The survival of salmonellas in shell eggs cooked under simulated domestic conditions

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(Accepted 1 March 1989)

SUMMARY

Strains of Salmonella enteritidis, S. typhimurium and S. senftenberg inoculated into the yolks of shell eggs were found to survive forms of cooking where some of the yolk remained liquid. Survival was largely independent of the size of the initial inoculum. The organisms also grew rapidly in eggs stored at room temperature and after 2 days the number of cells per gram of yolk exceeded $\log_{10} 8.0$. With this level of contamination viable cells could be recovered from eggs cooked in any manner.

INTRODUCTION

In 1988 there has been a large increase in the number of cases of human salmonellosis in England and Wales. Reported isolations of one organism Salmonella enteritidis phage type (PT) 4, far exceeded those of any other type.

Until recently, chicken meat was regarded as the main source of this organism (1), evidence has accumulated, however, that eggs are becoming increasingly important. Recent investigations (2,3) have suggested that S. enteritidis PT4 can be transmitted vertically by infected hens. Thus, it can be isolated from egg yolk as well as from the outside of the shell.

Most outbreaks have resulted from the use of raw eggs in sauces or mayonnaise. There have been, however, in both England and Wales with S. enteritidis PT4 (4)

and in the USA (5) with other phage types of S. enteritidis, outbreaks where cooked eggs or dishes derived from them were implicated.

A strain of *S. enteritidis* PT4 isolated from eggs used to prepare a cooked pudding implicated as the vehicle in an outbreak investigated by one of the laboratories in this study was found to survive in boiled, fried or scrambled eggs. The findings had potentially serious public health implications and it was decided to examine in detail, in a multi-laboratory study, the ability of *S. enteritidis* PT4 and other salmonellas isolated from eggs, to survive in shell eggs cooked under simulated domestic conditions.

Each laboratory followed the same protocol with the exception that they used their usual salmonella selective and enrichment media.

MATERIALS AND METHODS

Eggs

Eggs were purchased from retail shops in the locality of the laboratories involved in the study during the period 18–26 July 1988. These were stored at ambient temperature (c 23 °C) for up to 7 days before use. Details of the brand of eggs, packing and sell-by dates were recorded.

Cultures

The Division of Enteric Pathogens, Colindale (DEP) of the Public Health Laboratory Service (PHLS) provided six freeze-dried salmonella cultures. Five of these had previously been isolated from eggs: S. typhimurium PTs 141 and 110 and S. enteritidis PTs 4, 8 and 13a. The other strain was S. senftenberg 775W, believed to be the most heat resistant of the salmonellas. The cultures were resuscitated in nutrient broth (Oxoid No. 2) and inoculated on blood agar which was incubated overnight at 37 °C. A single colony was then inoculated into 10 ml of nutrient broth which was incubated for 24 h at 37 °C. These cultures were then used for the experiments described below.

The effects of boiling or frying on the survival of salmonellas inoculated into the yolks of shell eggs was examined using S. typhimurium PT110 and PT141, S. enteritidis PT4 and S. senftenberg 775W. When survival in scrambled eggs was determined S. enteritidis PT8 and 13a were also used in parallel with the above organisms. S. enteritidis PT4 was also used to assess the relationship between survival, inoculum size, type of cooking and cooking time.

Types of cooking investigated

The effects of boiling for between 4 and 10 min, frying, 'sunny-side up' or 'over-easy' and scrambling in either a microwave oven or in a saucepan, on the survival of salmonellas in the yolks of shell eggs, were investigated. The laboratory workers followed a detailed experimental protocol, although it was recognized that cooking under domestic conditions can be difficult to standardize, particularly in studies involving more than one laboratory.

Each type of cooking was carried out at least three times using normal cooking utensils. The time taken to cook the eggs, the temperatures of the cooked yolks and, where appropriate, their consistency were also recorded.

Inoculum

The inoculum size was varied; in initial experiments one of approximately \log_{10} 6·0 cells per gram of yolk was used. Thus, for boiled or fried eggs, 0·2 ml of a 24 h culture was injected into the yolk. Because two eggs and up to 20 ml of milk were used for scrambled eggs, the inoculum size was increased to 0·5 ml. In later experiments, in order to assess the influence of the size of the initial population, a culture of S. enteritidis PT4 was diluted as appropriate. Different volumes were then used so that the number of cells in the yolk varied between \log_{10} 2·0 and \log_{10} 8·0 cells per gram.

The inoculum for each individual experiment was estimated. With boiled or fried eggs this was achieved by diluting the broth culture and performing viable counts on blood agar which was incubated at 37 °C for 24 h. For scrambled eggs, 1 ml of the inoculated raw egg mixture was removed and diluted for viable counting on blood agar.

Boiled eggs

When the eggs were to be boiled, an area on the egg shell was disinfected with an alcohol wipe and a hole cut in the shell using a small needle or drill. The appropriate volume of culture broth was then inoculated in the yolk using a syringe and needle. To ensure that the inoculum had reached the yolk, some of the contents were drawn into the syringe before injection. The hole in the shell was sealed with a water-resistant glue or nail varnish. Once this had dried the egg was placed in boiling water and was boiled for up to 10 min. The egg was removed from the water and placed in an egg cup or similar container. The 'top' was removed within 30 s and the temperature of the yolk measured with a mercury thermometer or a thermocouple disinfected in alcohol. The volk was transferred to a weighed stomacher bag (Seward Medical Ltd) by use of a sterile spoon, weighed and a 1/10 dilution prepared in buffered peptone water (BPW). Following maceration this was further diluted using 0.1% peptone water and the number of salmonellas surviving cooking was estimated by performing counts on blood and McConkey agar plates incubated for 24 h at 37 °C. The count was expressed as colony forming units (cfu) per gram of yolk and as a percentage of the initial inoculum. The remainder of the 1/10 dilution was incubated overnight at 37 °C. When there was no growth on primary plating, 10 ml of the BPW was removed and added to 100 ml of either Selenite F Broth, Tetrathionate Broth or Rappaport Vassiliadis Broth (Oxoid). These were incubated for 24 h at either 37 °C or 43 °C and sub-cultured on two of either Deoxycholate, Xylose Lysine Deoxycholate, Brilliant Green or Bismuth Sulphite agars (Oxoid). The plates were incubated for at least 24 h at 37 °C.

Fried eggs

An egg was broken into a sterile Petri dish and the inoculum was injected into the centre of the yolk. The egg was then cooked in a frying pan containing vegetable oil at a temperature of approximately 120 °C. It was left to the discretion of the person doing the cooking as to when the eggs could be considered cooked. They were asked, however, to ensure that the white was solid and opaque.

This took approximately 1.5-2.0 min. In parallel experiments, the effect of 'basting' the eggs with the hot oil was also examined.

For 'over-easy' eggs, cooking was initially as described above but the eggs were then turned over and cooked for up to 1 min longer. The time taken to cook the egg was recorded, as was the temperature at the centre of the yolk at serving. The cooked yolk was examined for salmonellas as for boiled eggs.

Scrambled eggs

When the effects of scrambling were examined, two eggs were mixed with 10–20 ml of sterilized whole milk and 0·5 ml of a 24 h salmonella culture. The egg mixture was cooked either in a saucepan, using melted margarine or vegetable oil, or a microwave oven (Panasonic 600 w) to the personal preference of the member of the laboratory staff doing the cooking, provided no visible liquid remained. With eggs scrambled in a saucepan the mixture was stirred throughout the cooking procedure. The effects of cooking rapidly over a high flame and slowly over a low to moderate flame were also compared. For the microwave experiments, the mixture was stirred after approximately 30 s cooking. The cooking time and the temperature at the centre of the egg were recorded. Two samples, each of 25 g, were removed from different parts of the cooked mixture and added to separate stomacher bags. Buffered peptone water (225 ml) was added to each and the mixture macerated for 2 min. The macerate was transferred to a sterile jar and cultured for salmonellas using the pre-enrichment and enrichment techniques described for boiled eggs.

Identity of salmonellas surviving cooking

Cultures were checked on receipt and representatives of all isolates from cooked eggs were confirmed at the DEP.

The growth of salmonellas in whole or homogenized egg

Whole shell eggs were inoculated as for boiled eggs with 0.2 ml of a diluted broth culture of either S. enteritidis PT4 or S. typhimurium PT141 so that the number of cells per yolk was either approximately $\log_{10} 1.0$ or $\log_{10} 3.0$ per yolk. They were stored at either room temperature (23 °C) or 4 °C for up to 14 days. At daily intervals, eggs were removed and the number of salmonellas present estimated as previously described.

To assess the ability of salmonellas to grow in whole homogenized egg, two eggs were homogenized for 1 min in a blender previously disinfected by steaming. The homogenate was transferred to a sterile jar and 1 ml of a 24 h salmonella culture added. The inoculated mixture was allowed to stand at room temperature (c. 23 °C) or 4 °C for 24 h. Samples were removed at intervals and the number of salmonellas present were estimated as above.

Statistical analysis

The differences in the ability to survive cooking were compared using chi-square tests.

RESULTS

In initial experiments the effect of cooking on the survival of salmonellas in eggs was assessed using an initial inoculum of $\log_{10} 6.0-7.0$ per gram of yolk or egg/milk mix.

Survival in eggs boiled for 4 minutes

All four test strains survived in eggs boiled for 4 min in all experiments (Table 1). The percentage of the inoculum which could be recovered from the cooked yolk was significantly higher (P < 0.05), however, with S. senftenberg 775W.

Survival in fried eggs

Salmonella could be recovered from all samples of 'sunny-side up' fried eggs when an inoculum of approximately $\log_{10} 7.0$ cells per gram was used (Table 2). There were differences, however, in the abilities of the various organisms to survive this form of cooking. In four out of nine separate experiments with $S.\ typhimurium\ PT141$, its presence could only be demonstrated after enrichment culture. With the other salmonellas sufficient cells remained viable to permit enumeration in all experiments (Table 2). $S.\ enteritidis\ PT4$ generally survived better than the other egg-derived salmonellas in 'sunny-side up' fried eggs and in one experiment 75% of the inoculum remained viable. All egg-associated strains, however, survived less well than $S.\ senftenberg\ 775W$.

The additional cooking to which 'over-easy' fried eggs were subjected served to reduce further the numbers of surviving salmonellas. S. typhimurium PT141 could not be recovered from eggs cooked in this manner in any of nine separate experiments (Table 3). The other organisms were isolated only after enrichment culture (Table 3) indicating that the number present was below 10 per gram. Salmonella senftenberg 775W survived significantly better than the strains previously isolated from eggs (P < 0.05).

The effects of scrambling

When eggs were scrambled quickly at a high temperature it was possible to destroy all of the egg-associated strains of salmonellas when an inoculum of \log_{10} 7·0 cells per gram of mixture was used (Table 4). With slower cooking, using a low to moderate heat, the mean temperature reached at the centre of the mix when cooking was complete was $74\cdot3\pm0\cdot9$ °C, approximately 10 °C lower than that achieved with the more rapid form of cooking. With the exception of one experiment with S. typhimurium PT141, all salmonellas survived the slower form of cooking (Table 4). They were not present in sufficient numbers, however, to permit direct enumeration and could only be demonstrated by enrichment culture.

Cooking in a microwave oven for 1 min at high power destroyed all the inoculated salmonellas when the initial inoculum was $\log_{10} 7.0$ ml. It was important, however, that a clean form was used when the mixture was beaten after 30 s cooking. Many of the laboratory staff asked to cook eggs in this manner used the same fork with which they had initially mixed the eggs with the milk. This re-introduced contamination and allowed organisms to survive cooking.

Table 1. The effect of boiling for 4 min on the survival of salmonellas injected into the yolks of shell eggs.

	Strain			
	Egg-associated*	S. senftenberg		
Mean inoculum/g yolk,	6.81 ± 0.06	$7 \cdot 26 \pm 0 \cdot 05$		
$\log_{10} \pm \text{S.E.}$ Mean number of survivors/g	5.87 ± 0.27	6.72 ± 0.38		
volk, $\log_{10} \pm S.E.$	301 ±021	072 ± 0 38		
Mean number of survivors as	11.58 ± 4.17	28.84 ± 2.1		
% of inoculum ± S.E. Mean temperature °C of yolk	54.6 + 1.93	57.1 + 3.5		
post cooking ± S.E.	010 <u>-</u> 100	0.1 <u>-</u> 00		

^{*} The results for S. typhimurium PT110 and PT141 and S. enteritidis PT4 have been combined.

Table 2. Frying 'sunny-side up' and the survival of salmonellas in eggs.

	Strain					
	S. typhimurium PT141	S. typhimurium PT110	S. enteritidis PT4	S. senftenberg 775W		
Mean inoculum/g, $\log_{10} \pm S.E.$	6.68 ± 0.7	6.74 ± 0.7	6.90 ± 0.5	7.07 ± 0.6		
Number of salmonella- positive samples*	4/9	9/9	9/9	9/9		
Mean number of survivors/g log ₁₀ ± S.E.	N.D.	5.03 ± 0.2	5.14 ± 0.2	5.97 ± 0.3		
Mean numbers of survivors as % of inoculum ± S.E.	N.D.	1·95 ± 2·2	1.74 ± 2.6	8.0 ± 3.2		
Mean temperature °C of yolk post cooking ± S.E.	53.8 ± 1.7	53.9 ± 1.6	55.2 ± 1.0	51·1 ± 1·9		
Cooking time (min) ± S.E.	2.1 ± 0.3	1.7 ± 0.2	1.6 ± 0.2	$2 \cdot 0 \pm 0 \cdot 3$		

^{*} Each organism was tested in nine separate experiments. N.D. Not done.

The influences of inoculum size and type of cooking on the survival of S. enteritidis PT4

When the size of the inoculum was $\log_{10} 7.0$ or less per gram of yolk, the ability of S. enteritidis PT4 in survive in cooked eggs, as measured by the ability to recover organisms from 5 g of cooked yolk, was influenced more by the type of cooking than by the size of the inoculum. Where some of the yolk remained liquid as with 'sunny-side up' fried eggs or for eggs boiled for up to 8 min, it was possible to isolate salmonellas from the yolk from an initial inoculum of $\log_{10} 2.0$ cells per gram (Table 5). There was also essentially no difference between fried eggs which had been 'basted' with hot oil during cooking and ones which had not. The

	Strain					
	S. typhimurium PT141	S. typhimurium PT110	S. enteritidis PT4	S. senftenberg 775W		
Mean inoculum/g $\log_{10} \pm S.E.$	6.64 ± 1.0	6.14 ± 0.8	6.88 ± 0.4	$7 \cdot 10 \pm 0 \cdot 6$		
Number of salmonella- positive samples*	0/9	4/9	5/9	8/9		
Mean temperature °C of yolk post cooking ± S.E.	$69 \cdot 4 \pm 2 \cdot 1$	$71 \cdot 1 \pm 2 \cdot 0$	67·7 ± 1·4	67.2 ± 1.2		
Cooking time (min) ±S.E.	$2 \cdot 4 \pm 0 \cdot 2$	2.4 ± 0.2	$2\cdot 4\pm 0\cdot 2$	2.4 ± 0.3		

Table 3. Frying 'over-easy' and the survival of salmonellas in eggs

Table 4. The survival of salmonellas in scrambled eggs cooked in a saucepan

	Rapid cooki temper	-	Slow cooking at low/moderate temperature		
	Strain		Strain		
	Egg-associated*	S. senftenberg 775W	Egg-associated*	S. senftenberg 775W	
Mean inoculum/g $\log_{10} \pm S.E.$	6.09 ± 0.13	6.6 ± 0.5	5.9 ± 0.1	$6 \cdot 22 \pm 0 \cdot 2$	
Number of salmonella- positive samples	0/15	2/3	29/30	6/6	
Mean temperature °C of mixture post-cooking ±S.E.	82·8 ± 1·1	$82 \cdot 3 \pm 0 \cdot 3$	73.9 ± 0.8	74.7 ± 0.8	
Mean cooking time (min)	1.2	1.2	3·1	2.7	

^{*} The results for S. typhimurium PT110 and PT141, S. enteritidis PT4, PT8 and PT13a have been combined. With eggs cooked at low/moderate temperatures one experiment with S. typhimurium PT141 was negative after enrichment culture.

relationship between inoculum size and survival was essentially the same for both boiled and fried eggs and the results for the former only are shown in Table 5.

With harsher forms of cooking such as scrambling, frying 'over-easy' or boiling for 9–10 min, survival was, to an extent, inoculum related. Thus it was possible to destroy salmonellas when the inoculum was $\log_{10} 7.0$ or less per gram of yolk. Survivors could be isolated, however, from eggs cooked in any of the ways examined here when the inoculum exceeded $\log_{10} 8.0$ per gram.

The growth of salmonellas in whole or homogenized egg

Salmonellas inoculated into the yolks of shell eggs or into whole, homogenized egg could multiply rapidly at room temperatures, although not at refrigeration temperatures. Both the salmonellas tested, S. typhimurium PT141 and S. enteritidis PT4, behaved in essentially the same way at either level of inoculum. For clarity the results for S. enteritidis PT4 only are given in Fig. 1. At 23 °C,

^{*} Each organism was tested in nine separate experiments.

Table 5. The influence of inoculum size on the survival of Salmonella enteritidis PT4 in boiled eggs

Inoculum size, \log_{10}/g yolk	Boiling time (minutes)*						
19810/ 8 3 9 111	4	5	6	7	8	9	10
8.0	+†	+	+	+	+	+	± ‡
7.0	+	+	+	+	+	+	_ §
6.0	+	+	+	+	+	+	_ `
5.0	+	+	+	+	+	_	_
4.0	+	+	+	+	+	_	_
3.0	+	+	+	+	\pm	_	-
2.0	+	+	+	+	±	_	_
Mean							
temperature of							
cooked egg yolk °C	56.2	57.6	57.9	59·1	64.5	65.5	68.6

- * Eggs placed in boiling water and boiled for the appropriate time.
- † Salmonellas isolated from 5 g of cooked yolk by enrichment culture. ‡ Yolk samples salmonella-positive in some experiments. § All samples, in all experiments, salmonella-negative.

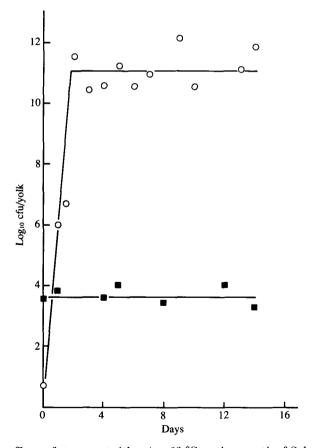


Fig. 1. The effects of storage at either 4 or 23 °C on the growth of Salmonella enteritidis PT4 in the yolks of shell eggs. ○, cfu/yolk at 23 °C; ■, cfu/yolk at 4 °C.

S. enteritidis PT4 had a generation time of 60 min in egg yolk and 45 min in homogenized egg. In the yolk of shell eggs, the numbers of this organism increased from $\log_{10} 0.7$ to approximately $\log_{10} 11.0$ per yolk within 48 h at room temperature (Figure 1). This level of contamination was maintained throughout the storage period. It was not possible to differentiate contaminated eggs from those that had not been inoculated with S. enteritidis PT4 either by the appearance of the shell or the colour, odour or consistency of the egg contents. By 4 days at 23 °C, however, the white of some eggs contaminated with salmonellas had become cloudy.

At 4 °C, the numbers of salmonellas in egg yolks or homogenized whole egg did not change significantly during 14 days.

DISCUSSION

Shell eggs play an important and valuable part in human nutrition and until recently were considered to be inherently safe. Evidence has accumulated in both the United Kingdom (6,7) and the USA (5,8), however, that eggs and dishes derived from them are becoming increasingly important vehicles for human salmonella infections with, in particular, S. enteritidis.

The finding that S. enteritidis PT4 had been implicated in outbreaks of food poisoning involving cooked eggs (4) suggests that it is able to survive in eggs after some cooking procedures and to support this contention, earlier work in the USA (9) showed that S. typhimurium could be isolated from boiled or fried eggs.

The survival of S. enteritidis PT4 in shell eggs was, therefore, examined and compared with that of other salmonellas derived from eggs and the heat-resistant strain S. senftenberg 775W. The earlier studies (9) had concentrated upon determining the presence or absence of S. typhimurium in cooked eggs. So that safe and effective cooking regimens could be identified it was decided to extend this earlier work to include the influences of inoculum size and a variety of forms of cooking.

The results presented in this paper show that salmonellas present in the yolks of shell eggs are able to survive in cooked eggs. In eggs boiled for up to 8 min or fried 'sunny-side up', where all or some of the yolk remained liquid and where cooked yolk did not reach temperatures lethal for salmonellas, survival was largely unaffected by the serotype or size of the inoculum. In some experiments with S. enteritidis PT4, however, up to 75% of the initial inoculum could be recovered.

When the various salmonellas were subjected to more extreme heat, as with scrambled or 'over-easy' fried eggs, differences between the organisms emerged and S. typhimurium PT141 was found to be more heat sensitive than the others even though it has been implicated in outbreaks involving eggs (7).

Provided that the number of cells of S. enteritidis PT4 was less than $\log_{10} 8.0$ per gram of yolk or egg/milk mix, it was possible to destroy all of the inoculum by cooking scrambled egg rapidly at high temperature, boiling for 9 min or longer or frying 'over-easy' until all the yolk had solidified. With scrambled eggs, it would seem to be important to reach a temperature of 80 °C in the cooked mix. Only 1 of 15 separate samples which reached this temperature was salmonella-positive.

With slower cooking, where the mean temperature was 75.2 ± 1.0 °C, salmonella survived in all 15 samples (P < 0.0001).

It could be suggested that the potential hazard associated with salmonellas in cooked eggs has been exaggerated in this study by the use of large inocula. It should be noted, however, that salmonellas are able to grow well in the yolk of experimentally infected eggs at room temperature. In experiments with S. enteritidis PT4 an initial inoculum of \log_{10} 0.7 cells per yolk increased to \log_{10} 9.7 per yolk within 24 h and \log_{10} 11.0 within 48 h at 23 °C. Eggs are usually stored at room temperature in retail outlets and are frequently given a shelf-life of 14 days.

Although S. enteritidis PT4 would appear to be more heat resistant than S. typhimurium PT141 this may not be a significant factor in its survival in cooked eggs. It would also not appear to differ significantly from PTs 8 and 13a in its ability to survive in scrambled egg. Given a sufficiently large initial population many salmonellas could be expected to survive forms of cooking common in domestic kitchens.

Recent guidelines to hospital caterers on the use of the shell eggs, issued by the Department of Health in England and Wales (10), advised that raw or lightly cooked eggs should not be served to hospital patients. Results presented in this paper show that any form of cooking, where all or some of the yolk remains liquid, can permit the survival of S. enteritidis PT4, even from a relatively small inoculum. Where a greater number of cells is present it would appear to be difficult to guarantee destruction with any of the common forms of cooking. Recent salmonella outbreaks involving cheese (11), dried milk (12) or chocolate (13) have demonstrated that with certain products the infective dose for salmonellas may be low. Thus, the presence of a few cells of S. enteritidis PT4 within cooked egg may well be significant.

ACKNOWLEDGEMENTS

We wish to thank Mrs E. Joslin, Miss D. Pope and Mr A. H. L. Gawler at the Exeter Public Health Laboratory and Miss J. Rodhouse and Miss M. Kendall of the Food Hygiene Laboratory for their excellent technical assistance and Miss S. Street of the Exeter Laboratory for typing the manuscript.

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