Peer-reviewed articles

Outbreak of *Salmonella* Typhimurium 44 related to egg consumption

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Abstract

ACT Health investigated an outbreak of gastroenteritis associated with a local restaurant in December 2008. The infecting agent was *Salmonella* serotype Typhimurium phage type 44. A case control study was conducted to identify the source of infection. A total of 22 cases and 9 controls were recruited to take part in the study. Both poached eggs (odds ratio [OR] 42.00) and hollandaise sauce (OR 19.00) had elevated odds ratios that were statistically significant. The major limitation of the study was the small sample size and small number of controls. Despite this, a strong association with illness and consumption of eggs and hollandaise sauce was detected and this was further supported by environmental evidence. The investigation concluded that the cause of the outbreak was putatively contaminated eggs, either on their own or as an ingredient used in hollandaise sauce. The investigation and control measures led to an improvement in hygiene practices at the restaurant and contributed to the voluntary recall of the contaminated batch of eggs from the Australian Capital Territory. The results of the study also build upon other evidence that egg-related salmonellosis is now common in Australia and attention to commercial practices at production and processing is overdue. *Commun Dis Intell* 2009;33(4):414–418.

Keywords: *Salmonella*, outbreak, eggs, hollandaise, case-control study

Introduction

On 8 December 2008, a general practitioner notified the Health Protection Service, ACT Health of a case of salmonellosis, with a local restaurant being implicated as the possible source of infection. Further reports of illness linked to the restaurant were received from the general public. Numerous *Salmonella* notifications were received from local laboratories. Interviews following these notifications further implicated the restaurant. An outbreak was declared and an investigation was undertaken incorporating environmental, laboratory and epidemiological evidence. Approximately 1 week after the initial report was received the outbreak strain was identified as *Salmonella* serotype Typhimurium phage type 44 (STm44).

Salmonella Typhimurium infection commonly results in symptoms such as abdominal pain, diarrhoea, fever, nausea and vomiting. The organism is transmitted via ingestion, usually of food contaminated by the faeces of an infected person or animal. The incubation period of *Salmonella* can range between 6 and 72 hours but is more commonly between 12 and 36 hours.[1] There have also been instances of longer incubation periods of up to 16 days.[1]

Salmonella is one of the most frequently notified foodborne pathogens in Australia with 8,281 notifications in 2008.[2] Historically in Australia, *Salmonella* Typhimurium is the most frequently notified *Salmonella* serotype associated with foodborne outbreaks. During 2006, 16 egg-related outbreaks were identified, with over 80% due to various *Salmonella* Typhimurium phage types.[3] One of the most common phage types causing infection in Australia is STm44, with previous studies and outbreaks showing a common association with the consumption of raw eggs or pre-prepared dishes containing raw egg as an ingredient (OzFoodNet outbreak register, 2009, unpublished data).

Methods

Epidemiological investigation

Initial interviews with both confirmed and probable cases of *Salmonella* were conducted to allow the generation of hypotheses and to guide further investigations. Cases were interviewed using the OzFoodNet *Salmonella* questionnaire investigating exposures in the 7 days prior to onset of illness. This led to the hypothesis that the outbreak was linked to a local restaurant, affecting those who attended for breakfast. To test this hypothesis, a case control study was developed based upon the restaurant's breakfast menu.

A case was initially defined as 'any person who ate at the restaurant on 29 November 2008 and developed symptoms of gastroenteritis, defined as two or more gastrointestinal symptoms'. Probable cases were those who met this definition and confirmed cases were those who met the definition and had a faecal sample that was positive for *Salmonella*. The case definition

was later revised to include 'any person who ate breakfast at the restaurant during the period between the 29 November and 14 December 2008 and who developed symptoms of gastroenteritis after exposure'. This was altered as more cases were identified within this time period. For the purposes of analysis, all confirmed and probable cases were included.

As a booking list was not available from the restaurant, controls were selected via convenience sampling. This involved asking cases if they ate with any other people whilst at the restaurant. This resulted in the recruitment of 9 controls. Participants took part in a structured questionnaire examining clinical illness and 40 different food exposures from the breakfast menu.

Data were stored in an Epi Info database, before analysis of odds ratios (OR) and stratification to remove the effects of confounding between some menu items. Case control analysis and stratification were performed using Stata 9.

Environmental investigation

Environmental Health Officers attended the restaurant on 9 December 2008. General hygiene practices, food storage and preparation procedures and fridge temperatures were all examined. Temperatures of the main storage fridge were tested using a data logger from 10 to 12 December 2008. Samples of hollandaise sauce, eggs and mint sauce were taken from the restaurant for testing and the egg supplier was contacted to explain egg sourcing and production procedures. The NSW Department of Health was also contacted to further the investigation into the practices of the New South Wales based egg supplier.

Laboratory investigation

Food samples taken during the environmental investigation were provided to the Australian Capital Territory Government Analytical Laboratory (ACTGAL) for testing for detection of *Salmonella*. Faecal samples collected from 17 suspected cases were tested by the local public health laboratory and other private pathology providers for the presence of *Salmonella*, *Campylobacter* and *Shigella*.

ACTGAL also performed tests to identify the replication times of these *Salmonella* isolates in hollandaise sauce. The sauce was made by the restaurant's chef with lemon juice, raw eggs, butter and dill. Due to the acidic nature of hollandaise sauce, replication times may be longer than normal. The sauce taken from the restaurant, which was made approximately 2 days prior to the environmental health inspection, was inoculated with STm44 and incubated at 37° C. The test was carried out twice on 2 separate samples. Both samples were then examined at 0, 3, 6 and 24 hours.

Results

Epidemiological results

A total of 24 people were identified as cases after consuming food at the restaurant. Twenty-two subsequently agreed to participate in the case-control study. The age of cases ranged from 3–53 years, with 61% of those affected being female. Cases ate breakfast at the restaurant between 29 November and 9 December 2008. Illness onset ranged from 30 November to 10 December 2008. The average incubation period of STm44 infection in this instance was 22.6 hours (range 15 to 35.5 hours). The average length of illness was 7.5 days, with diarrhoea (100%), fever (77.3%) and nausea (77.3%) the most frequently reported signs and symptoms. Of those ill, 19 (86.4%) people consulted a doctor and two people (8.3%) were hospitalised.

Table 1 shows the odds ratios calculated for each item on the breakfast menu with confidence intervals (CI) and *P* values. These results suggest that the most likely source of *Salmonella* infection was poached eggs or hollandaise sauce with odds ratios of 42.00 (CI 2.80–2017.00) and 19.00 (CI 1.90–243.00) respectively. Despite having large confidence intervals both these results were statistically significant. Cases were asked if eggs consumed were runny or hard. The subjective nature of the question however, caused some confusion and a combined variable for all egg consumption was used. The odds ratios for tomatoes (OR 8.00), sour dough rye toast (OR 6.13) and hash browns (OR 4.57) were also elevated but not statistically significant. A total of 24 infrequently eaten foods (and hence with no suggestion of association with illness) have not been shown in Table 1.

Stratification of foods with elevated odds ratios was performed to adjust for possible confounding. As shown in Table 2, after stratification, the poached eggs and hollandaise sauce could not be separated. This was because almost all those who ate poached eggs also had hollandaise sauce. The odds ratio for poached eggs remained elevated and statistically significant when stratified with tomato (OR 30.00) and sourdough rye toast (OR 42.00).

Similarly, the odds ratios for hollandaise sauce remained high when stratified with tomato (OR 13.50) and sourdough rye toast (OR 18.00). All of the odds ratios had confidence intervals higher than one and were statistically significant at the 5% level.

Tomatoes (OR 2.22) had elevated odds ratios when stratified against hollandaise sauce but this was no

Table 1: Univariate analysis of breakfast menu items

Food Item	Exposed cases	Exposed controls	Odds ratio	Confidence interval	*P* value
Butter	12	6	0.66	0.0–4.3	0.70
Cheese	2	1	0.80	0.0–53.0	1.00
Tomato	11	1	8.00	0.7–386.2	0.10
Sourdough rye toast	14	2	6.12	0.8–69.9	0.05
Plain toast	1	1	0.38	0.0–33.6	0.50
Maple syrup	2	2	0.35	0.0–5.9	0.56
Bacon	14	4	2.18	0.3–14.3	0.43
Baby spinach	8	2	2.00	0.2–23.8	0.67
Hollandaise sauce	19	3	19.00	1.8–243	0.003
Poached eggs	21	3	42.00	2.8–2017	0.0007
Mushrooms	6	2	1.40	0.1–17.4	1.00
Hash brown	8	1	4.57	0.4–227	0.22
Zucchini	4	2	0.77	0.0–10.5	1.00
Coffee	13	5	1.15	0.1–7.1	1.00
Water	17	4	4.00	0.5–28.4	0.11

Table 2: Stratification analysis

Poached eggs adjusted for:	Odds ratio	Confidence interval	*P* value
Tomato	30.00	2.2–405	0.006
Sourdough rye toast	42.00	2.1–825	0.009
Hollandaise sauce adjusted for:			
Tomato	13.50	1.4–123	0.02
Sourdough rye toast	18.00	1.2–255	0.03
Tomatoes adjusted for:			
Poached eggs	2.20	0.1–28.1	0.50
Hollandaise sauce	2.22	0.1–28.8	0.50
Sourdough rye toast adjusted for:			
Poached eggs	1.00	0.0–13.0	0.72
Hollandaise sauce	1.08	0.0–14.4	0.70

longer significant statistically, as shown in Table 2. The odds ratio for tomatoes, when stratified with poached eggs remained elevated at 2.20 but was no longer statistically significant. Adjusting hash browns for poached eggs and hollandaise could not be performed. Sourdough rye toast no longer showed an association when adjusted for with poached eggs or hollandaise sauce.

Environmental results

The possibility that raw eggs may have been the vehicle of infection was raised during the initial inspection of the restaurant. Environmental Health Officers advised of the dangers of serving raw egg dishes and provided information to reduce the risk. The restaurant subsequently ceased serving dishes containing raw eggs.

The data logger recorded an ineffective temperature range in the main storage fridge. The lowest reading during the duration of the test was 5.5° C, with the recommended temperature being 5° C or less. The highest temperature recorded during the 24 hour period was 10.3° C, with an average of 7° C. All food was labelled correctly with the date of preparation before storage, with the exception of the hollandaise sauce.

The process involving the storage and preparation of hollandaise sauce was identified as a possible problem. The dish was prepared with lemon juice, raw eggs, butter and dill and then stored in the

main refrigerator. The sauce was left under a heat lamp to soften before serving. The process for the preparation of poached eggs was also examined with all eggs cooked for approximately 2½ minutes, resulting in an egg that is almost completely cooked but not hard. However, this varies depending on customer preference.

Information from the egg supplier advised that they had investigated several other complaints, which had identified one batch of eggs that may have been responsible for the contamination. Their own investigation had identified that the eggs were not suitable for packing as first grade eggs and were supposed to be processed into liquid pulp eggs. However, a packing error resulted in the eggs being boxed and sold.

Laboratory results

Salmonella was not isolated from any of the food samples taken during the environmental health investigation. Faecal samples taken from symptomatic diners yielded a total of 16 positive results for STm44, with only 1 person found to be negative for *Salmonella*.

The 2 samples of hollandaise sauce were found to have a pH of 4.01 and 4.05. The tests regarding the incubation period of *Salmonella* in the hollandaise sauce showed that the number of *Salmonella* did not increase at any time in a 24 hour period. It is likely that the incubation temperature of 37° C was higher than the temperature of the heat lamp at the restaurant.

Public health action

After the initial environmental health investigation and hypothesis generating questionnaires, a letter was sent to the restaurant proprietor recommending the removal of dishes containing raw eggs. The restaurant subsequently ceased serving any dish containing raw egg. Improvements regarding a decrease in temperature of the main storage fridge were also advised to management of the restaurant. ACT Health was later informed via phone that the restaurant intended to remove all dishes from the menu that contain raw egg and temperature problems had been resolved. The restaurant also contacted their egg supplier who collected and replaced all eggs from the suspected batch. There were no further *Salmonella* infections related to the restaurant.

ACT Health liaised with the NSW Department of Health about the outbreak. It was found that several similar outbreaks associated with eggs had occurred in the region. ACT Health had not been aware of these outbreaks. This highlights the importance of communication between jurisdictions. The NSW Department of Health informed that a voluntary recall of the contaminated eggs was underway. Subsequently, notifications of STm44 infection in the Australian Capital Territory significantly reduced.

Discussion

The results from the epidemiological investigation suggest that the most likely cause of this outbreak was contaminated eggs served at the restaurant. The most common cause of *Salmonella* contamination of eggs within Australia comes from contamination of the egg shells, specifically when they are soiled or damaged.[4] Infection can also occur during the development of the egg in the hen (trans-ovarian infection). This type of infection is most commonly associated with *Salmonella* Enteritidis and is not endemic in Australia.[4]

The infection may have been transmitted by the eggs when served on their own or via the hollandaise sauce, which contained raw egg as an ingredient. The odds ratios for both the eggs and the sauce were extremely high, indicating a strong association. The strength of association remained following adjustment for possible confounding. However, the 2 items on the breakfast menu are usually served together as 'eggs benedict' and could not be separated with stratification.

Though the 2 items could not be statistically separated, the environmental investigation suggested the hollandaise sauce provided a more plausible explanation for the outbreak. The procedure for serving hollandaise sauce may have provided an opportunity for *Salmonella* present on the shell of the eggs to contaminate the sauce. The sauce is served over multiple breakfast sittings. Due to lack of dates on the bottles it was also possible the sauce was kept for longer than is hygienically responsible. In comparison, the poached eggs, even if contaminated from the shell, could possibly be sterilised during the cooking process, depending on the length and temperature of cooking.

This conclusion is not supported by other laboratory evidence as *Salmonella* was not isolated from other food samples. However, the high turnover of food at the restaurant means it is unlikely that the food tested was from the same batch as the food that caused the illness. It is also possible that the hollandaise sample collected from the restaurant was more acidic than the sauce that likely caused the infection, thus mitigating against achieving *Salmonella* growth in the laboratory test.

There is a body of evidence that links outbreaks of STm44 with contaminated eggs and food containing raw eggs. An analysis of the OzFoodNet outbreak register data from January 2001 to December 2008

identified 12 outbreaks of STm44 associated with consumption of eggs or foods with eggs as a key ingredient.[3] Of these egg-associated outbreaks, the majority have occurred since 2006. Hollandaise sauce has been previously associated in outbreaks caused by a variety of *Salmonella* serotypes, including *S.* Hessarek and *S.* Typhimurium phage type 9 in Australia,[5,6] and *S.* Enteriditis in the United States of America.[7]

Limitations

One of the major limitations of this study was the small sample size and disproportionate numbers of cases to controls. This may have affected the results leading to an erroneous exclusion of other foods as possible sources of the infection. However, this would seem less likely given the supporting environmental evidence and higher attack rates among persons eating poached eggs and hollandaise sauce. An association between illness and consumption of eggs was strong enough to be detected in this group of consumers.

As mentioned previously, there is strong evidence of confounding in these results. Both poached eggs and hollandaise sauce had high odds ratios but because the 2 items are usually served together they could not be separated with stratification. All people who became ill and who ate hollandaise sauce, also ate poached eggs as the sauce is served as a topping. Only 1 person who became ill ate poached eggs without hollandaise sauce while another could not recall. Hence epidemiological evidence was incorporated with environmental evidence to formulate conclusions.

The investigation concluded the most likely cause of this outbreak was consumption of undercooked eggs or raw egg containing sauce putatively contaminated by *Salmonella*. This evidence was then used by the restaurant's egg supplier to institute a voluntary recall of product from outlets in both the Australian Capital Territory and New South Wales. This action may have averted future infections, as well as increased general awareness about appropriate procedures for the distribution of uncontaminated eggs. The incident also led to improvements in hygiene and food storage procedures at the restaurant and serves to highlight the need for further education of food handlers in relation to the preparation of dishes containing raw eggs. In a wider context, this outbreak demonstrates the importance of exemplary hygiene and food storage practices in restaurant settings as a means of reducing the risk of egg-related salmonellosis. This adds to mounting evidence that contaminated eggs are a leading cause of outbreaks and often in restaurant settings. In addition to highlighting the importance of effective hygiene measures within commercial kitchen settings in Australia, this also suggests the need for more stringent regulation for the production of eggs. Health authorities should also consider prohibiting commercial outlets serving dishes containing raw eggs to further reduce the risk to the public of *Salmonella* infection.

Acknowledgements

We would like to acknowledge the following people for their assistance with the investigation: Staff from the Communicable Disease Control section and Environmental Health section of the Health Protection Service, ACT Health. Staff from the ACT Government Analytical Laboratory for testing of food samples and the Microbiological Diagnostic Unit Public Health Laboratory, University of Melbourne for serotyping and phage typing of human *Salmonella* isolates.

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References

1. Heymann DL, ed. *Control of Communicable Diseases Manual*. 19th ed. 2008: American Public Health Association: Washington USA.
2. Australian Government Department of Health and Ageing. National Notifiable Diseases Surveillance System. Accessed on 27 January 2009. Available from: http://www9.health.gov.au/cda/Source/CDA-index.cfm
3. The OzFoodNet Working Group. OzFoodNet annual report, 2006. *Commun Dis Intell* 2007;31(4):345–365.
4. Australian Egg Corporation Limited. Code of Practice for Shell Egg, Production, Grading, Packing and Distribution, 2008. Accessed on 4 February 2009. Available from: http://www.aecl.org/
5. The OzFoodNet Working Group. OzFoodNet quarterly report, 2005. *Commun Dis Intell* 2005;29(2):197–199.
6. The OzFoodNet Working Group. OzFoodNet quarterly report, 2004. *Commun Dis Intell* 2004;28(3):401–405.
7. Mintz ED, Cartter ML, Hadler JL, Wassell JT, Zingeser JA, Tauxe RV. Dose-response effects in an outbreak of *Salmonella* Enteritidis. *Epidemiol Infect* 1994;112:13–23.