

# AN OUTBREAK OF GASTROENTERITIS DUE TO *SALMONELLA* TYPHIMURIUM PHAGE TYPE 170 ASSOCIATED WITH CONSUMPTION OF A DESSERT CONTAINING RAW EGG

Anna Reynolds, Cameron RM Moffatt, Amalie Dyda, Rebecca L Hundy, Andrew L Kaye, Radomir Krsteski, Simon Rockliff, Riemke Kampen, Paul M Kelly, Eddie D O'Brien

## Abstract

Eggs are frequently implicated as a source of foodborne salmonellosis. In February 2009 an investigation was commenced following reports of gastrointestinal illness among diners at a Canberra restaurant. The investigation sought to confirm the existence of an outbreak, identify a source and implement public health measures to prevent more cases. Menus and booking lists were obtained from the restaurant and a case-control study was commenced. A suspected case was defined as a person who ate at the restaurant on 13 or 14 February 2009 and subsequently developed diarrhoea and/or vomiting. Twenty cases and 31 controls were enrolled in the study. Eating a tiramisu dessert containing raw egg had a highly statistically significant association with illness (crude odds ratio 130.50, 95% confidence interval 13.54–1605.28). Among the 20 cases, nine of 12 stool samples were positive for *Salmonella* Typhimurium phage type 170 (STm 170). No microbiological evidence of STm 170 was obtained from the restaurant or during the egg trace-back investigation. This report highlights the risk associated with consumption of foods containing raw or undercooked shell egg. *Commun Dis Intell* 2010;34(3):329–333.

**Keywords:** *Salmonella* Typhimurium; disease outbreak; foodborne disease; Australia; salmonellosis

## Introduction

On 20 February 2009, a general practitioner in the Australian Capital Territory notified the Health Protection Service (HPS) at ACT Health of a patient with gastroenteritis. The case reported that they had eaten at a local restaurant and that a number of others at the same table were also ill. A laboratory-confirmed case of salmonellosis was also notified to the Communicable Disease Control Section later that same day. This case implicated the same restaurant as a potential source of their illness, also reporting a number of

fellow diners as being unwell. In total, 7 cases of gastroenteritis from 2 separate tables were linked to the restaurant on 13 February. The venue was inspected by environmental health officers (EHOs) on 20 February to assess kitchen hygiene standards and identify any potential sources of infection. An Acute Response Team meeting was then held and an outbreak investigation launched to confirm that there was an outbreak associated with the restaurant, to identify the source of the illness and implement public health measures to prevent further illness.

## Methods

### Case-control study

A case-control study was conducted to test the hypothesis that gastrointestinal illness was associated with consumption of a particular food item at the restaurant. During this study, cases were identified using the following case definition.

**Confirmed** – a person who has a laboratory-confirmed case of *Salmonella* and ate at the restaurant on 13 or 14 February 2009.

**Suspected** – a person who ate at the restaurant on 13 or 14 February 2009 and developed diarrhoea (defined as three or more loose stools in a 24-hour period) and/or vomiting with onset of these symptoms on or after those dates.

A structured questionnaire was developed from the menu provided by the restaurant and was used during telephone interviews with cases and controls. The aim of the questionnaire was to obtain information on illness symptoms, onset date and time, and consumption of specific food and beverage items at the restaurant. Restaurant reservation lists for 13 and 14 February 2009 were also obtained and used for case ascertainment and to recruit controls for the study.

Cases were identified via the reservation lists, in conjunction with an examination of *Salmonella* notifications received following the suspected days of exposure. Some cases were identified through an examination of the notifications for people who lived in suburbs close to the restaurant. Controls were recruited using the reservation lists and via a convenience sampling process in which cases were asked who they dined with at the restaurant. Controls were defined as persons who ate at the restaurant and did not develop gastrointestinal illness. An unmatched analysis was conducted as there were often whole tables affected by illness and cases varied by age and gender.

### Data analysis

Data from the interviews were entered into Epi Info™ version 3.3.2 and analysed using STATA™ version 9.0, with both suspected and laboratory confirmed cases included in the analysis. Case and control demographic details such as sex were compared using Fisher's exact test, and age using Student's *t*-test. Univariate analysis was conducted to calculate crude odds ratios (OR) with 95% confidence intervals (CI) for all exposures. A multivariate logistic regression model was constructed to adjust for confounding using food items that had a *P* value of <0.01 in the univariate analysis. As around 50 food and beverage items were being examined, a conservative cut off for the *P* value was chosen to reduce the probability of a chance association between a food item and gastrointestinal illness.

### Environmental investigation

Environmental health officers conducted an inspection of the restaurant kitchen facilities and food preparation procedures. Advice regarding safe food handling practices and the preparation of foods containing egg was provided to restaurant staff. Samples of mascarpone cheese, raw shell eggs and tiramisu were taken, however there were no leftover foods from the suspected days of exposure. In this case, the specific batch of eggs used to make the tiramisu could not be determined. A trace-back investigation identified a specific supplier of fresh eggs to the restaurant. Because the Australian Capital Territory does not have a Department of Primary Industries, the NSW Food Authority was contacted as they have extensive experience in trace-back investigations. They provided advice on egg farm investigations, including a sampling protocol. The supplier/producer of the fresh eggs was inspected with a number of samples taken,

including ready for sale and fresh laid eggs, wash and rinse waters, swabs from cages, egg conveyor belts, and drag swabs from laying sheds.

### Laboratory investigation

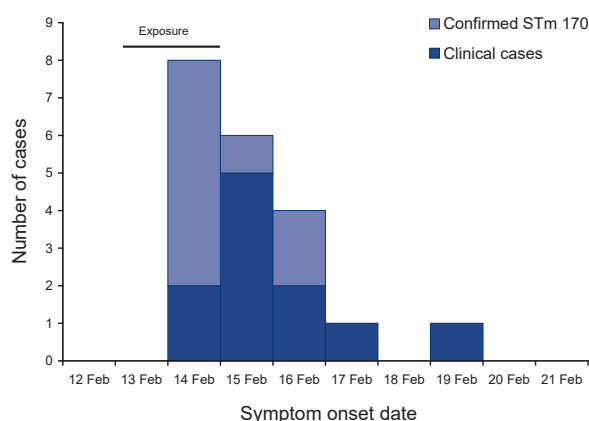
Stool samples were obtained from 12 cases and were tested for enteric pathogens using standard laboratory methods. Food samples taken from the restaurant and environmental samples from the local egg supplier were tested for the presence of *Salmonella*, by the Australian Capital Territory Government Analytical Laboratory using standard food and environmental laboratory methods. *Salmonella* isolates were serotyped, phage typed and identified by multiple locus variable-number tandem repeat analysis (MLVA) at the Microbiological Diagnostic Unit, Melbourne, Victoria.

## Results

### Epidemiological findings

A total of 20 cases that met the case definition were identified using *Salmonella* notification data and active case ascertainment. Eight cases reported that they ate at the restaurant on 13 February 2009 while 12 cases reported eating at the restaurant on 14 February 2009 (Figure). The median incubation period was 24 hours with an interquartile range of 15.5 hours. Symptom prevalence for the 20 cases was: watery diarrhoea 100%, lethargy 96%, abdominal pain 90%, fever 85%, headache 75%, nausea 70%, and vomiting 40%. None of the cases reported bloody diarrhoea. Fourteen cases (70%) consulted a doctor about their illness and 5 cases (25%) reported visiting a hospital

**Figure: Cases of gastrointestinal illness associated with a restaurant, Australian Capital Territory, 12 to 21 February 2009, by symptom onset date**



emergency department for treatment, although none of these was admitted. The duration of illness ranged from between 3 and 14 days.

All 20 cases identified during the investigation were included in the case-control study. A total of 31 controls were recruited and enrolled in the study. Controls were either nominated by cases or recruited through the restaurant booking list for the suspected dates of exposure. The mean age of the control group was 42 years (median: 43 years; range 9–71 years) and 56% were females. The sex distribution did not significantly differ between cases and controls (Fisher's exact 2-tailed test,  $P=0.39$ ) and there was no significant difference in the mean ages between the groups ( $t=0.80$   $P=0.43$ ).

The exposure of cases and controls to approximately 50 foods and beverages served at the restaurant during the potential exposure period was determined using a questionnaire based on the restaurant menu. The restaurant served black-board specials, which varied from day to day, but it was not possible to determine which specials were served on 13 and 14 February. However, cases and controls were asked if they ate a special and what that special was. Bread and coffee had elevated odds ratios but the association of illness with these items was not statistically significant. Tiramisu consumption was significantly associated with illness having a crude OR of 130.50 (95% CI 13.54–1605.28) (Table). Eighteen cases and 2 controls reported eating tiramisu, with some sharing a single serving and others reportedly eating very small amounts. The crude OR for carbonara sauce (16.15 95% CI 1.70–751.40) was also significantly elevated suggesting it could be

associated with illness. However, this food item appeared to be confounded by tiramisu as the adjusted OR was no longer significant as the 95% confidence interval included one (Table).

### Environmental findings

Investigation of the restaurant kitchen by EHOs determined that there was a good standard of general food hygiene and cleanliness. There were no sick food handlers working at the restaurant during the suspected exposure period. The tiramisu dessert was made on site in a large batch and did not undergo any cooking; it was cold set in the fridge and no temperature abuse was identified. However, raw fresh shell eggs were being used to prepare the tiramisu and the broken shells used to separate the egg yolk. The restaurant owners were advised that this practice increases the risk of *Salmonella* contamination and that in future they should either use an egg separator or pasteurised eggs during the dessert preparation. Investigation of the egg supplier/producer revealed production of eggs from both free range and caged chickens. The facility was semi-modern and fully automated, with eggs undergoing a two-stage washing procedure, followed by candling and ultrasonic detection of cracks.

### Laboratory findings

Twelve stools samples were obtained from cases and nine were positive for *Salmonella* Typhimurium phage type 170 (STm 170). All 9 samples were also MLVA typed and found to have the same MLVA pattern: 03-09-08-13-526/523 (Australian nomenclature). Environmental samples taken

**Table: Odds ratios for a selection of foods and beverages consumed, by cases and controls**

	Foods eaten				Crude OR	95% CI	Multivariate analysis			
	Cases		Controls				P value*	Adjusted OR	95% CI	P value*
	n	%	n	%						
Bread	17	85	20	65	3.12	0.66–19.83	0.20			
Amatriciana sauce	2	10	1	3	3.33	0.16–203.06	0.55			
Original pizza	4	20	1	3	7.50	0.64–381.29	0.07			
Fettuccine	9	45	4	13	5.52	1.18–28.91	0.02			
Carbonara sauce	7	35	1	3	16.15	1.70–751.40	<0.001†	4.70	0.17 – 127.97	0.36
Tiramisu	18	90	2	6	130.50	13.54–1605.28	<0.001†	100.90	12.72 – 800.16	<0.001*
Coffee	9	45	8	26	2.35	0.61–9.14	0.23			

Cases = 20 and Controls = 31

OR odds ratio

\* P value calculated using 2-tailed Fisher's exact Test.

† Statistically significant ( $P<0.01$ )

from the restaurant kitchen were all negative for *Salmonella*, including the raw shell egg samples. A drag swab collected on the supplier/producer's premises tested positive for both *Salmonella* Agona and *Salmonella* Infantis.

## Discussion

The results of this investigation demonstrate that this was a point source outbreak, with the epidemiological evidence supporting the hypothesis that the source of illness was tiramisu containing raw shell egg. The attack rate of the tiramisu was very high (18/20 cases) and the crude odds ratio associating consumption of tiramisu with illness was also high (130.50). The crude odds ratios for carbonara sauce (also made using raw shell egg) was also significantly elevated. However, this was likely confounded by tiramisu as all of the people who ate fettuccine with carbonara sauce also ate tiramisu. In this outbreak, the tiramisu was made using raw shell eggs, with the shells used for separation of the yolks. It is therefore possible that one or more of the eggs used was contaminated with STm 170, although cross contamination from another unknown source cannot be excluded. Nevertheless, the authors consider this unlikely. Raw egg containing tiramisu has been implicated in a number of Australian outbreaks caused by various *Salmonella* Typhimurium phage types.<sup>1-3</sup> STm 170 has previously been isolated in outbreaks associated with foods containing raw or undercooked egg.<sup>4,5</sup> The initial public health action taken in response to this outbreak was to provide the restaurant with advice on methods that could reduce the risk of *Salmonella* transmission, such as the use of an egg separator or pasteurised eggs. In addition, the restaurant voluntarily removed the tiramisu from the menu for a short period of time.

From January 2009, the number of notifications of laboratory-confirmed STm 170 began to increase significantly in a number of states, including New South Wales, Queensland, Victoria and the Australian Capital Territory. This prompted a multi-jurisdictional outbreak investigation by OzFoodNet. While analyses of food frequencies identified several foods of interest, none of the hypotheses were tested through a case-control study, due to decreasing case numbers following the declaration of the outbreak (personal communication, Katrina Knope, OzFoodNet). An MLVA profile of the STm 170 cases in this outbreak was obtained and compared with other recent sporadic cases in the Australian Capital Territory and cases in other jurisdictions. All of the outbreak STm 170 positive cases in the Australian Capital Territory had the same MLVA pattern (03-09-08-13-526/523), suggesting a common source, and

this profile was the predominant strain circulating in the Australian Capital Territory from January to March 2009.

During this investigation it was not possible to identify the specific batch of eggs used in the preparation of the tiramisu. However, the restaurant egg supplier/producer was traced and their facilities inspected for the presence of *Salmonella*. This outbreak highlighted an issue in the Australian Capital Territory regarding who has the authority to inspect food production facilities, as unlike other jurisdictions, the Australian Capital Territory does not have a Department of Primary Industries. Furthermore, inspections of any Australian Capital Territory egg supplier/producer as a direct response to a public health issue had not been previously conducted by the HPS. The inspection showed the egg supplier/producer had sophisticated processing facilities and no positive microbiological evidence of the presence of STm 170 was found. The investigators did have some difficulty in readily accessing the facility and obtaining a full quota of samples. This was in part due to the field investigators limited experience in egg farm investigation and also as a result of actions on behalf of the supplier/producer who initially refused access. A nationally agreed protocol for a program of auditing and investigation of primary production facilities would be useful for future outbreak investigations. Nevertheless, drag swabs taken from the floors of laying sheds were positive for both *S. Agona* and *S. Infantis*. This suggests that contamination of eggs could potentially occur at the facility and be passed on to consumers through unsafe food hygiene practices.

This outbreak investigation shows that despite advice from organisations such as the NSW Food Authority<sup>6</sup> and Food Science Australia<sup>7</sup> regarding the risks associated with eggs, the consumption of foods containing raw or undercooked shell egg continues to be associated with gastrointestinal illness. The risk of illness due to raw shell egg consumption must continue to be communicated to both the general public and hospitality industry. Improved communication between egg suppliers/producers and health departments, along with routine testing of egg supplier/producer facilities, could have public health benefits and may reduce the number of egg-related disease outbreaks.

## Acknowledgements

We would like to acknowledge the following for their assistance with the investigation: staff at the Communicable Disease Control section at the Australian Capital Territory HPS, Environmental

Health Officers at the Australian Capital Territory HPS; Craig Shadbolt at the NSW Food Authority; staff at the Australian Capital Territory Government Analytical Laboratory; Geoff Hogg, Mark Veitch and staff at the Microbiological Diagnostic Unit, Melbourne, Victoria; and OzFoodNet staff, Australian Government Department of Health and Ageing.

The Master of Applied Epidemiology program is funded by the Australian Government Department of Health and Ageing. Paul Kelly's salary is supported by the National Health and Medical Research Council.

## Author details

Anna Reynolds<sup>1,2,3</sup>  
 Cameron RM Moffatt<sup>1</sup>  
 Amalie Dyda<sup>1,2</sup>  
 Rebecca L Hundy<sup>1</sup>  
 Andrew L Kaye<sup>1</sup>  
 Radomir Krsteski<sup>1</sup>  
 Simon Rockliff<sup>1</sup>  
 Riemke Kampen<sup>1</sup>  
 Paul M Kelly<sup>4</sup>  
 Eddie D O'Brien<sup>1</sup>

1. Health Protection Service, Australian Capital Territory Health, Canberra, Australian Capital Territory
2. Master of Applied Epidemiology scholar, National Centre for Epidemiology and Population Health, Australian National University, Canberra, Australian Capital Territory
3. Office of Health Protection, Australian Government Department of Health and Ageing, Canberra, Australian Capital Territory
4. National Centre for Epidemiology and Population Health, College of Medicine, Biology and Environment, Australian National University, Canberra, Australian Capital Territory

Corresponding author: Mr Cameron Moffatt, Communicable Disease Control, Health Protection Service, Locked Bag 5, Weston Creek Australian Capital Territory 2611. Telephone: +61 2 6205 1734. Facsimile: +61 2 6205 1739. Email: cameron.moffatt@act.gov.au

## References

1. Hall R. Outbreak of gastroenteritis due to *Salmonella* Typhimurium phage type 135a following consumption of raw egg. *Commun Dis Intell* 2002;26(2):285–287.
2. OzFoodNet: enhancing foodborne disease surveillance across Australia: quarterly report, July to September 2004. *Commun Dis Intell* 2004;28(4):485–488.
3. Fullerton K. Monitoring the incidence and causes of diseases potentially transmitted by food in Australia: annual report of the OzFoodNet Network, 2007. *Commun Dis Intell* 2008;32(4):400–424.
4. OzFoodNet quarterly report, 1 October to 31 December 2006. *Commun Dis Intell* 2007;31(1):128–133.
5. OzFoodNet quarterly report, 1 January to 31 March 2008. *Commun Dis Intell* 2008;32(2):267–271.
6. NSW Food Authority. Enjoy eggs safely factsheet. Sydney, New South Wales. Accessed 15 April 2009. Available from: [http://www.foodauthority.nsw.gov.au/\\_Documents/consumer\\_pdf/enjoy\\_eggs\\_safely.pdf](http://www.foodauthority.nsw.gov.au/_Documents/consumer_pdf/enjoy_eggs_safely.pdf)
7. Food Science Australia. *Salmonella* in food containing raw or undercooked eggs – information for the food service industry. August 2008. Accessed on 14 April 2009. Available from: <http://www.foodscience.afisc.csiro.au/issues/salmonella-eggs.htm#6>