

AN OUTBREAK OF *SALMONELLA* TYPHIMURIUM PHAGE TYPE 135A GASTROENTERITIS LINKED TO EGGS SERVED AT AN AUSTRALIAN CAPITAL TERRITORY CAFE

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Abstract

Salmonella is an important foodborne pathogen, with eggs and egg-containing foods being frequently implicated in causing outbreaks of disease. In April 2012, an investigation was commenced after a number of cases of salmonellosis were linked to a Canberra café. The investigation sought to identify the cause of illness and to introduce public health measures to prevent further disease. A case control study was undertaken using the café's booking list to identify potential cases and controls. A structured questionnaire was developed using the café's menu, with information collected via telephone interview or email. A case was defined as any person who ate at the implicated café on 25 April 2012 and subsequently developed gastroenteritis. A total of 20 cases and 22 controls were recruited into the study. All 20 cases had faecal cultures positive for *Salmonella* Typhimurium phage type 135a (STm 135a). Eating eggs Benedict (odds ratio 63.00, 95% confidence interval 6.08–2771.66 $P < 0.001$) was significantly associated with illness. While no microbiological evidence of STm 135a was obtained from foods sampled from the café, STm 135a was recovered from swabs taken from the kitchen environment. This report illustrates an ongoing trend in Australia, where raw and minimally cooked egg-containing foods are identified as the responsible vehicles in a high proportion of foodborne *Salmonella* outbreaks. *Commun Dis Intell* 2012;36(3):E281–E287.

Keywords: eggs, *Salmonella* Typhimurium, disease outbreak, foodborne disease, case control study

Introduction

Responding to outbreaks of salmonellosis linked to cafés and restaurants serving foods containing raw or minimally cooked egg is an increasingly common action undertaken by public health and food safety authorities in Australia.¹ In south-east Australian jurisdictions (including the Australian Capital Territory, New South Wales and Victoria), the most common agent causing such outbreaks has been *Salmonella enterica* serovar Typhimurium (STm).¹ Investigation of these outbreaks has shown that consuming raw or minimally cooked egg-containing

foods, such as dressings like hollandaise sauce and desserts like tiramisu, is frequently associated with human illness.^{2,3}

On 1 May 2012 the Communicable Disease Control (CDC) section of the ACT Health Protection Service was alerted to a higher than expected number of laboratory diagnoses of *Salmonella* infection. These cases were rapidly interviewed by CDC staff to determine if a common exposure could be identified. Seven cases reported eating breakfast at the same café on the ANZAC Day public holiday on 25 April 2012. An acute response team meeting was held and an investigation into an outbreak linked to breakfast served at the café on the ANZAC Day public holiday was commenced. The investigation sought to identify the cause of illness and to implement appropriate public health measures to prevent further cases.

Methods

Epidemiological investigation

Initial interviewing of *Salmonella* cases was conducted using a hypothesis generating questionnaire, which included a detailed three-day food history. The results of these interviews led to the formation of a hypothesis that illness was linked to one or more egg-containing dishes eaten at a Canberra café, with exposure having occurred on 25 April 2012.

A case control study was undertaken to test this hypothesis. Ethics approval was not sought as the investigation was conducted as part of a public health response. Cases were identified either via routine public health investigation of any laboratory-confirmed *Salmonella* infection or via interrogation of contacts listed on the café's booking list for 25 April 2012. Controls were also recruited from the booking list or through their nomination by other cases and/or controls. A structured questionnaire was developed based on the café's menu listing. The questionnaire sought to confirm symptoms, onset and exposure dates and times, in addition to providing detail on specific food and beverages consumed by cases and controls during the postulated day of exposure.

A clinical case was defined as any person who ate at the implicated café on 25 April 2012 and subsequently developed gastroenteritis (defined as diarrhoea with or without abdominal pain). Clinical cases were encouraged to provide a faecal sample to assist with the epidemiological investigation. A confirmed case was defined as per a clinical case, in addition to having a faecal sample positive for *Salmonella* Typhimurium Phage type 135a (STm 135a).

Data obtained from the structured questionnaire were entered into a Microsoft Excel® database before analysis using Stata® version 9. Case and control demographic details, such as gender, were compared using a Fisher's exact test, while age was compared using a student's *t*-test. An unmatched analysis was performed with crude odds ratios (OR) and associated 95% confidence intervals (CI) calculated for all food exposures. A multivariate logistic regression model was then constructed to adjust for potential confounding using food items that had a *P* value < 0.10 in the univariate analysis.

Environmental investigation

Environmental Health Officers (EHO) inspected the café on 1 May 2012, with the kitchen facilities and the food preparation procedures being reviewed. Copies of the café's booking list for 25 April 2012, which included patron contact names and telephone numbers, were obtained, while details on staff illness and absenteeism were also requested. Although no remnant food items remained from the likely date of exposure, a number of statutory food samples were taken, including whole shell eggs (*n*=60), an egg yolk mix, egg mayonnaise, lemon mayonnaise, chickpea dip and a café prepared barbeque sauce. Extensive environmental swabbing of the kitchen was also undertaken from sites including food display trays, sinks, a chopping board, a tap handle (for staff hand washing), refrigerators, a walk-in cool room, a display bench, the floor, and a variety of other kitchen implements, including scales, eggs rings, a knife and takeaway containers.

As initial case interviews had identified egg containing dishes as plausible food vehicles for infection, an extensive review of the preparation of raw or minimally cooked egg-containing foods was conducted. Particular emphasis was placed on the café's preparation and use of hollandaise sauce. Trace-back of egg supply and production was conducted, with the New South Wales Food Authority (NSWFA) and the NSW Ministry of Health both being contacted regarding a New South Wales-based grading facility thought to process the brand of eggs used by the café.

Advice regarding the safe handling of egg-containing foods was provided to café staff. In addition, a public health message describing the potential risk

associated with use of raw eggs was incorporated into a routine correspondence with all Australian Capital Territory food businesses to alert them to the problem of salmonellosis and eggs.

A number of follow-up inspections of the café were conducted by EHO on 9 May and 30 May 2012, with additional swabbing performed at the latter visit. Sites tested included the cool room and refrigerator door handles, a food preparation bench, a cutting board and tongs.

Laboratory investigation

Stool samples were tested for enteric pathogens using standard laboratory methods. Food samples and environmental swabs taken from the restaurant were tested for the presence of *Salmonella* (and other pathogens), by the Australian Capital Territory Government Analytical Laboratory using standard food and environmental laboratory methods. *Salmonella* isolates were serotyped and identified by multi-locus variable number of tandem repeats analysis (MLVA) at the Microbiological Diagnostic Unit (MDU), University of Melbourne, Victoria or the New South Wales Enteric Reference Laboratory, Institute for Clinical Pathology and Medical Research (ICPMR), Sydney. Isolates that were received by the MDU initially underwent phage typing prior to MLVA results being specifically requested.

Results

Epidemiological findings

Descriptive epidemiology

A total of 20 cases were identified using *Salmonella* notification data or active case ascertainment via the café booking list (Figure). All cases confirmed that they ate at the café on 25 April 2012. The median incubation period was 13.5 hours (range 7.5 hours to 42 hours, interquartile range 4.5 hours). Symptom prevalence for cases was: diarrhoea (100%), abdominal pain (95%), fever (85%), headache (85%), nausea (80%), myalgia (60%), vomiting (50%), lethargy (45%) and bloody diarrhoea (30%). Nineteen cases (95%) consulted a doctor (either a general practitioner or a hospital emergency department) about their illness and 2 cases (10%) were hospitalised. The length of stay for the hospitalised cases was 3 days each. Detail on the duration of illness was unable to be reported as the majority of cases were still unwell at the time of data collection.

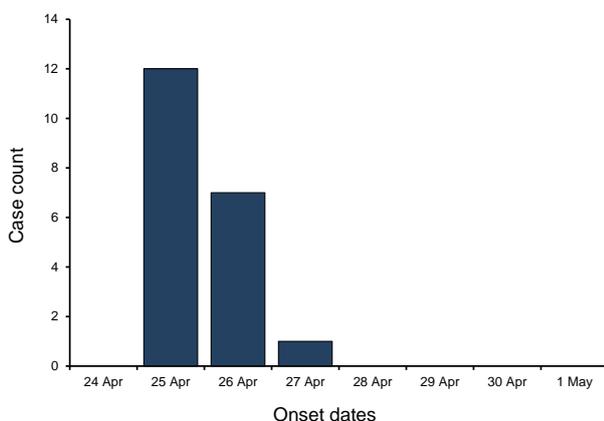
All 20 cases identified during the investigation were included in the case-control study. A total of 22 unmatched controls were enrolled in the study. Controls were either nominated by cases or recruited through the café booking list from

25 April 2012. The mean age of cases was 34 years (median = 31 years; range 19–62 years), with 65% being male. For the control group, the mean age was 33 years (median = 31 years; range 2–77 years) and 59% were females. The sex distribution did not differ significantly between cases and controls (Fisher's exact test, $P = 0.14$) and there was no significant difference in the mean ages between the groups ($t = -0.21$ $P = 0.84$).

Analytical epidemiology

The results of the univariate and multivariate analyses are shown in the Table. Eating eggs Benedict (OR 63.00, 95% CI 6.08–2771.66 $P < 0.001$) or any poached eggs (OR 8.19, 95% CI 1.57–53.60 $P = 0.003$) were both significantly associated with illness. However, only eggs Benedict (aOR 101.92, 95% CI 4.91–2112.97 $P = 0.003$) maintained a significant association with illness after adjustment via multivariate analysis.

Figure: Onset dates for cases of *Salmonella* Typhimurium PT 135a gastroenteritis linked to an Australian Capital Territory café, April 2012



Environmental findings

Inspection by EHO on 1 May showed that the café kitchen itself was generally clean and well maintained, with staff being able to demonstrate a good knowledge of food safety. Temperature checks for refrigerators were within accepted limits, with the exception of the main kitchen preparation fridge, which had a recorded temperature of 8.0°C at the time of inspection. Hand washing facilities were however deemed unsatisfactory due to the absence of paper towels for hand drying. The need for additional cleaning in non-food preparation areas, as well as some minor structural repairs, was also reported. As a result of these findings, an improvement notice was issued. A second inspection on 9 May 2012 showed that the previously identified issues with temperature control, hand hygiene, cleaning and minor repairs had been remedied. A final visit to the café was conducted on 31 May 2012 to exclude on-going contamination following the isolation of the outbreak strain of *Salmonella* from environmental samples taken on 1 May 2012. No further issues were identified.

One staff member, a barista, was identified as being unwell with gastroenteritis at the time of the investigation. However this person's illness onset did not occur prior to other cases. Additionally, this staff member also reported eating eggs Benedict during a meal break on 25 April 2012. No other sick food handlers or staff members were identified as working during the exposure period.

Hollandaise sauce

The hollandaise sauce is made daily at the café and used only in breakfast items, specifically with eggs Benedict. In general, a single batch is made on weekdays and 2 batches are prepared on Saturdays and Sundays. The first batch is reported as always

Table: Odds ratios for selected breakfast items consumed by cases and controls

Foods consumed	Cases		Controls		Crude OR	95% CI	P-value	Multivariate analysis		
	n	%	n	%				Adjusted OR	95% CI	P-value
Eggs Benedict	15	75	1	5	63.00	6.08–2771.66	<0.001	101.92	4.91–2112.97	0.003
Any poached eggs	17	85	9	41	8.19	1.57–53.60	0.003	1.25	0.08–19.99	0.87
Any eggs	20	100	15	68	undefined	2.21–undefined	0.006			
Any extras	9	45	4	18	3.68	0.76–19.90	0.06	5.38	0.40–72.06	0.20
Any eggs on toast	4	20	2	9	2.50	0.31–30.32	0.31			
Fried eggs	2	10	1	5	2.33	0.11–144.09	0.49			
Scrambled	1	5	0	0	–	–	0.28			
Poached eggs	1	5	1	5	1.11	0.01–90.83	0.95			
Breakfast burger	2	10	4	18	0.50	0.04–4.07	0.45			
Breakfast platter	1	5	1	4	1.11	0.01–90.83	0.95			

being used before the second is made, with any left-over sauce being discarded prior to the lunch menu commencing. On the ANZAC day public holiday, the breakfast menu, which included eggs Benedict, was available to customers throughout the day. On that particular day, two batches of hollandaise sauce were prepared due to high customer demand. The eggs poached for eggs Benedict are always served soft, unless otherwise requested by a customer. The hollandaise sauce is made in approximately 1 litre batches. It comprises 15 egg yolks, vinegar and clarified butter. The ingredients are whisked in a bowl over a pot of boiling water until the correct consistency is achieved. The chef estimated the process to take around 15 minutes. Once made the sauce is stored directly above the stove, on a shelf, in a stainless steel container. The chef stated that the sauce can remain on the shelf for up to 2.5 hours.

Trace-back of eggs

The café used free range eggs, which were delivered weekly by a local distributor. The café reported using approximately 1,800 to 2,500 eggs per week (i.e. 10–14 boxes, with each box containing 15 dozen eggs). All eggs were stored under refrigeration on receipt. The chef advised that the wholesaler usually supplied the same brand of eggs (packed at a New South Wales-based grading facility). However, on occasions different egg brands were received from the wholesaler. Details of the suspected grading facility were forwarded to New South Wales Food Authority and the NSW Ministry of Health to determine if this facility had been implicated in investigations in that jurisdiction. No batch details or leftover cartons from the delivery prior to the outbreak were available and New South Wales agencies reported the company had not been previously implicated in the supply of contaminated eggs. The café also ceased using the local distributor to supply eggs. The distributor was unable to be contacted.

Laboratory findings

Twenty stool samples were obtained from cases and all were positive for *Salmonella* Typhimurium (STm). Of the 14 samples received by MDU, all were phage typed as STm 135a. MLVA typing of these 14 isolates showed a common pattern, 03-13-11-10-523. For the 6 STm isolates typed at ICPMR, five shared a common MLVA pattern, 03-13-11-09-523. The 6th isolate was closely related, with a single digit difference at the second loci (MLVA 03-12-11-09-523). This isolate was also considered to be a case. The observed differences between the MDU and ICPMR generated MLVA patterns are most likely not real and relate to the latter laboratory's shift to the use of adjusted fragment sizes (personal communication Mary Valcanis and Karolina Dimovski). Positive environmental

swabs taken from a refrigerator door handle and a walk-in cool room door handle on 1 May 2012 were also tested at MDU. Both were positive for STm 135a, with MLVA pattern 03-13-11-10-523. All other statutory food samples taken on 1 May were negative for *Salmonella*. Follow-up swabs taken from the kitchen on 30 May 2012 were negative also for *Salmonella*.

Discussion

The results of the epidemiological investigation support the hypothesis that the outbreak's probable cause was contaminated eggs used in the preparation of hollandaise sauce. Having eaten any poached eggs or eggs Benedict (which comprises both poached eggs and hollandaise sauce) showed significant associations with illness but after multivariate adjustment only the eggs Benedict remained significantly associated with illness. The findings and observations from the environmental investigation provide further support to the epidemiological evidence. On the day of exposure, the café reported a very high level of business, with in excess of 350 orders. This demand had an impact on the hollandaise sauce used that day, with staff needing to prepare an additional batch to meet demand. It is therefore plausible that a breakdown in food handling practices occurred, involving insufficient temperature during the hollandaise preparation. In addition, the description of the sauce being left above the stove during service, at likely ambient temperatures, could also have assisted the survival and potential proliferation of any bacteria present.

While the recovery of the outbreak strain on door handles indicates pathogen transfer via hands, it does not assist in determining whether the *Salmonella* may have been transferred from contaminated shell eggs (or some unknown source) to the hands of staff and then into the wider kitchen environment. It is possible that the eggs were free of bacteria prior to their arrival at the café and that the hollandaise sauce became contaminated during or after its preparation. Although STm 135a was not recovered from food items or eggs sampled from the café this is not an unsurprising finding as the café reported making fresh hollandaise sauce daily. Furthermore, the inherent delays between case exposure, illness onset and authorities identifying an outbreak and undertaking an inspection of the premises means that left over eggs, egg products, cartons and packaging from the time of exposure are unlikely to be recovered. Nevertheless, egg-associated outbreaks, including those both with and without positive food microbiology, are being identified with increasing frequency across Australia.⁴⁻⁶

The use of booking lists and recruitment of controls via known cases was an appropriate and practical strategy to use in response to this outbreak. The time from exposure to commencement of the analytical study was only 1 week, which may have assisted in reducing exposure misclassification. While a matched analysis was not performed for practical reasons, it is unlikely that the characteristics of cases and controls differed significantly, with whatever indefinable factors led cases to eat at the café likely applying to controls. This would have assisted with management of potential confounding and bias. The descriptive epidemiology lends support to this with no significance differences for gender and age between the 2 groups. The effect size and width of confidence intervals does however reveal some imprecision around the study's primary findings, a factor that can be difficult to control for in analysis conducted as part of a rapid public health response.

Although restaurants and cafés remain the most common settings for egg-associated outbreaks of salmonellosis,⁷ more concerted efforts are required to reduce infection pressure at the primary production level. Arguably, if pathogen reduction is increased on farm, there should be a decrease in the sale of contaminated product, resulting in fewer cafés and restaurants being identified as settings for *Salmonella* outbreaks. With eggs being so frequently implicated as a cause of such outbreaks, the resulting impact of the new Food Standards Australia New Zealand *Primary Production and Processing Standard for Eggs* on this significant public health problem is eagerly awaited.

Conclusion

Egg-associated outbreaks of *Salmonella* are becoming an all too common problem for public health authorities across Australia, with this outbreak investigation contributing to the mounting body of evidence highlighting salmonellosis and its links to eggs. Effective control of this issue remains elusive. It requires a more concerted effort by public health and regulatory authorities to not only address issues with egg handling and use by food premises but also to improve consumer understanding of potential risk and to increase the egg industry's awareness of this as a public health issue.

Acknowledgements

The authors acknowledge the following persons and organisations for their assistance with the investigation: staff at the Communicable Disease Control section at the ACT Health Protection Service; Environmental Health officers at the ACT Health Protection Service; staff at the ACT Government Analytical Laboratory; Geoff Hogg, Mary Valcanis, Karolina Dimovski and staff at the Microbiological Diagnostic Unit, Melbourne, Victoria; Peter Howard

and staff at the NSW Enteric Reference Laboratory, Sydney, NSW; and Craig Shadbolt, NSW Food Authority, Sydney.

The OzFoodNet program is funded by the Australian Government Department of Health and Ageing.

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