

## Microbial health risk posed by table eggs in Trinidad

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### SUMMARY

A survey of the microbial quality of table eggs sold in Trinidad was conducted. For 23 poultry layer farms each visited twice approximately 1 month apart, 25 pooled eggs constituted a composite sample, for 14 shopping malls each visited twice approximately 1 month apart, six pooled eggs made a composite sample and for a total of 102 other retailers across the country each visited once over a 4-month period, six pooled eggs constituted a composite sample. Swabs of egg shells and egg content were tested for selected bacteria. Twenty-four (13·0%), 68 (37·0%), and two (1·1%) of a total of 184 composite eggs (shells, egg content or both) sampled were positive for *Salmonella*, *Escherichia coli*, and *Campylobacter* respectively. All 184 samples tested were negative for *Listeria* spp. *Salmonella* was recovered from seven (3·8%) egg shell samples only compared with 14 (7·6%) egg content samples only positive for the pathogen. Fifty-two (28·3%) egg shell samples and seven (3·8%) egg content samples were positive for *E. coli*. Both isolates of *Campylobacter coli* originated from egg contents. Of a total of 24 composite egg samples positive for *Salmonella*, eight different serotypes of *Salmonella* were isolated from a total of 24 *Salmonella*-positive composite eggs of which *S. Enteritidis* was the most prevalent, 58·3% (14/24). *Salmonella* Georgia was isolated for the first time in Trinidad. Failure to properly handle or heat table eggs sold in Trinidad poses a potential health hazard to consumers because of their poor microbial quality.

### INTRODUCTION

Worldwide, table eggs are used in the preparation of numerous commercial and homemade products [1, 2]. During the period 1980–1989 several egg-borne epidemics of salmonellosis were reported in the United Kingdom that caused a widespread reduction in egg consumption [3, 4]. The fact that eggs could be contaminated or infected by pathogens such as

*Salmonella* horizontally in the environment where they are laid or vertically through trans-ovarial transfer makes eggs an important potential source of pathogens [5, 6].

Of all bacterial pathogens, egg-borne *Salmonella*, particularly *S. Enteritidis* appears to be the most important cause of foodborne outbreaks [7, 8]. Other enteric pathogens such as *Campylobacter* spp., particularly *C. jejuni*, *Listeria* spp. and *Escherichia coli* have been isolated from eggs, egg products or egg washing facilities [9–12].

In Trinidad and Tobago, table eggs are used in several dishes and drinks. In 1992, an outbreak

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involving 300 patients of a mental hospital, which resulted in 10 deaths, was confirmed as foodborne salmonellosis allegedly due to consumption of contaminated egg-nog [13]. Subsequently, Indar et al. [14] demonstrated trans-ovarial transmission of *Salmonella* in table eggs sampled from poultry farms in Trinidad. In that layer farm-based study [14], *Salmonella* was found in egg shells' surfaces from all 10 farms with a predominance of *S. Typhimurium*, and in the egg contents from three farms with *S. Enteritidis* being the most frequent. Currently, information is unavailable on the potential for table eggs consumed in Trinidad to serve as sources of other enteric pathogens. The effect of practices and storage conditions on the microbial load of table eggs is also unknown.

In view of the fact that some retailers import table eggs, coupled with an increase in the number of layer farms in the country within the last few years, the study was conducted to determine the prevalence of *Salmonella*, *E. coli*, *Campylobacter* and *Listeria* on shells and in the contents of table eggs sold at outlets across Trinidad. The study also investigated the possible effects of management practices at the poultry layer farm level, as well as storage conditions at sale outlets, on the prevalence of these enteric pathogens in the eggs.

## MATERIALS AND METHODS

### Study design and source of samples

Table eggs from layer farms and sale outlets (shopping malls and other retailers) were sampled. Shopping malls are medium to large shopping complexes each with numerous shops including a supermarket. All supermarkets in the malls had refrigeration facilities for their eggs. Other retail outlets for eggs include kiosks, roadside vendors, supermarkets (small, medium or large) selling table eggs where few had refrigeration facilities for the eggs. All layer farms and shopping malls in the island of Trinidad were sampled while other retailers of table eggs across the country were included in the study.

Initially, through the assistance of the Poultry Surveillance Unit, all layer farms in operation were identified and a questionnaire was administered to each farm. The questionnaire addressed management practices, production and sales on each farm. Twenty-three layer farms in operation at the time of the study served as sources of table eggs from farms. Each farm

was visited twice, approximately 1 month apart, during which time a total of 46 composite egg samples were processed. All 14 large shopping malls across Trinidad, each with a supermarket and representative of various regions of the island, served as mall samples of table eggs (with refrigeration facilities). Each mall was visited twice for sampling, approximately 1 month apart. Mall samples accounted for a total of 31 composite eggs. A total of 102 other retail outlets (kiosks, roadside vendors and supermarkets) across Trinidad were sources of a total of 107 composite egg samples (different local and imported sources) processed from this source. Each outlet was sampled once over the study period. At the farms, a total of 25 eggs were randomly collected from each farm with samples proportionally distributed based on bird population and number of poultry houses. At the shopping malls and other retail outlets, six eggs were randomly sampled from different producers that were available for sale during the visit and pooled to constitute a composite sample. Overall, for the three sources studied, 1978 table eggs were pooled and processed for bacterial isolation.

### Sample collection

All egg samples from the malls and other retailers were collected in the crates that were used for their sale to consumers. The temperature of storage at each sale outlet (room temperature, ambient temperature or refrigeration temperature) was noted and eggs were kept at that temperature until processed. For farm egg samples, eggs were collected in sterile crates and kept at the same temperature before processing. The investigators could not personally collect eggs from the farms because of restrictions by farm owners. Eggs were therefore collected by the farmers or their assistants. Samples were transported to the laboratory within 2 h of collection. All eggs were processed in the laboratory within 24 h of collection and when impracticable, they were stored overnight at the temperatures at which they were sold.

### Processing of egg samples

Sterile gloves were worn to handle egg samples from each source. For egg shells, from either a pool of six or 25 eggs, one sterile swab, moistened in sterile saline, was applied to the surface of each egg. The swabs applied to six egg shells (mall and other retailers), were submerged in 6 ml of sterile saline as 'shell wash' while swabs applied to 25 egg shells from

farms were dipped in 20 ml of saline also as 'shell wash'. The contents were mixed with a VWR Mini-vortexer (Henry Troemner, Thorofare, NJ, USA) and used to inoculate appropriate enrichment broths or media. For egg content (yolk and albumen) samples, a pool of six or 25 eggs, was submerged in 75% ethanol for 5 min after which the pointed end of each egg was further disinfected by flaming for 5–10 s with a Bunsen burner. A sterile scalpel blade was then used to break a small hole on the shell through which the egg content (yolk and albumen) was aseptically emptied into a Stomacher bag (Seward, London, UK). The contents of each composite sample were blended for 30 s at normal speed in a Stomacher 400 (Seward). The resulting mixture was used to inoculate enrichment broths or media.

### Isolation and identification of bacteria

To isolate *Salmonella*, 1 ml of 'shell wash' of pooled eggs and 10 ml of the pooled egg contents were each used to inoculate 90 ml of lactose broth (LB) (Oxoid Ltd, Basingstoke, Hampshire, UK) which was incubated at 37 °C overnight for pre-enrichment. Samples were enriched in selenite cystine (SC) broth and tetrathionate (TT) broth, growths were plated on xylose lysine desoxycholate (XLD) agar, Brilliant Green agar (BGA) and bismuth sulphite (BS) agar; and identification of isolates followed standard procedures [15]. Isolates of suspected *Salmonella* were serologically identified by slide agglutination test using the *Salmonella* Polyvalent antiserum (A-I∞Vi) (Difco, Detroit, MI, USA). The Caribbean Epidemiology Centre (CAREC), Port of Spain, the regional laboratory for *Salmonella*, kindly confirmed and serotyped the isolates of *Salmonella*.

For qualitative detection of *E. coli*, 0.1 ml each of 'shell wash' and egg content was used to inoculate eosin Methylene Blue (EMB) plates which were incubated overnight aerobically at 37 °C. To quantify the number of *E. coli* in pooled egg contents, 0.1 ml of egg content was added to 9.9 ml of sterile saline (100-fold dilution) and further serial 100-fold dilutions were made. Thereafter, 0.1 ml of undiluted egg content and 0.1 ml of 100-fold serial dilutions were inoculated onto EMB plates which were incubated overnight at 37 °C. Standard procedure was used to isolate and identify *E. coli* [16]. Counts were expressed as colony-forming units (c.f.u.) of *E. coli* per ml of egg content.

To detect *Campylobacter* spp. 1 ml of 'shell wash' and 25 ml of egg content were inoculated into 90 and

225 ml respectively of Bolton broth containing antibiotics and lysed horse blood. The broths were incubated at 37 °C in 8% CO<sub>2</sub> in a CO<sub>2</sub> incubator (Forma Scientific, Marietta, OH, USA) for 4 h for pre-enrichment and then incubated at 42 °C in 8% CO<sub>2</sub> for 28–29 h for enrichment as described earlier [17]. Plates of *Campylobacter* blood-free selective agar, containing CCDA (charcoal cefoperazone deoxycholate agar) (Oxoid), were inoculated with 0.1 ml of growth in the enrichment broth and incubated at 42 °C in 8% CO<sub>2</sub> for 48 h. Gram-negative curved or seagull-appearing colonies were subjected to identification protocol as proposed by Lior [18].

To isolate *Listeria* spp., 1 ml of 'shell wash' and 10 ml of egg content were inoculated into 9 and 90 ml respectively of *Listeria* enrichment broth (LEB) containing nalidixic acid and acriflavine (Oxoid). The broths were incubated aerobically for 48 h at 30 °C after which they were plated in *Listeria* selective agar containing antimicrobial supplements: cycloheximide, colistine sulphate, acriflavine, cefotetan and fosfomycin (Oxoid) and incubated at 35 °C for 24–48 h. Greyish-black colonies were subcultured on blood agar and incubated at 30 °C overnight. Gram-positive, short rod colonies were subjected to biochemical tests as described [19]. Poly O antisera, for serotypes 1 and 4 (Difco) were used to serologically identify *Listeria*.

### Statistical analysis

The prevalence and counts of microorganisms on shells and/or in egg contents were compared for various sources (farms, malls, other retailers) and storage temperatures (room temperature, ambient temperature and refrigeration temperature); and farm and sale outlet practices were also related to the frequency of isolation of selected bacteria by the  $\chi^2$  tests using SPSS version 10 (SPSS Inc., Chicago, IL, USA). All statistical analyses were two-tailed and interpreted at the 5% level of significance.

## RESULTS

### Frequency of detection of bacteria in egg shell and egg content

The frequency of isolation of *Salmonella* from the egg content alone was 7.6% (14/184) compared with the isolation rate of 3.8% (7/184) from egg shell alone (Table 1) *E. coli* was recovered at a higher frequency

Table 1. Distribution of enteric pathogens in table egg shells and contents. Values are the numbers (%) of positive samples

Source	No. of composite† eggs tested	No. (%) of samples* positive for					
		Shell only‡		Egg content§		Shell/Egg content	
		<i>Salmonella</i>	<i>E. coli</i>	<i>Salmonella</i>	<i>E. coli</i>	<i>Salmonella</i>	<i>E. coli</i>
Farm	46	3 (6.5)	27 (58.7)	3 (6.5)	2 (4.3)	0 (0.0)	4 (8.7)
Mall	31	1 (3.2)	8 (25.8)	3 (9.7)	2 (6.5)	2 (6.5)	3 (9.7)
Other retailers	107	3 (2.8)	17 (15.9)	8 (7.5)	3 (2.8)	1 (0.9)	2 (1.9)
Total	184	7 (3.8)	52 (28.3)	14 (7.6)	7 (3.8)	3 (1.6)	9 (4.9)

\* Two (1.1%) of 184 samples were positive for *Campylobacter* spp., both isolated from contents of eggs from farms. All samples were negative for *Listeria* spp.

† Pooled egg samples, 6 each from mall and supermarkets, and 25 each from farms.

‡ Shells only were positive for the bacteria.

§ Egg contents only were positive for bacteria.

|| Shells and egg contents were positive for bacteria.

from the shells than from the egg content. Eggs sampled directly from farms had a significantly ( $P < 0.05$ ,  $\chi^2$ ) higher prevalence of *E. coli* on their shells compared with eggs purchased from shopping malls and other retailers. Both isolates of *Campylobacter coli* originated from egg contents.

Overall, of 184 composite eggs (shell and content) tested from the three sources, 24 (13.0%), 68 (37.0%) and two (1.1%) were positive for *Salmonella*, *E. coli* and *Campylobacter* respectively. Six (13.0%) of 46 composite egg samples from farms, six (19.4%) of 31 from malls and 12 (11.2%) of 107 from other retailers were positive for *Salmonella*. However, 33 (71.7%), 13 (41.9%) and 22 (20.6%) of composite eggs from farms, malls and other retailers respectively were positive for *E. coli* and the differences were statistically significant for each comparison ( $P < 0.05$ ,  $\chi^2$ ). *Campylobacter coli* was isolated from two (1.1%) of 184 composite samples both originating from farm sources.

The frequency of detection of *Salmonella* and *E. coli* on egg shell and in egg content was not statistically significantly ( $P > 0.05$ ,  $\chi^2$ ) affected by the presence of faeces or blood on their shells, the presence of cracks, or storage temperature.

#### Distribution of *Salmonella* serotypes by source of table eggs

The distribution of *Salmonella* serotypes by source and location in composite eggs is shown in Table 2. For the 46 pooled composite egg samples from farms, four serotypes (*S. Enteritidis*, *S. Mbandaka*, *S. Javiana*

and *S. Caracas*) were recovered compared with only three serotypes (*S. Enteritidis*, *S. Javiana* and *S. Ohio*) from the 31 mall samples and five serotypes (*S. Enteritidis*, *S. Braenderup*, *S. Georgia*, *S. Ohio* and Group C1) from the 107 samples from supermarkets. Overall, a total of eight different serotypes were recovered with *S. Enteritidis* constituting 42 (56.8%) of the 74 isolates of *Salmonella*. *S. Enteritidis* was recovered from 14 (58.3%) of 24 *Salmonella*-positive samples, *S. Ohio* from three (12.0%) and *S. Javiana* from two (8.3%), and *S. Caracas*, *S. Mbandaka*, *S. Georgia*, *S. Braenderup* and Group C1 were each isolated from one composite egg sample.

For eggs with identified farm sources, *Salmonella* of the same serotype was isolated from the shells and contents of eggs from three sources: *S. Ohio* from farm A, *S. Enteritidis* from farm B and *S. Enteritidis* from farm C. However, for two farm sources, different serotypes of *Salmonella* were isolated from the shells and yolk/albumen mixture of the same composite egg pool: *S. Javiana* from the shell and *S. Enteritidis* from the egg content on farm D and *S. Ohio* from the shells and *S. Enteritidis* from the egg content of farm E.

Of a total of 10 identified farm sources which supplied table eggs to sale outlets, eight (80.0%) had eggs contaminated by *Salmonella* and six (60.0%) were positive for *S. Enteritidis*.

#### DISCUSSION

Contamination of shell eggs with *Salmonella* and other enteric pathogens remains an important public

Table 2. *Distribution of Salmonella serotypes by source and part of eggs (values within parentheses are the number of isolates)*

Source	Composite eggs from outlets			Egg							
	No. of composite eggs tested	No. of <i>Salmonella</i> * isolates recovered	Serotypes of <i>Salmonella</i> (n)	Shell alone			Egg content alone		Shell and egg content		
				No. of composite eggs tested	No. of <i>Salmonella</i> * isolates recovered	Serotypes of <i>Salmonella</i> (n)	No. of <i>Salmonella</i> * isolates recovered	Serotypes of <i>Salmonella</i> (n)	No. of <i>Salmonella</i> * isolates recovered	Serotypes of <i>Salmonella</i> (n)	
Farm	46	18	Enteritidis (7) Mbandaka (6) Javiana (4) Caracas (1)	46	7	Mbandaka (6) Caracas (1)	6	Enteritidis (6)	5	Javiana (4) Enteritidis (1)	
Mall	31	25	Enteritidis (11) Ohio (13) Javiana (1)	31	5	Ohio (5)	9	Enteritidis (8) Javiana (1)	11	Ohio (8) Enteritidis (3)	
Other retailers	107	31	Enteritidis (24)  Braenderup (3) Group C (2) Georgia (1) Ohio (1)	107	6	Braenderup (3)  Group C1 (2) Georgia (1)	21	Enteritidis (20)  Ohio (1) Ohio (1)	4	Enteritidis (4)	
Total	184	74		184	18		36		20		

\* Based on enrichment in tetrathionate (TT) broth and selenite cystine (SC) broth and plating on xylose lysine desoxycholate (XLD), Brilliant Green agar (BGA) and bismuth sulphite (BS) agar.

health concern in Trinidad. Although the frequency of isolation of *Salmonella* (26.1%) from shell and content of table eggs from 23 farms studied is considerably lower than a prevalence of 100.0% reported by Indar et al. [14] in a study of ten layer farms in Trinidad, there were potentially important differences between these studies. For pre-enrichment, Indar et al. [14] inoculated swabs of 25 egg shells constituting a pool into 225 ml LB while in the present study where other pathogens including *Salmonella* were assayed for, 1 ml of the 'shell wash' of 25 egg shell swabs was used to inoculate 10 ml LB. For the egg contents, in the earlier study [14] 25 ml was added to 225 ml LB (1:10) while in this study 10 ml of egg content was inoculated into 90 ml LB (1:10). These differences would have resulted in a larger sample being analysed in the first study. For both studies, the same enrichment procedure, isolation technique and selective media were used. The difference in prevalence may, therefore, reflect a difference in the pre-enrichment protocol or a change in the pattern of *Salmonella* infection in layers or contamination of table eggs in the country.

Several observations suggest that these differences reflect real changes. First, table eggs sampled from shopping malls had a significantly higher prevalence for *Salmonella* than eggs sampled from farms and other retailers despite the fact that six eggs per producer constituted a pool for mall sources while 25 eggs from farms made up a composite sample. This suggests that eggs, from sources other than the 23 farms studied, are sold at outlets in the malls. There is evidence that some retailers import their table eggs (Poultry Surveillance Unit, Trinidad and Tobago, personal communication). Second, the overall prevalence of 9.2% for *Salmonella* in table egg contents in the present study is considerably higher than the prevalence of 1.2% reported earlier for egg contents in Trinidad [14] and the range of prevalence from 0.7% to 3.8% reported for various countries [20–22]. Finally, the changing distribution of leading serotypes, from *S. typhimurium* to *S. Enteritidis* is consistent with the other observations.

In the present study, from the three sources *Salmonella* was isolated at a much higher frequency from egg contents alone (7.6%) compared with a prevalence of 3.8% for isolation of the organism from egg shell alone. For eggs sampled from the farms, the frequency of isolation of *Salmonella* was the same (6.5%) each for egg shells alone and egg contents alone. In contrast, Indar et al. [14] had reported that the prevalence of *Salmonella* on egg shell (4.7%) was

significantly higher than that found in egg contents (1.2%) from layer farms in the country. The difference may be explained in part by the possibility of increased trans-ovarial contamination of table eggs in the local industry, a change in the pattern of infection of layers by serotypes of *Salmonella* and the difference in enrichment procedures. Trans-ovarial transmission of *Salmonella* is well documented in the literature [5, 6, 14]. It is also known that *S. Enteritidis* is the most important serotype for egg-borne salmonellosis in humans having been implicated in several outbreaks [3, 4, 23].

The finding of *S. Enteritidis* on egg shells and contents of some samples also suggests that these eggs may have been contaminated in the environment particularly in egg nests and from intestinal carriage [16, 14, 24]. However, no genetic fingerprinting was performed to conclusively establish the relatedness of the same serotypes of *Salmonella* [25, 26]. Washing of shelled eggs followed by pasteurization has been reported to eliminate bacteria from egg shells without causing coagulation of egg contents [27–29].

It is not clear whether differences in serotype distribution between this and Indar's study reflect a change in the pattern of infection of layer birds or could be due in part to differences in the pre-enrichment procedures used in the studies. Isolation techniques are known to affect the serotypes of *Salmonella* recovered [30]. Of the six serotypes isolated from the previous study [14] only *S. Ohio* and *S. Enteritidis* were recovered in the present study. *S. Georgia* was isolated for the first time from any source in Trinidad. The overwhelming predominance of *S. Enteritidis* amongst the isolates recovered from the egg contents in both studies probably has epidemiological significance since there has been an increase in the frequency of isolation of *S. Enteritidis* from human salmonellosis in Trinidad [31–33]. The other serotypes isolated, *S. Mbandaka*, *S. Javiana*, *S. Caracas* and *S. Braenderup* have all been isolated from patients with gastroenteritis in the country [31–33].

It is evident from this investigation that the risk of egg-borne campylobacteriosis and listeriosis is minimal, a finding in agreement with published reports [9, 10, 34]. It is difficult to assess the risk posed by the *E. coli* strains isolated since their virulence and pathogenicity were not determined. However, the frequent evidence of faecal contamination of shell eggs at retail further establishes their microbial risk.

In conclusion, there is a significant risk of table egg-borne gastroenteritis, particularly due to salmon-

ellosis, in Trinidad associated with the consumption of raw or improperly cooked eggs or egg products [35, 36]. It is imperative that the population be enlightened on the need to handle eggs with good sanitary practices and to consume only properly cooked eggs or egg products.

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