Epidem. Inf. (1988), **101**, 75–82
Printed in Great Britain

Salmonella typhimurium phage type 141 infections in Sheffield during 1984 and 1985: association with hens' eggs

By P. A. CHAPMAN

Public Health Laboratory, Northern General Hospital, Herries Road, Sheffield S5 7AU

P. RHODES and WENDY RYLANDS

Food Safety Section, Environmental Health Department, Town Hall Chambers, Barkers Pool, Sheffield S1 1EN

(*Accepted 8 February 1988*)

SUMMARY

Food poisoning due to *Salmonella typhimurium* phage type 141 was unusual in the Sheffield area before 1984. The sudden increase in incidence of this phage type during 1984 and 1985, and its causative role in several small outbreaks in this period have been investigated. Epidemiological and laboratory investigations suggested that hens' eggs were the most likely source of *S. typhimurium* phage type 141.

INTRODUCTION

During 1984, 68 human infections with *Salmonella typhimurium* phage type 141 were confirmed amongst the population of Sheffield, and a further 73 cases in surrounding districts. In the same year 546 cases of the same phage type were confirmed in the UK (518 in England and Wales), 320 (59%) of them occurring in the Northern, Yorkshire and Trent Regional Authorities. Before this, infections with *S. typhimurium* phage type 141 had been uncommon, though the phage type was associated with outbreaks from bovine sources in Scotland in the 1970s (Anderson *et al.* 1978). In 1982 and 1983 there were only 21 and 43 isolations of this phage type notified in the UK (Palmer & Rowe, 1986), of which 3 and 27 were in Sheffield.

It was therefore decided to study in detail the Sheffield cases occurring between 1 January 1984 and 31 July 1985. During this 19-month period five outbreaks and several sporadic cases occurred in and around Sheffield.

OUTBREAKS

Outbreak 1 (June 1984) and outbreak 2 (November 1984)

The first outbreak of gastroenteritis occurred after a child's birthday party and affected 14 children and 1 adult. Twelve of the children and the adult attended the party and ate a variety of foods; all ate birthday cake. The remaining two children, who did not attend the party, ate birthday cake (and no other foods) which had been brought home from the party by their brothers. It therefore seems likely that the birthday cake was the vehicle of infection. *S. typhimurium* phage type 141 was isolated from faecal samples from 10 children who attended the party, the two who did not, and from the adult.

Outbreak 2 affected 10 children at a small school in an outlying district of Sheffield. All 10 had eaten cake served as part of a school meal; children who had not eaten the cake were unaffected. *S. typhimurium* phage type 141 was isolated from the 10 children with gastroenteritis.

In both outbreaks, the preparation of the cake was unusual in that raw shell eggs were used in making the butter icing for the cake. The icing was not cooked and was added to the cakes after they were baked. No cake from either incident was available for bacteriological examination. In outbreak 1, the eggs were purchased from a supermarket in Sheffield and marketed by a co-operative of egg producers in Lincolnshire. The source of the eggs in outbreak 2 could not be traced.

Outbreak 3 (August 1984)

Six people were ill after eating vanilla slices manufactured by a small bakery in Sheffield. The practice in the bakery was to use the same mixing bowl for products which were to be baked, such as egg custards, and those which were to receive no heat treatment such as cold-mix custards, without properly cleaning the bowl between mixes. Eggs which were mixed in the bowl were shell eggs obtained from the same marketing co-operative as in outbreak 1. *S. typhimurium* phage type 141 was isolated from six patients. The vanilla slices were not available for examination; vanilla slices from another batch did not reveal salmonellae.

Outbreak 4 and outbreak 5 (July 1985)

In outbreak 4, at least 18 people were ill after attending a christening celebration, at which food was provided by a small Sheffield-based caterer. Three foods (savoury quiche, banana pie and a fruit flan) which were made using raw shell eggs were found to contain large numbers of *S. typhimurium* phage type 141. Salmonellae were not isolated from another seven dishes made without eggs. *S. typhimurium* phage type 141 was isolated from eight people, each of whom had eaten at least one of the suspect foods.

Outbreak 5 affected nine people, all of whom had eaten a meringue pudding at a restaurant served by the same caterer as in outbreak 4. Raw shell eggs were used in the meringues which were presumed to be the vehicle of transmission, though none were available for examination. Meringues generally do not receive high heat treatment. *S. typhimurium* phage type 141 was isolated from nine patients. The eggs used by the caterer were from the same co-operative as those implicated in outbreaks 1 and 3.

Sporadic cases

A descriptive epidemiological study was undertaken of the sporadic cases that had occurred during the 19-month period to identify any common factors. Of the 48 such cases identified during 1984, comprehensive food histories were obtained from only 21, and incomplete food histories from a further 5 cases. Sixteen of the

21 cases had eaten eggs (either boiled, scrambled, fried or poached) in the 48 h prior to onset of symptoms, and in 12 the eggs could be traced back to the same co-operative as that associated with the outbreaks. No other food link was established between the cases. The study was not case-controlled.

MATERIALS AND METHODS

Examination of eggs

A co-operative of 11 Lincolnshire egg producers supply nearly 500000 eggs weekly, mostly to the Sheffield area, through a marketing depot in Doncaster.

One thousand whole shell eggs were examined for salmonellae over a period of 5 weeks. Eggs in batches of 10 were cracked into a sterile container and blended until of a uniform consistency. A 10 ml volume of the whole egg was then transferred to 100 ml of tetrathionate broth and incubated at 37 °C. This was subcultured to brilliant green MacConkey agar at 24 and 48 h and colonies resembling those of salmonellae were further investigated by standard techniques.

Each individual farm in the co-operative produced bulk liquid egg which was also examined for salmonellae. Five 10 ml portions were added to individual 100 ml amounts of tetrathionate broth and processed as above.

Survival of salmonellas in cooked eggs

Two small experiments were devised to determine the feasibility of salmonella infection occurring after consumption of a lightly cooked egg.

In the first the inside temperature of eggs was measured using a digital thermometer. Using a steel spike, a hole about 4 mm diameter was made in the shell at the airspace end of the egg. While viewing the egg against a strong light, a piece of plastic tubing (internal diameter about 3 mm) was inserted until the end was about 5 mm above the centre of the egg. After trimming to leave 10 mm of tube outside the egg, this exposed tube was cut lengthways and folded into a small flange which was fastened to the egg with adhesive. The probe from a digital thermometer was then inserted through the tubing until the sensor protruded about 5 mm from the end of the plastic tube (see Figure 1). The egg was then held in a wire support, placed in boiling water, and the water level adjusted to be just below the hole in the top of the egg. Water was kept boiling and temperature was recorded initially and at 30 s intervals for 8 min. Twelve size 2 eggs were examined, 6 which had been stored at ambient temperature (21 °C) and 6 which had been stored at larder refrigerator temperature (8 °C).

Survival of S. typhimurium *phage type 141 during heating*

The survival of *S. typhimurium* phage type 141 in whole egg or phosphate buffered saline (PBS) was examined at temperatures of 50 and 65 °C.

Fresh whole egg was prepared by disinfecting the shell exteriors of 12 eggs using 70% ethanol in water, removing the contents into a sterile container and aseptically blending until of a uniform consistency. Then 10 ml portions of whole egg and 10 ml portions of PBS were preheated to the test temperature in a thermostatically controlled water bath. Also 10 μl volumes of an overnight broth culture of *S. typhimurium* were added simultaneously to whole egg and PBS. As

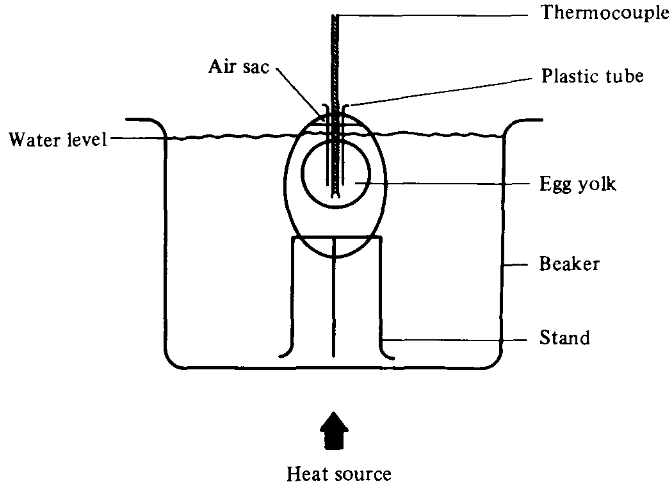

Fig. 1. Measurement of inside temperature of eggs.

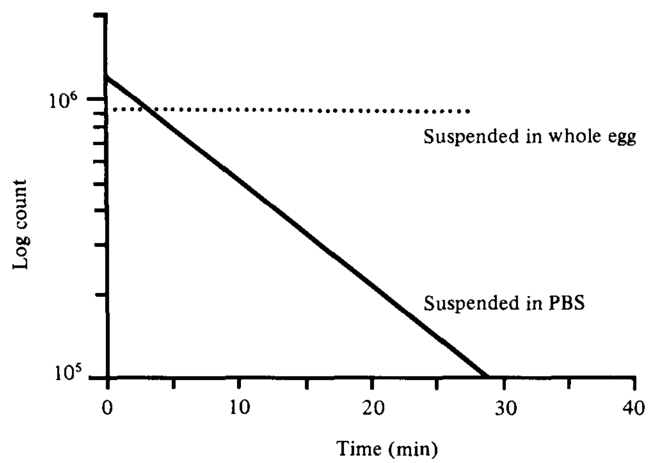

Fig. 2. Survival of *S. typhimurium* phage type 141 in phosphate buffered saline (PBS) and whole egg at 50 °C.

quickly as possible, these were mixed and 100 μl volumes transferred to 900 μl volumes of PBS at refrigerator temperature. This sampling procedure was then repeated at 1 min intervals for 30 min. Salmonellae in these suspensions were then enumerated using a spiral inoculator and nutrient agar plates.

RESULTS

Examination of eggs

Salmonellae were not isolated from the 1000 shell eggs examined. *S. typhimurium* phage type 141 was isolated from bulk egg produced by 5 of 11 farms; *S. typhimurium* phage type 104 and *S. virchow* were also isolated from some samples of bulk liquid egg.

After 4 min in boiling water, the temperature in the centre of the eggs stored at

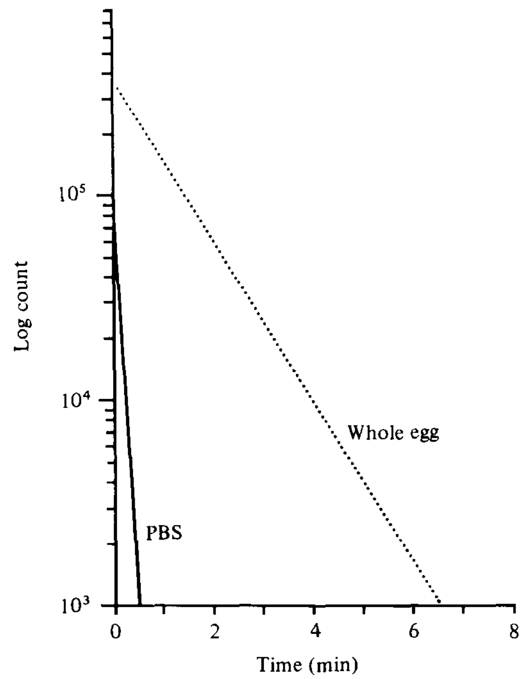

Fig. 3. Survival of *S. typhimurium* phage type 141 in phosphate buffered saline (PBS) and whole egg at 65 °C.

ambient temperature (21 °C) did not rise above 40 °C, the centre of eggs stored in a larder refrigerator (8 °C) did not rise above 28 °C.

Survival of S. typhimurium *phage type 141 during heating*

Suspensions of *S. typhimurium* phage type 141 in saline were quickly killed by heat, but suspensions in whole egg were much more stable (see Figs 2 and 3). Suspended in whole egg, viable counts remained unaltered after 30 min at 50 °C. Using suspensions in whole egg at 65 °C, 3·5 min were needed to reduce viable counts from 5×10^5 to 10^4/ml and more than 6 min to further reduce counts to $< 10^3$/ml.

DISCUSSION

The ease with which salmonellae in feedstuffs may colonize poultry flocks and lead to contamination of eggs with salmonellae is well documented (Gordon & Tucker, 1965; Crabb & Walker, 1971). There are several ways in which shell eggs can become contaminated, externally and internally, with microorganisms. Normally the oviduct of the hen, and therefore also the shell and interior of the hens' egg, are free from microorganisms (Stuart & McNally, 1943). Rarely the ovaries and oviduct may be infected with salmonellae which may be deposited through the shell membrane inside the egg (Scott, 1930). *S. typhimurium* does not usually cause illness in chickens (Kirby, 1985*a*,*b*). However, using chickens from specific pathogen-free flocks, Barrow and colleagues (1987) used oral inoculation to study the virulence of 10 different phage types of *S. typhimurium*. *S. typhimurium* phage type 141 was the most virulent type studied; mortality varied from 25 to 100% depending upon the breed and at autopsy there were signs of systemic spread of the organism. It is possible that such high virulence may increase the frequency of infection of ovaries and oviduct and lead to more eggs becoming contaminated with *S. typhimurium* phage type 141. More frequently, however, eggs become contaminated after they are laid (Stokes, Osborne & Bayne, 1956). Bacterial contamination from egg washwater in batteries may be a problem (Pearson, Southam & Holley, 1987).

The hens' egg has several natural defences against bacterial penetration and multiplication, but these may be breached in certain conditions. The shell is not impervious to salmonellae; the time taken to penetrate depends on individual shell porosity, moisture, number and type of salmonellae and temperature (Haines & Moran, 1940; Stokes, Osborne & Bayne, 1956; Board, 1966; Sparks & Board, 1985). Penetration may also be aided by cracking or abrasion of the shell, and by poor cuticle layer formation, both of which are common features of battery-produced eggs. Subsequent multiplication may be enhanced by rough handling and/or storage at high ambient temperature (Ayres, Mundt & Sandine, 1958; Board, 1966; Anderson, Carter & Morley Jones, 1970*a*,*b*). Early studies demonstrated only a low incidence of salmonellae in hens' eggs (Bernstein, 1952; Watts & Mander, 1953). Our failure to isolate salmonellae from 1000 individual shell eggs should therefore be interpreted with caution. *S. typhimurium* is now the commonest serotype responsible for asymptomatic colonization of poultry flocks, the incidence having increased markedly recently (Kirby, 1985*a*,*b*). Battery production has risen from 17·1% of eggs produced in England in 1960 to over 80% presently (Oosterwoud, 1987), and it is quite likely that the incidence of salmonellae in eggs may also have increased concurrently.

Imported egg products have frequently been associated with salmonellosis (Newell, 1955*a*,*b*; Newell, Hobbs & Wallace, 1955; Report 1955, 1958; Smith & Hobbs, 1955), although reports of infection from shell eggs are rare. Watt (1945) confirmed contaminated hens' eggs as the source of *S. montevideo* in a food-poisoning outbreak affecting merchant seamen, and Crowe (1945) confirmed butter icing prepared using hen shell eggs as the source of *S. typhimurium* food poisoning. Our work, and that of others (Clarenburg & Burger, 1950) has shown that normal cooking procedures applied to eggs may not destroy all salmonellae. Recently the thermal resistance of *S. typhimurium* has been shown to be greater if temperature is increased gradually (Mackey & Derrick, 1987), as is true of most light cooking procedures applied to eggs. The infective dose of salmonellae is usually high (DuPont & Hornick, 1973). However, a high fat content in food can protect salmonellae against stomach acid, thus making the infective dose much lower. Infective doses as low as 1–6 organisms have been recorded in salmonellosis due to chocolate (Craven *et al.* 1975; D'Aoust, 1977; Gill *et al.* 1983) or cheese (D'Aoust, 1985); it is possible therefore that similarly low infective doses may be a feature of egg-borne salmonellosis.

Nevertheless, outbreaks of food poisoning associated with eggs and egg products are rare in the UK, even though hens' eggs are often eaten only lightly cooked, and food preparations containing eggs may receive little or no heat treatment. It is likely that the proportion of laying hens excreting salmonellae is low because infection tends to be shed naturally between the growing and laying periods. Those hens remaining infected are almost always clinically healthy carriers and are unlikely to be detected by veterinary investigation (Kirby, 1985*a*,*b*). Egg-associated salmonellosis has been reported from other countries (Todd, 1985), and the large quantities of shell eggs consumed annually by the British public, 12288 million in 1984, (Central Statistics Office, 1986) means that even very low levels of contamination present a sizeable risk.

However, with the exception of outbreak 4, the association of eggs with the outbreaks is presumptive though the circumstantial evidence is convincing even in the absence of case controls. Examination of chickens or litter from battery cages was unfortunately not possible in this study. Further work is needed to clarify the role, if any, of eggs in the marked increase of *S. typhimurium* infections in man in recent years.

We thank Dr B. Rowe, Division of Enteric Pathogens, Central Public Health Laboratory, Colindale, London for phage typing the isolates of *S. typhimurium*, Mr A. Devonald and Miss A. MacFarlane, Sheffield Public Health Laboratory for technical assistance, and the staff of Doncaster Environmental Health Department, Sheffield Environmental Health Department and Sheffield Public Health Laboratory for their contribution to these investigations.

REFERENCES

Anderson, G. B., Carter, T. C. & Morley Jones, R. (1970*a*). Some factors affecting the incidence of cracks in hens' egg shells. *British Poultry Science* **11**, 103–116.

Anderson, G. B., Carter, T. C. & Morley Jones, R. (1970*b*). Some factors affecting dynamic fracture of egg shells in battery cages. In *Factors Affecting Egg Grading* (ed. B. M. Freeman and R. F. Gordon), pp. 53–69. Edinburgh: Oliver and Boyd.

Anderson, E. S., Ward, L. R., De Saxe, M. J., Old, D. C., Barker, R. & Duguid, J. P. (1978). Correlation of phage type, biotype and source in strains of *Salmonella typhimurium*. *Journal of Hygiene* **81**, 203–217.

Ayres, J. C., Mundt, J. O. & Sandie, W. E. (1980). Eggs and egg products. In *Microbiology of Foods* (ed. J. C. Ayres *et al.*), pp. 504–527. San Francisco: W. H. Freeman & Co.

Barrow, P. A., Huggins, M. B., Lovell, M. A. & Simpson, J. M. (1987). Observations on the pathogenesis of experimental *Salmonella typhimurium* infection in chickens. *Research in Veterinary Science* **42**, 194–199.

Bernstein, A. (1952). Salmonella infection of hen eggs. *Monthly Bulletin of the Ministry of Health and the Public Health Laboratory Service* **11**, 64–67.

Board, R. G. (1966). Review article: the course of microbial infection of the hen's egg. *Journal of Applied Bacteriology* **29**, 319–341.

Central Statistics Office (1986). *Annual Abstract of Statistics*. HMSO.

Clarenburg, A. & Burger, H. C. (1950). Survival of salmonellae in boiled ducks' eggs. *Food Research* **15**, 340–341.

Crabb, W. E. & Walker, M. (1971). The control of salmonella in broiler chickens. In *Hygiene and Food Production* (ed. A. Fox), p. 119. Edinburgh and London: Churchill Livingstone.

Craven, P. C., Mackel, D. C., Baine, W. B., Barker, W. H., Gangarosa, E. J., Goldfield, M., Rosenfield, H., Altman, R., Lachapelle, G., Davies, J. W. & Swanson, R. C. (1975). International outbreak of *Salmonella eastbourne* infection traced to contaminated chocolate. *Lancet* i, 788–792.

Crowe, M. (1945). A localised outbreak of *Salmonella* food poisoning apparently transmitted by a hen's egg. *Journal of Hygiene* **44**, 342–345.

D'AOUST, J. Y. (1975). *Salmonella* and the chocolate industry: a review. *Journal of Food Protection* **40**, 718–727.

D'AOUST, J. Y. (1985). Infective dose of *Salmonella typhimurium* in cheddar cheese. *American Journal of Epidemiology* **122**, 717–720.

DUPONT, H. L. & HORNICK, R. B. (1973). Clinical approach to infectious diarrhoeas. *Medicine* **52**, 265–275.

GILL, O. N., SOCKETT, P. N., BARTLETT, C. L. R., VAILE, M. S. B., ROWE, B. & GILBERT, R. J. (1983). Outbreak of *Salmonella napoli* infection caused by contaminated chocolate bars. *Lancet* i, 574–577.

GORDON, R. F. & TUCKER, J. F. (1965). The epizootiology of *Salmonella menston* infection and the effect of feeding poultry food artificially infected with salmonella. *British Poultry Science* **6**, 251–264.

HAINES, R. B. & MORAN, T. (1940). Porosity of, and bacterial invasion through, the shell of the hen's egg. *Journal of Hygiene* **40**, 453–461.

KIRBY, F. D. (1985*a*). Surveillance of animal salmonella infection 1976–1984. *Veterinary Record* **2**, 456–457.

KIRBY, F. D. (1985*b*). Zoonoses in Britain. *Journal of the Royal Society for Health* **105**, 77–87.

MACKEY, B. M. & DERRICK, C. M. (1987). Changes in the heat resistance of *Salmonella typhimurium* during heating at rising temperatures. *Letters in Applied Microbiology* **4**, 13–16.

MURDOCK, C. R. (1954). The occurrence of salmonella in liquid egg. *Monthly Bulletin of the Ministry of Health and the Public Health Laboratory Service* **13**, 43–45.

NEWELL, K. W. (1955*a*). Outbreaks of paratyphoid B fever associated with imported frozen eggs. I. Epidemiology. *Journal of Applied Bacteriology* **18**, 462–470.

NEWELL, K. W. (1955*b*). Paratyphoid B fever possibly associated with chinese frozen eggs. *Monthly Bulletin of the Ministry of Health and the Public Health Laboratory Service* **14**, 146–154.

NEWELL, K. W., HOBBS, B. C. WALLACE, E. J. G. (1955). Paratyphoid fever associated with chinese frozen whole egg: outbreaks in two bakeries. *British Medical Journal* **2**, 1296–1298.

OOSTERWOUD, A. (1987). Effect of egg handling on egg quality. In *Egg Quality, Current Problems and Recent Advances* (ed. R. G. Wells and C. G. Belyavin), pp. 283–291. Poultry Science Symposium No. 20. London: Butterworths.

PALMER, S. R. & ROWE, B. (1987). Trends in *Salmonella* infections. *P.H.L.S. Microbiology Digest* **3**, 18–22.

PEARSON, J., SOUTHAM, G. G. & HOLLEY, R. A. (1987). Survival and transport of bacteria in egg washwater. *Applied and Environmental Microbiology* **53**, 2060–2065.

REPORT (1955). Salmonella in liquid egg, including frozen liquid egg. A report of the Public Health Laboratory Service. *Monthly Bulletin of the Ministry of Health and the Public Health Laboratory Service* **14**, 65–69.

REPORT (1958). The contamination of egg products with salmonellae, with particular reference to *Salm. paratyphi* B. *Monthly Bulletin of the Ministry of Health and the Public Health Laboratory Service* **17**, 36–51.

SCOTT, W. M. (1930). Food poisoning due to eggs. *British Medical Journal* **2**, 56–58.

SMITH, M. E. & HOBBS, B. C. (1955). *Salmonella* in chinese frozen egg. *Monthly Bulletin of the Ministry of Health and the Public Health Laboratory Service* **14**, 154–160.

SPARKS, N. H. C. & BOARD, R. G. (1985). Bacterial penetration of the recently oviposited shell of hens' eggs. *Australian Veterinary Journal* **62**, 169–170.

STUART, L. S. & MCNALLY, E. H. (1943). Bacteriological studies on the shell egg. *U.S. Egg and Poultry Magazine* **49**, 28–47.

STOKES, J. L., OSBORNE, W. W. & BAYNE, H. G. (1956). Penetration and growth of salmonella in shell eggs. *Food Research* **21**, 510–518.

TODD, E. C. D. (1985). Foodborne and waterborne disease in Canada – 1978 annual summary. *Journal of Food Protection* **48**, 990–996.

WATT, J. (1945). An outbreak of *Salmonella* infection in man from infected chicken eggs. *Public Health Reports* **60/2**, 835–839.

WATTS, P. S. & MANDER, S. (1953). An investigation into the incidence of salmonellae in South Australian eggs and egg pulp. *Monthly Bulletin of the Ministry of Health and the Public Health Laboratory Service* **12**, 127–129.