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The role of home-made ice cream as a vehicle of *Salmonella enteritidis* phage type 4 infection from fresh shell eggs

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SUMMARY

A family outbreak of *Salmonella enteritidis* PT4 infection is described in which home-made ice cream was identified as the vehicle of infection. The ice cream contained approximately 10^5 *S. enteritidis* PT4 organisms per gm and was probably contaminated by an infected shell egg containing between 10^5–10^8 organisms. The continued relevance of the Chief Medical Officer's warning on the use of raw shell eggs is highlighted.

Home-made ice cream using the same recipe as ice cream that had been incriminated as the cause of the family outbreak of *S. enteritidis* PT4 infection was used to study the growth of the organism that might have occurred in the 3–4 h it took to prepare the product. When the inoculum was in the stationary phase, as it would be from shell or other cross contamination, there was a lag phase of 3 h before growth occurred at room temperature. Even when actively multiplying organisms were introduced, as may be found in an infected egg, there was less than 3 $\log_{10}$ increase in the salmonella count in 4 h at room temperature. It was, therefore, given the high *S. enteritidis* count, unlikely that the ice cream was cross-contaminated.

By contrast, raspberry sorbet at pH 3·73 proved to be lethal to a large inoculum of *S. enteritidis* and may be a relatively safe raw egg containing product.

INTRODUCTION

Outbreaks and sporadic cases of *Salmonella enteritidis* PT4 have frequently been attributed to the consumption of a foodstuff containing fresh shell eggs [1]. Although epidemiological and microbiological studies have identified vehicles of infection that contained fresh shell eggs [2–4], and shell eggs have been found to contain *S. enteritidis* PT4 [5], their exact role in the current *S. enteritidis* epidemic has been debated [6].

Many recipes [7], including that for home-made ice cream, appearing in current recipe books and magazines, which require little or no heating contain fresh shell eggs [8]. These recipes usually produce only small quantities which are almost completely consumed by the time their causal relationship to illness has been

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established. Commercial production of ice cream and the heat treatment of its ingredients is controlled by statute [9] and has not been associated with any outbreak of salmonella infection for many years. In September 1991 the Public Health Laboratory Service, Communicable Disease Surveillance Centre (CDSC) received three reports of family outbreaks of *S. enteritidis* PT4 infection associated with the consumption of home-made ice cream [10]. One of those outbreaks presented an opportunity to examine an incriminated vehicle of *S. enteritidis* PT4 infection in detail. We describe the events relating to an incident in which poor storage, kitchen hygiene and culinary practices may be discounted and the importance of the infection of shell eggs is highlighted.

Sufficient ice cream contaminated with a pure culture of *S. enteritidis* PT4 and raspberry sorbet, made at the same time, and uncontaminated ice cream made to the same recipe were available to enable a series of experiments to be undertaken. These were designed to determine the rates of growth of *S. enteritidis* PT4 in home-made ice cream. The experiments were carried out in a manner resembling the conditions prevailing at the time the ice cream was made.

THE OUTBREAK

Two sisters suffered gastroenteritis within 24 h of consuming home-made ice cream. The first case, A, ate three tablespoonsful (approximately 100 gm) of home-made chocolate chip ice cream at lunchtime on the day after its preparation. Her symptoms began that evening. Thirteen days later, her sister, B, visited and ate 'a large quantity', probably more than 200 gm of the chocolate chip ice cream followed by home-made raspberry sorbet. The following morning she had severe diarrhoea and as she was a hospital nurse she submitted a specimen of faeces to Leeds Public Health Laboratory (PHL) from which *S. enteritidis* PT4 was isolated.

The two sisters suspected the ice cream as the cause of their illness because only they had eaten the ice cream and both were ill within 24 h. *S. enteritidis* PT4 was isolated from a subsequent faecal sample from A. Neither sister had eaten poultry in the 72 h before their onset of illness. Leeds PHL isolated *S. enteritidis* PT7 (a variant which is usually derived from PT4) [11] from a 25 gm sample of the chocolate chip ice cream and *S. enteritidis* PT4 from a 25 gm sample of the raspberry sorbet. The samples were taken by case B, first from the chocolate chip ice cream and then the raspberry sorbet with a spoon which was not cleaned or disinfected in-between.

Preparation of ice cream

During a period of warm weather, when maximum daily air temperatures were between 20 °C and 26 °C, A, who was 36 weeks pregnant, and her friend C purchased the ingredients for home-made ice cream at about 11.00. from a nearby large supermarket of a national chain. These included eggs and whipping cream. The eggs were the supermarket's own brand class A, size 2 in three cardboard boxes of six. In the supermarket the eggs were held at ambient temperature. The cream was a pint pot of the supermarket's own brand pasteurized whipping cream, which had a plastic cover over the foil lid, and had

been stored in a refrigerated display. Two of the boxes of eggs were stored by A in her refrigerator. The other box was left in C's car. Around 15.30 C washed her hands thoroughly and made ice cream and sorbet in A's kitchen.

The ice cream consisted of 8 oz. (227 gm) icing sugar, 8 egg yolks, 8 egg whites and 1 pint (560 ml) whipping-cream. The eggs, from A's refrigerator, were separated by hand and the yolks were put into the bowl of a food-mixer, and the icing sugar added from a previously unopened packet. The cream was then placed in a clean bowl and whipped with an electric hand-mixer until thick. The hand-mixer was cleaned in hot water and detergent and was then used to whip the egg whites until they were stiff and then they were added to the yolks and icing sugar mix in the food mixer bowl. Half this mixture was put in a plastic container and chocolate chips were added. The other half, vanilla ice cream, was put in a clean 1 l container which had previously held commercial ice cream. The ice cream took about 20 min to prepare. It was immediately put into the freezer and took about 3 h to freeze. A knew this because the container of chocolate chip ice cream was turned until it was frozen. C then made a raspberry sorbet with $\frac{1}{2}$ pint (280 ml) water, 6 oz. (170 gm) granulated sugar, juice $\frac{1}{2}$ lemon, 1 pound (454 gm) home grown frozen raspberries and 2 egg whites. Eggs from the boxes in A's refrigerator purchased earlier that day were separated by hand and the yolks were later discarded. After preparation, C tasted a teaspoonful (approximately 5 gm) of the ice cream before it was frozen but did not suffer any symptoms. A faecal sample from C was examined and found to be salmonella negative by another laboratory some 4 weeks after making the ice cream.

All utensils were carefully cleaned in hot water and detergent before and after each stage of preparation. Work surfaces were also disinfected with a quaternary ammonium preparation. C's kitchen practice appeared to be of a high hygienic standard. There was no poultry stored in the refrigerator or freezer.

The following day C made rum and raisin ice cream at her own home using the eggs she had bought the previous day and left in her car whilst she prepared A's ice cream. *S. enteritidis* was not isolated from this ice cream.

MATERIALS AND METHODS

Laboratory investigations of the ice creams

Samples of the chocolate chip ice cream and sorbet, taken by B were examined qualitatively by Leeds PHL. All of the vanilla (470 gm) and the remaining contaminated chocolate chip ice cream (241 gm), the remaining sorbet and C's rum and raisin ice cream were stored in domestic freezers and were kept in the frozen state until they were transported to Hull PHL. The ice creams and sorbet were allowed to thaw and soften for aliquots to be taken for the salmonella counts and growth experiments. All of the growth experiments were conducted with the ice creams and sorbet in the liquid state.

The vanilla, chocolate chip ice cream, raspberry sorbet and C's rum and raisin ice cream were examined qualitatively and quantitatively by Hull PHL. Portions of the three ice creams and the sorbet were sent for confirmation of the results to the Food Hygiene Laboratory, Central Public Health Laboratory, London.

For qualitative salmonella isolation, 25 gm aliquots of the ice cream were pre-enriched by overnight incubation in Buffered Peptone Water (BPW) at 37 °C and enriched in Rappaport-Vassiliadis R10 broth [12] at 41 ± 1 °C overnight. Each broth culture was plated on Xylose Lysine Desoxycholate (XLD) and Bismuth Sulphite (BS) agars [13]. After overnight incubation, typical salmonella colonies were identified using antisera provided by PHLS Laboratory of Microbial Reagents and were confirmed and phage typed by the PHLS Laboratory of Enteric Pathogens, Central Public Health Laboratory, London.

The first quantitative salmonella estimation was by a Most Probable Number (MPN) technique initially by enriching 10 × 10 gm aliquots in 120 ml volumes of BPW. After overnight incubation at 37 °C the broths were plated on XLD and BS agars and after overnight incubation at 37 °C typical salmonella colonies were identified by slide agglutination. Subsequently, successive decimal dilutions of the ice-cream were made in Peptone Salt Diluent (PSD) to give the equivalent of 5 × 1 gm, 5 × 0·1 gm, 5 × 0·01 gm, 5 × 0·001 gm and 5 × 0·0001 gm amounts until no *S. enteritidis* was isolated from any of the 5 tube series. The original 10^{-1} dilution was frozen and thawed between each stage. The MPN of *S. enteritidis* PT4 was estimated from modified tables [14].

For subsequent quantitative salmonella estimations, 0·1 ml of a series of decimal dilutions of the ice cream in PSD was spread onto XLD agar and the number of salmonella colonies counted after 18 h incubation at 37 °C. The ice creams and sorbet were also plated on non-selective agars for the isolation of other organisms that may have been present.

For the growth experiments, salmonella counts were performed by inoculating two decimal dilutions of the ice creams onto XLD Agar using a Spiral Plater (Don Whitley Scientific Limited).

Growth of Salmonella enteritidis *in home-made ice cream*

For these experiments the salmonella-free rum and raisin ice cream and raspberry sorbet were used as the culture media. The inoculum of *S. enteritidis* was either a portion of the contaminated vanilla ice cream or a 10^{-1} dilution of that ice cream in salmonella-free pasteurized liquid egg (PLE).

Growth in ice cream: inoculum without pre-incubation

A 10^{-1} dilution was made by adding 2 ml of the contaminated vanilla ice cream to 18 ml of the salmonella-free rum and raisin ice cream. This ice cream was thoroughly mixed by repeated inversion. This 10^{-1} dilution of contaminated ice cream was incubated at ambient temperature (22 °C) and the *S. enteritidis* enumerated at 1, 2, 3, 4 and 5 h from the start of incubation. This was repeated nine times.

Growth in ice cream: pre-incubated inoculum of multiplying organisms in egg

Two ml of vanilla ice cream was added to 18 ml of PLE known to be salmonella-free. The mixture was incubated at 37 °C. After 2 h incubation, 2 ml of the mixture was added to 18 ml of rum and raisin ice cream. The mixture of contaminated ice cream and PLE was incubated at ambient temperature and salmonella counts were performed as above. The experiment was repeated four times.

Table 1. *Counts of* S. enteritidis *PT4 in the ice cream and sorbet samples*

	Leeds PHL	Hull PHL	Food Hygiene Laboratory, London
Chocolate chip	+ve in 25 gm*	$3{\cdot}2 \times 10^5$/gm (MPN 8×10^2/gm†)	$2{\cdot}1 \times 10^5$/gm
Vanilla	NT‡	$4{\cdot}0 \times 10^5$/gm (MPN 8×10^2/gm†)	$1{\cdot}5 \times 10^5$/gm
Sorbet	+ve in 25 gm	ND§ in 300 gm	ND in 25 gm
Rum and raisin	NT	ND in 300 gm	ND in 25 gm

* *S. enteritidis* PT7.
† 95% confidence limits 300–2400.
‡ NT, not tested.
§ ND, not detected.

Incubation in raspberry sorbet

A 10^{-1} dilution was made by adding 2 ml of ice cream contaminated with *S. enteritidis* to 18 ml of raspberry sorbet. This mixture was incubated at ambient temperature (22 °C) and salmonella counts were performed as above at 0, 1, 2, 3, 4 and 5 h. This experiment was repeated four times with additional qualitative salmonella cultures from 25 gm at 2, 3, 4, 5 and 18 h. The pH of the raspberry sorbet was measured with a Jenway 3020 pH meter, accurate to two decimal places.

RESULTS

Laboratory investigations of the ice creams

S. enteritidis PT7 was isolated from a 25 gm sample of chocolate chip ice cream and *S. enteritidis* PT4 from a 25 gm sample of raspberry sorbet examined by Leeds PHL. *S. enteritidis* PT4 was isolated by Hull PHL from the remaining chocolate chip and the previously untested vanilla ice cream but not from the raspberry sorbet nor the rum and raisin ice cream. Plate counts by Hull PHL and the Food Hygiene Laboratory indicated that the *S. enteritidis* count was approximately 10^5 per gm in pure culture. An initial MPN estimation of the *S. enteritidis* present in the chocolate chip and vanilla ice creams suggested that the count was approximately 800 *S. enteritidis* per gm with 95% confidence limits of 300–2400. The results of the salmonella cultures on the ice creams are summarized in Table 1. The raspberry sorbet was at pH 3·73.

Growth in ice cream: without pre-incubation

From an initial count of approximately 10^4 *S. enteritidis* per gm (Fig. 1), there was a lag phase of 3 h. Thereafter growth occurred with a mean generation time of 21 min.

Growth in ice cream: inoculum organisms pre-incubated in egg

Where the organism was pre-incubated in PLE (Fig. 2), there was no lag phase and growth occurred with a mean generation time of 17 min. There was more

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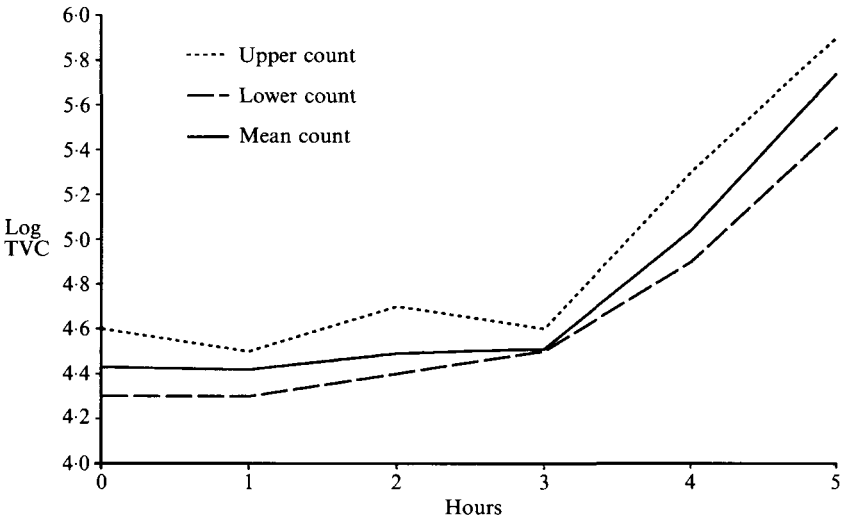

Fig. 1. Growth of *S. enteritidis* PT4 in ice cream: inoculum of contaminated ice cream without preincubation.

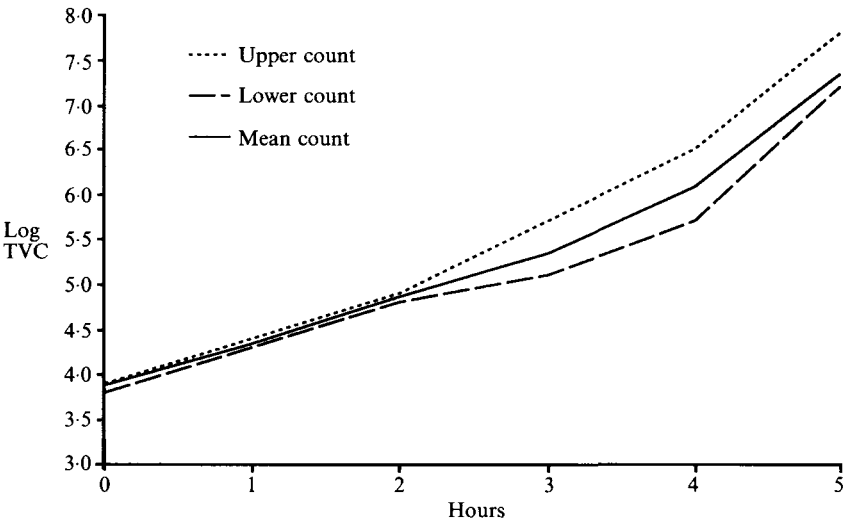

Fig. 2. Growth of *S. enteritidis* PT4 in ice cream: inoculum of ice cream with liquid egg previously incubated for 2 hours.

variability in the counts at each stage than in the experiment with the inoculum in ice cream.

Incubation in raspberry sorbet

From an initial count of approximately 10^4 *S. enteritidis* per gm, there was a decline to less than 10^2 organisms per gm in 2 h. The subsequent experiments demonstrated a decline to undetectability in 25 gm in 5 h at room temperature as shown in Table 2.

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Table 2. *Counts of* S. enteritidis *PT4 in raspberry sorbet incubated at room temperature*

		Start	1 h	2 h	3 h	4 h	5 h	18 h
Aliquot 1	TVC*	$2{\cdot}2 \times 10^4$	$4{\cdot}0 \times 10^3$	$1{\cdot}0 \times 10^3$	$< 10^3$	$< 10^3$	$< 10^3$	NT†
	25 g	NT	NT	+	+	−	−	−
Aliquot 2	TVC	$1{\cdot}8 \times 10^4$	$4{\cdot}0 \times 10^3$	$< 10^3$	$< 10^3$	$< 10^3$	$< 10^3$	NT
	25 g	NT	NT	+	+	+	−	−
Aliquot 3	TVC	$2{\cdot}8 \times 10^4$	$1{\cdot}0 \times 10^3$	$1{\cdot}0 \times 10^3$	$< 10^3$	$< 10^3$	$< 10^3$	NT
	25 g	NT	NT	+	+	−	−	−
Aliquot 4	TVC	$1{\cdot}2 \times 10^4$	$3{\cdot}0 \times 10^3$	$1{\cdot}0 \times 10^3$	$< 10^3$	$< 10^3$	$< 10^3$	NT
	25 g	NT	NT	+	+	−	−	−

* TVC, Total viable count.
† NT, Not tested.

DISCUSSION

This episode presented a rare opportunity to examine an incriminated vehicle of a *S. enteritidis* PT4 outbreak in detail. *S. enteritidis* was present in pure culture in both the chocolate chip and vanilla ice creams, probably at about 10^5 organisms per gm. The *S. enteritidis* PT7 found by Leeds PHL in the chocolate chip ice-cream was probably a spontaneously occurring lipopolysaccharide deficient variant of the *S. enteritidis* PT4 present [11]. As the ice cream had remained frozen from the time of consumption, the number of *S. enteritidis* present at the time of testing would be expected to be little changed from then. The cases, A and B, would therefore have ingested $\geqslant 10^7$ organisms, whereas C, who probably ingested 10^5–10^6 organisms in the small amount of the ice cream she tasted, remained symptom free. These findings do not, however, preclude the possibility that small numbers of *S. enteritidis* in a suitable vehicle may be infective in susceptible persons. The initial finding of 800 organisms per gm by the MPN method, with its wide 95% confidence limits of 300–2500, was probably an under-estimate caused by the lethal effect of the repeated freezing and thawing of the 10^{-1} dilution of the ice cream in PSD used in this method.

The preparation of the ice cream and sorbet has been described in detail and shows that cross contamination of the ice cream during preparation was unlikely. Since the quantity of ice cream produced weighed approximately 1 kg, the total number of *S. enteritidis* in the ice cream was about 10^8 organisms. A series of experiments were undertaken to mimic as closely as possible the conditions that might occur during the preparation of home-made ice cream. The source of *S. enteritidis* for these experiments was the contaminated vanilla ice cream containing approximately 10^5 *S. enteritidis* per gm in pure culture. By using organisms which had not been cultivated on artificial laboratory media, the effects of adaptation to those media were avoided. Contaminated PLE was used to mimic the effect of adding the contents of a shell egg in the albumen of which *S. enteritidis* was growing as described by Humphrey [5].

Home-made ice cream proved to be a nutritious medium for the growth of *S. enteritidis* PT4 giving mean generation times of 17–21 min at kitchen ambient temperature (22 °C). There was, however, a lag phase of 3 h indicating that the

inoculum was in the stationary phase. The vanilla ice cream had been kept frozen since the outbreak and the organisms were probably in the stationary phase due to the effects of the cold shock. When the inoculum in PLE had been pre-incubated, growth was continuous but, in 4 h, the increase in number of organisms was less than 3 $\log_{10}$.

A small inoculum would be unable to increase in number sufficiently to explain the high salmonella content of the original ice cream, unless the preparation time at ambient temperature was very protracted. The inoculum into the ice cream mixture was, therefore, probably between 10^5 and 10^8 organisms. It is unlikely that such large numbers of *S. enteritidis* could have been introduced from the egg shells or other sources of cross-contamination. Furthermore, such contamination would probably have introduced a mixture of other faecal and environmental organisms. Similarly contamination of the ice creams by C, if she had been a symptomless excreter, is equally implausible.

S. enteritidis PT4 has been isolated from the contents of clean unbroken shell eggs [5, 15–17] and fresh shell eggs have been incriminated in several outbreaks of *S. enteritidis* PT4 infection [2, 15]. In these outbreaks, contamination of the food vehicles, home-made ice cream, almond parfait and chocolate mousse, by large numbers of *S. enteritidis* must have occurred as there would have been little or no opportunity for bacterial multiplication to have taken place during their preparation prior to chilling or freezing. Shell eggs containing 10^5 organisms to $> 10^7$ *S. enteritidis* per gm have previously been described [5, 17]. Humphrey reported the growth of *S. enteritidis* PT4 in the albumen of eggs stored for over 3 weeks at ambient temperature [5] and the occasional egg in which multiplication of artificially introduced *S. enteritidis* begins soon after laying (Humphrey, TJ, personal communication). The supermarket chain from which the eggs were purchased set a sell-by date for their eggs of 2 weeks after the packing date. It was, unfortunately, not possible to trace the source and packing date of the eggs used as the cartons has been discarded. It is possible that a high ambient temperature due to the warm weather had accelerated the growth process in the egg(s).

Of the other ingredients, only cream has previously been associated with salmonella infections. The pasteurized whipping cream was the brand of a national chain of supermarkets and if that had been heavily contaminated there would have been a large community outbreak.

The results reported here are consistent with the contents of eggs, or more likely in view of their low incidence [5, 18] of infection a single egg being the source of contamination. The individuals involved in this outbreak said that since there had been no recent publicity about eggs and salmonella they thought the problem was over and used a recipe for the ice cream from a popular cookery book. This incident demonstrates that the Chief Medical Officer's warning [19] about the use of uncooked and partially cooked eggs should still be heeded. The use of raw eggs in such products will remain a hazard until all egg laying flocks are free from *S. enteritidis* PT4.

By contrast the raspberry sorbet was lethal at room temperature to even large numbers of *S. enteritidis*. It is probably a relatively safe product providing that the pH is sufficiently acid and there is a sufficient contact time with the organisms during preparation. The frozen sorbet did not appear to have a bactericidal effect comparable to that of its liquid state. The contamination of the sorbet sample.

however, by the spoon used to sample the chocolate chip ice cream illustrates the ease with which cross contamination can occur with heavily contaminated egg-containing dishes.

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