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A comparative study of the heat resistance of salmonellas in homogenized whole egg, egg yolk or albumen

T. J. HUMPHREY[1], P. A. CHAPMAN[2], B. ROWE[3] AND R. J. GILBERT[4]

[1] *Public Health Laboratory, Church Lane, Heavitree, Exeter EX2 5AD, UK*
[2] *Public Health Laboratory, Northern General Hospital, Sheffield S5 7AU, UK*
[3] *PHLS Division of Enteric Pathogens, Central Public Health Laboratory, 61 Colindale Avenue, London NW9 5HT, UK*
[4] *PHLS Food Hygiene Laboratory, Central Public Health Laboratory, 61 Colindale Avenue, London NW9 5HT, UK*

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SUMMARY

Salmonella enteritidis PT4 was found to be more resistant to heat in egg than some other common egg-associated salmonellas. This organism was significantly more heat sensitive than *S. senftenberg* 775W, however, and should not survive in pasteurized liquid egg.

INTRODUCTION

A comparative study to examine the ability of salmonellas to survive in the yolks of shell eggs cooked under simulated domestic conditions [1] demonstrated that they could be isolated from cooked eggs even when the measured temperature was in excess of that used to pasteurize liquid egg, 64 °C. The differences in heat transfer properties between liquid and in-shell egg mean that organisms which survive domestic cooking may not survive pasteurization. Given the importance of human salmonellosis in England and Wales and its recent association with eggs [2], however, it is clearly important to ascertain the possible practical implications of the heat resistance of the common egg-associated salmonellas and to assess their potential for surviving pasteurization.

Salmonellas grow rapidly in the yolks of artificially contaminated shell eggs stored at room temperature [1] and reach stationary phase within two days. They remain in this state for the shelf-life of the egg [1]. This may also happen in naturally contaminated eggs. Stationary phase cells of *Streptococcus faecalis* have been shown to be more heat resistant than log phase cells [3]. It is likely that salmonellas will behave in a similar manner and thus might survive treatments which could destroy more heat-sensitive organisms.

In order to assess the heat resistance of some common egg-associated salmonellas their death rates were compared to those of *S. senftenberg* 775W in homogenized whole egg, egg yolk or albumen. The effect of phase of growth was also examined.

MATERIALS AND METHODS

Cultures

The Division of Enteric Pathogens (DEP), Colindale, 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 either 24 or 48 h at 37 °C. These cultures were used for the experiments described below.

Preparation of homogenized whole egg, egg yolk or albumen

Eggs were purchased from retail shops in the locality of the Exeter and Sheffield Public Health Laboratories and were stored at room temperature (*c.* 21 °C) for a maximum of 7 days before use.

The shells of the above eggs were disinfected with alcohol as previously described [4] and the contents were removed into a sterile glass jar. Homogenized whole egg was prepared as in earlier work [1]. Ten millilitres of either egg yolk, albumen or homogenized egg was then transferred to a sterile 25 ml screw-capped bottle and used immediately in the manner described below.

Measurement of heat resistance

Each screw-capped bottle was inoculated with 1 ml of a different 24 h salmonella culture. After thorough mixing, the inoculated egg was distributed in 1 ml amounts to each of six 46 mm screw-capped glass vials (Trident Ltd). These were then placed in a rack in a water bath set at the appropriate temperature. The level of the water was above the top of the vial to ensure even distribution of heat within the egg mix. After allowing time for the contents to reach the required temperature, 5 min at 55 °C, 2 min at 60 °C and 1 min at 64 °C, vials were removed at intervals and immediately placed in chilled water. The sampling times varied with the organisms being tested and the temperature of the water bath. As a general rule, however, vials were removed at 2 min intervals at 55 °C, 20 s intervals at 60 °C and every 10 s at 64 °C. On completion of each set of experiments the contents of the vials were removed, diluted as appropriate with 0·1 % peptone water and the number of surviving salmonellas estimated by performing counts on blood agar plates incubated for 24 h at 37 °C.

Comparative heat resistance in homogenized whole egg, egg yolk or albumen

To compare the heat resistance of the strains named above, their death rates at 55 and 60 °C in homogenized whole egg were determined using the technique described above. *Salmonella enteritidis* PT4, *S. typhimurium* PT141 and *S. senftenberg* were also used to compare death rates in egg yolk, albumen or homogenized whole egg at the above temperatures.

The results of the above experiments were used to produce death curves and decimal reduction (*D*) values. The latter figure is the time taken to kill 90 % of the population.

Table 1. *Death rates of some salmonellas in homogenized whole egg*

Strain	*D* value* (minutes ± S.E.) 55 °C	60 °C	64 °C
S. enteritidis PT4	6·4 ± 0·6	0·44 ± 0·1	0·22 ± 0·02
S. enteritidis PT8	5·9 ± 0·2	0·30 ± 0·2	NT
S. enteritidis PT13a	3·9 ± 0·6	0·22 ± 0·3	NT
S. typhimurium PT110	4·7 ± 0·7	0·26 ± 0·2	NT
S. typhimurium PT141	2·3 ± 0·3	0·20 ± 0·1	0·15 ± 0·03
S. senftenberg 775W	34·3 ± 1·2	5·60 ± 0·1	2·80 ± 0·2

* *D* value is the time taken to kill 90% of the population.
NT, not tested.
The results are the mean values of at least three separate experiments.

Heat resistance in log phase and stationary phase cells

To examine the effects of phase of growth on heat resistance, *S. enteritidis* PTs 4, 8 and 13a and *S. typhimurium* PT141 were grown in nutrient broth at 37 °C for either 24 h for log phase or 48 h for stationary phase cells. These cultures were then used to measure death rates in homogenized whole egg at 60 °C. Death rates of *S. enteritidis* PT4 at 64 °C were also determined.

Statistical analysis

Each experiment was performed on at least three separate occasions. The differences in *D* values were compared using *t* tests.

RESULTS

Comparative heat resistance in homogenized whole egg

In homogenized whole egg, *S. enteritidis* PT4 was more heat resistant than the other egg-associated salmonellas (Table 1). The differences in the death rates at 55 and 60 °C of this organism from those of *S. typhimurium* PT141 and *S. enteritidis* PT13a were significant ($P < 0·05$). *Salmonella enteritidis* PT4 was significantly ($P < 0·0001$) more heat sensitive, however, than *S. senftenberg* 775W (Table 1). For example, at 55 °C the *D* value for *S. enteritidis* PT4 was 6·4 min while for *S. senftenberg* it was 34·3 min.

The influence of the suspending medium on heat resistance of salmonellas in eggs

All three strains were significantly ($P < 0·001$) more heat resistant in egg yolk than in either homogenized whole egg or albumen (Table 2). Death rates were particularly rapid in albumen and at 60 °C it was difficult to differentiate the death rates of *S. enteritidis* PT4 from those of *S. typhimurium* PT141.

Heat resistance in log and stationary phase cells

Cells in stationary phase were significantly more heat resistant ($P < 0·01$) than those in logarithmic phase (Table 3). At 64 °C the *D* value for logarithmic phase cells of *S. enteritidis* PT4 in homogenized whole egg was 5·2 ± 0·6 s. The equivalent figure for stationary phase cells was 12·5 ± 0·8 s ($P < 0·01$).

Table 2. *The influence of the suspending medium on the heat resistance of salmonellas in eggs*

	D value (minutes ± S.E.)					
	55 °C			60 °C		
	Homogenized Egg	Egg yolk	Albumen	Homogenized egg	Egg yolk	Albumen
S. typhimurium PT141	2·3 ± 0·3	8·0 ± 0·1	1·0 ± 0·2	0·3 ± 0·1	0·8 ± 0·1	0·3 ± 0·1
S. enteritidis PT4	6·4 ± 0·6	21·0 ± 1·5	1·5 ± 0·2	0·4 ± 0·1	1·1 ± 0·2	0·2 ± 0·1
S. senftenberg 775W	34·3 ± 1·2	42·0 ± 1·0	3·0 ± 0·2	5·6 ± 0·1	11·8 ± 0·2	0·8 ± 0·1

Table 3. *The effect of culture age on the death rates of salmonellas in homogenized whole egg*

	D 60 °C value (min ± S.E.)	
Strain	Stationary phase* cells	Log phase* cells
S. typhimurium PT141	0·33 ± 0·06	0·21 ± 0·04
S. enteritidis PT13a	0·50 ± 0·1	0·25 ± 0·07
S. enteritidis PT8	0·62 ± 0·1	0·29 ± 0·06
S. enteritidis PT4	0·90 ± 0·08	0·41 ± 0·06

* Log phase cells were from a 24 h broth culture and stationary phase cells from a 48 h culture.

DISCUSSION

Salmonella enteritidis PT4 has been shown to be more resistant to heat than some other common egg-associated salmonellas (Table 1). This confirms earlier observations [1]. Previous work has also shown that when the inoculum exceeds 10^8 cells per egg, *S. enteritidis* PT4 can be isolated from eggs cooked in a variety of ways, including boiling for up to 10 min [1], even though the measured temperature of the cooked yolk exceeds that used to pasteurize liquid egg. This could have led to the belief that *S. enteritidis* PT4 is exceptionally heat resistant. The results presented in this paper show that this is not the case. The organism was significantly more heat sensitive than *S. senftenberg* 775W and at 55 °C, for example, there was a fivefold difference in the *D* values for the two organisms (Table 1).

Salmonella enteritidis can be isolated from raw liquid eggs entering pasteurizing plants [2]. The death rates of this organism in homogenized whole egg indicate that even if large numbers of organisms were present in a batch of egg prior to pasteurization, and this is extremely unlikely given the prevalence of contaminated egg [2], *S. enteritidis* PT4 would not survive the heating process. Thus salmonellas should not be isolated from properly pasteurized liquid egg.

Cooked eggs were identified as sources of *S. enteritidis* PT4 infection in England

and Wales in 1988 [2]. The information presented in this paper (Table 2) coupled with the earlier observations that salmonellas in the yolks of shell eggs stored at room temperature rapidly reach stationary phase [1] provide an explanation for the ability of *S. enteritidis* PT4 to survive cooking. Egg yolk is protective and significantly increases the heat resistance of salmonellas (Table 2). Cells grown at 37 °C for 48 h and in stationary phase are also much less heat sensitive than cells grown for 24 h even though many of the latter population would have been in late log phase (Table 3). Thus, a combination of these two factors may have permitted the survival of sufficient viable cells to constitute an infective dose. Despite this, the results presented in this paper show that, whilst *S. enteritidis* PT4 is more heat resistant than some other salmonellas, it is not exceptional. What sets it apart from other organisms is its presence in egg contents [4–6] particularly in the yolks of intact shell eggs.

Although *S. enteritidis* PT4 should not survive pasteurization in liquid egg the very small risk of it doing so can be further reduced by the use of fresh eggs and storage at temperatures low enough to inhibit growth, prior to pasteurization.

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