Salmonella Contamination of Hatching and Table Eggs: A Comparison

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ABSTRACT

This study determined and compared *Salmonella* contamination rates of pools of surplus, early and culled hatching eggs from layer and broiler breeder flocks, and of pools of early and regular table eggs from layer flocks. Each pool contained 6 eggs. Five methods were used for the isolation of *Salmonella*. Nine of 126 pools of culled layer hatching eggs, 2 of 126 pools of surplus layer hatching eggs, and one of 126 pools of early layer hatching eggs were contaminated with *Salmonella*. All 126 pools of broiler breeder surplus, and early and culled hatching eggs tested negative for *Salmonella*. All 168 pools of regular table eggs tested negative for *Salmonella*, whilst one of 84 pools of early table eggs contained *Salmonella agona*. The pools of culled layer hatching eggs and surplus layer hatching eggs that contained *S. typhimurium* were derived from the same breeder operation. Similarly, the pools of culled and early layer hatching eggs that contained *S. heidelberg* were derived from one breeder operation. Pools of culled hatching eggs were more frequently contaminated with *Salmonella* than other hatching or table eggs. Pools containing eggs that were both cracked and dirty were more frequently contaminated with *Salmonella* than all other pools of eggs. The overall *Salmonella* contamination rate of the table eggs was 0.07 to 0.4%. Critical control points (macroscopic classification of the eggs as cracked and dirty) were validated microbiologically.

RÉSUMÉ

Les taux de contamination par *Salmonella* spp. ont été déterminés et comparés pour des œufs à éclore en surplus, ceux du début de la période de ponte et ceux rejetés provenant de troupeaux de reproducteurs de poules pondeuses et de poulet de chair, ainsi que dans des œufs du début de la période de ponte et ceux mis en marché provenant de troupeaux de poules pondeuses. Un échantillon était constitué de six œufs et chaque échantillon était analysé à l'aide de cinq méthodes permettant l'isolement de *Salmonella* spp. Une contamination par *Salmonella* spp. fut retrouvée dans neuf des 126 échantillons d'œufs à éclore rejetés, deux des 126 échantillons d'œufs à éclore en surplus, et 1 des 126 échantillons d'œufs à éclore du début de la période de ponte provenant des reproducteurs de pondeuses. Les 126 échantillons d'œufs à éclore rejetés, d'œufs à éclore en surplus et d'œufs à éclore du début de la période de ponte des troupeaux de reproducteurs de poulet à griller ont tous été trouvés négatifs. Les 168 échantillons d'œufs mis en marché étaient négatifs pour *Salmonella* spp., alors qu'un des 84 échantillons d'œufs en début de période de ponde provenant d'une pondeuse était positif pour *S. agona*. Les échantillons d'œufs à éclore en surplus et ceux rejetés provenant de troupeaux de reproducteurs de poules pondeuses qui étaient positifs pour *S. typhimurium* provenaient tous du même éleveur. Les échantillons d'œufs à éclore du début de la période de ponte et ceux rejetés provenant de troupeaux de reproducteurs de poules pondeuses et qui étaient contaminés par *S. heidelberg* provenaient tous d'un seul producteur. Les échantillons d'œufs à éclore rejetés étaient plus souvent contaminés par *Salmonella* spp. que les autres types d'œufs à éclore ou de consommation. Les échantillons qui contenaient des œufs craqués et sales étaient plus souvent contaminés par *Salmonella* spp. que tous les autres types d'échantillons d'œufs. Dans son ensemble, le taux de contamination des œufs de consommation par *Salmonella* spp. variait de 0,07 à 0,4 %. Des points critiques de contrôle (classification macroscopique des œufs comme craqués et sales) ont été validés de manière microbiologique.

(Traduit par docteur Serge Messier)

INTRODUCTION

In many countries there has been such a dramatic increase in the number of *S. enteritidis* infections in humans and animals that *S. enteritidis* has overtaken *S. typhimurium* to become the most commonly isolated serovar (1,2). Outbreaks of *S. enteritidis* infections in humans have been associated with the consumption of eggs, foods that contain eggs (3,4), poultry meats and other poultry products contaminated with *S. enteritidis* (5,6). The reasons for the increased number of human infections and outbreaks may include infection of breeder flocks and subsequently of layer flocks (5,7,8), the ability of *S. enteritidis* to cause infection of the ovaries and oviduct and subsequently of the eggs (9,10), increased

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TABLE I. Sampling plan for the surplus, early and culled hatching eggs, and for the regular and early table eggs

Type of flock	No. of hatcheries (H) or farms (F)	Type of eggs	No. of pools of 6 eggs each	No. of flocks	No. of pools from each flock	Total number of eggs
Layer hatching	7 H	Surplus	18	N.D.[a]	N.D.[a]	7 × 18 × 6 = 756
	7 H	Early	18	"	"	7 × 18 × 6 = 756
	7 H	Culled	18	"	"	7 × 18 × 6 = 756
					Layer Hatching Subtotal	2268
Broiler hatching	7 H	Surplus	6	N.D.[a]	N.D.[a]	7 × 6 × 6 = 252
	7 H	Early	6	"	"	7 × 6 × 6 = 252
	7 H	Culled	6	"	"	7 × 6 × 6 = 252
					Broiler Hatching Subtotal	756
Layer table	1–40 F	Regular	168	40	3–9	168 × 6 = 1008
	37–61 F	Early	84	25	3–6	84 × 6 = 504
					Layer Table Subtotal	1512
					Grand Total	4536

[a] N.D. = Not Determined

consumption of poultry and poultry products (11), and temperature abuse of eggs allowing *Salmonella* bacteria to increase in numbers (12,13). The vertical transmission of the infection from the ovaries to the eggs is called transovarian infection or transovarian transmission (9). Not only the host-adapted serovars *S. pullorum* and *S. gallinarum*, but also non-host-adapted *Salmonella*, such as *S. enteritidis, S. typhimurium* and *S. heidelberg*, have been shown to infect eggs by transovarian transmission (9,10, 14). However, this is not the only route by which eggs may become infected. The surface of the eggs may be contaminated with *Salmonella* present in feces and in other matter such as yolk, fluff, dust and other debris present in poultry houses. *Salmonella* belonging to a variety of serovars may contaminate the eggs by penetration of the egg shell (15).

Hatching eggs from poultry breeder flocks may not be incubated because they are surplus, early or culled. Surplus hatching eggs are the normal eggs that are surplus to the need for incubating hatching eggs. The early hatching eggs are eggs that are not set for hatching because they are laid in the early production phase of the breeder flock and are considered too small for hatching. The culled hatching eggs are those hatching eggs that are culled because they are cracked, dirty, have irregular shells, have poor shells, have double yolks, or for other reasons. The culling is done at the egg grading and washing stations. The surplus and early hatching eggs are usually washed, graded and diverted to the table egg trade. The culled eggs are usually washed and graded at the end of the day, after removal of eggs with large cracks, eggs that leak or eggs with large patches of dirt and fecal matter. After washing and grading, they are dyed and used for processed egg products (16). There is a concern that hatching eggs that are not incubated and diverted to the table egg trade may be more commonly contaminated with *Salmonella* than the regular and the early or small table eggs. Some of the reasons for the diversion of hatching eggs to the table and further processing egg trade are measurable without microbiological examination and, if validated microbiologically, may constitute critical control points (17) in the handling, washing and marketing of eggs.

The purposes of the present study were 1) to determine the *Salmonella* contamination rates of surplus, early, and culled eggs from layer breeder and broiler breeder flocks and the rates occurring among early and regular table eggs from layer flocks, 2) to compare the contamination rates of the different categories of eggs, 3) to validate microbiologically whether classifying the eggs as being cracked and/or dirty constitutes critical control points, and 4) to compare 5 different methods of isolation of *Salmonella* from eggs.

MATERIALS AND METHODS

STUDY DESIGN

Unwashed and ungraded eggs were either obtained from egg washing and grading stations or directly from the hatchery. The hatching eggs collected were derived from 7 layer and 7 broiler hatcheries which constitute the majority of the large registered layer and broiler hatcheries in Ontario, Canada. Comparisons of *Salmonella* contamination rates were made between hatching and table eggs, between different categories of hatching and table eggs, between cracked and non-cracked, between dirty and non-dirty and between other categories of eggs. The table eggs were obtained at the same time as the hatching eggs from the same egg washing and grading stations. The eggs were collected in pools consisting of 6 eggs per pool according to the sampling plan shown in Table I. The eggs were collected by a systematic random sampling procedure applied within each category of eggs. Clean gloves were used between the pools from the same producer and the eggs were collected once or twice weekly and shipped on new fibre trays. The study was conducted during the months June until September of the year 1996. This period may be representative for the whole year since the eggs were cooled at the farm and hatcheries, transported in refrigerated trucks, and refrigerated at the egg washing and grading stations.

MACROSCOPIC EXAMINATION OF EGGS

At the laboratory, before being cultured, all pools of eggs were examined visually for any adherence of fecal matter, dirt or any other signs of contamination of the outer surface of the shell. The eggs were candled with an egg candler (Richard Brancker Research Ltd., Ottawa, Ontario) to determine if there were any cracks in the shell. Since the aim of the study was to compare the *Salmonella* contamination rates of different categories of eggs and to examine critical control points such as "cracked" or "dirty" that influence the categorizing of eggs before washing, the eggs were collected before being washed and they were not surface-sterilized as is the practice when studying transovarian transmission.

CULTURE AND ISOLATION OF *Salmonella* FROM EGGS

After arrival at the laboratory and before being cultured, the eggs were kept at room temperature for 4–7 d in order to promote multiplication and facilitate detection of *Salmonella*, if present (18). Clean gloves were used after handling 6 pools or between pools from different producers. Six whole eggs were put in double bagged plastic bags of 15 × 20″ (S3500 Sterilized Paddle Bags, QA Life Sciences Inc., San Diego, California, USA), and 1.2 L of double strength buffered peptone water (BPW) (BBL, Becton Dickinson and Co., Cockeysville, Maryland, USA) was added to each bag. The eggs were broken by pressure from the outside of the bags, while being careful not to puncture the plastic bags, and the contents were mixed by shaking. The bags were then incubated for 20–24 h at 37°C. The samples were selectively enriched for *Salmonella* in 3 ways. One mL of the pre-enriched sample in BPW was transferred to 9 mL of tetrathionate brilliant green (TBG) broth (BBL, Becton Dickinson and Co.), to which 0.2 mL of potassium iodide solution had been added, just prior to use. One mL of the pre-enriched BPW was added to 9 mL of selenite cystine (SC) broth (BBL, Becton Dickinson and Co.). Also, 0.1 mL of the pre-enriched sample was dropped onto the periphery of a modified semisolid Rappaport Vassiliadis (MSRV) (Difco, Detroit, Michigan, USA) agar plate. The TBG and the MSRV plates were incubated at 42°C, and the SC plate at 37°C, each for 20–24 h.

A loopful from each of the TBG and the SC was streaked onto brilliant green sulfa (BGS) agar (BBL brilliant green agar with sulfadiazine, Becton Dickinson and Co.) and onto bismuth sulfite (BS) agar (Difco, Detroit, Michigan, USA). The agar plates were incubated at 37°C for 20–24 h. The MSRV plates were examined for selective migration of *Salmonella* for a distance of ≥ 20 mm into the semisolid agar, and a loopful from the migrated bacteria was streaked onto Luria-Bertani (LB) agar, (Miller; Difco, Detroit, Michigan, USA). The MSRV plates that were negative for migrating *Salmonella* at 42°C were incubated for another 20–24 h. Putative *Salmonella* colonies from the BGS and BS agar plates were picked and streaked out for isolated colonies on McConkey agar plates. The plates were incubated at 37°C for 20–24 h. Isolated colonies were streaked onto LB agar. All putative *Salmonella* colonies were further examined by slide agglutination tests for agglutination with polyvalent anti-*Salmonella* antisera. They were also examined for typical biochemical reactions by streaking a urea slant and by stabbing a triple sugar iron agar slant, and, if they could possibly be *Salmonella*, they were biotyped and serotyped. They were phagetyped if they belonged to serovars for which typing phages and a typing scheme were available. Thus, *Salmonella* were isolated by the use of 5 methods: 1) BPW-->TBG-->BGS, 2) BPW--> TBG-->BS, 3) BPW-->SC-->BGS, 4) BPW-->SC-->BS, and 5) BPW-->MSRV.

BIOTYPING

Biochemical reactions were performed on each isolate using Gram-negative identification (GNI) cards and the automated microbial identification system of bioMérieux-Vitek, Hazelwood, Missouri, USA (19).

SEROTYPING

The O, or somatic antigens of *Salmonella* isolates were determined with slide agglutination tests as described by Ewing (20), whereas the H, or flagellar antigens were identified by using a microtechnique (21) that employs microtitre plates. The antigenic formulas of *Salmonella* serovars as listed by Le Minor and Popoff (22) were used to name the serovars.

PHAGETYPING

The standard phagetyping technique described by Anderson and Williams (23) was employed throughout this investigation. Strains that did not conform to any recognized phage type were considered atypical (AT). The designation of the phage types of *S. typhimurium* was that of Anderson et al (24). The phages and type strains of *S. typhimurium* were obtained from the International Centre for Enteric Phage Typing (ICEPT), Central Public Health Laboratories, Colindale, UK. *Salmonella heidelberg* strains were phagetyped with the phages isolated and the phagetyping scheme developed at the Laboratory Centre for Disease Control (LCDC) in Ottawa, Ontario (25).

STATISTICAL ANALYSIS

Fisher's exact test applied to two independent proportions was used to determine whether there were significant differences between the *Salmonella* contamination rates among the different categories of eggs, and between the *Salmonella* recovery rates with the different isolation methods (26).

RESULTS

Salmonella CONTAMINATION RATES OF DIFFERENT CATEGORIES OF EGGS, AND THE SEROVARS AND PHAGETYPES ISOLATED

Two of 126 pools of surplus layer hatching eggs contained *S. typhimurium*, one of 126 pools of the early layer hatching eggs contained *S. heidelberg*, 2 pools of culled layer hatching eggs contained *S. typhimurium* and 7 pools of the 126 pools of the culled layer hatching eggs contained *S. heidelberg* with or without a *Salmonella* with the antigenic formula I:4,12:–:1,2 (subspecies I, O antigens 4, and 12, and flagellar antigens of the 2nd phase of 1,2 like *S. heidelberg* but lacking the 1st phase of the flagellar antigen) (Table II). None of the 42 pools of each of surplus, early and culled broiler hatching eggs contained *Salmonella*. One of 84 pools of early layer table eggs contained *S. agona*, but none of 168 pools of regular layer table eggs contained *Salmonella*.

The *Salmonella* contamination rates of the pools of the layer breeder hatching eggs was 3.2%, of the pools of broiler breeder eggs it was 0.0% and of the pools of layer table eggs it was 0.4% (Table II). The overall rate of *Salmonella* contamination of the pools of eggs was 1.7%. Since the pools each consisted of 6 eggs, the above percentages could have varied between 0.5% and 3.2% of the layer breeder hatching eggs and between 0.07% and 0.4% of the layer table eggs. Since it is unlikely that more than 1 egg per pool would have tested positive for *Salmonella*, these percentages would likely have been at the lower end of the scale: thus a *Salmonella* contamination rate of 0.5% of the layer breeder hatching eggs and 0.07% of the layer table eggs.

The 1 pool of layer breeder surplus eggs and the 1 pool of culled layer breeder eggs containing *S. typhimurium* PT66 (Table II) were derived

TABLE II. Number of pools of hatching and table eggs positive or negative for *Salmonella*, and serovar, phagetype and biotype of the *Salmonella* isolates

Hatchery type	Category of eggs	Hatchery number	No. of pools examined	No. of pools *Salmonella* positive (%)	*Salmonella* serovar and (number)	Phage type (PT) and (number)	Biotype and (number)
Layer	Surplus	1–5, 7	108	0	N.A.[a]	N.A.	N.A.
		6	18	2	Typhimurium (8)	PT 66 (6) PT 3 (2)	A[b] (8)
	Early	1–7	108	0	N.A.	N.A.	N.A.
		7	18	1	Heidelberg (5)	PT 8 (5)	A (5)
	Culled	1–5	90	0	N.A.	N.A.	N.A.
		6	18	2	Typhimurium (8)	PT 66 (7) PT 193 (1)	A (8)
		7	18	7	Heidelberg (35)	PT 8 (31) AT[c] (4)	A (31) A (4)
					I:4,12:-:1,2 (4)	N.A. (4)	A (4)
	Hatching layer subtotal		378	12 (3.2%)			
Broiler hatching	Surplus	8–14	42	0	N.A.	N.A.	N.A.
	Early	8–14	42	0	N.A.	N.A.	N.A.
	Culled	8–14	42	0	N.A.	N.A.	N.A.
	Hatching broiler subtotal		126	0 (0.0%)			

Flock type	Table eggs	Farm number	No. of pools examined	No. of pools *Salmonella* positive	*Salmonella* serovar and (number)	Phage type (PT) and (number)	Biotype and (number)
Layer	Regular	1–40	168	0	N.A.	N.A.	N.A.
	Early	37–60	81	0	N.A.	N.A.	N.A.
		61	3	1	Agona (5)	N.A. (5)	A (5)
	Layer Table Subtotal		252	1 (0.4%)			
Grand Total (hatching + table eggs)			756	13 (1.7%)			

[a] N.A.: Not Applicable
[b] Biotype: The biochemical tests to determine biotypes have been described (Poppe et al 1993); biotype A is the common biotype
[c] AT: Atypical

TABLE III. Comparison of *Salmonella* contamination rates of pools of eggs

	Salmonella +	*Salmonella* −	Total
All hatching	12	492	504
All table	1	251	252
Total	13	743	756
P value = 0.07 (two-tailed), the differences are not significant (Fisher's exact test for 2 × 2 tables).			
Layer hatching	12	366	378
Broiler hatching	0	126	126
Total	12	492	504
P value = 0.08 (two-tailed), the differences are not significant (Fisher's exact test for 2 × 2 tables).			
Early table	1	83	84
Regular table	0	168	168
Total	1	251	252
P value = 0.33 (two-tailed), the differences are not significant (Fisher's exact test for 2 × 2 tables).			
All non-culled hatching	3	333	336
All table	1	251	252
Total	4	584	588
P value = 0.64 (two-tailed), the differences are not significant (Fisher's exact test for 2 × 2 tables).			
Culled hatching	9	159	168
Other hatching and table	4	584	588
Total	13	743	756

P value = 0.0003 (two-tailed), the differences are significant (Fisher's exact test for 2 × 2 tables).
Odds Ratio (OR) = 8.26

from the same commercial operation. In addition to *S. typhimurium* PT66, 1 pool of layer breeder surplus eggs contained *S. typhimurium* PT3 and 1 pool of culled layer breeder eggs contained *S. typhimurium* PT193. The 7 pools of culled layer breeder eggs and the 1 pool of layer breeder early eggs that were contaminated with *S. heidelberg* (Table II), were all derived from the same breeder operation. The *S. heidelberg* isolates from the pool of early layer breeder eggs were all PT8 strains. *Salmonella heidelberg* PT8 was isolated from 3 of the pools of culled layer breeder eggs, *S. heidelberg* PT8 and an atypical PT of *S. heidelberg* were isolated from 2 pools, and PT8, an atypical PT and a serovar with the antigenic formula I:4,12:-:- were isolated from another 2 pools of the culled layer breeder eggs. *Salmonella enteritidis* was not isolated from any of the pools of eggs.

Comparison of *Salmonella* contamination rates of all pools of hatching eggs with those of all table eggs showed that the hatching eggs were not significantly more often contaminated than the table eggs (*P* value = 0.07, two-tailed) (Table III). Similarly, within the category of hatching eggs, the pools of layer hatching eggs were not significantly more frequently contaminated than those of broiler hatching eggs (*P* = 0.08). The differences in contamination rates of pools of early and regular table eggs were not significant (*P* = 0.33). The contamination rates in pools of all non-culled hatching eggs (which are the hatching eggs that are, like the table eggs, washed, graded and marketed) were not significantly higher than those of all (regular and early) table eggs (*P* = 0.64). The *Salmonella* contamination rates of pools of culled hatching eggs (which are the eggs destined for further processing) were significantly higher than those of other hatching and table eggs (P = 0.0003); they were 8 times more often contaminated with *Salmonella*.

The pools of surplus hatching eggs were not significantly more often contaminated with *Salmonella* than other hatching eggs (P = 0.35), and the pools of early hatching eggs were not significantly more often contaminated than those of other hatching eggs (P = 0.12) (Table IV). However, pools of culled hatching eggs were significantly more often contaminated than pools of other hatching eggs (P = 0.003; OR = 6.28).

COMPARISON OF *Salmonella* CONTAMINATION RATES OF POOLS CONTAINING CRACKED AND DIRTY *VERSUS* THOSE CONTAINING WHOLE AND CLEAN EGGS

Pools containing 1 or more cracked eggs were not significantly more often contaminated with *Salmonella* than pools of whole eggs (P = 0.12) (Table V). Similarly, pools containing 1 or more dirty eggs were not significantly more often contaminated with *Salmonella* than pools of clean eggs (P = 0.40). Pools containing 1 or more eggs that were both cracked and dirty were not significantly more often contaminated than pools of eggs that were both whole and clean (P = 0.08). However, pools containing 1 or more eggs that were both cracked and dirty were significantly more often contaminated than all other pools of eggs (P = 0.03); they were 3 times more likely to be contaminated with *Salmonella*.

COMPARISON OF METHODS TO ISOLATE *Salmonella* FROM POOLS OF EGGS

Pre-enrichment in BPW followed by selective enrichment in SC and plating onto BGS and BS (methods 3 and 4) resulted in the isolation of *Salmonella* from 11 of the 13 *Salmonella* contaminated pools of eggs, whereas all 13 pools were positive with the methods 1 and 2, and with method 5 (Table VI). The differences were not significant (P = 0.84). The methods 1 and 2, which consisted of pre-enrichment in BPW followed by selective enrichment in TBG and plating onto BGS (method 1) or by

TABLE IV. Comparison of *Salmonella* contamination rates of pools of hatching eggs

	Salmonella +	*Salmonella* −	Total
Surplus hatching	2	166	168
Other hatching	10	326	336
Total	12	492	504
P value = 0.35 (two-tailed), the differences are not significant (Fisher's exact test for 2 × 2 tables).			
Early hatching	1	167	168
Other hatching	11	325	336
Total	12	492	504
P value = 0.12 (two-tailed), the differences are not significant (Fisher's exact test for 2 × 2 tables).			
Culled hatching	9	159	168
Other hatching	3	333	336
Total	12	492	504

P value = 0.003 (two-tailed), the differences are significant (Fisher's exact test for 2 × 2 tables).
Odds Ratio (OR) = 6.28

TABLE V. Comparison of *Salmonella* contamination rates of pools of cracked and whole, dirty and clean, cracked and dirty versus all other eggs, and of cracked and dirty versus whole and clean

	Salmonella +	*Salmonella* −	Total
Cracked	7	227	234
Whole	6	516	522
Total	13	743	756
P value = 0.12 (two-tailed), the differences are not significant (Fisher's exact test for 2 × 2 tables).			
Dirty	9	406	415
Clean	4	337	341
Total	13	743	756
P value = 0.40 (two-tailed), the differences are not significant (Fisher's exact test for 2 × 2 tables).			
Cracked and dirty	6	149	155
Whole and clean	3	259	262
Total	9	408	417
P value = 0.08 (two-tailed), the differences are not significant (Fisher's exact test for 2 × 2 tables).			
Cracked and dirty	6	149	155
All other eggs	7	594	601
Total	13	743	756

P value = 0.03 (two-tailed), the differences are significant (Fisher's exact test for 2 × 2 tables).
Odds Ratio (OR) = 3.42

TABLE VI. Isolation rates of *Salmonella* with 5 isolation and identification methods

Salmonella isolated from egg pool no.	*Salmonella* serovar and phagetype isolated with method				
	1[a]	2	3	4	5
40	SH, PT 8[b]	SH, PT 8	SH, PT 8	SH, PT 8	SH, PT 8 + SH, AT[c]
41	SH, PT 8	SH, PT 8	SH, PT 8	SH, PT 8	SH, PT 8
42	SH, PT 8	SH, PT 8	SH, PT 8	SH, PT 8	SH, PT 8 × 2[d]
43	SH, PT 8	SH, PT 8	SH, PT 8	SH, PT 8	SH, PT 8 + SH, AT
44	SH, PT 8	SH, PT 8	SH, PT 8	SH, PT 8	SH, PT 8
47	SH, AT	SH, AT	O:4,12:-:1,2[e]	0:4,12:-:1,2	SH, PT 8
49	0:4,12:-:1,2	SH, PT 8	SH, PT 8	SH, PT 8	SH, PT 8 + 0:4,12:-:1,2
375	SH, PT 8	SH, PT 8	SH, PT 8	SH, PT 8	SH, PT 8
410	Agona	Agona	Agona	Agona	Agona
686	ST, PT 66[f]	ST, PT 66	—[g]	—	ST, PT 66
687	ST, PT 66	ST, PT 66	ST, PT 3[h]	ST, PT 3	ST, PT 66
704	ST, PT 66	ST, PT 66	—	—	ST, PT 66
711	ST, PT 66	ST, PT 66	ST, PT 66	ST, PT 66	ST, PT 193

[a] The 5 methods are those described under Material and Methods
[b] SH, PT8 = *S. heidelberg*, phagetype 8
[c] SH, PT8 + SH, AT = Two colony types were isolated: one was *S. heidelberg* PT8; the other *S. heidelberg* of an atypical PT
[d] SH, PT8 × 2 = Two colony types were isolated: both were *S. heidelberg* PT 8
[e] O:4,12:-:1,2 = The serovar of this isolate could not be determined as the 1st phase of the flagellar antigen was lacking
[f] ST, PT66 = *S. typhimurium*, phagetype 66
[g] No *Salmonella* was isolated with this method
[h] Isolated after 2 subcultures on BGS

plating on BS (method 2), resulted in the isolation of *Salmonella* from 13 of the 13 *Salmonella* positive pools. The only difference between method 1 and 2 was the isolation of an untypeable serovar (O:4,12:-:1,2) by method 1 from pool no. 49, and *S. heidelberg* PT8 by method 2 from the same pool. The methods 1 and 2 differed from methods 3 and 4 in that *S. heidelberg* of an atypical PT was isolated from pool 47 and *S. typhimurium* PT66 from pool 687, respectively, by methods 1 and 2, but the serovar O:4,12:-:1,2 and *S. typhimurium* PT3, respectively, by methods 3 and 4. Method 5 (pre-enrichment in BPW and selective enrichment in MSRV) resulted in the same number of pools being positive for *Salmonella* as method 1 and 2; however, method 5 resulted in the additional recovery of *S. heidelberg* of an atypical PT from pools 40 and 43, the additional isolation of the atypical serovar O:4,12:-:1,2 from pool 49, and the isolation of a different PT of *S. typhimurium* (PT193, in place of PT66) from pool 711.

DISCUSSION

Seven of the 9 pools of the culled hatching eggs that were contaminated with *S. heidelberg* came from the same layer breeder operation (hatchery no. 7, Table II). The one pool of early layer hatching eggs that was also contaminated with *S. heidelberg*, was derived from the same layer breeder operation (hatchery no. 7). The eggs from this pool would likely have been washed, graded and marketed as table eggs. The 2 pools of culled hatching eggs and the 2 pools of surplus hatching eggs that were contaminated with *S. typhimurium* came from the same breeder operation (hatchery 6, Table II). These pools of the surplus layer hatching eggs would likely have entered the egg washing and grading facilities and entered the food chain as table eggs.

It should be noted that eggs from different pools and categories of eggs contaminated with *S. heidelberg* were traceable to and being produced in one layer breeder operation, whereas, in a similar manner, contamination of pools of eggs with *S. typhimurium* was traceable to and occurred at another layer breeder farm. Both these serovars (*S. heidelberg* and *S. typhimurium*) are known to be able to infect the ovaries of laying hens and to cause transovarian transmission of infection (9,14). Since there was a significant association between *Salmonella* contamination and the eggs being cracked and dirty, contamination of the culled eggs with *S. heidelberg* may have been caused primarily by egg shell contamination with fecal matter and penetration of the cuticle and shell (13,15). However, transovarian transmission may also have been a significant cause of contamination of the eggs with *S. heidelberg*, and especially with *S. typhimurium*, since half of the pools contaminated with *S. typhimurium* were surplus hatching eggs. Shell eggs contaminated with *S. typhimurium* or *S. heidelberg* have been associated with large and smaller outbreaks of food-borne salmonellosis (27,28). Food-borne disease caused by the consumption of eggs contaminated with *S. typhimurium* particularly, is a significant public health concern, especially among infants, the elderly, and those who are immunologically compromised (29,30). The pools of culled hatching eggs had a significantly higher contamination rate with *Salmonella* than the pools of other hatching and table eggs. This suggests that the practise of culling hatching eggs at the egg washing and grading station because of characteristics such as readily visible cracks or gross contamination with dirt or fecal matter is an effective way of eliminating most of the eggs that are contaminated with *Salmonella* from the table egg trade. Washing, grading and dying of such eggs at the end of the day for further processing would perhaps cause contamination with *Salmonella* of surfaces, equipment and the environment of the egg washing and grading station and may possibly cause cross-contamination of product not destined for further processing and pasteurization. Perhaps culled hatching eggs should for this reason not be allowed to enter the washing, grading and shipping area of the egg grading stations, and not be marketed for further processing. The same observation can be made for eggs that are both cracked and dirty.

Todd (16) conducted a risk analysis on cracked eggs and found that cracked eggs are 3 to 93 times more likely than uncracked shell eggs to cause outbreaks of salmonellosis. In this study, we were unable to show that pools containing one or more cracked eggs were significantly more often contaminated with *Salmonella* than whole eggs, and similarly, that pools containing dirty eggs were significantly more often contaminated than clean eggs, although pools of culled eggs were significantly more often contaminated than all other pools of eggs. The likely reason is the classification of eggs as cracked or dirty after a detailed examination in the laboratory during which process even eggs with a small speck of dirt or which upon candling showed a small crack were categorized as dirty or cracked. This resulted in larger numbers of pools being categorized as cracked or dirty than would have been the case at the egg washing and grading station where only eggs with obvious cracks or that were obviously dirty would have been classified as such and culled. Another reason for being unable to show that pools containing one or more cracked or dirty eggs were significantly more often contaminated with *Salmonella* than whole or clean eggs, respectively, is that some of the eggs may have been infected by the transovarian route. In that case, being more often contaminated with *Salmonella* would not have been associated with being cracked or dirty.

Classification of eggs in macroscopically identifiable groups and microbiological examination of the eggs resulted in the finding that being classified as both cracked and dirty was statistically significantly related to being contaminated with *Salmonella*. Culling of the eggs is triggered by the eggs having obvious cracks and or dirty spots or having other macroscopically definable undesirable characteristics. These characteristics, which are macroscopically identifiable and measurable, could be classified as critical control points in the handling, washing and marketing of eggs (17). This study validated microbiologically the use, namely culling, of these critical control points (31,32).

One pool of the early type of layer table eggs and none of the regular table eggs were contaminated with *Salmonella agona*. The overall contamination of pools of the table eggs was 0.4%, and if only one of the 6 eggs from the pool would have tested positive for *Salmonella*, the percentage would have been 0.066% or about 7 eggs per 10 000. *Salmonella agona* has, to our knowledge, not been reported to cause a transovarian transmission and the egg(s) would likely have been contaminated because the shell had been contaminated by feces, dirt or other *Salmonella*-containing matter.

The lower but not significantly different isolation rate of methods 3 and 4 *versus* methods 1 and 2 is likely related to the use of SC broth as selective enrichment medium in method 3 and 4, in comparison with TBG in methods 1 and 2. Selective enrichment for *Salmonella* with TBG has previously been shown to result in significantly higher number of *Salmonella* isolations compared to selective enrichment by use of SC (33). Method 5 resulted in the same number of pools being positive as method number 1 and 2. Use of method 5 resulted in the isolation of 3 additional strains from the 13 pools. This method, which uses BPW for pre-enrichment and the MSRV medium as selective enrichment medium, is less costly and labour intensive than the other methods employed as it does not require the use of 2 enrichment procedures and 2 plating media. These results agree with a previous study which showed that use of MSRV as the selective enrichment procedure resulted in higher isolation rates than when using selenite enrichment broth (34).

In summary, the main findings of this study are: 1) culled layer hatching eggs were more frequently contaminated with *Salmonella* than other hatching eggs and table eggs; 2) the classification of eggs as cracked and dirty was significantly associated with *Salmonella* contamination, 3) the *Salmonella* serovars isolated from the layer hatching eggs are known to cause transovarian transmission of *Salmonella* to eggs; 4) the broiler hatching eggs tested negative for *Salmonella*, 5) the overall contamination rate of the table eggs was 0.07 to 0.4%, and 6) no *S. enteritidis* bacteria were isolated from any of the pools of eggs.

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