A multi-jurisdiction outbreak of *Salmonella* Typhimurium phage type 135 associated with purchasing chicken meat from a supermarket chain

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Abstract

A multi-jurisdiction case control study was conducted after an increase of *Salmonella* Typhimurium phage type 135 notifications (a local designated subgroup) was observed throughout Australia. Hypothesis generating interviews conducted in three jurisdictions identified consumption of chicken, eggs, beef and bagged carrots as common among cases and that a high proportion of cases (>80%) reported purchasing their groceries from a particular supermarket chain (Supermarket A). We conducted a case control study to test whether *S.* Typhimurium 135 infections were associated with these food items and the purchasing of these products from Supermarket A. The study comprised 61 cases and 173 controls. Cases were younger than controls (p=0.003) and their distribution by jurisdiction was also significantly different (p<0.001). In multivariate analysis, cases had significantly higher odds of having eaten chicken purchased from Supermarket A (OR=3.2, 95% CI 1.2,9.0) or having eaten chicken from a fast food outlet (OR=2.8, 95% CI 1.0,7.7) compared to controls. Two positive *S.* Typhimurium 135 results were obtained through a chicken sampling survey conducted at four Supermarket A stores in Victoria. The results of this study were presented to industry and retail representatives, which facilitated better communication between these groups. *Commun Dis Intell* 2006;30:449–455.

Keywords: Salmonella *Typhimurium, outbreak, chicken, case-control study*

Introduction

In September 2005, Tasmania, Victoria and New South Wales reported increased notifications of a locally designated strain of *S.* Typhimurium 135 (*S.* Typhimurium 135a). Nationally, the number of notifications in November 2005 was six times higher than in November 2004 (unpublished data: National Notifiable Diseases Surveillance System, Australian Government Department of Health and Ageing). In the period October to December 2005, Tasmania investigated four point source outbreaks of *S.* Typhimurium 135a associated with egg-based dishes prepared by bakeries, restaurants and caterers. These eggs were all sourced from the same egg farm. However, Tasmania also observed an increase in sporadic cases of *S.* Typhimurium 135a that were not associated with a defined point source outbreak. In addition, South Australia, New South Wales, Queensland and the Australian Capital Territory also received notifications during this time, although not above expected levels.

Previously in Australia, *S.* Typhimurium 135 and *S.* Typhimurium 135a had been associated with chicken,[1,2] raw egg products,[3–6] bakery products,[7]

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pork-filled bread rolls from Asian bakeries,[8–10] and a commercial fruit juice.[11] *S.* Typhimurium 135a is a sub-type of *S.* Typhimurium 135 that is used in Australia to more confidently identify epidemiological links between cases, but is not recognised internationally. Initially, the increase in cases was observed for *S.* Typhimurium 135, yet after further typing the focus became *S.* Typhimurium 135a. The National Enteric Pathogen Surveillance System, a voluntary system which collects data on human and non-human enteric pathogens in Australia,[12] reported isolating *S.* Typhimurium 135a from chicken meat samples from New South Wales and Victoria in October 2005. The Australian *Salmonella* Reference Centre also reported *S.* Typhimurium 135a in chicken meat samples from New South Wales (n=20), Queensland (n=19) and South Australia (n=10) in September 2005.[13]

Hypothesis generating interviews with people infected with *S.* Typhimurium 135a were conducted independently in Victoria, Tasmania and New South Wales to identify risk factors for illness. The food exposures most commonly reported by cases were chicken, eggs, beef and bagged carrots. A high proportion of cases (>80%) also reported purchasing their groceries from a particular supermarket chain (Supermarket A). As a result, OzFoodNet, a collaborative initiative of Australia's state and territory health authorities that aims to investigate and understand foodborne disease at a national level, coordinated a multi-state investigation into *S.* Typhimurium 135a infections. Given the similarities in food frequencies for a concurrent investigation of *S.* Typhimurium 44 cases, both phage types were included in the case control study.

The aim of this study was to determine whether there was an association between infection with *S.* Typhimurium 135a and *S.* Typhimurium 44 and the food products identified, in order to prevent further infections and inform future food safety measures. In this paper we report on the *S.* Typhimurium 135a component of the case control study.

Methods

Epidemiological investigation

We conducted a case control study to test whether *S.* Typhimurium 135a infections were associated with eggs, chicken, chicken products, bagged carrots and minced beef, and the purchasing of these products from Supermarket A. The investigation was conducted in the context of a public health intervention as per state and territory legislation. Consent was obtained from participants after being advised that participation in the study was voluntary and responses were confidential.

Study population

A case was defined as a resident of New South Wales, the Australian Capital Territory, South Australia, Victoria or Tasmania who had *S.* Typhimurium 135a isolated from a faecal specimen and notified to the respective jurisdiction between 2 November and 23 December 2005. Queensland also reported cases, but due to the notification rate not exceeding the expected threshold and competing demands, did not recruit cases into this study. Cases were excluded if they were co-infected with another enteric pathogen, returned from overseas travel within four days of onset of illness, were not the primary case in the household or were not interviewed within 40 days of specimen collection. Cases were also excluded if they had been part of the point-source outbreak investigations in Tasmania.

To identify controls we used progressive digit dialling, whereby phone numbers either side of each case's phone number were telephoned sequentially until an eligible household was contacted. The person in the household with the next birthday was invited to participate in the study. Attempts were made to recruit two controls for every case. Controls were excluded if they had returned from overseas travel in the previous four days or if a household member had diarrhoea in the previous two weeks. To compare another method of control selection, South Australia recruited an additional two controls per case using their control bank, a list of people that had participated in a previous health study that were willing to be contacted again.

Data collection

Cases and controls were interviewed over the telephone using a tailored questionnaire developed by OzFoodNet epidemiologists. Up to six attempts were made to contact each case and control phone numbers were called six times before a new number was attempted. Interviews were conducted on weekdays between 12 and 23 December, during the day and in the evenings. The interview included questions about food items consumed in the four days prior to onset of illness for cases or in the four days prior to interview for controls. Each jurisdiction was responsible for interviewing cases that resided in their jurisdiction and two controls per case. Completed interview data were entered by each jurisdiction onto a national NetEpi Case Manager (New South Wales Health) database, a web-based reporting system for which data entry can be conducted at multiple sites. OzFoodNet Central was responsible for maintaining the NetEpi system and downloading the data for analysis. No identifying information was entered onto the NetEpi system.

Data analysis

We obtained data from the National Notifiable Diseases Surveillance System to review the descriptive epidemiology of all cases of *S.* Typhimurium 135a. Statistical analysis was conducted using Microsoft Excel and Intercooled STATA version 8.[14] Demographics were compared using Pearson's chi-squared test for trend. Food exposures were compared using odds ratios with 95 per cent confidence intervals. Controls selected for both *S.* Typhimurium 135a and *S.* Typhimurium 44 were included in the analysis. A maximum-likelihood logistic regression model was constructed to analyse the association between chicken consumption and illness. This was adjusted for age and state of residence as well as those food items significantly associated with cases at the univariate level (p<0.05).

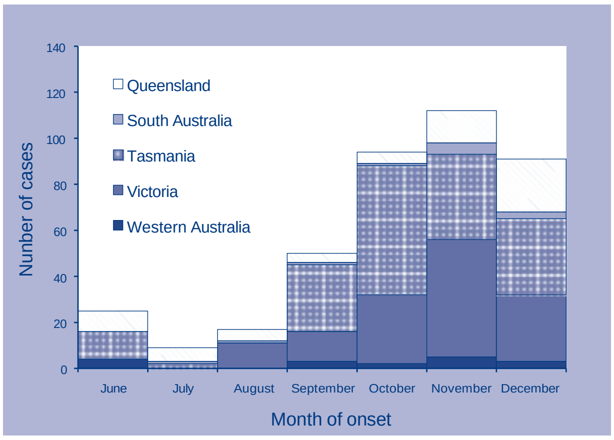
Environmental investigation

The Victorian Communicable Disease Control Unit conducted a food sampling survey, whereby chicken samples were obtained from four Supermarket A stores where cases reported purchasing chicken. Local government environmental health officers were requested to purchase chicken wings, a whole chicken and chicken breasts from the fresh pre-packaged section of the supermarket. These were then sent to the Microbiological Diagnostic Unit for *Salmonella* testing using standard methods of analysis.

Results

The epidemic curve (Figure 1) shows the increase in notifications of *S.* Typhimurium 135a from September 2005, which continued through the study period (December 2005). Most cases were from Victoria and Tasmania (point source outbreak cases included), with cases from New South Wales and Queensland increasing from October and November respectively.

Figure 1. Notifications of *S.* Typhimurium 135a, June to December 2005, by state or territory



The case control study was conducted between 12 and 23 December 2005 and comprised 61 cases and 173 controls (including those selected for the *S.* Typhimurium 44 study). From all jurisdictions 98 cases were enrolled in the study. Of these, 23 (23%) were ineligible and one did not have a phone number. Of the remaining 74 eligible cases, 11 (15%) were unable to be contacted; two (3%) refused and 61 (82%) completed the interview. For control recruitment using progressive digit dialling, calls were made to 729 individual phone numbers. Of these 293 (40%) were ineligible phone numbers (faxes or business numbers) and 19 (3%) were contacted but ineligible for the study. Of the 417 remaining, 200 (48%) were unable to be contacted, 70 (18%) refused and 147 (35%) completed the interview. South Australia, using their control bank, made an additional 40 calls, of which four (10%) were to ineligible phone numbers and three (7.5%) were contacted but ineligible. Of the remaining 33, six (18%) were unable to be contacted; one refused; and 26 (79%) completed an interview.

Symptoms reported by cases included diarrhoea (100%), cramps (88%), fever (86%), nausea (71%), headache (63%), vomiting (57%), muscle and body aches (49%) and blood in the stool (42%). The median duration of illness was 8 days (range 3–21 days) and 10 cases (17%) were hospitalised. The median age of cases was 23 years compared to 45 years for controls. The proportion of cases and controls by age group and by jurisdiction was significantly different (p=0.002 and p<0.001 respectively). There was no difference by sex (Table 1).

Table 1. Characteristics of cases and controls

	Cases		Controls		
Characteristic	**n**	**%**	**n**	**%**	**p value†**
Total	61	100	173	100	
Age group*					
1–9	22	36.1	23	13.3	0.002
10–19	6	9.8	10	5.8	
20–39	14	23.0	39	22.5	
40–59	11	18.0	51	29.5	
60+	8	13.1	47	27.2	
State					
ACT	1	1.6	5	2.9	<0.001
NSW	8	13.1	39	22.5	
Qld	0	0.0	21	12.1	
SA	6	9.8	51	29.5	
Tas	16	26.2	18	10.4	
Vic	30	49.2	39	22.5	
Sex*					
Females	25	41.0	74	42.8	0.802
Males	35	57.4	96	55.5	

* These variables may not add up to the total due to missing responses.

† P values calculated using Pearson's chi-squared test for trend.

In univariate analysis, when compared to controls, cases had significantly higher odds of having consumed chicken eaten outside the home; chicken eaten at a fast food chicken outlet; kebabs eaten at own home; other meat products purchased from Supermarket A; mince purchased from Supermarket A and; lamb purchased from Supermarket A (Table 2). Cases had significantly lower odds compared to controls of consuming lettuce, tomatoes, berries and carrots, as well as a combined fruit and vegetable variable.

Figure 2 schematically shows the different hypotheses tested for the association between illness and the consumption of chicken. Different combinations of chicken consumption categories were used to create the multivariate models, which were adjusted for age, state of residence, and lamb, mince and fruit and vegetable consumption (Table 3). After adjusting for these factors, cases had significantly higher odds of having eaten chicken purchased from Supermarket A (groups 6 and 8 compared to all others, OR=3.2) or having eaten chicken from a fast food outlet (OR=2.8) compared to controls. The odds of fruit and vegetable consumption were independently and significantly lower for cases compared with controls in all four multivariate models.

When selection of controls was restricted to controls selected for *S.* Typhimurium 135a cases only, the univariate results were similar, except that the odds ratios for any and meat and mince purchased at Supermarket A increased (data not shown). A similar increase in odds ratios was observed in multivariate analysis for chicken consumption category 1 (chicken versus no chicken) and category 3 (chicken eaten at home only, Supermarket A purchased versus other purchased), although they were not significant. The odds ratio for category 4 (home only and both at home and out, Supermarket A purchased versus all others) decreased.

When selection of cases was restricted to those from Victorian and Tasmanian cases only (the jurisdictions where most cases were reported), the univariate odds ratio for chicken purchased at Supermarket A became significantly associated with illness (OR=2.4, 95% CI=1.0,5.7). All the odds ratios decreased in the multivariate analysis, with wider confidence intervals and none were significant.

Environmental results

There were seven positive *Salmonella* results from the chicken sampling survey of Supermarket A in Victoria. A breast sample from one store and a thigh sample from another were positive for *Salmonella* Typhimurium 135a. Four samples, three breast and one drumstick from four different stores were positive for *Salmonella* Sofia and two, a thigh and wing sample from the one store, were positive for *Salmonella* Infantis.

Discussion

The results from this case control study suggest there was an association between infection with *S.* Typhimurium 135a and chicken consumption, in

Figure 2. Schema of hypotheses tested for association between chicken consumption and illness

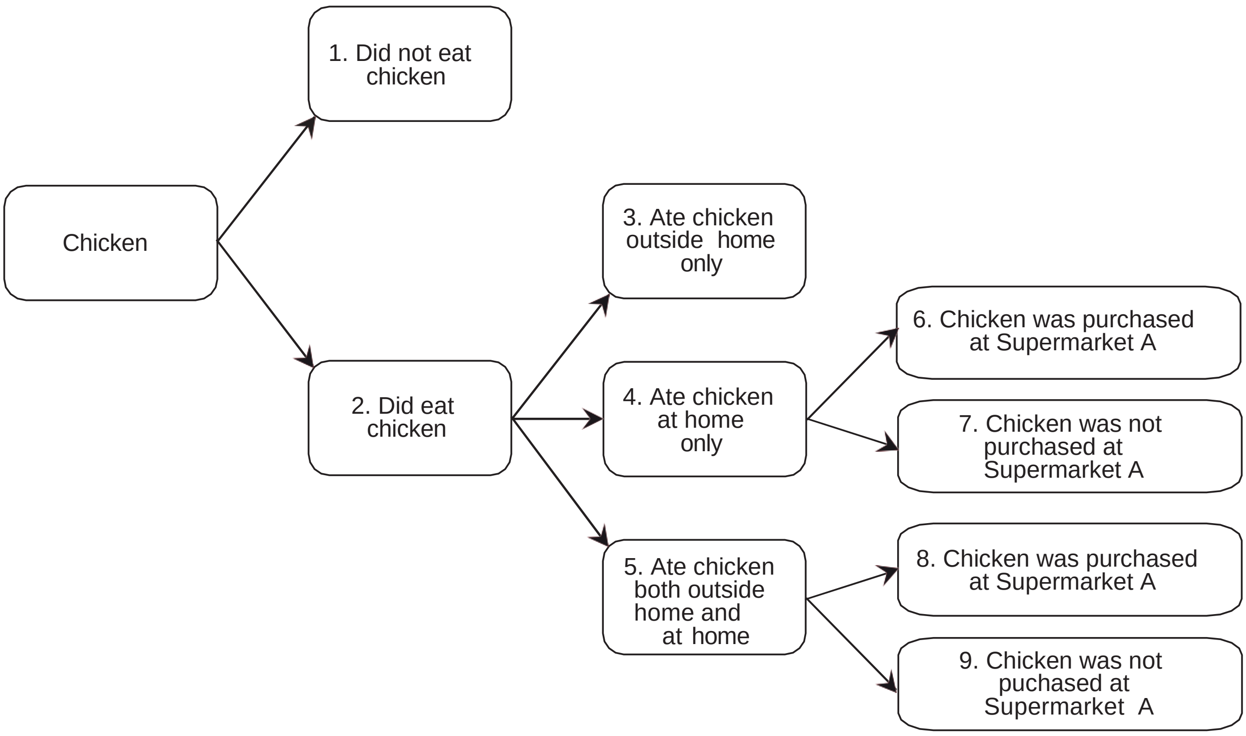


Table 2. Univariate analysis of exposure to selected foods amongst cases and controls

Exposure	Cases Exposed /total*	Cases %	Controls Exposed/ total*	Controls %	Odds ratio	95% CI	P value
Chicken							
Chicken	42/52	80.8	117/168	69.6	1.8	0.8-4.4	0.12
Chicken eaten outside home	30/50	60.0	72/168	42.9	2.0	1.0-4.0	0.02
Fast food chicken outlet	12/61	25.0	18/172	14.0	2.5	1.0-6.3	0.03
A la carte restaurant	4/61	8.7	9/172	7.1	1.3	0.3-4.8	0.70
Meal at another house	4/61	8.5	6/172	4.7	1.9	0.4-8.5	0.31
BBQ from supermarket	9/61	18.8	25/172	19.4	1.0	0.4-2.4	0.97
Chicken eaten at own home	25/51	49.0	63/166	38.0	1.6	0.8-3.1	0.16
Purchased at Supermarket A	13/61	52.0	22/173	12.7	1.9	0.8-4.2	0.11
Whole	3/51	5.9	10/168	8.8	1.0	0.2-4.1	1.00
Breast	13/51	25.5	35/168	20.8	1.4	0.6-3.2	0.46
Pieces	6/51	11.8	23/168	13.7	0.8	0.2-2.3	0.61
Kebab	4/49	8.2	2/168	1.2	8.1	1.1-91.6	0.01
Value added	3/50	6.0	5/169	3.0	2.1	0.3-11.7	0.30
Other meat							
Other meat total	46/54	85.2	148/170	87.1	0.9	0.3-2.4	0.76
Purchased at Supermarket A	19/61	31.1	31/172	18.0	2.1	1.0-4.2	0.03
Mince	19/47	40.4	45/163	27.6	1.8	0.8-3.7	0.07
Purchased at Supermarket A	9/61	17.3	10/163	6.1	2.8	1.0-8.2	0.03
Lamb	14/46	30.4	34/166	20.5	1.7	0.7-3.7	0.15
Purchased at Supermarket A	8/61	13.1	4/173	2.3	6.4	1.6-29.8	0.00
Pork	5/48	10.4	30/162	18.5	0.5	0.1-1.4	0.17
Hamburger	1/50	2.0	10/163	6.1	0.3	0.0-2.3	0.24
Ham	18/50	36.0	68/165	41.2	0.8	0.4-1.6	0.52
Salami	2/49	3.9	20/162	12.3	0.3	0.0-1.3	0.10
Eggs							
Any eggs	32/54	59.3	99/166	59.6	1.0	0.5-2.0	0.97
Eggs eaten outside the home	6/55	10.9	21/166	12.7	0.8	0.3-2.3	0.70
Eggs eaten at own home	25/53	47.2	88/167	52.7	0.8	0.4-1.6	0.50
Any raw egg product	21/60	35.0	53/173	30.6	1.0	0.5-2.1	0.47
Fruit and vegetables							
Lettuce	28/52	53.8	128/170	75.3	0.4	0.2-0.8	0.00
Tomatoes	24/53	45.3	123/169	72.8	0.3	0.2-0.6	0.00
Cucumber	23/55	41.8	80/166	48.2	0.8	0.4-1.5	0.39
Sprouts	2/59	3.4	11/168	6.5	0.4	0.0-2.1	0.29
Strawberries	18/52	34.6	61/169	36.1	1	0.5-2.0	0.97
Berries	2/57	3.5	26/168	15.5	0.2	0.0-0.9	0.02
Carrots	24/58	41.4	100/169	59.2	0.5	0.3-0.9	0.02
Apples	26/58	44.8	93/173	53.8	0.7	0.4-1.3	0.25
Kiwi	7/58	12.1	26/168	15.5	0.7	0.2-1.8	0.43
Any fruit/vegetables	50/60	83.3	166/173	96.0	0.2	0.1-0.7	0.00
Juice	18/58	31.0	54/168	32.1	1	0.5-1.9	0.88
Combined Supermarket A†	34/61	55.7	86-172	50.0	1.3	0.47-2.4	0.44

CI Confidence interval.

* Totals include all cases and controls in study, excluding those that answered, 'Don't know/unsure.' For example, the total for 'Chicken eaten outside home' includes those that said 'no' to having eaten chicken.

† Includes those purchasing fruit, vegetables, chicken and any meat from Supermarket A.

Table 3. Univariate and multivariate analysis of exposure to various chicken categories amongst cases and controls

Chicken category	Cases (%)	Controls (%)	Univariate analysis		Adjusted analysis*	
			OR	95% CI	OR	95% CI
Chicken vs no chicken (2 vs 1)	80.8	69.6	1.8	0.8–4.3	1.2	0.5–3.0
Out only chicken, from a chicken fast food outlet	25.0	14.0	2.5	1.0–6.3	2.8	1.0–7.7
Home only chicken, Supermarket A purchased vs other purchased (6 vs 7)	9.8	8.6	2.1	0.7–5.9	2.2	0.6–8.6
Home only and both at home and out, Supermarket A purchased vs all others (6 and 8 vs all other categories)	21.3	12.7	2.2	0.9–5.1	3.2	1.2–9.0

OR=Odds ratio, CI=Confidence interval

* Maximum likelihood logistic regression adjusted by age, state of residence, lamb, mince and fruit and vegetable consumption.

particular chicken purchased from Supermarket A. Cases were three times more likely to have eaten chicken purchased at Supermarket A and three times as likely to have eaten chicken purchased at a chicken fast food outlet. Analysis using controls selected for *S.* Typhimurium 135a cases only, and that restricted to Victoria and Tasmania, also supported the association between chicken and illness. The corroborating evidence of the two positive *S.* Typhimurium 135a chicken samples purchased at Supermarket A identified by cases is consistent with the findings of the case control study.

This study has several limitations. There were a high proportion of controls unable to be contacted and those recruited were significantly different to cases in respect of age and jurisdiction of residence. Controls were used from all jurisdictions in the combined *S*. Typhimurium 135a and *S*. Typhimurium 44 investigation regardless of whether there were cases in these jurisdictions, yet not all jurisdictions recruited two controls per case. These issues suggest that the controls may be an unrepresentative sample of the general population, which may have affected the results, although it is difficult to speculate in which direction bias may have occurred. Also, as the greatest increase in cases occurred in Victoria, and *S*. Typhimurium 135a cases from other states may represent background sporadic cases unrelated to the outbreak, using cases from other states might have reduced the power of the study to detect an association between exposure and illness.

Factors for *S*. Typhimurium 135a contamination or infection of retail chicken in this outbreak are unclear. It is plausible that the *Salmonella* infection of chicken that led to this outbreak occurred at the farm level. Poultry are exposed to *Salmonella* via sources such as feed or through environmental contamination and when introduced, *Salmonella* can spread rapidly throughout the flock.[15] Anecdotal evidence indicated that several farms, particularly in Victoria, had outbreaks of *S.* Typhimurium 135a in chickens during September and November 2005 that occurred concurrently with mice plagues.

It is also unclear why Supermarket A was more strongly associated with illness than other chicken retailers. The link may be due to the supermarkets purchasing chicken from affected suppliers. Discussions with food safety and retail specialists did not identify any hypotheses that would adequately explain this finding.

Salmonellosis outbreaks resulting from the consumption of chicken comprised 13 per cent of all outbreaks investigated in Australia from 1995 to 2000.[16] The incidence of human salmonellosis has increased in most industrialised countries in the 1980s and 1990s.[17] Sweden is an exception as authorities introduced voluntary testing for *Salmonella* and destruction of positive flocks between 1970 and 1984, after which the practice became mandatory. As a result the prevalence of *Salmonella* infection in chickens was reduced to 0.2–0.7 per cent in 1994 with a corresponding low prevalence of domestically acquired salmonellosis in humans.[18] The results of our investigations were communicated to industry, regulatory and retail representatives to improve longer-term objectives of reducing illness associated with chicken meat.

The results of the case control study indicated that cases were less likely to have reported eating fruit and vegetables in both the univariate and all the multivariate models. This was a consistent finding in this study, even when the results were adjusted for by age. Reasons for this protective effect of fruit and vegetables are unclear, although a possible explanation is that frequent consumers of fruit and vegetables are generally healthier and therefore less likely to become ill after eating contaminated chicken.

This outbreak demonstrates the importance of multi-jurisdiction cooperation and coordination for outbreak investigations. It was conducted over a short period of time, with most interviews completed within two weeks. Having a central web-based database for data entry allowed for efficient data analysis as current data could be downloaded directly on a daily basis. This combined effort from staff in most jurisdictions in Australia, co-ordinated by the OzFoodNet central office, should be repeated for future studies of this nature.

Acknowledgements

The authors wish to thank the Outbreak Investigation Team (in alphabetical order): Jim Adamopoulos (Vic), Leanne Cleaver (Tas), Michelle Cretikos (NSW), David Coleman (Tas), Frank Dawood (Vic), Victor Di Paola (Vic), Simon Firestone (Tas and DoHA), Rachel Hanson (Vic), Eric Johnson (Tas), Fiona Jones (Vic), Martyn Kirk (DoHA), Karin Lalor (Vic), Jeremy McAnulty (NSW), Tony Merritt (NSW), Avner Misrachi (Tas), Sally Munnoch (NSW), Lillian Mwanri (SA), Heather O'Donnell (Vic), Rhonda Owen (DoHA), April Roberts (NSW), Cameron Sault (Tas), Chris Sturrock (FSANZ), Kaye Sturge (Vic), Graham Tallis (Vic), Roscoe Taylor (Tas), Kate Turner (Tas), Tory Worgan (NSW) and the Disease Surveillance and Investigation Unit of the Communicable Disease Control Branch (SA), Salmonella Reference Laboratory of the Institute of Medical and Veterinary Science, Food Unit of the SA Environmental Health Service, Microbiological Diagnostic Unit and the Knox, Glen Eira, Greater Shepparton, and La Trobe Victorian Local Government Councils.

The Master of Applied Epidemiology program and OzFoodNet are funded by the Australian Government Department of Health and Ageing.

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