8	Beef	90	+	+
S. infantis	1	Beef	None	+	–
S. agona	2	Beef	None	+	–
S. heidelberg	16	Chicken	90,45,7.2,2.1	+	–
S. heidelberg	1	Chicken	None	+	–
S. infantis	10	Chicken	None	+	–
S. schwarzengrund	11	Chicken	None	+	–

[a] *spvC* — positive isolates possessed no plasmid.

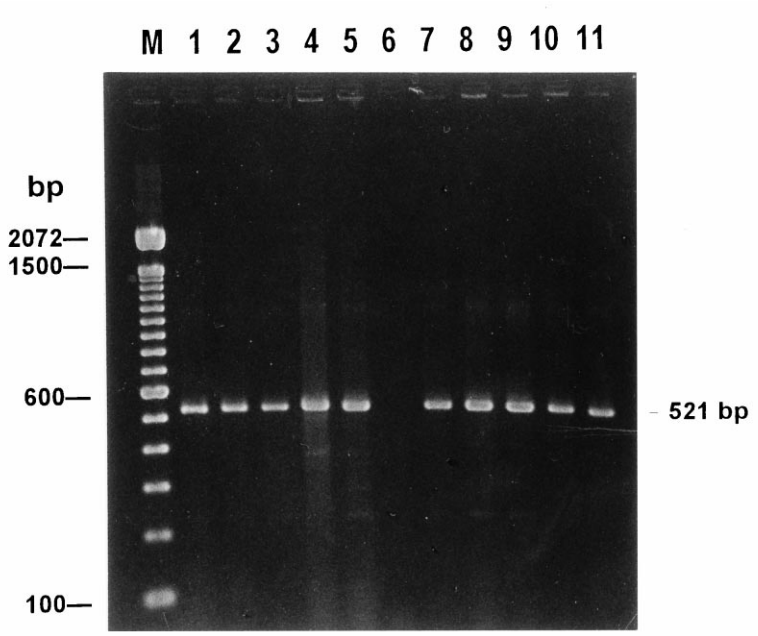

Fig. 1. Analysis of PCR products of the *invA* gene of multiple *Salmonella* serovars on 1.5% agarose gel electrophoresis. Lane M: 100 bp ladder, lanes 1–4: *S. typhimurium*, lane 5: *S. typhimurium* SR 11, lane 6: *E. coli* 101, lanes 7–8: *S. heidelberg*, lane 9: *S. enteritidis*, lane 10: *S. infantis* and lane 11: *S. agona*.

4. Discussion

During the study period the prevalence of *Salmonella* in slaughtered beef cattle on PEI was 4.6%. Although, the rate of *Salmonella* positive cattle was not high in the present study, the presence of even a single carrier animal can be a potential source of contamination of the environment, carcasses and personnel. However, isolation rate as high as 61% from cecal contents of slaughter cattle has been reported in a study in Australia [23]. The isolation of *S. typhimurium* as the predominant serovar in this study is consistent with the finding of at least one study with regard to beef cattle [24]. *S. typhimurium* continues to be an important cause of human foodborne disease in Canada [2,25], and in a recent survey 37% of this serovar from humans were DT104 [26]. High prevalence of *S. typhimurium* in slaughter cattle support the notion that beef could be a reservoir of human infection by *S. typhimurium*. Furthermore, DT104 which was predominant in cattle in the present study was also identified once among human strains from PEI. It is noteworthy that in the United Kingdom *S. typhimurium* DT104 was found to be the predominant type in beef cattle, and it also has been recognized as the second most common cause of human salmonellosis in that country

recently [27]. Despite these observations, more studies are needed to link *Salmonella* in beef cattle, with human cases on PEI.

Salmonella heidelberg, S. schwarzengrund and *S. infantis* were the only serovars found in broiler chickens in the present study. Isolation of these serovars from chickens was reported in Canada by Poppe et al. [10]. All isolates of *S. heidelberg*, which was the predominant serovar, with one exception belonged to an atypical phage type. On the other hand, phage types 8 and 10 were predominant among human isolates, suggesting that chickens slaughtered in PEI may not have been the source of human infection with *S. heidelberg*. However, sampling during different seasons of the year, and sampling of retail chickens are necessary to shed more light on the role of broiler chickens in human salmonellosis. During the study period *S. enteritidis* was not recovered from broiler chickens. This is not surprising as *S. enteritidis* has been associated with eggs, and less frequently with poultry meat and other animal sources [28]. Human isolates of *S. enteritidis* studied on PEI belonged to phage types 4, 8 and 29, however phage type 29 is uncommon in Canada [2]. Any possible association between the isolation of *S. infantis* from broiler chickens, and human cases due to this serovar needs further study.

Antimicrobial drug resistance to one or more drugs was seen among 10/11 (91%) of beef cattle isolates, whereas it was 28/39 (71.8%) among those from

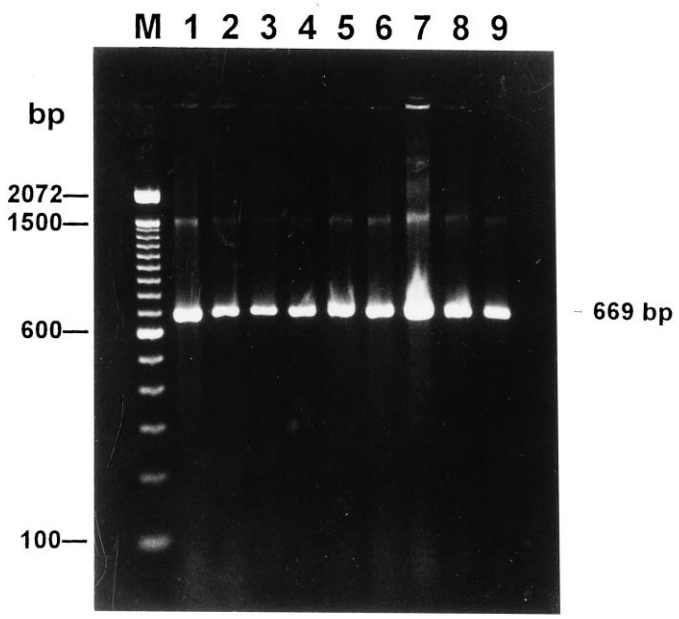

Fig. 2. Analysis of PCR products of the *spvC* gene of multiple *Salmonella* serovars on 1.5% agarose gel electrophoresis. Lane M: 100 bp ladder, lanes 1–4: *S. typhimurium*, lanes 5–6: *S. heidelberg*, lane 7: *S. infantis*, lane 8: *S. enteritidis* and lane 9: *S. typhimurium* SR 11.

chickens. The most common resistance pattern among beef cattle isolates was ACSSuT which was present among 73% of beef isolates, and among the chicken isolates it was SSuTG, the rate being 41%. All *S. typhimurium* DT104 isolates were of the typical R-type ACSSuT. This R-type has recently emerged in the United States and Canada [26,29]. In the UK, 45 to 50% of *S. typhimurium* isolates from cattle have been reported to be DT104 R-type ACSSuT [30]. Plasmid analysis of *S. typhimurium* R-type ACSSuT strains investigated here revealed that all isolates possessed a 90 kbp plasmid DNA, which usually does not encode drug resistance [10,31]. However, no attempt was made to investigate the role of this plasmid in drug resistance. The genes encoding ACSSuT resistance pattern has been shown to become integrated into the chromosome [30]. It is possible that the additional resistance to kanamycin and neomycin in the DT104 strain of human origin may be due to the 3.6 kbp plasmid.

The investigation using PCR for the presence of *invA* gene in this study demonstrated its presence in all *Salmonella* isolates irrespective of the serovar or source. This finding was consistent with previous reports [19,32] that established the presence of *invA* gene in nearly all *Salmonella* irrespective of serovar or source. The *invA* gene is important in the invasion of nonprofessional phagocytic epithelial cells, and entry into the intestinal mucosa [32,33].

Examination of *Salmonella* for *spvC* gene sequence by PCR revealed its presence only in 21/75 (28%) isolates. All *spvC*-positive isolates originated from beef cattle and human sources; none of the chicken isolates were positive. The majority (14/21 or 67%) of the *spvC*-positive isolates were *S. typhimurium*. Plasmid DNA analysis of the *spvC*-positive isolates using 3 different protocols revealed that 18 of the 21 *spvC*-positive isolates possessed a large size (90 kbp) plasmid. This suggests that the amplified sequences in the three isolates, all of human origin (Table 6), which did not possess the large molecular size plasmid were of chromosomal origin. The presence of virulence gene *spvC* in *Salmonella* strains which do not have plasmid DNA suggests that the plasmid had integrated into the chromosome. Since the exact location of the *spvC* gene sequence of the 18 positive isolates with the large plasmid was not determined, it is possible that some or all of these isolates also have the *spvC* sequence on the chromosome rather than the plasmid. It has been hypothesized by Jones et al. [34] that the virulence plasmid (90 kbp) could exist in both autonomous and integrated states. The integration of the virulence plasmid into the chromosome may allow for the expression of integrated *spv* genes as a stable character that could be lost if the plasmid had not been integrated. This finding has important implications for comparative studies of the virulence within different *Salmonella* serovars. The *spv* genes are primarily responsible for virulence, and it has been suggested that spvC gene enhances the growth and survival of *Salmonella* in reticuloendothelial cells [35,36], and its expression is induced after *Salmonella* is being phagocytosed [35–37].

This study yielded hitherto unpublished information on the characteristics and drug resistance patterns of *Salmonella* serovars in beef cattle, broiler chickens and humans on PEI, which will serve as a baseline for future surveillance. *Salmonella*

typhimurium DT104 with multiple drug resistance was the predominant type in beef cattle, and it was also found among human isolates. This study also highlights the adverse effects of fasting cattle prior to slaughter, with regard to cecal carriage of *Salmonella*. Examination of the *Salmonella* isolates in this study at the molecular level gave indirect evidence on the occurrence of the *spvC* virulence plasmid gene sequence in the bacterial chromosome.

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