Contamination of hands and work surfaces with *Salmonella enteritidis* PT4 during the preparation of egg dishes

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SUMMARY

Salmonella enteritidis PT4 was recovered from fingers following the breaking of intact shell eggs artificially contaminated in the contents with the bacterium. Kitchen utensils used to mix egg dishes were salmonella-positive, sometimes after washing. Following the preparation of batter or the mixing of eggs, *S. enteritidis* was recovered from work surfaces over 40 cm from the mixing bowl. The bacterium survived well in thin, dry films of either batter or egg and, from an initial level of one cell per cm², could be recovered from formica work surfaces 24 h after contamination.

INTRODUCTION

Roberts [1] reported that cross-contamination was an important contributory factor in 57 out of 396 (14·4%) outbreaks of human salmonellosis in the UK. Studies in a number of other countries, involving either outbreak investigation [2–4] or using food products contaminated either naturally or artificially with micro-organisms [5–7], have revealed that raw meat, and particularly poultry meat, are important in the dissemination of potential human pathogens, including salmonellas, in the kitchen. Less attention has been paid to the role of other foods.

Intact shell eggs can be contaminated, in the contents, with *Salmonella enteritidis* and a few eggs have been found to contain high levels of contamination [8–9; de Louvois, personal communication]. The consumption of such eggs may pose a direct risk to health. There is also the possibility that their use in the preparation of foods may bring about the contamination of the kitchen environment. This was investigated using eggs artificially contaminated with an isolate of *S. enteritidis* PT4. Information is presented on the contamination of kitchen utensils, work surfaces and hands and on the survival of *S. enteritidis* on surfaces.

MATERIALS AND METHODS

Eggs

Eggs were obtained from a local commercial battery unit. Repeated testing by Exeter Public Health Laboratory (PHL) and Ministry of Agriculture, Fisheries and Foods (MAFF) had shown that the chickens appeared to be free from infection

with *S. enteritidis*. Each egg was inspected individually and only those that were intact and free from faeces were used.

Bacterial strain

Experiments were performed with a strain of *S. enteritidis* PT4, previously isolated from the contents of an intact egg, which was grown in Lemco broth for 18 h at 37 °C. Cultures were diluted in Ringer's solution before being inoculated into either intact or homogenized eggs.

Investigations using intact contaminated eggs

Six separate experiments were performed using intact eggs. On each occasion, 20 eggs were inoculated, using previously published techniques [10], into the albumen with between $\log_{10} 3.0$–$\log_{10} 6.0$ cells of *S. enteritidis*. Each egg was then cracked, by hand, against the edge of a glass bowl and the egg contents removed into the bowl. After each egg had been processed, the fingers of both hands of the staff member performing the task (either A.W. or K.M.) were dipped in 60 ml buffered peptone water (BPW) in a sterile 60 ml screw-capped plastic container. The BPW was incubated at 37 °C for 18–24 h and then plated onto XLD agar (Oxoid) incubated at 37 °C for 18–24 h. Salmonella-like colonies were identified using standard laboratory procedures. Hands were washed with toilet soap and hot water immediately after rinsing in BPW. The presence of salmonellas on washed fingers was investigated using the protocol outlined above.

After 10 eggs had been cracked, the edge of the glass bowl was swabbed using a sterile cotton wool ball moistened with BPW. The swab was added to 50 ml BPW, cultured overnight at 37 °C and plated onto XLD incubated at 37 °C for 18–24 h. Salmonella-like colonies were confirmed using standard procedures. The interior surfaces of the mixing bowl were also examined for salmonellas, using the above technique, both before and after washing with hot water (48 °C) and domestic liquid detergent.

In separate experiments, fingers were examined for the presence of salmonellas following the handling, for 30 s, of 20 eggs which contained approximately 10^6 cells of *S. enteritidis* but which remained intact. The techniques described above were used.

Investigations where egg contents were homogenized

The contents of three uncontaminated eggs were placed in a clean, disinfected glass bowl with either 70 ml of pasteurized semi-skimmed milk or milk plus 75 g flour and 25 g sugar. The above ingredients were inoculated with between 10^5–10^8 cells of *S. enteritidis* and blended, for approximately 1 min, with a fork or a hand-held electric mixer (Kenwood Ltd, Havant, UK; Model No. A177) operating at top speed.

During mixing, the bowl was placed on a marked position on the laboratory bench and, in some experiments, was surrounded by up to 43 open XLD plates which were incubated at 37 °C for 24 h following exposure. In other experiments, areas of bench in a circle either 0–30 or 30–60 cm from the bowl were swabbed after egg mixing and examined for salmonellas using the techniques described above for the examination of the mixing bowl.

Survival of Salmonella enteritidis *on contaminated surfaces*

Two experimental protocols were used. In the first, 4 cm² squares of formica, previously disinfected with Industrial Methylated Spirits (IMS), were inoculated with 0·02 ml of either batter or homogenized whole egg containing approximately 10^3 cells of *S. enteritidis*. The inoculated squares were placed in sterile petri dishes and held at 20–21 °C for up to 24 h. The egg or batter droplets dried within 2 h. Each hour, five squares were removed and each placed in 10 ml sterile BPW. The dried droplet was loosened using a sterile, disposable plastic loop and dispersed in the BPW by vigorous shaking. Using a sterile, disposable plastic pipette an XLD plate was inoculated with 15 drops of 0·04 ml of BPW. Plates were incubated at 37 °C for 24 h and salmonella-like colonies identified and counted using standard procedures.

In the second set of experiments, a 10000 cm² square of formica was divided into 40 squares each of 25 cm². Approximately 1 ml of either batter or egg containing *c.* 10^3 cells of *S. enteritidis* was placed in the centre of the piece of formica and spread over the entire area using a sterile, disposable plastic spreader. This gave a level of contamination of approximately 1 cell per cm². The formica was held at 20–21 °C and at intervals, for up to 24 h, five squares were chosen at random and swabbed with a cotton-wool tipped wooden swab moistened in BPW. The swabs were placed in 10 ml BPW which was incubated at 37 °C for 18–24 h. These cultures were streaked onto XLD which was incubated at 37 °C for 24 h. Salmonella-like colonies were identified using standard protocols.

RESULTS

Contamination of fingers

Before each experiment the fingers of those involved in the study were examined for the presence of salmonellas. All cultures were salmonella-negative. The breaking of contaminated eggs led to contamination of fingers and *S. enteritidis* was cultured from finger rinses particularly when levels of egg contents contamination exceeded $\log_{10}$ 5·0 cells per egg (Table 1). A total of 170 samples were taken from fingers after washing with soap and hot water. Three (1·8 %) were positive for *S. enteritidis*.

Salmonella enteritidis was isolated from the edge of the unwashed bowl from 2 of 21 samples (10 %). All samples taken from the bowl interior before washing were salmonella-positive. Four of 19 swabs (21 %) taken from the bowl interior after washing yielded *S. enteritidis*.

Contamination of work surfaces when eggs containing Salmonella enteritidis *are homogenized*

A total of 70 separate experiments were performed, 19 using hand whisking and 51 using an electric mixer. The results, which are shown in Table 2, demonstrate that contamination of surfaces around a mixing bowl was a common occurrence, particularly when levels of contamination in the egg/batter mix exceeded $\log_{10}$ 3·7 cells of *S. enteritidis* per ml (Table 2). Comparison of the data from egg mixes containing either $\log_{10}$ 2·7 or 3·7 cells per ml of egg or batter mix revealed that

Table 1. *Contamination of fingers during the cracking of eggs artificially contaminated in the contents with* Salmonella enteritidis

No. of cells of S. enteritidis per egg	No. of finger rinses salmonella+ve (%)*/ No. examined
10^3	1/20 (5%)
10^4	6/50 (12%)
10^5	10/40 (25%)
10^6	8/40 (20%)

* Finger rinses were taken after each egg was cracked.

Table 2. *Contamination of bench surfaces with* Salmonella enteritidis *during the mixing of pancake batter or egg**

Log$_{10}$ no. of cells of Salmonella enteritidis per ml of egg/batter mix	No. of bench surfaces salmonella-positive (%)/ No. tested	
	Hand-mixing	Electric mixer
2·7	1/13 (8%)	3/10 (30%)
3·7	2/5 (40%)	12/20 (60%)
4·7	1/1 (—)	21/21 (100%)

* Data from egg or batter have been combined.

homogenization using an electrix mixer created more contaminated droplets (15/30 [50%] bench surfaces salmonella-positive) than hand whisking (3/18 [16.7%] bench surfaces (28%) salmonella-positive, $\chi^2 = 5.1$, $P < 0.05$).

In two of the experiments when egg mixes were contaminated with either log$_{10}$ 4·5 or 5·7 cells of *S. enteritidis* per ml, open XLD plates were placed around the mixing bowl while eggs or batter were homogenized. After incubation, the plates were returned to their original place on the bench. The distance of the salmonella colonies from the bowl was measured. The results demonstrated the widespread distribution of contaminated droplets and *S. enteritidis* was found over 40 cm from the mixing bowl. In none of the experiments was contamination of either bench surfaces or plates associated with obvious splashing. Many contaminated droplets were invisible to the naked eye.

Survival of Salmonella enteritidis *on contaminated work surfaces*

Once present on formica work surfaces, *S. enteritidis* survived well. In 0·02 ml droplets of either batter or homogenized egg, viability was unaffected over a 24-h sampling period (Table 3) even though the droplets dried within 30–60 min of placing on the formica. For the sake of clarity the results from the three experiments using either contaminated egg or batter have been combined to give those shown in Table 3.

The batter or egg mix spread over the 10000 cm² area of formica dried almost immediately to produce a thin film which, in places, was invisible to the naked eye. *Salmonella enteritidis* survived well in the dried films and could be recovered after 24 h even though the initial inoculum was only approximately one cell per cm². The results of a typical experiment are shown in Table 4.

Table 3. *Survival of* S. enteritidis *in 0·02-ml batter droplets exposed to air at 20 °C*

Time post-inoculation (hours)	Log_{10} no. of *S. enteritidis* per 4 cm² square ($\pm$ s.e.)
1	2·80 ± 0·03
2	2·51 ± 0·13
3	2·81 ± 0·04
4	2·72 ± 0·04
5	2·69 ± 0·05
6	2·78 ± 0·02
24	2·70 ± 0·05

Table 4. *Survival of* S. enteritidis *in a thin film of dried batter on a formica work surface*

Time (h) after contamination*	No. of 25 cm² squares salmonella-positive/ No. tested
1	5/5
2	4/5
3	3/5
4	5/5
5	5/5
6	4/5
8	4/5
24	5/5

* Approximately 1 cell per cm².

DISCUSSION

The potential importance of raw meat and poultry in kitchen cross-contamination is well understood [2–7]. In the United Kingdom, Government [11] and egg industry [12] advice on the handling of shell eggs suggests that eggs may be a potential source of cross-contamination. It appears, however, that there has been little or no scientific work carried out on cross-contamination with *S. enteritidis* when contents-positive eggs are used in cooking.

The results presented in this report clearly show that cross-contamination can occur during the handling or processing of eggs contaminated in the contents with *S. enteritidis*. The bacterium was isolated from fingers, after eggs were cracked and from utensils in which egg dishes were prepared. In addition, the homogenization of eggs or the preparation of batter, where eggs were mixed with milk, sugar and flour, resulted in the production of contaminated droplets which meant that *S. enteritidis* could be isolated from work surfaces over 40 cm away. *Salmonella enteritidis* was also capable of prolonged survival in either droplets or a film of egg or batter. There would appear to be a strong relationship between levels of contamination and either the production of contaminated aerosols or the contamination of hands or kitchen utensils. Thus, when intact eggs containing 10^3 cells of *S. enteritidis* were cracked only 5% of finger rinses were found to be salmonella-positive (Table 1). Fresh eggs are known to contain only low levels of contamination [8, 13–14]. *Salmonella enteriditis* has been found to be able to grow,

however, in eggs stored in simulated kitchen conditions before inoculation [15], and industry [12] and Government advice [11] stresses the need to refrigerate eggs after purchase. This advice is primarily aimed at reducing the direct hazard from the consumption of heavily contaminated eggs. Results presented in this paper indicate that egg refrigeration would also reduce indirect hazards resulting from cross-contamination. Some naturally contaminated eggs, not stored under refrigeration [8, 9], have been found to contain levels of *S. enteritidis* in excess of some of those used in this work and shown to result in cross-contamination.

Egg refrigeration and the careful handling of eggs in the kitchen would do much to reduce the potential public health hazards described in this paper. Some of the data demonstrate, however, that even the use of eggs containing a relatively low inoculum resulted in the contamination of fingers or the production of contaminated aerosols. For example, contamination of fingers occurred with eggs containing $c.$ 10^3 cells of *S. enteritidis*. If one assumes that size two eggs contain approximately 50 ml of contents 10^3 cells per egg would be equivalent to 20 cells per ml. The mixing of eggs and milk containing $c.$ 500 cells of *S. enteritidis* per ml led to the production of contaminated aerosols. These results demonstrate the difficulties of controlling cross-contamination in the kitchen and support the view that prevention of food poisoning cannot be left solely to the consumer but requires action at every point of the food chain from farm to home.

Whether the presence of salmonella on kitchen surfaces results in food poisoning depends, in part, on the survival of the bacterium. This was addressed in this paper and the results (Tables 3 and 4) demonstrate that the isolate of *S. enteritidis* PT4 used in this study was capable of prolonged survival either in small droplets or thin films of either homogenized egg or batter. Viability was largely unchanged over a 24 h period at 20 °C. These results contrast markedly with other investigations with other salmonellas [16] and may suggest that either egg is particularly protective or that *S. enteritidis* is especially resistant. This is being investigated.

The isolation of *S. enteritidis*, on a few occasions, from washed hands and utensils is in contrast to previous reports [17]. This may be a reflection of the enhanced heat resistance of *S. enteritidis* PT4 [18]. This is also being investigated.

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