

## The use of sequential studies in a salmonellosis outbreak linked to continental custard cakes

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### SUMMARY

We investigated an outbreak of 54 cases of *Salmonella* Typhimurium phage type 9 (STM9) with a specific antibiotic resistance pattern. We used sequential analytic studies: two retrospective cohort studies, a case-control study, and a modified case-control study. An outbreak of salmonellosis due to *Salmonella* Typhimurium PT9 SSu (resistant to streptomycin and sulphafurazole) was identified. Fifty-four cases had illness onset from November 1998 to March 1999. Notifications commenced following a restaurant birthday party in December 1998. An initial cohort and case control study found no association with consumption of custard cake. However, case follow-up identified another cohort of people who had attended a birthday party in February at which 8/27 people who consumed a continental custard cake were ill compared to 0/10 who did not ( $P = 0.07$ ). A revised case control study found illness was strongly associated with consumption of a particular continental custard cake (Mantel–Haenszel matched OR  $\infty$ ,  $P = 0.00004$ ). This report highlights the epidemiological value of using sequential study types, and persisting with the investigation of apparently sporadic food-borne outbreaks.

### INTRODUCTION

In Australia, reports of food poisoning are increasing and are estimated to cost the community more than 2.6 billion dollars every year [1]. *Salmonella* Typhimurium is one of the major serovars causing infection in Australia and is the most common *Salmonella* serovar notified in Victoria, accounting for 61.5% of salmonella notifications in 1998 [2].

Cases of laboratory confirmed salmonella are routinely notified to the Department of Human Services Notifiable Infectious Diseases Surveillance System (NIDS). In January 1999, an outbreak of *Salmonella* Typhimurium phage type 9, resistant to

streptomycin and sulphafurazole (*S.* Typhimurium PT9 SSu) was identified among people in the south eastern suburbs of Melbourne. In this report, we highlight the utility of using hypotheses modification and sequential epidemiological studies to identify the source of the outbreak.

### METHODS

#### Case definitions

A laboratory confirmed case was defined as a person with a diarrhoeal illness who had *S.* Typhimurium PT9 SSu isolated from a faecal specimen. Clinically compatible cases were those persons in a cohort investigation who defined themselves as being ill with gastrointestinal symptoms (three or more loose stools

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in a 24 h period). Only laboratory confirmed cases were included in the case control studies.

### Retrospective cohort 1 – restaurant party

In December 1998, 1 month prior to the identification of the geographical clustering of cases, a retrospective cohort study was conducted to investigate an outbreak of *S. Typhimurium* PT9 SSu which occurred among 19 people who attended a party at a restaurant. Following the investigation of this outbreak, all subsequent notifications of *S. Typhimurium* PT9 SSu cases were interviewed.

### Initial case control study

In February 1999, we commenced a case-control study to identify risk factors associated with illness. A hypothesis-generating questionnaire was developed including demographic characteristics of the case, country of birth, date of illness' onset, foods consumed in the 3 days before illness' onset, foods consumed in their routine diet, food handling practices, ethnic background and recent travel.

Control households were matched to case households by area of residence and were selected by sequentially adding one to the last digit of the case household's telephone number. Individual controls were selected from within the household by interviewing the person who was having the next birthday. Where the selected control was aged less than 16 years, the parent/guardian was interviewed on their behalf. Calls to telephone numbers that were not answered, busy, or connected to answering machines were repeated a minimum of five times with at least one call after 18.00, before being rejected. If the identified control was not at home at the time of the call, a mutually convenient time was arranged to conduct the interview. Potential controls were contacted using this method until two controls per case were recruited. Potential controls were excluded if they had experienced a gastrointestinal illness in the previous month or if travel history indicated they had been absent during the week prior to onset of symptoms in the case.

### Retrospective cohort study 2 – family party

In the process of conducting the initial case control study, an outbreak of *S. Typhimurium* PT9 SSu was identified among guests at a family birthday party. The host family provided a menu of the foods served

at the party. This formed the basis of the food history questionnaire.

### Second stage case control

Based on the investigation results from the family birthday party, a sequential case control study was undertaken to test the hypothesis that illness was associated with the consumption of a continental custard cake. We re-interviewed all cake eaters from the initial case control study and assumed that both cases and controls who were non-cake eaters in the initial case control study were non-cake eaters for the purposes of this stage. Laboratory confirmed cases from the family birthday party and their matched controls were included in this stage. We asked about consumption of a variety of foods including various types of cake.

### Statistical methods

In order to measure associations between exposures and illness, Epi Info version 6.04 was used to calculate Mantel–Haenszel summary odds ratios, relative risks, exact 95% confidence intervals (CI), *P* values, Yates and two-tailed Fisher's exact tests where appropriate [3]. A stratified analysis was used to identify ethnicity as a confounder.

### Environmental investigation

A specific cake shop was identified as the source of the suspected continental custard cakes. Food handling procedures and practices were reviewed, product and ingredient samples were collected and environmental swabbing was conducted. Where available, food samples were collected from the homes of cases. Systematic community sampling was also conducted, with specimens collected from egg farms and retail food outlets.

### Microbiological investigation

All Victorian provisional human and non-human salmonella isolates were sent to the Microbiological Diagnostic Unit (MDU), University of Melbourne, for confirmation, serotyping, subtyping and antibiotic resistance profiles.

## RESULTS

A total of 54 cases had *S. Typhimurium* PT9 SSu isolated from stool specimens. Illness onset for the

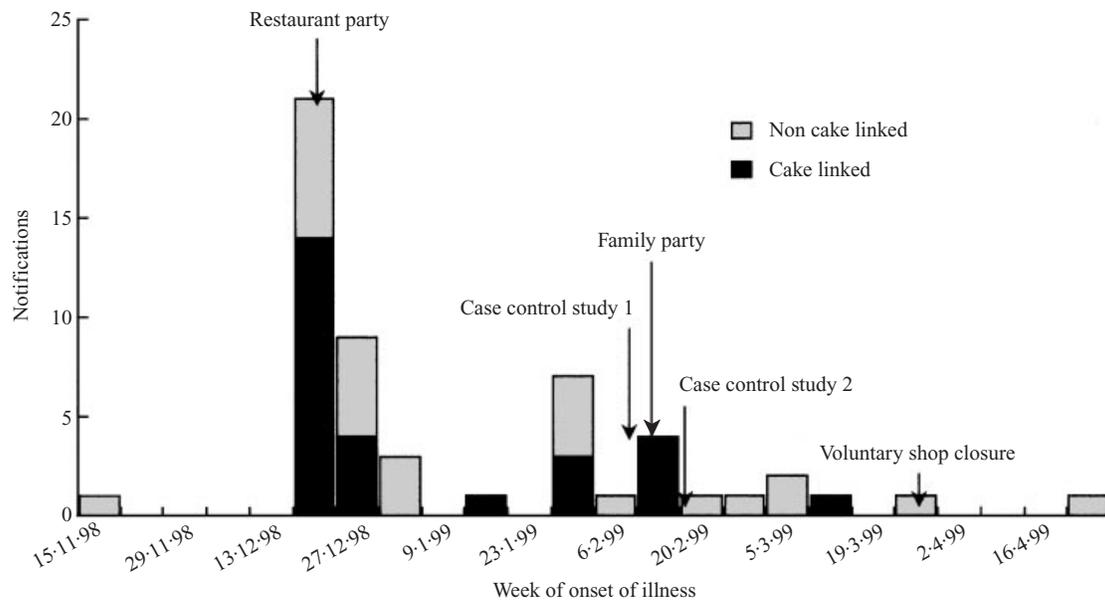


Fig. 1. *Salmonella* Typhimurium PT9 SSu by week of onset November 1998 to April 1999 (laboratory confirmed cases).

first case was 15 November 1998 and the last was 15 March 1999 (Fig. 1). The median age was 17 years (range 1–72 years). Of the 54 cases, 47 were resident around the south-eastern suburbs of Melbourne. Clinical presentation included diarrhoea (100%), abdominal pain (92%), fever (92%), nausea (76%) and vomiting (56%).

#### Retrospective cohort 1 – restaurant party

Of the 19 people who attended the restaurant birthday party in December 1998, 7 had *S. Typhimurium* PT9 SSu isolated from stool specimens. Initial investigation found no association between the provided menu items and illness (Table 1).

#### Initial case control study

Nine cases with 1 matched control and 16 cases with 2 matched controls have been enrolled in the first stage case control study by the time the birthday party cohort came to the attention of the Department of Human Services.

Analysis conducted at this time showed illness was strongly associated with the consumption of any dried pasta (Table 2). By analyzing by brand of pasta, we identified one particular brand to be linked with illness [Matched OR  $\infty$ ,  $P = 0.006$ ]. Stratified analysis of dried pasta consumption by ethnicity, reduced the association with a loss of statistical significance [OR 4.5, 95% CI 0.23, 129.65,  $P = 0.52$ ].

#### Retrospective cohort study 2 – family party

Of the 37 people who attended the family birthday party, two had *S. Typhimurium* PT9 SSu isolated from stool specimens. A further six people had a clinically compatible illness but were not laboratory confirmed. The clinically compatible cases were included as cases in the cohort investigation but not in the case control study.

Mortadella was the only food which had a statistically significant association with illness but only accounted for illness in half of the cases (Table 3). Although the association with illness was not statistically significant, all eight cases had eaten the continental custard cake. The place of purchase of the cake was identified and subsequently investigated.

#### Second stage case control study

Ten cases with 2 matched controls and 4 cases with 1 matched control were enrolled in the second stage case control study. Questions regarding consumption of continental custard cake were nested amongst questions about other types of cakes and other foods. Those who were ill were more likely to have eaten continental custard cakes than those who were not ill [Mantel–Haenszel matched OR  $\infty$ ,  $P = 0.00004$ , AR% = 71%]. No other foods, including other types of cakes, were associated with illness. Stratified analysis by ethnicity confirmed that ethnicity remained a confounder for pasta and pasta sauce consumption.

Table 1. *Retrospective cohort 1 – restaurant party. Association between illness and foods consumed by restaurant birthday party attendees, in a Salmonella Typhimurium PT9 SSu outbreak, Melbourne, 1998*

Food	Attack rate – exposed %	Attack rate – non exposed %	*RR (95% CI)
Singapore noodles	70	55	1.26 (0.62, 2.57)
Chicken spring rolls	80	50	1.60 (0.78, 3.27)
Prawns in batter	67	63	1.07 (0.44, 2.59)
Sweet and sour sauce	67	56	1.20 (0.53, 2.71)
Fried rice	64	60	1.07 (0.47, 2.42)
Ice cream	71	40	1.79 (0.58, 5.49)

\* RR, Relative risk – likelihood of disease in those exposed compared with the unexposed.

Table 2. *Initial case control study. Association between illness and foods consumed by cases and controls in a Salmonella Typhimurium PT9 SSu outbreak, Melbourne, 1999*

Food	Cases exposed (n = 25 (%))	Controls exposed (n = 41 (%))	Matched OR	95% CI	P value
Chicken	19 (76)	36 (88)	0.56	0.15, 2.08	0.59
Raw eggs	2 (8)	3 (7)	0.24	0.06, 0.96	0.05
Raw eggs mixed in food	5 (20)	3 (7)	3.2	0.6, 17.13	0.24
Egg custard	2 (8)	1 (2)	5.33	0.46, 61.8	0.46
Meat crumbed with egg	9 (36)	6 (15)	3.26	0.85, 12.45	0.11
Pasta	24 (96)	23 (56)	∞		0.002
Pasta, brand X	13 (52)	32 (78)	∞		0.01
Cake	14 (56)	24 (59)	1.2	0.38, 3.83	0.98

Table 3. *Retrospective cohort study 2 – family party. Association between illness and foods consumed by family birthday party attendees, in a Salmonella Typhimurium PT9 SSu outbreak, Melbourne, 1999*

Food	Attack rate – exposed %	Attack rate – non exposed %	*RR (95% CI)
Corn chips	26	14	1.83 (0.43, 7.83)
Roast chicken	24	12	1.93 (0.28, 13.49)
Lasagne	25	11	2.25 (0.32, 15.91)
Chicken schnitzel	30	12	2.55 (0.59, 11.02)
Mortadella	57	13	4.29 (1.4, 13.07)
Continental custard cake	30	0	∞ (P = 0.07)

\* RR, Relative risk – likelihood of disease in those exposed compared with the unexposed.

### Review of retrospective cohort 1 – restaurant party

On completion of the second stage case control study, further investigation revealed that one of the guests had taken a continental custard cake to the restaurant party in December 1998 but this had been inadvertently left off the menu at the time of the original

investigation. The custard cake had been purchased from the same shop implicated in the subsequent family birthday party outbreak. All seven laboratory confirmed cases had eaten continental custard cake. Most of the people who did not become ill could not remember whether they had eaten continental custard cake. Further statistical analysis was not conducted

because of the time lapse and the substantial impact of recall bias.

### Cases linked to continental custard cake

Of the 54 cases of *S. Typhimurium* PT9 SSu notified during the epidemic, 28 cases were known to have eaten continental custard cakes purchased from the same bakery (Fig. 1).

### Environmental investigation

The premises responsible for the production of the custard cakes were a retail and wholesale outlet. Food handling errors in the method of egg custard preparation, a common ingredient in most of the cakes prepared at the premises, were identified. Dry ingredients and hot water were mixed in a large bowl which was then placed on the concrete floor near the floor drain. Eggs or raw egg products were then added to the mixture. The custard, having been made in the morning, was left unrefrigerated and uncovered to cool in the bowl for the rest of the day. Some of the custard was put into reusable cloth pipers that were then used to fill cakes. During the hot summer months, hot custard was made and scooped from the bowl with bare hands. It was then placed directly onto a marble bench top to cool.

The proprietor indicated that the eggs used in preparation of the custard were usually frozen egg yolks and whites purchased in 10 kg bags from a wholesale outlet. Samples obtained direct from the wholesaler were negative for *S. Typhimurium* PT9 SSu. The proprietor also indicated that eggs were also occasionally purchased from a nearby supermarket and that he had received 2–3 dozen eggs a week from a friend who had chickens in her backyard. The proprietor refused to identify the friend but assured the investigators that he would not use these eggs in the future. There were some shelled eggs in a plastic container at the premises but the brand could not be identified. Samples were negative for *S. Typhimurium* PT9 SSu. Powdered eggs were rarely used and there were none available at the cake shop.

Twenty-five other food samples and 12 environmental swabs collected from the cake shop were negative for *S. Typhimurium* PT9 SSu. No leftover custard cakes from any of the parties were available. Thirteen food samples and six environmental swabs from the homes of cases were also negative for *S.*

*Typhimurium* PT9 SSu. In addition, 12 food samples and three environmental swabs collected from retail and wholesale outlets were negative for *S. Typhimurium* PT9 SSu.

*S. Typhimurium* PT9 SSu was detected in home made fresh pasta that was sampled from the household of a case in the north eastern suburbs. The pasta was made with raw eggs, flour, and water. The eggs were from household chickens; chicken manure samples were negative for *S. Typhimurium* PT9 SSu.

### DISCUSSION

Despite the lack of definitive microbiological linkage, strong epidemiological evidence from the sequential studies and supporting environmental evidence identified the cake shop as the likely source of the outbreak. The descriptive epidemiology showed that geographical clustering of cases in the south eastern suburbs was consistent with the distribution outlets serviced by the implicated premises. Furthermore, as illustrated in Figure 1, continental custard cake purchased from the same premises was eaten by laboratory confirmed cases at two separate birthday parties held months apart in different suburbs. After refinement of the hypotheses through sequential studies, the second stage case-control study established that illness was strongly associated with the consumption of the continental custard cake. The environmental investigation supported this finding, identifying poor food handling practices during preparation of the custard that might have resulted in cross-contamination. Once contaminated, the non refrigerated custard cake provided a warm moist environment for the growth of *S. Typhimurium* PT9 SSu. Voluntary closure and extensive cleaning of the shop was followed by a prompt fall in the numbers of *S. Typhimurium* PT9 SSu notifications.

The lack of microbiological evidence at the time of the cake shop investigation suggests that *S. Typhimurium* PT9 SSu outbreaks may be an intermittent problem. Because the investigation occurred several weeks after the majority of cases had become ill, representative samples of continental custard cake were not available for microbiological analysis, further limiting our ability to culture the pathogen from the suspected source. Other food samples and environmental swabs collected at the time of the cake shop inspection were also negative. However, the evidence from the two separate birthday parties

suggests that there may have been sporadic small outbreaks of *S. Typhimurium* PT9 SSu.

This investigation highlights the importance of obtaining the complete menu at the outset of the outbreak. The source of this outbreak may have been detected and further cases may have been prevented if it had been known that there was a continental custard cake at the restaurant birthday party in December 1998.

The distinct cluster of continental cake linked *S. Typhimurium* PT9 SSu cases did not explain all the cases notified during the outbreak. Thirty of the 54 cases were notified over 6 weeks following the Christmas/New Year period. Given that many of cases had attended parties during this period where cake may have been served, it is plausible that more cases were linked to consumption of continental custard cakes than these studies identified. The unrelated sporadic case who had consumed home-made pasta contaminated with microbiologically confirmed *S. Typhimurium* PT9 SSu was not a resident of the south eastern suburbs. The pasta was made of flour, eggs, and water. It is of interest that instructions for use of the home pasta machine in question recommended it not be washed between uses. *S. Typhimurium* PT9 SSu has also been found in other food sources (4) and there have been intermittent singular notifications of *S. Typhimurium* PT9 SSu since this outbreak.

Salmonella outbreaks have previously been linked to raw egg ingredients in desserts and confectionery [5–8]. In Australia there have been published reports of salmonella outbreaks linked to products made with eggs [9], but none linked with custard. *Salmonella* Typhimurium phage type 9 has previously been identified in samples of egg products, egg wash water, and egg process equipment in Australia [4].

Different forms of bias can affect the results of sequential case-control studies in a foodborne outbreak investigation. Interviewer bias and selection bias was minimized by recruiting experienced interviewers who used standardized questionnaires and followed standardized protocols for the selection of cases and controls. In the second stage case control study, we limited the potential problem of differential recall amongst cases by asking questions about a variety of foods including various types of cake. There is potential bias in cohort studies where only a few cases are culture confirmed. In the second birthday party cohort, we also included probable cases; people that met the clinical component of the case definition

but did not have faecal samples collected and were therefore not laboratory confirmed. To minimize the risk of bias we ensured that these people had attended the same birthday party and that other descriptive epidemiologic and clinical features could be linked to the cases.

The initial case control study found dried pasta was strongly associated with illness but the association appeared to be confounded by ethnicity. Cases and controls had been asked their country of birth but this was a poor proxy for cultural practices and eating habits. Further analysis by ethnic background identified 52% of cases being from the same ethnic background. This compared with only 10% of controls. Infections with antibiotic resistant strains of salmonella are of concern because although the majority of infections cause self limiting gastrointestinal illnesses that do not require antibiotics, antibiotics can be lifesaving for patients with systemic infections.

Knowing the salmonella serovar/phage type does not generally impact on the treatment or outcome of a case but it does provide important public health information that can enable identification of outbreaks and action to curtail further spread. The identification of antibiotic resistance markers allowed further discrimination beyond the phage type. The resistance profile SSu is found in other salmonellae, but in this instance the resistance profile SSu was not demonstrated by all of the *S. Typhimurium* PT9 that were isolated in the same time frame. The *S. Typhimurium* PT9 SSu was a specific cluster. Analysis of the plasmid profiles may have been useful to distinguish the outbreak associated isolates from the *S. Typhimurium* PT9 isolates but this was not performed. Without antibiotic sensitivity testing results it would have been difficult to differentiate these cases from the additional 70 cases of *S. Typhimurium* PT9 that had also been notified in the same time frame. The differentiation of cases enhanced the identification of clusters of cases in the south eastern suburbs.

Hypothesis modification and sequential case control studies are important for following up apparently sporadic cases of particular salmonella serovars. Case control studies are relatively simple and economical but their success is often dependent upon the degree of specificity in questioning. Sequential case control studies have infrequently been used in the investigation of foodborne outbreaks and they are an underutilized tool in the clarification and refining of the

hypothesis [10]. It may be important to continue an investigation where the results of the preliminary investigations are unrewarding. The use of a range of epidemiological tools enabled us to detect this pattern amongst the background of sporadic unrelated infections. Routine subtyping of some foodborne pathogens combined with sequential analytic epidemiological studies provide powerful mechanisms for investigating such outbreaks. Had this approach not been used contaminated food may have continued to be distributed.

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