
LARGE OUTBREAKS OF *SALMONELLA* TYPHIMURIUM PHAGE TYPE 135 INFECTIONS ASSOCIATED WITH THE CONSUMPTION OF PRODUCTS CONTAINING RAW EGG IN TASMANIA

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Abstract

This report describes one of the largest egg-associated outbreaks of foodborne illness in Australia for many years. Between June and December 2005, five outbreaks of *Salmonella* Typhimurium phage type 135 were identified in Tasmania, leading to 125 laboratory-confirmed cases. Public health investigations included case and food handler interviews, cohort studies, environmental health investigations of food businesses, microbiological testing, traceback, and inspections and drag swabbing of an egg farm. These investigations enabled identification of foods containing raw egg or foods contaminated through inadequate food handling and/or storage procedures as possible vehicles for infection. A particular poultry farm was reported as the common source of eggs. Interventions targeting the general public and food handlers to promote better handling of egg products, and advice to egg producers regarding harm minimisation strategies led to the series of outbreaks being brought under control. *Commun Dis Intell* 2007;31:118–124.

Keywords: salmonellosis, foodborne illness, outbreak, cohort studies, surveillance, eggs, Typhimurium 135

Introduction

Foodborne illness is a public health concern in all parts of the world. In Australia, an estimated 32% of gastroenteritis is foodborne, causing around 5 million illnesses, 4,000 hospitalisations and approximately 76 deaths annually.¹ Among known pathogens, enteropathogenic *Escherichia coli*, noroviruses, *Campylobacter* spp. and *Salmonella* spp. accounted for 88% of all foodborne disease in Australia in 2000.¹

From 2001 to 2004, the average yearly rate of laboratory-confirmed *Salmonella* infections in Tasmania was less than that for Australia as a whole (31.2 versus 37.6 cases per 100,000 population respectively). During these 4 years, *S. Mississipp*i was the most commonly reported *Salmonella* serotype in Tasmania, comprising 52% of the *Salmonella* notifications from this State. *S. Mississipp*i (a group G *Salmonella*) is considered an environmental serovar occupying an ecological niche in native Tasmanian animals, and is commonly acquired from exposure to those animals and/or drinking untreated water.² *S. Typhimurium* (a group B *Salmonella*) was the next most commonly reported *Salmonella* serotype in Tasmania, comprising a further 22% of the

Salmonella notifications. Amongst these *S. Typhimurium* cases, phage types 9 and 135 were most commonly reported, with a combined average of 17 cases per year (3.5 cases per 100,000 population), of which an average of 8 were *S. Typhimurium* 135 (STm135) cases (1.7 cases per 100,000 population). Previous outbreaks of STm135 in Australia have been associated with the consumption of desserts containing raw egg products,^{3,4,5} a bakery's cream piping-bag,⁶ egg sandwiches,⁷ chicken,^{8,9} and a commercial orange juice.¹⁰

In June 2005, an increase in the number of laboratory notifications of salmonellosis was observed by the Communicable Diseases Prevention Unit of the Department of Health and Human Services Tasmania (DHHS). These were subsequently identified as STm135 but investigations at the time were unable to pinpoint a source. Several months later, there were a series of point-source outbreaks which caused a dramatic increase in *Salmonella* notifications.

An outbreak investigation consisting of a series of community case interviews and cohort studies was conducted in order to identify the source of infection and to enable public health intervention to occur.

Methods

There were a total of 5 outbreaks between June and December 2005, some occurring in group functions and others in restaurant or similar settings (Table 1). Methods used to investigate each outbreak varied

and included case interviews, microbiological assessments, environmental investigations, and cohort studies where appropriate.

Case reports of salmonellosis in Tasmania are routinely investigated by local government Environmental Health Officers. However, given the magnitude of notifications in this outbreak, the DHHS outbreak investigation team conducted the majority of the case interviews. All case interviews were carried out by telephone. Community case interviews were conducted following the first peak in notifications, using a hypothesis-generating questionnaire. The questionnaire included a detailed 3-day food history and general questions about activities and foods consumed in the seven days prior to illness. The questionnaire was later expanded to include a 7-day food history. When the evidence from interviews and other lines of investigation were able to confirm a food business as the source of the outbreak, questionnaires were then modified to focus on the consumption of implicated products.

Faecal specimens were collected from individuals suffering from gastroenteric symptoms and tested for the presence of a range of pathogens including *Salmonella*. Limited *Salmonella* antigenic testing was conducted by laboratories in Tasmania. The isolates from cases of salmonellosis were then sent to the Microbiological Diagnostic Unit (MDU) Public Health Laboratory in Victoria for serotyping and phage typing. The tests conducted in Tasmania on the somatic (O) antigen of isolates collected during the initial increase in notifications showed that they were of group B *Salmonellae*. From this information

Table 1. Five outbreaks of *Salmonella* Typhimurium phage type 135 cases, Tasmania, June to December 2005

Outbreak number	Notification date	Number of cases	Method of investigation	Case definition*
1	16–27 June 2005	11	Case interviews, traceback	STm135 isolated from a faecal specimen
2	3–19 October 2005	63	Case interviews, cohort studies, food handling review, microbiological testing, traceback	STm135 isolated from a faecal specimen 'Probable cases' included in cohort if reported being ill following function attendance, with diarrhoea and two other clinically compatible symptoms*
3	28 October–2 November 2005	10	Case interviews, food handling review, microbiological testing, traceback	STm135 isolated from a faecal specimen
4	18–21 November 2005	5	Case interviews, food handling review, microbiological testing, traceback	STm135 isolated from a faecal specimen
5	1–20 December 2005	36	Case interviews, cohort studies, food handling review, microbiological testing, traceback	STm135 isolated from a faecal specimen 'Probable cases' were included if they reported being ill with symptoms of gastroenteritis following function attendance.

* Vomiting, nausea, abdominal cramps, lethargy, headache, fever or rigors.

the investigation focussed on group B *Salmonellae* in a timelier manner until more detailed typing information was obtained.

Case definitions were developed from information collected at interview and from laboratory specimens as shown in Table 1.

Environmental investigations

All food businesses identified in food histories of more than 1 case were investigated by environmental health and food safety officers. Food handling practices were reviewed and samples were collected from food products, raw ingredients, food preparation surfaces and equipment, for microbiological investigation.

Microbiological assessments

All human and non-human *Salmonella* isolates were sent to the MDU for confirmation, serotyping, subtyping and antibiotic resistance profiles.

Cohort studies

Of the 5 outbreaks, cohort studies were conducted in two: outbreaks 2 and 5 (Table 1). Two groups of individuals in outbreak 2 (cohorts 1 and 2) and 5 groups of individuals in outbreak 5 (cohorts 5, 8, 9, 10 and 11, Table 2) were investigated. The cohorts were assembled from guest lists and verbal consent was obtained from subjects prior to interview. Questionnaires were developed to collect data on food consumption, basic demographic data and symptoms.

Statistical methods

Data were entered into a Microsoft® Excel spreadsheet and analysed using Stata® version 8.0 (Stata Corporation, College Station, TX, USA) (Stata).

Food exposures were expressed as dichotomous variables, and crude relative risks (RR) with 95% confidence intervals (CI) were calculated.

For cohort 1 of outbreak 2, a logistic regression model was fitted to the cohort study data and adjusted odds ratios (OR_{adj}) were calculated with 95% confidence intervals to adjust for possible confounding. Food exposure variables were only included in the model if they showed a strong univariate association with the dependant variable (illness due to STm135). Model fit was optimised by categorising age into 20-year age groups.

Following univariate statistical analysis of cohort study data from outbreak 5, a stratified analysis was conducted to clarify the influence of confounding and effect modification on results.

Results

Descriptive epidemiology

A total of 125 laboratory-confirmed cases of STm135 were notified across the 5 outbreaks (Figure 1). The age of cases ranged from <1 year to 86 years, with a median of 37 years (Figure 2). Females accounted for 55% of all laboratory-confirmed cases.

Outbreak 1

The first outbreak consisted of 11 sporadic community cases in the north of Tasmania (Table 1). The cause of illness in this outbreak could not be identified. Geographical clustering of cases suggested that a local food business or ingredient may have been the source however investigators were unable to link cases to a common source.

Outbreak 2

Outbreak 2 consisted of 53 sporadic cases in the community in the north of Tasmania and of 2 cohorts

Table 2. Food exposures associated with illness due to *Salmonella* Typhimurium phage type 135, from cohorts attending catered functions, outbreak 5, Tasmania, December 2005*

Food exposure	Cases/ people exposed	Cases / people not exposed	Relative Risk (95 % CI)	
Cohort 5				
Smoked salmon with potato salad and mayonnaise	18 / 25	6 / 15	1.8	(0.9, 3.5)
Cohort 8				
Chicken sandwich	12 / 15	0 / 2	undefined	
Beef sandwich	10 / 12	2 / 5	2.08	(0.7, 6.3)
Cohorts 9, 10 and 11 combined				
Cajun chicken / avocado sandwich	22 / 23	1 / 13	12.4	(1.9, 81.9)
Leg ham sandwich	18 / 25	5 / 15	2.2	(1.0, 4.6)
Scones	13 / 14	13 / 31	2.1	(1.4, 3.3)

* Analysis includes laboratory-confirmed and probable cases.

Figure 1. Epidemic curve of *Salmonella* Typhimurium phage type 135 cases, Tasmania, Australia, 1 January 2005 to 31 January 2006, by outbreak

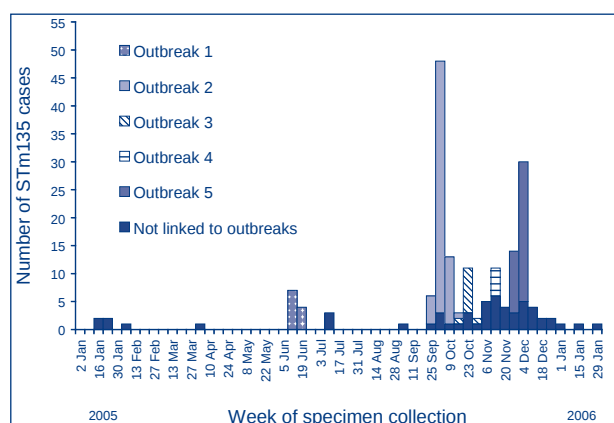
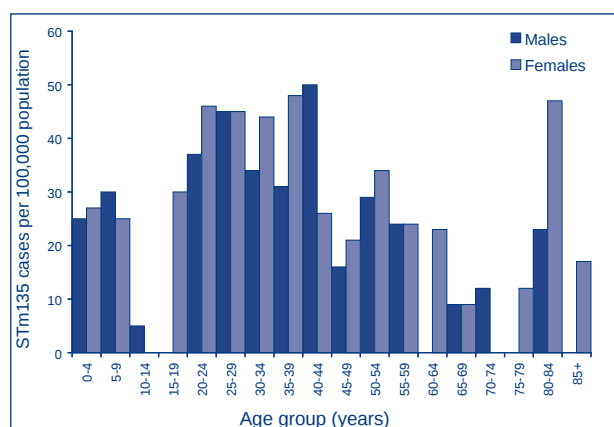


Figure 2. *Salmonella* Typhimurium phage type 135, all outbreak cases, Tasmania, 1 June to 31 December 2005, by age group and sex



(1 and 2) made up of 5 cases each, linked to two separate birthday party functions in the south of Tasmania (Table 1). Laboratory-confirmed cases ($n = 10$) and probable cases ($n = 15$) were included in both cohort studies. The community cases reported consuming products originating from 2 co-owned bakeries (Bakeries A and B) in the same city. Various sweet and savoury products, particularly cream-based products such as chocolate éclairs, were consumed. Cases associated with the functions all ate at least one product that had been prepared by the implicated bakeries. Table 3 shows the results of the univariate analysis of data collected from cohort 1. Three food items were associated with illness: a sponge-cream cake from Bakery A, and egg/bacon pies and pin-wheels (small savoury scrolls) supplied by other unassociated bakeries. However, logistic regression showed that sponge-cream cake from Bakery A was the only food item significantly associated with illness ($OR_{adj} = 7.7$, 95% CI: 1.7, 35.0).

Outbreak 3

All 10 cases in this outbreak had eaten at least one product at the same café in the south of Tasmania (Table 1). Products consumed included meals containing raw egg sauces and home-made hamburgers that some cases reported were obviously undercooked.

Outbreak 4

All 5 cases in this outbreak had eaten at least one product prepared at a third bakery in the north of Tasmania (Bakery C) (Table 1). A variety of products were reported as being consumed by cases.

Table 3. Association between food item consumption and illness due to *Salmonella* Typhimurium phage type 135, cohort 1, outbreak 2, Tasmania, 1 October 2005*

Food exposure	Cases/ people exposed	Cases/ people not exposed	Relative risk (95 % CI)		Adjusted odds ratio [†] (95 % CI)	
Foods from Bakery A						
Sponge cake	11 / 37	3 / 52	5.2	(1.5, 17.2)	7.7	(1.7, 35.0)
Mud cake	6 / 42	8 / 48	0.8	(0.3, 2.3)		
Savoury Toasts	6 / 30	8 / 57	1.4	(0.5, 3.7)		
Party-pies	5 / 21	9 / 67	1.8	(0.7, 4.7)		
Quiches	3 / 19	10 / 67	1.1	(0.3, 3.5)		
Foods from other bakeries						
Egg/bacon pies	8 / 25	5 / 58	3.7	(1.3, 10.2)	3.2	(0.7, 13.6)
Pinwheels	6 / 17	8 / 68	3.0	(1.2, 7.5)	1.1	(0.2, 5.2)

* Analysis includes laboratory-confirmed and probable cases.

† Adjusted for age, sex, mud cake and savoury foods from Bakery A. The potential confounders (sex and all age group categories) were not strongly associated with the dependant variable.

Outbreak 5

Eleven separate groups of individuals including 36 cases ate at functions catered for by the same restaurant in the south of Tasmania over a period of 8 days (Tables 1 and 4). A summary of exposures found to be associated with illness for catered functions, by cohort, is shown in Table 2. Cohorts of cases who did not eat catered food were excluded due to unreliable recall of foods consumed, therefore results are reported only for 5 cohorts (5, 8, 9, 10 and 11). Consumption of an entrée of smoked salmon with potato salad and mayonnaise was associated with gastroenteritis in cohort 5. Consumption of chicken sandwiches in cohort 8 was associated with gastroenteritis. Three food exposures were associated with illness in cohorts 9, 10 and 11, chicken and avocado sandwiches, scones and leg ham sandwiches. Stratified analysis demonstrated that chicken and avocado sandwiches was the only food item consistently associated with gastroenteritis. Investigations by Food Safety Officers identified that raw egg had been used in the preparation of mayonnaise and mixed with avocado in the implicated food items in each cohort. Laboratory confirmed cases (n=36) and probable cases (n=40) were included in the analyses.

Environmental investigations

Extensive environmental investigations were undertaken at each implicated food business, and a number of raw ingredients and food products were collected for microbiological testing. STm135 was isolated from a disposable cream piping bag and a bench surface swab in Bakery A associated with outbreak 2. Staff interviews revealed that the disposable cream piping-bag had been re-used a number

of times, potentially contaminating the cream layer of sponge cakes distributed to both functions in early October. STm135 was also isolated from the cream of a chocolate éclair distributed from Bakery A to another retail outlet. Bakeries A and B were found to share both the ingredients and preparation of their ready-to-eat foods. Samples collected during outbreaks 3 and 4 were negative for *Salmonella*. At the restaurant implicated in outbreak 5, STm135 was isolated from mayonnaise, tartare sauce and lettuce mix. These products were used in the making of the food items associated with illness.

Cross contamination issues were identified in all food businesses implicated in the series of outbreaks. Production demands which exceeded the physical capacity of the food businesses, combined with inadequate sanitisation and hygiene practices, were also contributing factors. The handling of products containing raw egg was identified to be a major issue. Both the café in outbreak 3 and the restaurant/caterer in outbreak 5 produced a variety of sauces made with raw eggs. Investigations revealed that these sauces were being kept and reused for up to 7 days. Raw egg was a component of the mayonnaise and tartare sauce which tested positive for STm135 in outbreak 5. Raw eggs were also used in the meat of hamburgers implicated in outbreak 3, the centres of which were reported to be undercooked, possibly due to their thickness.

Traceback of ingredients used by the food businesses implicated in these outbreaks confirmed that they all purchased their eggs from the same farm in Tasmania. This first became apparent during outbreak 3 when investigations demonstrated that eggs used by the café originated from the same farm that supplied Bakeries A and B identified during

Table 4. Eleven cohorts linked to either a function catered by, or with dining at, the same food business between 25 November and 2 December 2005 (outbreak 5), Tasmania

Cohort	Date of function	Number attended	Reported ill at interview		Tested positive for STm135	
			n	% attendees	n	% attendees
1	25 Nov	2	2	100	2	100
2	25 Nov	90	3	NA	2	2
3	25 Nov	3	3	100	2	67
4	25 Nov	2	2	NA	1	50
5	26 Nov	41	24	59	5	12
6	26 Nov	13	2	15	2	15
7	29/30 Nov	1	1	100	1	100
8	30 Nov	17	12	71	4	29
9*	2 Dec	6	5	83	5	83
10*	2 Dec	10	9	90	7	70
11*	2 Dec	28	12	43	5	21

* Analysed as one group due to identical menu.

NA Not applicable, not all attendees were interviewed.

outbreak 2. Traceback during outbreaks 4 and 5 also found that the eggs originated from the same farm. Visible external contamination of a number of eggs and packaging (dirt and other debris) was observed during the investigations of food businesses in outbreaks 2, 3 and 5, however microbiological samples from these raw eggs and exterior shells tested negative for *Salmonella*.

Management

In late October 2005, drag swabbing and a Hazard Analysis and Critical Control Point assessment (HACCP) were undertaken at the egg farm associated with the point source outbreaks. The drag swabbing methodology followed the New South Wales guidelines for voluntary *Salmonella* Enteritidis-free accreditation of egg farms.¹¹ Thirteen samples were submitted for microbial investigation. None were positive for *Salmonella*. Farm management issues that might have increased the risk of egg contamination included egg washing, storage, vermin control, and procedures for packaging and transportation. These issues were addressed in a 'whole of farm' quality assurance program that was being developed for the egg farm with the assistance of the Department of Primary Industry and Water (DPIW) at the time of the investigation. This quality assurance program has subsequently been implemented. Farm assessments (HACCP) and drag swabbing were repeated in December 2005 and January 2006 to check for the possibility of intermittent shedding of *Salmonella* by poultry. STm135 was subsequently isolated from samples taken from poultry faeces, spilled feed, and an egg conveyor belt in December, as well as from the surface of pulp grade eggs tested in January.

DHHS and DPIW sent a letter to all Tasmanian egg producers with recommendations on ways to reduce *Salmonella* contamination of eggs. As a precaution, DHHS issued media releases advising the community not to consume raw or under-cooked eggs. In a separate letter to all Tasmanian food businesses, DHHS made recommendations regarding the use of clean eggs ('free of visible external contamination'), refrigerated storage of eggs, and the safe preparation and handling of foods containing raw eggs. Following these media releases, a national supermarket chain changed to using single-use piping bags in their in-store bakeries (personal communication, M Kirk, OzFoodNet, December 2005). DHHS also sent information to general practitioners and emergency departments across the state to keep them informed about the outbreak and to request increased microbiological testing of patients presenting with gastroenteritis.

Following these interventions, the rate of notifications decreased with only 3 cases of STm135 notified in Tasmania during January 2006.

Microbiology

All *Salmonella* isolates from human and non-human sources were found to be antigenically identical and exhibited a phage reaction pattern that is designated as *S. Typhimurium* 135a by the Institute of Medical and Veterinary Science in South Australia.

Discussion

This report describes one of the largest egg-associated outbreaks in Australia for many years. Investigations identified several food businesses associated with point source outbreaks. Subsequently, traceback and farm level investigations supported the hypothesis that eggs were likely to be the food vehicle, although *Salmonella* was not isolated from market eggs. Interventions targeting food handlers and members of the public to raise awareness about safe handling of raw egg products and harm minimisation strategies on the farm from which eggs were reported to be sourced, led to the outbreak being controlled.

Ninety-one per cent of the cases in the 5 outbreaks (Table 1) were linked to food businesses supplied by a single egg farm. Each of these food businesses was found to have inadequate food handling and/or storage procedures that led to a potential *Salmonella* hazard from unclean eggs becoming an actual risk. Higher food handling standards with an emphasis on avoidance of cross-contamination could have prevented most if not all of these cases.

The food items from which positive microbial isolates were obtained were mostly dessert ingredients or sauces used in salad-based dishes. Even when contaminated by very small doses of *Salmonella*, these ingredients provide a fertile growth medium, especially if stored outside the refrigerator.

The available evidence from this outbreak suggests that, over a period of 6 months, a series of farm management issues led to eggs and egg containers leaving the identified farm carrying enough poultry faecal material to contaminate raw ingredients at the food businesses. However, no positive isolate from the outside of eggs actually in the human food chain was obtained, and the first series of drag swabs was negative. In spite of this, a low level of *Salmonella* intermittently shed by the farm's poultry, in conjunction with faecal contamination of eggs, is a likely explanation for our findings.

These findings illustrate the shared responsibility of primary food producers and food handlers to ensure the hygiene of the finished food product. Farm products such as eggs should never be regarded as 'sterile'.

The cooperation of the egg producer in these investigations was vital to understanding and ensuring a good public health outcome.

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