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The prevalence of *Salmonella enteritidis* and other *Salmonella* spp. among Canadian registered commercial layer flocks

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SUMMARY

A survey was conducted to estimate the prevalence of *Salmonella enteritidis* and other salmonellas among Canadian commercial egg producing flocks. Environmental (faecal and eggbelt) samples from 152 of 295 (52·9%) randomly selected flocks were contaminated with salmonellas. Thirty-five different salmonella serovars were isolated. Eggbelt samples were more often contaminated with salmonellas than faecal samples (25·7 *v.* 10·1%). The most prevalent serovars were *S. heidelberg*, *S. infantis*, *S. hadar*, and *S. schwarzengrund*; they were isolated from samples of 59/295 (20%), 18/295 (6·1%), 17/295 (5·8%), and 15/295 (5·1%) flocks, respectively. Feed samples of 21/295 (7·2%) flocks were contaminated with salmonellas. *Salmonella enteritidis* was isolated from the environmental samples of 8/295 (2·7%) flocks. *Salmonella enteritidis* phage type (PT) 8 was isolated from 5 flocks, PT 13a from 2 flocks, and PT 13 from 1 flock.

INTRODUCTION

In several countries, notably the UK, Spain, and the US, there has been a dramatic increase of *Salmonella enteritidis* infection in humans during the last 5–10 years. In England and Wales isolations of *S. enteritidis* from humans increased from 1087 in 1981 to 15427 (56% of a total of 27478 isolates of salmonella) in 1989, whereas infections due to all other serovars combined increased by about half [1, 2]. Similarly, in Scotland, *S. enteritidis* infections in humans increased from 11% of all isolates of salmonella in 1982 to 52% for the first 11 months of 1988 [3]. In Spain, outbreaks of food-borne disease in humans due to *S. enteritidis* increased from 8% in 1977 to 40% in 1984, whereas food-borne disease due to *S. typhimurium* remained stable at 8% [4]. In the US, reported *S. enteritidis* infections in humans increased from about 6% of all human isolates of salmonella before 1976 to more than 51% in 1987 [5]. In Canada, *S. enteritidis* was the third commonest isolate of salmonella (8·3% of a total of 10646 isolates of salmonella) from people in 1987, and the fourth commonest isolate (9·2% of 9957 isolates of salmonella) in 1988 [6].

In Europe most human *S. enteritidis* isolates belong to phage type (PT) 4 [1, 4].

Salmonella enteritidis strains isolated in Canada and the US belong primarily to PT 8: 64% of the human isolates of *S. enteritidis* in Canada [6], 48% of human and animal [7] and 64% of animal isolates of *S. enteritidis* in the US were phage type 8 [8].

Outbreaks of disease by *S. enteritidis* in humans have been associated with the consumption of eggs or foods that contain eggs [1, 4, 9–13]. It has been suggested that *S. enteritidis* may infect eggs by transovarian transmission [12, 14]. The prevalence of isolation of *S. enteritidis* from hens and ovaries and eggs of hens varied considerably among different studies [15–19]. Hopper and Mawer [17] isolated *S. enteritidis* from 13 of 50 dead hens taken from a commercial layer flock of 60000 hens that was epidemiologically identified as the source of raw shell eggs that caused an outbreak of human food poisoning by *S. enteritidis* PT 4. *Salmonella enteritidis* was cultured from the caecal contents of 13, the oviduct of 8, and the ovaries of 6 of the 50 hens [17].

Examination of the egg contents of 1119 eggs derived from two small flocks of 12 and 23 egg-laying hens each, showed that 11 eggs were positive for *S. enteritidis* [18]. The production of infected eggs was clustered though intermittent. The positive eggs which were produced by 10 of the 35 hens all contained fewer than 10 salmonellas [18]. Examination of 15000 eggs derived from more than 1300 layer flocks in the US resulted in the detection of only one flock infected with *S. enteritidis* [19]. Tracebacks from infected people to three egg-producing flocks resulted in the isolation of *S. enteritidis* from yolk of eggs in one flock, from a pooled ovarian sample of another flock, and no isolations in the third flock [15]. In orally infected and contact-exposed hens, intestinal colonization persisted for as long as 18 weeks, some strains of *S. enteritidis* caused significant decreases in egg production, and *S. enteritidis* was found in a high percentage of the yolks and albumens of eggs laid during the first 2 weeks after inoculation [16].

The purpose of this study was to estimate the prevalence of salmonellas, and of *S. enteritidis* in particular, among Canadian registered commercial layer flocks. The study was prompted by concerns about the rapid increase of *S. enteritidis* infection in people in countries such as the UK and the US, by the possibility that eggs contaminated with *S. enteritidis* may be a significant source of human infection, and by concerns about the effect these problems may have on the Canadian poultry industry.

METHODS

Flocks and samples

Three hundred flocks from which samples were to be collected were randomly chosen from a list of 1682 producing commercial egg-laying operations registered with the Canadian Egg Marketing Agency. The flock size ranged from 200 to 57200 layers, and the average flock size was 8810 laying hens. Flocks, not premises, were the unit of interest. Assuming that *S. enteritidis* would be present in 5% of flocks, it was calculated that a sample size of 300 would give a 95% confidence interval with a 2·5% error limitation for our prevalence estimate. Sampling kits were sent to Agriculture Canada District Offices in each province and the samples were collected by Agriculture Canada inspectors from May 1989, until September 1989, inclusive. To ensure a 95% probability of detecting at least one infected sample, and assuming a 5% within-flock prevalence, 60 randomly

selected faecal droppings were collected from within each flock. In addition, 12 dust/fluff samples were collected from eggbelts (in the case of automated collection systems), or vents, fans, and walls (when producers collected the eggs by hand). Samples of faeces and eggbelts were pooled into groups of three. The eggbelt samples consisted of dust, faecal matter, egg shell matter, yolk, fluff, feathers, and other debris that falls off the eggbelt when it turns around at the end of a row of layer cages. The eggbelt and/or dust samples are hereafter collectively called eggbelt samples. A one kg sample of feed was collected from each flock. The feed samples were taken from the bins or augers but not from the feed troughs. The samples were collected in sterile plastic bags with the aid of sterile tongue depressors. The faecal samples weighed about 10–25 g, the eggbelt samples weighed about 5–10 g and the feed sample weighed about 1 kg. The samples were kept cool in containers by ice-packs and shipped by air or car to the laboratory.

Culture of salmonellas from faecal and eggbelt samples

On arrival at the laboratory the faecal and eggbelt samples were weighed and nine times the weight of the sample of Buffered Peptone Water (BPW) (Difco) was added to the contents of the plastic bags. Not only the eggbelt but also the faecal samples were pre-enriched in BPW because laying hens shed salmonellas only intermittently and in low numbers in the faeces [20], and about 10^2 of salmonellas per ml must be obtained by the end of the pre-enrichment period [21, 22]. The samples were stomached and incubated at 37 °C for 18–24 h. One-tenth of a ml of the BPW was then transferred with a sterile 1 ml pipette and inoculated at the periphery of a Modified Semisolid Rappaport Vassiliadis (MSRV) agar [21] plate. Overgrowth of salmonellas by competing organisms in the pre-enrichment medium was not considered to be an impeding factor in isolation of salmonellas because a few salmonella bacteria in the pre-enrichment culture are capable of motility and growth in MSRV medium even if they are outnumbered by competing bacteria by a factor of 10^7 [21]. The inoculated MSRV medium was incubated overnight at 42 °C for 18–24 h. A loopful of the semisolid agar was taken from the outer edge of migration and streaked onto MacConkey (MC) agar (Difco). The plates were discarded if, after a total incubation period of 72 h, no zone of migration into the MSRV was observed.

Serological procedures

Up to five colonies were picked from the MC agar plate and examined by a slide-agglutination test using salmonella polyvalent O antiserum (Bacto salmonella O antiserum poly A-I and Vi; Difco) for agglutination. To determine whether the isolate that gave a positive test with the salmonella polyvalent O antiserum could possibly be a *Salmonella enteritidis* strain, a slide-agglutination test with salmonella O group D_1 antisera (Bacto salmonella O antiserum group D_1 factors 1, 9, 12; Difco) was performed.

Lysis by polyvalent bacteriophages

To obtain additional evidence that the isolates were indeed salmonellas, all putative isolates of salmonella were examined for lysis by bacteriophages using a mixture of polyvalent salmonella phage O-1 and a bacteriophage specific for the O groups E_1–E_4 with the methods described by Fey and others [23, 24].

Plasmid DNA profiles

To limit the number of isolates of salmonella that needed to be serotyped, the plasmid profiles of all isolates were determined. Plasmid DNA preparations were made from the isolates by the method of Portnoy and White as cited by Crosa and Falkow [25], except that the isolates were grown overnight on LB agar [26], scraped off the surface of the agar with a sterile toothpick, and suspended in lysis buffer. The plasmid DNA was subjected to electrophoresis in a horizontal 0·7% agarose gel in Tris-acetate buffer, then stained with ethidium bromide and photographed [26]. To compare plasmid profiles of all isolates of salmonella from one flock, plasmid DNA preparations from isolates from one flock were electrophoresed together on one agarose gel. Plasmids used as molecular mass standards were: pSLT2, 60 MDa [27]; pDT285, 96 MDa, and pDT369, 23 MDa (both obtained from D. Taylor, Medical Microbiology, University of Alberta, Edmonton, Alberta); and the eight plasmids of *E. coli* V517 with molecular masses of 1·4–35·8 MDa [28].

Biochemical testing and serotyping

All salmonella cultures isolated from the faecal and eggbelt samples collected at one farm that displayed different plasmid profiles, and all salmonella cultures isolated from the feed, were biotyped and serotyped. Thirty biochemical tests were performed on each isolate by using the Gram-Negative Identification (GNI) card and the automated diagnostic bacteriology system of Vitek Systems (Hazelwood, MO). Procedures used for serotyping of salmonella isolates have been described previously [29, 30].

Phage typing of S. enteritidis *strains*

S. enteritidis strains were phage typed with typing phages obtained from the Division of Enteric Pathogens at the Central Public Health Laboratory in London, England [31].

Culture of feed samples for salmonellas

Feed samples were cultured for salmonellas at the Laboratory Services Division of Agriculture Canada in Ottawa. The method used was a modification of the Health Protection Branch Method MFHPB-20 [32]. Briefly, 200 ml of BPW was added to 100 g of feed, the mixture was incubated for 1 h at 35 °C, another 700 ml BPW was added, and the mixture was stomached for 2 min. Then the pH was adjusted to 7·0, and the sample was incubated at 35 °C overnight [33]. Selective enrichment was performed by transferring 1 ml of the BPW to 9 ml of tetrathionate brilliant green (TBG) broth. The TBG was incubated for 24 h at 43 °C and a loopful of the selective enrichment broth was streaked onto bismuth sulfite and brilliant green sulpha agar (BGS) [32].

Statistical analyses

The variability of the prevalence estimates was expressed as the standard error of the mean and was calculated by considering flocks as clusters of equal size. Flocks were designated as positive if one or more samples from a flock were culture positive for salmonellas. The unconditional association between the presence of

salmonellas and the type of sample taken (faecal *v.* eggbelt) was assessed for statistical significance using the χ^2 test [34].

RESULTS

Number of flocks in which salmonellas were isolated from faecal and/or eggbelt samples

Table 1 enumerates the number of faecal and eggbelt samples received for analysis.

The results of testing layer flocks for the presence of salmonellas in faecal and eggbelt samples are shown in Table 2. Samples were received from 295 flocks. Salmonella were isolated from faecal and/or eggbelt samples of 156 of 295 ($52{\cdot}9 \pm 2{\cdot}9\,\%$) flocks. One or more faecal samples of 104 of 295 ($35{\cdot}3\,\%$) flocks were positive for salmonella. Salmonella were cultured from one or more eggbelt samples of 127 of 295 ($43{\cdot}1\,\%$) flocks. *S. enteritidis* was recovered from eight of 295 ($2{\cdot}7 \pm 0{\cdot}9\,\%$) flocks.

Six hundred and eleven strains of salmonella were isolated from faecal samples. Seventeen of the faecal samples contained two different colony types (large and small) of salmonella. These colony types isolated from one sample belonged in each case to the same serovar. Three hundred and sixteen salmonella strains were isolated from eggbelt samples. Two different colony types of salmonella were isolated from 12 of the eggbelt samples. The colony types isolated from one sample belonged in each case to the same serovar. From one sample three different colony types (of large, medium and small size) were isolated, two were the same serovar (*S. enteritidis*), the third colony type was a different serovar (*S. agona*). In total, salmonellas were isolated from 302 of 1176 ($25{\cdot}7\,\%$) eggbelt samples and from 594 of 5897 ($10{\cdot}1\,\%$) faecal samples. The frequency of isolation of salmonella from eggbelt samples was significantly higher ($P < 0{\cdot}001$) than from faecal samples.

Number of flocks in which salmonella serovars were isolated from faecal and eggbelt samples

The number of flocks in which a salmonella serovar was isolated from faecal, eggbelt, faecal and eggbelt, and faecal and/or eggbelt samples is shown in Table 3. Only salmonella serovars that occurred in three or more flocks are listed. *Salmonella heidelberg* was the commonest serovar isolated from flocks (from samples of 59 of 295 ($20{\cdot}0\,\%$) of the flocks) followed by *S. infantis*, *S. hadar*, and *S. schwarzengrund*. *Salmonella enteritidis* and *S. typhimurium* were each isolated from the environmental samples of eight flocks. A total of 35 serovars, and five rough antigenically different strains, were isolated. Examples of isolations of two or more salmonella serovars from environmental samples within flocks are given in Table 4.

Number of flocks in which salmonellas were isolated from the feed samples

Salmonellas were isolated from the feed samples of 21 of 295 ($7{\cdot}2\,\%$) flocks. The serovars isolated from the feed samples were compared to those isolated from the faecal and/or eggbelt samples of the same flocks (Table 5). In 5 of 295 ($1{\cdot}7\,\%$) flocks the same salmonella serovar was isolated from feed, faecal and/or eggbelt samples, whereas in 3 of 295 ($1{\cdot}0\,\%$) flocks the same salmonella serovar was isolated from

Table 1. *Number of faecal and eggbelt samples received for analysis*

Faecal			Eggbelt		
Number of flocks	Number of samples per flock	Total	Number of flocks	Number of samples per flock	Total
285	20	5700	290	4	1160
5	19	95	3	3	9
1	18	18	1	2	2
4	21	84	1	5	5
295		5897	295		1176

Table 2. *Numbers of flocks in which salmonellas were isolated from faecal and/or eggbelt samples, and number of samples positive for salmonellas*

Total number of flocks examined	295
With salmonellas	
In faecal and/or eggbelt samples	156 (52·9%)
In one or more faecal samples	104 (35·3%)
In one or more eggbelt samples	127 (43·1%)
In faecal but not eggbelt samples	29 (9·8%)
In eggbelt but not faecal samples	52 (17·6%)
With *S. enteritidis* in faecal and/or eggbelt samples	8 (2·7%)
Faecal samples	
Total number examined	5897
Contaminated with salmonellas	594 (10·1%)
Eggbelt samples	
Total number examined	1176
Contaminated with salmonellas	302 (25·7%)

feed, faeces and eggbelt samples. *Salmonella tennessee* was the commonest serovar isolated from feed followed by *S. infantis*, *S. heidelberg*, and *S. senftenberg*.

Frequency of isolation of S. enteritidis *from faecal and eggbelt samples, and phage types of the* S. enteritidis *strains*

The frequency of isolating *S. enteritidis* from faeces and/or eggbelts of the eight flocks that were positive for *S. enteritidis* and the phage types of these isolates is shown in Table 6. *S. enteritidis* strains that were isolated from the samples of five of the layer flocks were phage typed as PT 8, those of two flocks were PT 13a and those of one flock were PT 13.

Serotyping and plasmid profile analysis

Six hundred and eleven salmonella strains were isolated from the faecal samples, 316 from the eggbelt samples, and 22 from the feed samples. Thus a total of 949 salmonella strains were isolated. The plasmid profiles of all of the strains were determined and 455 of the strains that displayed different plasmid profiles within isolates from one flock or that did not harbour any plasmids were serotyped.

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Table 3. *Prevalence of the 20 most common salmonella serovars in flocks as determined by isolation rates from environmental samples*

		Flocks		Sample type		
Rank	Serover	Affected flocks*	Percentage of flocks	Faecal only	Eggbelt only	Faecal and eggbelt
1	*heidelberg*	59	20·0	9	18	32
2	*infants*	18	6·1	5	9	4
3	*hadar*	17	5·8	3	8	6
4	*schwarzengrund*	15	5·1	5	6	4
5	*enteritidis*	8	2·7	3	3	2
	havana	8	2·7	3	3	2
	typhimurium	8	2·7	2	5	1
6	*mbandaka*	7	2·4	1	3	3
	thompson	7	2·4	0	4	3
7	*anatum*	4	1·4	0	4	0
	johannesburg	4	1·4	3	1	0
	muenster	4	1·4	1	2	1
8	*agona*	3	1·0	1	2	0
	braenderup	3	1·0	0	0	3
	montevideo	3	1·0	1	2	0
	newington	3	1·0	2	1	0
	ohio	3	1·0	0	3	0
	oranienburg	3	1·0	1	0	2
	rough 0:r:2	3	1·0	3	0	0
	rough 0:r:5	3	1·0	2	1	0

* Number of flocks in which a salmonella serovar was isolated from faecal and/or eggbelt samples.

Note: *S. berta*, *S. blockley*, *S. bredeney*, *S. haardt*, *S. rubislaw*, *S. saintpaul*, *S. tennessee*, *and S. typhimurium* were isolated from two flocks, and *S. alachua*, *S. arizona*, *S. indiana*, *S. livingstone*, *S. newbrunswick*, *S. newport*, *S. orion*, *S.* rough 0:k:5, *S.* rough 0:l, v:z_{15}, *S.* rough 0:m:–, *S. senftenberg*, and *S. urbana* were each isolated from one flock.

Table 4. *Examples of isolations of two or more salmonella serovars per flock**

No. of flocks	Serovars isolated from faecal and/or eggbelt samples			
2	*S. braenderup*	*S. heidelberg*		
2	*S. hadar*	*S. heidelberg*		
3	*S. heidelberg*	*S.* rough 0:r:2		
2	*S. infantis*	*S. typhimurium*		
3	*S. mbandaka*	*S. schwarzengrund*		
1	*S. agona*	*S. enteritidis*	*S. hadar*	
1	*S. berta*	*S. hadar*	*S. heidelberg*	
1	*S. blockley*	*S. hadar*	*S. heidelberg*	
1	*S. enteritidis*	*S. heidelberg*	*S. typhimurium*	
1	*S. heidelberg*	*S. mbandaka*	*S. newport*	
1	*S. heidelberg*	*S. oranienberg*	*S. schwarzengrund*	
1	*S. enteritidis*	*S. hadar*	*S. heidelberg*	*S. infantis*
1	*S. hadar*	*S.* rough 0:r:5	*S. senftenberg*	*S. thompson*

* One serovar was isolated from 111 flocks, 2 serovars were isolated from 37 flocks, 3 serovars were isolated from 6 flocks, and 4 serovars were isolated from 2 flocks.

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Table 5. *Salmonella serovars isolated from feed samples compared with those isolated from faecal and/or eggbelt samples of the same flocks*

Flock no.	Serovar isolated from		
	Feed	Faecal	Eggbelt
053	*S. brandenburg*	*S. heidelberg*	—
072	*S. bredeney*	—	—
362	*S. heidelberg*	*S. heidelberg*	*S. heidelberg*
282	*S. infantis*	—	—
322	*S. infantis*	—	—
323	*S. infantis*	—	—
023	*S. mbandaka*	—	—
251	*S. montevideo*	*S. agona*	—
280	*S. montevideo* & *S. saintpaul*	*S. schwarzengrund*	—
327	*S. rubislaw*	*S. typhimurium* var. *cop.**	—
149	*S. saintpaul*	*S. saintpaul*	*S. saintpaul*
146	*S. schwarzengrund*	—	*S. schwarzengrund*
005	*S. senftenberg*	—	*S. anatum*
232	*S. senftenberg*	—	—
304	*S. tennessee*	—	—
319	*S. tennessee*	—	*S. ohio*
333	*S. tennessee*	—	—
335	*S. tennessee*	*S.* rough 0:k:5	*S. ohio*
393	*S. typhimurium*	*S. haardt*	—
328	*S. typhimurium* var. *cop.*	*S. typhimurium* var. *cop.* & *S. thompson*	—
145	*S. urbana*	*S. urbana*	*S. urbana*

* The *copenhagen* variety of *S. typhimurium*.

Table 6. *Frequency of isolation of* S. enteritidis *from faecal and eggbelt samples, and phage types of the* S. enteritidis *strains*

Flock no.	Faecal	Eggbelt	Phage type
29	4/20*	0/4	8
74	0/20	2/4	13a
155	0/20	1/4	8
161	2/20	1/4	8
310	9/20	3/4	13
379	0/20	1/4	8
383	1/20	0/4	8
400	1/20	0/4	13a

* *S. enteritidis* was isolated from 4 of 20 faecal samples.

Thirty-five different salmonella serovars and five serovars that had rough LPS and different flagellar antigenic formulas were isolated from the samples.

DISCUSSION

A survey to estimate the prevalence of salmonellas, in particular of *S. enteritidis*, in 295 randomly chosen layer flocks was carried out. The study showed that 52·9% of the flocks were environmentally contaminated with salmonellas.

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Considering the fact that the environment of adult hens was examined, it was expected that the percentage would be lower because chickens rapidly become resistant to salmonella infection with increase in age [35, 36]. The finding that such a high percentage of the environmental samples were contaminated with salmonellas may be explained by persistence of contamination in poultry houses for long periods of time and for consecutive generations of birds. Snoeyenbos and co-workers [20] noted that residual house contamination was frequent following depopulation, cleaning, and disinfection. Higgins and co-workers [37] found that dust was contaminated with salmonellas in 6 of 9 houses after disinfection and suggested that defects in the cleaning and disinfection of air inlets and fans seemed to be an important factor for recontamination of the house.

Although five times more faecal than eggbelt samples were taken, a larger number of flocks were found to be salmonella-positive by examining the eggbelt than the faecal samples (43·1 % *v.* 35·3 %). Possible explanations could be the fact that the eggbelt samples were by nature pooled samples, and the observation that salmonellas may persist in dust for long periods of time [37].

Isolation of *S. heidelberg* from faecal and/or eggbelt samples occurred more often than of any other serovar. The reasons for this finding are not known. One explanation may be a more prolonged or a higher degree of colonization of the caeca of chickens by *S. heidelberg* and subsequent prevention of colonization by other salmonella serovars that are antigenically closely related [38]. Other possible explanations are a higher degree of egg shell penetration by *S. heidelberg* resulting in a higher percentage of day-old chicks and subsequently a higher percentage of hens that are infected with this serovar, or perhaps a higher degree of extraintestinal infection or transovarian transmission by *S. heidelberg* in chickens [14]. Barnhart and colleagues [39] reported that *S. heidelberg* was the predominant serovar isolated from pooled ovaries collected from layer hens at the time of slaughter. Eggs were associated with, but not proved to be, the cause of a large outbreak of *S. heidelberg* infection in persons attending a conference in New Mexico in 1985 [40]. A relationship between the high frequency of *S. heidelberg* isolation from environmental samples in layer flocks and the prevalence of *S. heidelberg* infections in people has not been established. The frequency of *S. heidelberg* isolations from humans in Canada has not changed much during the last decade. In 1987 and 1988 *S. heidelberg* was the fourth and the third most common salmonella serovar isolated from humans (7·5 % and 9·2 % of all salmonella isolates, respectively) [6].

Salmonella enteritidis and *S. typhimurium* were each isolated from the environmental samples of 8 (2·7 %) of 295 flocks. Both *S. enteritidis* and *S. typhimurium* may cause a transovarian infection in hens [14, 41]. They were less frequently isolated than *S. heidelberg*, *S. infantis*, *S. hadar*, and *S. schwarzengrund*. *Salmonella typhimurium* and *S. hadar* are the most common salmonella serovars isolated from humans in Canada (accounting for 25·4 and 15·8 % of all human isolates, respectively) [6]. However, because of the paucity of information on the vehicle of transmission in outbreaks of salmonellosis in humans in Canada, it is impossible to draw definite conclusions about a possible link between salmonella-positive flocks of laying hens and infection of people.

The isolation rates of salmonella serovars from faecal and eggbelt samples

appear to correlate. This suggests that when faeces are contaminated with a serovar also the environment such as dust, feathers, shells, fluff, and other debris will be contaminated with the same serovar, and conversely, when the environment is contaminated with a certain serovar, the hens may become infected and shed the same serovar in the faeces.

Salmonellas were not often isolated from the feed samples (7·1% of the samples were positive for salmonellas) and the same salmonella serovar was isolated from feed, faecal, and eggbelt samples in only 3 (1·0%) of the 295 flocks. This finding may suggest that laying hens became infected or the environment became contaminated only occasionally because the feed was contaminated with salmonella. The lower isolation rate of salmonella from feed samples as compared to those from eggbelts and faeces, may be explained by the following reasons: (i) the feed sample was taken from the augers or feed bins and not from the feed troughs, which would tend to preclude previous contamination from the hens, faeces, feathers, and other extraneous matter within the poultry house; (ii) only one feed sample per flock was taken; and (iii) salmonellas may not be as easily recovered from feed than from faeces and dust because of low numbers of salmonellas present and poor viability of the bacteria.

The *S. enteritidis* strains that were isolated from the samples of five of the layer flocks were phagetyped as PT 8, those of two flocks were PT 13a and those of one flock were PT 13. In comparison, the *S. enteritidis* phage types isolated from people in Canada during 1988 were PT 8 (64·2%), PT 13 (27·4%), PT 4 (6·3%), PT 1 (1·6%), and PT 13a (0·5%) [6].

S. enteritidis PT 4 strains were shown to be invasive for day-old chicks (17) and laying hens, causing a bacteraemia with infection of many body sites including peritoneum, ovules and oviduct [43]. Timoney and co-workers [43] observed major differences in invasive abilities of *S. enteritidis* PT 8 strains for laying hens: one strain was as invasive as a *S. enteritidis* PT 4 that invaded many internal organs, whereas other *S. enteritidis* PT 8 strains were shed in the faeces but did not cause invasive infections. Determination of virulence properties of phage types of *S. enteritidis* isolated from poultry and their environment in Canada is in progress. This will allow further assessment of the significance of isolation of the different phage types of *Salmonella enteritidis* from poultry and their environment.

In summary, the main findings of this national survey to estimate the prevalence of salmonellas among commercial layer flocks in Canada were that (i) environmental samples of more than half of the flock contained salmonellas; (ii) *S. heidelberg* was the most common salmonella serovar isolated from the flocks; (iii) *S. enteritidis* was isolated from less than 3% of the flocks; and (iv) *S. enteritidis* phage type 8 was the most prevalent phage type isolated from the layer flocks.

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