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

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SUMMARY

A nation-wide survey was conducted to estimate the prevalence of *Salmonella enteritidis* and other salmonellas among Canadian commercial broiler flocks. Environmental (litter and/or water) samples from 226 of 294 (76·9%) randomly selected flocks were contaminated with salmonellas. Litter samples were more often contaminated with salmonellas than water samples (47·4 *v.* 12·3%). Fifty different salmonella serovars were isolated. The most prevalent serovars were *S. hadar*, *S. infantis*, and *S. schwarzengrund*; they were isolated from samples of 98/294 (33·3%), 26/294 (8·8%), and 21/294 (7·1%) flocks, respectively. Feed samples of 39/290 (13·4%) flocks were contaminated with salmonellas. *Salmonella enteritidis* was isolated from the environmental samples of 9/294 (3·1%) flocks. *Salmonella enteritidis* phage type (PT) 8 was isolated from seven flocks, and PT 13a from two flocks.

INTRODUCTION

During the last 5–10 years in European countries and the United States, human *Salmonella enteritidis* infections have increased to such an extent that *S. enteritidis* has become the most commonly isolated *Salmonella* serovar [1–5]. In England and Wales, 56% of all human salmonella isolates in 1988 were *S. enteritidis* [1], and in the United States 51% of human salmonella isolates in 1987 were *S. enteritidis* [3]. In Canada, the prevalence of *S. enteritidis* is much lower: *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 belonged to phage type (PT) 4 [1, 4], whereas in Canada and the United States they were mostly PT 8 [6–8].

It has been shown that *S. enteritidis* may infect eggs by transovarian transmission [9, 10], and outbreaks of disease caused by *S. enteritidis* in humans have been associated with the consumption of eggs or foods that contain eggs [1, 4, 11–15].

The consumption of broiler chickens has been suggested to be another important source of human infection with *S. enteritidis* [16]. Broilers may become infected with *S. enteritidis* PT 4 via vertical transmission from infected hens in broiler breeding stock [17], and by eating contaminated feed [18]. In the United Kingdom it has been shown that *S. enteritidis* PT 4 caused increased mortality in chicks of less than 1 week of age and stunted growth in up to 5% of the flock [17, 19]. Pathological lesions including toxic indurated yolk sac, pericarditis, and necrotic foci in the liver have been demonstrated in infected broilers of 2 weeks of age [19]. Lesions of *S. enteritidis* PT4 infection, characterized by a distended pericardial sac containing mucopurulent exudate, have also been observed in market-age broiler chickens at slaughter [16, 19]. *Salmonella enteritidis* PT 4 was isolated in pure culture from pericardial sacs from 22 of 30 (73%) broilers [19]. At broiler processing plants in the United Kingdom an average of 1 in 1000 birds was condemned because of gross pericarditis due to *S. enteritidis* [16]. *Salmonella enteritidis* PT 4 has been isolated from tissues such as heart, spleen and caecum of broilers with a macroscopic pericarditis, and from swabs of the carcass cavity [16].

Salmonellas have been isolated from rinse samples of 60·9% of chicken carcasses in Canada [20], and poultry is often identified as one of the main sources of human salmonellosis [21]. Serovars most frequently isolated from broiler carcasses are also most frequently found in humans [22].

The purpose of this study was to estimate the prevalence of salmonellas, and of *S. enteritidis* in particular, among Canadian registered broiler flocks.

The study was prompted by concerns about the rapid increase of *S. enteritidis* infection in people in the European countries and the United States, by the possibility that broilers contaminated with *S. enteritidis* and other salmonella serovars may be a significant source of human infection, and by concerns about the effect these problems may have on the Canadian poultry industry.

MATERIALS AND METHODS

Flocks and samples

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. Three hundred flocks from which samples were to be collected were randomly chosen from a list of 2319 commercial broiler operations registered with the Canadian Chicken Marketing Agency. Flocks, not premises, were the unit of interest. Sampling kits were sent to Agriculture Canada District Offices in each province and the samples were collected by Agriculture Canada inspectors from December, 1989, until May, 1990, inclusive. Twelve pooled litter samples were collected randomly from within each flock. The litter samples were collected in sterile plastic bags with the aid of sterile tongue depressors. Three pooled water samples were collected randomly from the drinkers using sterile syringes. 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 pooled litter samples weighed about 10–20 g, and the pooled water samples measured about 12 ml. The samples were kept cool in containers by ice-packs and shipped by air or ground transportation to the laboratory.

Culture of salmonellas from litter and water samples

The litter samples were weighed and diluted 1 to 10 (w/v) in Buffered Peptone Water (BPW) (Difco) and then stomached. The amount of water in the syringes was measured, the samples were expressed into sterile plastic bags, and diluted 1 to 10 in BPW. The litter and water samples were incubated at 37 °C for 18–24 h. One-tenth of an 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 [23] plate. The medium was incubated overnight at 42 °C for 18–24 h. The MSRV plates were examined for a zone of migration. If the putative salmonellas had migrated 20 mm or more into the MSRV agar, a loopful of the growth was taken from the outer edge of the zone 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–1 and Vi; Difco). Isolates that give a positive result were further tested with salmonella O group D_1 antisera (Bacto salmonella O antiserum group D_1 factors 1, 9, 12; Difco).

Lysis by polyvalent bacteriophages

All putative isolates of salmonella were examined for lysis by bacteriophages using a mixture of polyvalent salmonella phage 0–1 and a bacteriophage specific for the O groups E_1–E_4 with the methods described by Fey and colleagues [24] and Gudel and Fey [25].

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 by the method of Portnoy and White as cited by Crosa and Falkow [28], except that the isolates were grown overnight on LB agar [27], 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, and then stained with ethidium bromide and photographed [27]. 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 Mdal [28]; pDT285, 96 Mdal, and pDT369, 23 Mdal (both obtained from Diane Taylor, Medical Microbiology, University of Alberta, Edmonton, Alberta); and the eight plasmids of *Escherichia coli* V517 with molecular masses of 1·4–35·8 Mdal [29].

Biochemical testing and serotyping

All salmonella cultures, isolated from the litter and water 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 [30, 31].

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 [32].

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 [33]. 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. The pH was adjusted to 7·0, and the sample was incubated at 35 °C overnight [34]. Selective enrichment was performed by transferring 1 ml of the BPW to 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) [33].

Statistical analyses

The variability of the prevalence estimates was expressed as the standard error of the mean. Flocks were designated as positive if any litter or water samples (hereafter denoted as 'environmental samples') were culture positive for salmonellas. The unconditional association between the presence of salmonellas and the type of sample taken (litter *v.* water) was assessed for statistical significance using the Chi-square test [35].

RESULTS

Number of flocks in which salmonellas were isolated from litter and/or water samples, and number of samples positive for salmonellas.

Samples were received from 294 flocks. Salmonellas were isolated from the environmental samples of 226 of 294 (76·9 % ± 2·5 %) flocks. One or more litter samples of 223 of 294 (75·9 %) flocks were positive for salmonella. Salmonellas were cultured from one or more water samples of 63 of 292 (21·6 %) flocks.

In total, salmonellas were isolated from 1674 of 3534 (47·4 %) litter samples and from 108 of 875 (12·3 %) water samples. The frequency of isolation of salmonella from litter samples was significantly higher ($P < 0·001$) than from water samples. Twenty of the litter samples contained two different colony types (large and small) of salmonella. The colony types isolated from 18 of these 20 litter samples belonged to the same serovar. Colony types of the other two samples belonged to different serovars: from both samples *S. hadar* and *S. heidelberg* were isolated. Two litter samples contained three colony types (large, medium and small) of

salmonella. The colony types isolated from one sample belonged in each case to the same serovar. No different colony types were isolated from the water samples.

Prevalence of the 15 most commonly occurring salmonella serovars within flocks as determined by isolation rated from environmental samples

The prevalence of the most commonly occurring salmonella serovars within flocks is shown in Table 1. Only the 15 most frequently isolated salmonella serovars are listed. *Salmonella hadar* was the most common serovar isolated from flocks (from samples of 98 of 294 (33·3 %) of the flocks). *S. enteritidis* was isolated from the environmental samples of 9 of 294 (3·1 % ± 1·0 %) flocks. Thirty-nine serovars, 9 rough strains with different antigenic formulas, and 2 untypable strains, for a total of 50 antigenically different strains, were isolated. *S. typhimurium* var. *copenhagen* was isolated from 6 flocks.

Frequency of isolation of one or more salmonella serovar(s) per flock

The frequency of isolation of one or more salmonella serovars per flock as determined by isolation rates from environmental samples is shown in Table 2. Up to eight different serovars were isolated from one flock.

Commonly occurring combinations of serovars within flocks

Common combinations of salmonella serovars that were isolated from environmental samples within flocks are listed in Table 3. The most common combination of serovars isolated from flocks was *S. hadar* and *S. heidelberg*. These serovars were isolated from environmental samples of eight flocks. *S. anatum* and *S. hadar* were together isolated from environmental samples of five flocks. Ninety-four different combinations of serovars were isolated from environmental samples within flocks.

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

Salmonellas were isolated from the feed samples of 39 of 290 (13·4 %) flocks. The serovars isolated from the feed samples were compared to those isolated from the environmental samples of the same flocks (Table 4). In 13 of 290 (4·5 %) flocks the same salmonella serovar was isolated from feed, and litter and/or water samples. In 1 of 290 (0·3 %) flocks the same salmonella serovar was isolated from feed, litter, and water samples.

Frequency of isolation of S. enteritidis *from litter and water samples, and phage types of the* S. enteritidis *strains*

In the 9 *S. enteritidis* – positive flocks 42 of 108 (39 %) of litter samples and 4 of 27 (15 %) of water samples were positive. *Salmonella enteritidis* strains that were isolated from the samples of seven of the broiler flocks were phage typed as PT 8, whereas those of the other two flocks were PT 13a.

Serotyping and plasmid profile analysis

A total of 1845 salmonella strains were isolated: 39 from feed samples, 1698 from litter samples, and 108 from water samples. The plasmid profiles of all of the strains were determined and 1185 of the strains that displayed different plasmid

Table 1. *Prevalence of the 15 most common salmonella serovars in flocks as determined by isolation rates from environmental samples*

Rank	Serovar	Flocks		Sample type		
		Affected flocks*	Percentage of flocks	Litter only	Water only	Litter and water
1	*hadar*	98	33·3	82	1	15
2	*infantis*	26	8·8	21	0	5
3	*schwarzengrund*	21	7·1	19	0	2
4	*anatum*	17	5·8	15	0	2
	thompson	17	5·8	16	0	1
	typhimurium	17	5·8	12	0	5
5	*agona*	15	5·1	11	2	2
6	*berta*	14	4·8	9	0	5
	heidelberg	14	4·8	6	0	8
7	*mbandaka*	11	3·7	8	0	3
8	*enteritidis*	9	3·1	6	1	2
	kentucky	9	3·1	6	2	1
	ohio	9	3·1	7	0	2
	senftenberg	9	3·1	8	1	0
9	*montevideo*	8	2·7	5	0	3

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

Table 2. *Frequency of isolation of one or more salmonella serovar(s) from environmental samples within flocks*

	Number of serovars								
	1	2	3	4	5	6	7	8	Total
Number of flocks	110	66	34	13	1	1	0	1	226

Table 3. *Common combinations of salmonella serovars isolated from environmental samples within flocks*

No. of flocks	Salmonella serovars isolated from litter and/or water samples		
8	*hadar*	*heidelberg*	
5	*anatum*	*hadar*	
2	*agona*	*schwarzengrund*	
2	*berta*	*hadar*	
2	*berta*	rough O:fgt:-	
2	*hadar*	*mbandaka*	
2	*hadar*	*schwarzengrund*	
2	*hadar*	*typhimurium*	
2	*heidelberg*	*infantis*	
2	*infantis*	*thompson*	
2	*mbandaka*	*schwarzengrund*	
2	*anatum*	*hadar*	rough O:z_{10}:x
2	rough O:d:7	*schwarzengrund*	*typhimurium*

profiles within isolates from one flock, or that did not harbour any plasmids, were serotyped. Thirty-nine different salmonella serovars, nine serovars that had rough LPS and different flagellar antigenic formulas, and two strains that were untypable serologically, were isolated from the samples.

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Table 4. *Salmonella serovars isolated from feed samples compared with those isolated from litter and/or water samples of the same flocks*

Flock no.	Serovar isolated from: Feed	Litter	Water
162	*agona*	—	—
211	*agona*	*infantis*, *ohio*	—
133	*anatum*	—	—
054	*anatum*	*anatum*, *hadar*	—
358	*anatum*	*anatum*, *heidelberg*, *kentucky*	—
121	*anatum*	*haardt*, *infantis*, *ohio*, *thompson*	—
167	*anatum*	*indiana*	—
120	*binza*	—	—
216	*binza*	—	*senftenberg*
159	*binza*	*anatum*, *hadar*	—
132	*binza*	*anatum*, *hadar*	—
126	*binza*	*berta*, *binza*	—
209	*binza*	*binza*, *orion*, *schwarzengrund*, *senftenberg*	—
192	*binza*	*ohio*	—
370	*hadar*	*hadar*, *heidelberg*, *typhimurium*	*typhimurium*
353	*infantis*	*berta*	*hadar*
359	*infantis*	*berta*, *infantis*, rough O:f,g,t:-, rough O:r:5	—
357	*kentucky*	*heidelberg*, *infantis*, *manhattan*	—
069	*ohio*	*anatum*, *hadar*, *ohio*	—
068	*ohio*	*hadar*	—
143	*mbandaka*	*anatum*, *hadar*	—
199	*montevideo*	*enteritidis*	*enteritidis*
145	*montevideo*	*hadar*	—
157	*newington*	—	—
142	*newington*	*anatum*, *hadar*	*hadar*
408	*newington*	*berta*, *newington*	*berta*
134	*newington*	*newington*	*newington*
152	*schwarzengrund*	—	—
189	*schwarzengrund*	*agona*, *berta*	—
183	*schwarzengrund*	*agona*, *schwarzengrund*	—
299	*schwarzengrund*	*berta*	*berta*
191	*schwarzengrund*	*hadar*	*hadar*
161	*schwarzengrund*	*infantis*, rough O:r:5, *thompson*	—
263	*taksony*	—	—
252	*taksony*	*heidelberg*	—
378	*thomasville*	*braenderup*, *schwarzengrund*, *thomasville*	—
376	*thomasville*	*heidelberg*, rough O:r:2, *thomasville*	*heidelberg*
391	*worthington*	*hadar*, *heidelberg*, *infantis*, *schwarzengrund*, *typhimurium*, *worthington*	*infantis*
394	*worthington*	*kentucky*	*kentucky*

DISCUSSION

Salmonella enteritidis was recovered from environmental samples of only 9 of 294 broiler (3·1%) flocks and amongst layer flocks there was a similar low degree of contamination – 8/295 (2·7%) [36]. *S. enteritidis* PT 8, which was found in

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environmental samples of 7 of 9 broiler flocks, is also the most common phage type of *S. enteritidis* strains isolated from people in Canada [6]. Phage type 13a, found in samples of two broiler flocks, is rarely isolated from humans in Canada [6].

How the prevalence of *S. enteritidis* in environmental samples of broiler flocks relates to *S. enteritidis* infection in people in Canada is not well understood. Many variables may influence such a relationship. Prevalence of salmonellas in litter is a good indication of flock infection [37] and it is known that infected flocks introduce salmonellas to the processing plants [38]. Surveys conducted in Canada during 1988 and 1989 showed that *S. enteritidis* was isolated from only 1·2% of broiler carcase rinses that cultured positive for salmonellas (Moir, unpublished).

Salmonellas were present in the environmental samples (litter and/or water) from 226 of 294 (76·9%) commercial broiler flocks which is substantially higher than that found in commercial layer flocks (52·9%) [36]. Factors that may contribute to these contrasting figures are age, housing, and residual contamination in barns. Broiler flocks were sampled when the flock was less than 8 weeks of age against layer flocks which were sampled between the ages of 20–72 weeks. Young chickens, particularly those of 1–14 days of age, are much more susceptible to infection with salmonella [39] than pullets and hens which are often able to eliminate infection by 16–22 weeks of age [37]. Broiler birds housed on the floor have ample opportunity to spread infection and re-contaminate their environment, while layer flocks are raised in cages where cross-contamination between birds is minimized. Further, residual contamination, after cleaning and disinfection, of metal cages is likely to be lower than on wooden, dirt, or concrete floors [40].

Within the flocks, 47·4% of litter samples were contaminated with salmonellas and up to eight different serovars were isolated from samples from any one flock. The large numbers of birds allowed free movement in a confined space probably contributed largely to this high figure. Salmonellas have been found to spread rapidly from infected day-old chicks to pen mates reared on litter [41] and when 2 infected day-old chicks were placed with 98 non-infected pen mates, infection of the contact chicks approached 100% by the seventh day of exposure [41].

No comparable nation-wide study on the prevalence of salmonellas in the environment of broiler flocks has been carried out previously in Canada. A study on litter samples from 60 broiler houses in Nova Scotia, Canada, showed lower rates of contamination than in the present study, i.e. 55% from broiler houses and 30% from litter samples [42]. The lower rates of contamination may be because the houses were owned and operated by single and possibly superior management [42].

Isolations from water from 21·6% of the flocks and the proportion of water samples contaminated with salmonellas (12·3%) were much lower than those from litter. As the broilers grow the drinkers are raised off the floor and the water is less liable to contamination from the litter. Contamination of drinking water also depends on the kind of drinker used in the barns. The drinker most commonly used in Ontario, Canada is the red bell-shaped drinker, and the second most commonly used drinker was the trough drinker [43]. Both these drinkers are more prone to contamination than other less frequently used waterers such as cup drinkers and nipple drinkers [44]. Clearly litter is a better indicator of environmental contamination of flocks than water.

S hadar was the most frequently isolated serovar and was found in environmental samples of 33·3% of the broiler flocks. In 1973, *S. hadar* became established in flocks of the largest turkey breeder in England [45]. The infection spread to rearing units throughout the country and, in ensuing years, consumption of turkey contaminated with *S. hadar* caused a sharp increase in *S. hadar* infection in humans [45, 46]. *S. hadar* also became prevalent in broiler chicken flocks in the United Kingdom [47]. Exportation of turkey breeder stock from England to Canada [48] and the United States caused infection of turkeys and chicken in these countries, and a rapid increase in reported isolation rates of *S. hadar* from humans, first in Canada, and shortly thereafter in the United States occurred [47]. During the last decade the isolation rate of *S. hadar* from humans has increased steadily and *S. hadar* is now the second most common serovar isolated from people in Canada [6]. In Canada there is a high prevalence of *S. hadar* in the environment of broiler flocks, in the broilers themselves [37, 41] and in broiler carcasses [38]. During the years 1988 and 1989, of 427 salmonellas found on broiler carcasses, 240 (56·2%) were *S. hadar* (Moir, unpublished). Although *S. hadar* has been the second most commonly reported serovar isolated from humans in Canada during 1987 and 1988 [6] and is the most commonly isolated serovar from broiler carcass rinses, conclusive epidemiological evidence linking human disease with the contaminated poultry is still lacking.

The same serovar was isolated from feed and litter samples in 13 of 39 flocks, and in only one flock from feed, litter, and water samples. In 10 of 11 flocks in which salmonellas were isolated from feed, litter, and water samples, the same salmonella serovar was isolated from litter and water samples. Since the feed samples were taken from the augers or feed bins and not from the feeding troughs, feed must be presumed to be the origin of at least some contamination.

Why the prevalence of *S. enteritidis* in broiler and layer flocks in Canada appears to be lower than that in the United States and Western Europe is not understood. Perhaps the low prevalence in Canadian broiler and layer flocks is likely a reflection of the low prevalence of *S. enteritidis* in the breeder flocks supplying the Canadian industry. A detailed survey of salmonella contamination of breeder flocks is currently in progress.

In summary, the main findings of this national survey were: (i) *S. enteritidis* was isolated from 3% of the flocks; (ii) environmental samples of 77% of the flocks contained salmonellas; and (iii) *S. hadar* was the most common salmonella serovar isolated from the broiler flocks.

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