A National Survey to Estimate the Prevalence of *Salmonella* species Among Canadian Registered Commercial Turkey Flocks

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ABSTRACT

In 1990–1991, a national survey was conducted to estimate the prevalence of *Salmonella* species among Canadian commercial turkey flocks. Two hundred and seventy flocks were randomly selected across Canada. The proportion sampled from each province was selected according to each province's share of the national turkey market. Samples, consisting of 12 pooled litter and four pooled dust samples, were used to determine the *Salmonella* status of the environment of each flock. Additionally, a one kilogram sample of feed was taken from each flock premise. *Salmonella* was recovered from environmental samples in 234/270 (86.7%) of flocks and from feed samples in 26/266 (9.8%) of flocks. Forty-eight different *Salmonella* serovars were isolated from flock environmental samples. The most prevalent serovars were *S. anatum*, *S. hadar*, *S. agona*, *S. heidelberg* and *S. saintpaul* which were isolated from 53/270 (19.6%), 49/270 (18.1%), 49/270 (18.1%), 42/270 (15.6%) and 34/270 (12.6%) flocks, respectively.

RÉSUMÉ

Une enquête à l'échelle nationale a été effectuée en 1990–1991 afin d'évaluer la prévalence des salmonelles dans les troupeaux canadiens de dindons de consommation. Deux cent soixante-dix troupeaux ont été choisis au hasard à travers le Canada. Le nombre de troupeaux échantillonnés dans chaque province était en relation avec la part du marché national. Afin de déterminer le statut de l'environnement de chaque troupeau quant à la présence possible de *Salmonella*, des échantillons composites de litières et de poussières ont été analysés. De plus, un échantillon de 1 kg de nourriture était prélevé à chaque troupeau visité. À partir des échantillons de l'environnement, *Salmonella* a été isolé dans 234 des 270 troupeaux (86,7 %) et à partir de 26 des 266 (9,8 %) échantillons de nourriture. Les isolats provenant de l'environnement représentaient 48 sérovars différents de *Salmonella*. Les sérovars les plus prévalents étaient S. anatum, *S. hadar*, *S. agona*, *S. heidelberg* et *S. saint-paul* qui ont été isolés, respectivement, de 53/270 (19,6 %), 49/270 (18,1 %), 49/270 (18,1 %) 42/270 (15,6 %) et 34/270 (12,6 %) troupeaux.

INTRODUCTION

Foodborne salmonellosis in humans continues to be a major public health problem in many countries. In Canada, *Salmonella* is the most frequently reported cause of foodborne disease (1). In the U.S.A., *Salmonella* accounted for 57% of the bacterial disease outbreaks for the period 1983–1987 and was the most frequently reported bacterial pathogen for each year (2). Estimates of the total patient-related cost of salmonellosis in 1988 in the U.S.A. range from $275 million to $1.1 billion (3).

Consumption of poultry products is recognized as one of the main implicated sources of human salmonellosis. In Canada, human foodborne *Salmonella* disease statistics for the 1975–1984 period show that poultry was associated with 10% of the incidents, and 20% of the cases (1). Statistics from the U.S. Centers for Disease Control in Atlanta, Georgia identified turkey products as being the food vehicle in 763 (7.1%) of cases and seven (4.1%) of outbreaks of salmonellosis over the period 1983-1987 (3). In an overview of human salmonellosis in England and Wales, over the period 1981–1986, the number of strains submitted for serotyping increased by 66%, with poultry being the main source of the increase in infections (4).

International concern over rising isolations of *Salmonella*, and in particular *Salmonella enteritidis* among humans, and the association with poultry and poultry products, prompted Agriculture and Agri-Food Canada to initiate a program to control *Salmonella* within the Canadian poultry industry (5,6). The initial phase of the program has been devoted to collecting current information on the level and extent of *Salmonella* contamination on poultry farms. Results of surveys to estimate the prevalence of *S. enteritidis* and other *Salmonella* spp. in the environment of Canadian commercial layer and broiler flocks have been published (7,8).

Salmonella enteritidis has become a significant human pathogen and has surpassed *S. typhimurium* in frequency of isolation in humans in many countries(9, 10). Outbreaks of human disease have generally been associated with the consumption of eggs (11, 12) and broiler chicken meat (13). There have also been reports in the U.K. of human *S. enteritidis* infection

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TABLE I. Measure of agreement between litter and dust samples for the recovery of *Salmonella*

		Dust			
		Positive[a]	Negative[b]	Missing[c]	Total
Litter	Positive	199	18		217 (80.4%)
	Negative	16	36	1	53
Total [kappa= 0.6]		215 (79.6%)	54	1	270

[a] Flocks designated as positive for *Salmonella*
[b] Flocks designated as negative for *Salmonella*
[c] Flocks in which information is missing

associated with the consumption of turkey meat (14).

The purpose of the present study was to estimate the prevalence of *Salmonella* and *S. enteritidis* among commercial turkey flock premises and to collect information on *Salmonella* contamination of feedstuffs on farms.

MATERIALS AND METHODS

SAMPLE SIZE

A sample size of 300 flocks was calculated to detect the prevalence of individual serovars to the 5% level, with an error of 2.5% and a confidence of 95% (15). A total of sixty litter samples was collected from each flock unit. This sample size was calculated to be 95% certain to detect at least one positive sample if *Salmonella* was present in 5% of the samples (16). The samples were pooled into composites of five sub-samples for a total of twelve pooled litter samples. In addition, four pooled dust samples were collected from within each flock. Pooling of samples has been identified as a method to reduce the number of samples to be tested and to assure a relatively small standard error for the prevalence estimate (17).

One kilogram of feed was collected from each flock premise.

FLOCK UNIT SELECTION

For the purpose of this survey, a flock unit is defined as any area where birds are housed together and not separated by a solid wall. Flock units were randomly selected from a sampling frame of 525 commercial turkey producers registered with the Canadian Turkey Marketing Agency. One flock was selected from each farm. Where producers had multiple flocks, flock units were selected randomly. The number of flock units selected from each province was proportional to the provincial market share (quota) of the total 1990 Canadian turkey production.

SAMPLE COLLECTION

Sampling kits were sent to Agriculture and Agri-Food Canada district offices across the country. A training package was included with the kits to provide instructions on sampling procedures. Samples of litter, dust and feed were collected from flock units by Agriculture and Agri-Food Canada inspectors during the period October 1990 to May 1991 inclusive. Inspectors collected five litter samples from each of twelve randomly selected areas and pooled them into sterile plastic bags using sterile tongue depressors. Dust samples were collected from the walls, inlets, fans and other surfaces that collect dust. The feed sample was collected from either the outside storage bin or inside hoppers, but not from feed troughs.

Samples were shipped to one of five federal laboratories in containers with ice packs.

SALMONELLA CULTURE

Environmental (litter/dust) samples were processed by Agriculture and Agri-Food Canada laboratories in Calgary, Alberta, Guelph, Ontario, Saint-Hyacinthe, Quebec and Sackville, New Brunswick. All feed samples were processed by the Laboratory Services Division of Agriculture and Agri-Food Canada in Ottawa, Ontario.

Environmental and feed samples were processed by two different culture methods, respectively. The environmental (litter/dust) samples were processed using a method incorporating modified semi-solid Rappaport Vassiliadis (MSRV) agar as a selective enrichment medium (18). Feed samples were processed according to a modification of the Health Protection Branch (HPB) method MFHPB-20 (19, 20). These methods have been reported previously (7,8).

SALMONELLA SEROTYPING

All dust, fecal and feed isolates of *Salmonella* were serotyped. The serological procedures utilized for this survey have been reported previously (21,22).

STATISTICAL ANALYSES

Flock unit and culture results were sent to the laboratory in Guelph, Ontario where they were entered into a computer database (dBase III Plus, Ashton Tate, Torrance, California). Flock units were identified as environmentally contaminated with *Salmonella* if one or more samples of litter, and/or dust, were culture-positive. The variability of the prevalence estimates was expressed as the standard error of the mean and was calculated by considering flock units as clusters of equal size (23). The kappa statistic was utilized to estimate the level of agreement between litter and dust samples for the detection of *Salmonella* (24).

RESULTS

Of the 300 flock units initially targeted for sampling, dust samples were received from 269 flocks, litter samples were received from 270 flock units and feed samples were received from 266 out of the 270 flock units that were litter sampled. Thirty of the original flock units targeted were not included in the study because the premises were out of production at the scheduled time of sampling. Flock units were identified as environmentally contaminated with *Salmonella* in 234/270 (86.7 $\pm$ 2.1%) of flocks. Of the *Salmonella* contaminated flock units, 217/270 (80.3%) were culture-positive in the litter and 215/269 (79.9%) flock units were culture-positive in the dust. The measurement of agreement between litter and dust for the recovery of *Salmonella* demonstrated a good agreement (kappa=0.6, Table I). A comparison of the serovars recovered from litter or dust revealed that the majority of serovars were common to both.

A total of 2663 isolates of *Salmonella* were recovered from litter and dust samples: 1932/3240 (59.6%) from litter and 731/1076 (67.9%) from dust. The prevalence of the top 12 ranked serovars from environmental samples is shown in Table II. *Salmonella anatum* was the most frequently occurring serovar

contaminating 53/270 (19.6%) of flocks. *Salmonella enteritidis* was not recovered from any samples cultured in this survey. Multiple serovars were frequently isolated from individual flock units; environmental (litter and dust) samples of three flock units were contaminated with as many as five different serovars (Table III). Fifty-one different serovars were identified in this survey; forty-eight were recovered from environmental and feed samples and an additional three serovars were recovered from feed samples only. Forty of the 48 serovars isolated from the environmental samples were recovered from both the litter and dust samples. Serovars of *Salmonella* that were unique to the individual sample types included: *kiambu*, *bareilly*, Group E1 and rough O:eh:2 in litter; *johannesburg*, *borreze*, *livingstone* and Subspp.IIIa in dust; and *oranienburg*, *give* var 15+ and *taksony* in feed.

Feed samples were found to be contaminated with *Salmonella* in 26/266 (9.8 ± 0.85%) of flock units. In 14/26 (53.8%) of the flock units in which *Salmonella* was recovered from feed, the serovars isolated were not recovered from the litter or dust samples taken from the same flock unit.

TABLE II. Prevalence of top 12 *Salmonella* serovars in flocks determined by isolation rates from environmental samples

Rank	Serovar	Affected flocks[a]	Percentage of flocks[b]
1	*anatum*	53	19.6
2	*hadar*	49	18.1
	agona	49	18.1
3	*heidelberg*	42	15.5
4	*saintpaul*	34	12.6
5	*bredeney*	20	7.4
6	*senftenberg*	18	6.6
7	*typhimurium*	15	5.5
8	*schwarzengrund*	14	5.2
	anatum var. 15+	14	5.2
9	*reading*	12	4.4
	muenster	12	4.4
10	*indiana*	9	3.3
	kentucky	9	3.3
11	*mbandaka*	7	2.6
12	*havana*	5	1.8
	typhimurium var. *copenhagen*	5	1.8
	albany	5	1.8
	broughton	5	1.8

[a] Number of flocks in which a *Salmonella* serovar was isolated from litter or dust samples
[b] Multiple serovars were often isolated from single flocks

TABLE III. Number of flocks by frequency of isolation of *Salmonella* serovars from environmental samples within flocks

Number of serovars	1	2	3	4	5	Unknown[a]	Total
Number of flocks	102	81	31	13	3	4	234

[a] Because of incomplete flock coding of isolates, the number of serovars isolated from samples of four flocks could not be determined

DISCUSSION

This survey demonstrates an estimated prevalence of environmental contamination with *Salmonella* among commercial turkey flocks in Canada of 86.7%. This contrasts with prevalence estimates for *Salmonella* of 52.9% and 76.9% in Canadian commercial layer and broiler flocks, respectively (7,8). Examining the results of the three poultry surveys, shows that commercial turkeys experience a relatively higher level of environmental contamination with *Salmonella* than commercial layer and broiler poultry flocks. The differences in reported prevalence estimates may be a reflection of the actual *Salmonella* status of flocks or due to a difference in sampling methodology. The layer survey utilized fecal dropping and egg-belt scrapings and the broiler survey utilized litter and water samples. Litter and dust samples were used to evaluate *Salmonella* contamination of the turkey flock environment. These sample types were chosen for two reasons. Firstly, litter samples have been identified as an easy and practical method to detect both flock and carcass contamination (8,25). Secondly, in the national survey of commercial layers, it was determined that dust/fluff samples recovered from egg-belts provided a better estimate of flock-level *Salmonella* contamination than samples of fecal droppings (7). However, on turkey flock premises we found good agreement between litter and dust samples with regard to flock-level contamination. Additionally, 40 of the 48 different serovars isolated from environmental samples were common to both litter and dust sample types. These findings indicate that both sample types provide equal representation of the *Salmonella* status of the flock premises.

The high prevalence of contamination with *Salmonella* among commercial flocks of turkeys was suspected. Our results were comparable to a previous survey conducted in Canada that estimated the prevalence of *Salmonella* in turkey carcass rinse samples (26). That study reported *Salmonella* contamination in 36/37(97.3%) lots and in 159/239 (69.1%) carcasses tested. The higher contamination levels of the lots in the carcass rinse study, as compared to the environmental samples in the present study, is likely a reflection of cross-contamination within the slaughter facilities.

Commercial turkey flocks are susceptible to *Salmonella* contamination from many sources. Breeder flocks are known to be contaminated (27) and can pass *Salmonella* to their progeny (28). Residual contamination in the barn will often result in contamination of the new flock (28,29). Although it is a common practice in Canada to place birds in facilities that have been thoroughly emptied, cleaned and disinfected, if these procedures are not conducted rigorously, contamination of the in-coming flock may occur (30). Commercially reared poults become infected with *Salmonella* at a young age, usually within the first two weeks of life (31). The *Salmonella* can then spread rapidly among pen mates once introduced into a flock (32). Researchers have reported a high incidence and long duration of *Salmonella* infection in young poults and their environment in forced air-ventilated enclosed facilities (33). Commercial turkeys in Canada are frequently raised in this type of housing because of harsh weather conditions.

Feed has also been recognized as a vehicle for *Salmonella* infection of

flocks (34). Our study revealed that 9.8% of flock feed samples tested were contaminated with *Salmonella*, and that of the 26 flocks where positive feed samples were identified, 12 contained the same serovar in feed and environmental samples. In these flocks the feed samples may have become contaminated on the farm, or the birds may have become infected from consuming the feed resulting in subsequent environmental contamination. Similar levels of *Salmonella* contamination of feedstuffs have been obtained in surveys of feed mills conducted by Agriculture and Agri-Food Canada. Testing of samples obtained during the period April 1988 to March 1990 demonstrated that 40/592 (6.8%) of complete feedstuffs were positive for *Salmonella* (Plant Products Division, Agriculture and Agri-Food Canada).

Salmonella enteritidis was not isolated from any of the samples collected during this survey. This serovar has been previously recovered from a single litter sample of a turkey flock in Canada (35). *Salmonella enteritidis* may be present in Canadian commercial turkey flocks, but in such low numbers that our sample size was not large enough to detect it. Our results suggest that turkeys are not a significant source of *S. enteritidis* for humans in Canada.

It is interesting to note the differences in serovar distribution among the flocks of commercial poultry in Canada. Whereas *S. heidelberg* and *S. hadar* were the most prevalent serovars in the environments of layer flocks (7) and broiler flocks (8) respectively, we identified *S. anatum*, *S. hadar*, *S. agona* and *S. heidelberg* as the most common isolates from environmental samples of turkey flocks. In Israel, a summary of turkey flock submissions to regional poultry laboratories identified *S. anatum* as the second most common isolate of *Salmonella* (36). *Salmonella anatum* was also listed in the top five isolates recovered from poultry carcass rinse water samples in a national surveillance study conducted in Canada (26). Consumption of contaminated feed was identified as the source of introduction of *Salmonella hadar* (37) and *Salmonella agona* (38) serovars into the poultry industry in the United Kingdom. Reports from the U.K. indicated that consumption of turkey meat was identified in 46% of all foodborne outbreaks of *S. hadar* during the period 1975–1979 (39). Importation of infected turkey poults from England into Canada has resulted in the contamination of our domestic poultry with *S. hadar* (40). *Salmonella hadar* is now commonly isolated from poultry and was identified as the most common isolate from the environment of Canadian commercial broiler flocks and the third most common isolate from the environment of layer flocks (7,8).

In summary, the findings of this survey demonstrate a high prevalence of *Salmonella* among commercial turkey flocks in Canada; it shows that turkeys are not a significant source of *S. enteritidis* for humans in Canada; and it suggests that future sampling of flocks may incorporate the use of dust or litter samples as a simple means of detecting environmental contamination.

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