

## A *Salmonella* Typhimurium 197 outbreak linked to the consumption of lambs' liver in Sydney, NSW

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### SUMMARY

We identified an increase in the number of cases of *Salmonella* Typhimurium phage type 197 in New South Wales in February 2005. Cases were predominantly of Lebanese descent. To identify risk factors for illness, we conducted an unmatched case-control study including 12 cases and 21 controls. Eight of 12 cases (67%) and no controls reported eating lambs' liver (OR incalculable,  $P < 0.05$ ), and seven of nine cases (78%) and one of 21 controls (5%) reported eating fresh fish (OR 70.0,  $P < 0.05$ ). Among participants who did not eat liver, there was a strong association between eating fish and illness (OR 60.0,  $P < 0.05$ ). The fish was from divergent sources. Five cases had bought the liver from two different butcher's shops, which obtained the lambs' liver from a single abattoir. Consumption of liver is a risk for salmonellosis. Traditional dishes may place some ethnic groups at increased risk of foodborne disease.

### INTRODUCTION

Salmonellosis is a bacterial infection most commonly transmitted by contaminated food. The disease is characterized by a sudden onset of headache, abdominal pain, diarrhoea, nausea and sometimes vomiting. There are numerous serotypes of *Salmonella enterica* subsp. *enterica*, the most common being Typhimurium and Enteritidis; both are pathogenic for animals and humans [1]. In Australia, *Salmonella* Typhimurium was the most common agent identified for causing foodborne disease outbreaks during 1995–2004 [2–6].

Previously published studies have identified various meats as the vehicle for salmonellosis, including raw or undercooked ground beef [7–9], cooked beef [10, 11], salami [12], ham [13], pork meat [14–16], turkey [17], chicken [18, 19] and lambs' meat [20–22]. Two studies have found a link between offal and salmonellosis including chicken liver [23] and cows' and pigs' offal [24]. We were unable to find any reports of outbreaks linked to consumption of liver.

In New South Wales (NSW), Australia, laboratories are required to notify cases of salmonellosis to the NSW Health Department. Stool samples are routinely tested for salmonella when submitted for microbiology. A salmonella-positive sample in NSW is referred to one reference laboratory for serotyping. Once *S. Typhimurium* is identified, the sample is sent on for phage-typing to one of two laboratories in Australia. Surveillance and outbreak investigations

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are carried out by staff of public health units (PHU), which (at the time of the outbreak) were located in 17 area health services (AHS) across the state. PHU staff collaborate with food inspectors from the NSW Food Authority in these investigations.

In late February 2005 during routine analysis of surveillance data, we identified an increase in the number of cases of *S. Typhimurium* phage type (PT) 197 in NSW. By the end of the month, there were 33 cases notified, whereas the average background rate of *S. Typhimurium* PT 197 in NSW from 2002 to 2004 was five cases per month. At least two thirds of the cases resided in the Sydney metropolitan area and had a Lebanese surname. We investigated the apparent outbreak to identify its extent and cause.

## METHODS

### Descriptive epidemiology

We defined a case as a person with a diarrhoeal illness in whose stool *S. Typhimurium* PT 197 was identified between 17 January and 28 February 2005 and who was notified to the NSW Department of Health.

Initial cases were interviewed using a hypothesis-generating questionnaire covering a wide variety of foodstuffs including all foods eaten in the 3 days prior to illness. Because a large proportion of cases were found to be of Lebanese descent, cases were also asked about consumption of common Lebanese foodstuffs identified with the help of a Lebanese cookbook, several websites of Lebanese restaurants or caterers and discussion with a person of Lebanese background. All cases were contacted by telephone. From the hypothesis-generating interviews we developed a questionnaire to use in a case-control study. The interviews indicated that only a small number of foods (i.e. raw and cooked lambs' liver and fish) appeared more commonly eaten by the cases than might be expected and these were included in the case-control questionnaire. In addition, traditional Lebanese foods considered to be high risk for salmonella contamination (i.e. raw meats) were included in the case-control study questionnaire.

### Case-control study

We conducted an unmatched case-control study to examine risk factors for illness. Because a large proportion of cases belonged to the Sydney Lebanese community, the case-control study population was

restricted to people from this community. Cases were eligible for the case-control study if they were of Lebanese descent, resided in the Sydney metropolitan area, and had a stool specimen collection date between 17 January and 28 February 2005. We considered a case to be of Lebanese descent, if they reported that they, or one of their parents or grandparents were born in Lebanon. Cases were excluded if they reported symptoms of gastroenteritis whilst overseas during the study period or if a member of the case's household was ill within a few days before the case (secondary cases). Where the person was under the age of 16 years, their parent or carer was interviewed on their behalf. All cases who had completed the hypothesis-generating questionnaire were included in the case-control study. Cases were interviewed by a single interviewer in English. Where the interviewer reported that a case did not speak English well, a second interview was conducted in Arabic by an Arabic-speaking interviewer. Cases were interviewed between 28 February and 21 May 2005.

Controls were chosen from the Sydney metropolitan area based on the five local government areas (LGA) with the highest proportion of people aged  $\geq 15$  years born in Lebanon. This data was generated through the database collection warehouse HOIST [25]. All but one case resided in one of the identified LGA. Random telephone lists were generated from two AHS: Sydney South West and Sydney West. Records were then selected based on postcodes of the five LGA and where the record contained a surname. A person familiar with Lebanese names examined the list of surnames in order to identify of Lebanese surnames. People with Lebanese surnames were then contacted for interview.

Controls were included if they reported that they were of Lebanese descent. Controls were excluded if they had travelled interstate or overseas or if they had suffered from symptoms of gastroenteritis between 15 January 2005 and the interview date. Interviews were conducted in English or Arabic if the initial interviewer reported that the control did not speak English well. We attempted to contact potential controls up to seven times. Controls were interviewed between 19 April and 13 May 2005.

A short questionnaire was developed for cases and controls. Cases were asked about foods eaten in the week before onset of illness. For controls, food history was established for a typical week in February. Cases and controls were asked about their history of illness, travel, and consumption of various meats,

including lamb, beef, chicken, sliced meats, mince and sausages as well as liver and fresh fish. Details of where the food was bought and when it was eaten were recorded, if known.

### Environmental investigation

The source of the cases' meat and offal were traced to determine methods of handling and distribution. Two butcher's shops that supplied some of the cases were inspected to assess the potential for cross-contamination from other sources. Samples of raw lambs' liver were taken for laboratory analysis. Two environmental swabs were taken at one premise. The abattoir that supplied liver to the two butcher's shops and to one other case not included in the case-control study had been audited on 10 February 2005 as part of regular audits conducted on all businesses licensed by the NSW Food Authority and included a review of procedures during slaughter, preparation and handling of finished products.

### Statistical analysis

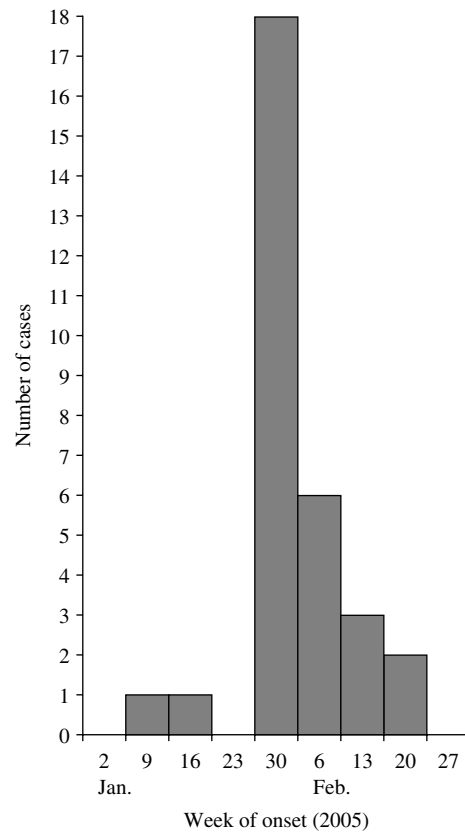
Statistical analyses were performed using SAS version 8.2 [26]. Univariate analysis was performed for place of residence, sex, age and all food exposures. Respondents who could not remember the food exposure were excluded from the analysis. Odds ratios (OR) were calculated where possible. We used the  $\chi^2$  test to calculate *P* values and normal confidence intervals (CI). Where the expected cell value was <5, a two-sided Fisher's exact test was used and exact 95% CI calculated. Because age was not normally distributed differences between cases and controls were analysed using the Wilcoxon Rank Sum test. For foods that were significantly related to illness, stratified analysis was performed.

## RESULTS

### Descriptive epidemiology

We identified 37 cases of salmonellosis caused by infection with *S. Typhimurium* PT 197. Of these, 24 (65%) had a surname consistent with Lebanese heritage. Of all 37 cases, information was obtained from 31, including onset of illness (Fig.). The remaining six cases could not be contacted or refused to participate, including two known to be of Lebanese descent.

Of the 31 cases, 20 (65%) were female, and the mean age was 28 years (median 23 years, ranging from



**Fig.** Epidemic curve of *S. Typhimurium* 197 outbreak: number of cases by week of onset (January–February 2005).

11 months to 81 years). Twelve cases (39%) resided in Sydney South West AHS, 11 (35%) in Sydney West AHS, six (19%) in North Coast AHS and one each in South East Sydney/Illawarra AHS and Greater Western AHS. The mean duration of illness was 9 days (median 8 days, range 4–28 days), excluding one case who reported illness lasting for 2.5 months. Cases most commonly reported diarrhoea and tiredness (97%), followed by watery diarrhoea (93%), fever (90%), abdominal pain (79%), nausea (70%), vomiting (52%), headaches (48%) and blood in stools (38%).

Twenty cases (65%) were of Lebanese descent and of these, 13 were born in Lebanon. Seven cases had either a parent or a grandparent who was born in Lebanon.

### Case-control study

We identified 20 potential cases who were eligible for the case-control study; eight (40%) cases were excluded, including four cases who refused to be

Table. Characteristics and food consumption of cases and controls participating in the case-control study

Demographics	Case-patients <i>n</i> = 12 (%)	Controls <i>n</i> = 21 (%)	Crude OR (95 % CI)*	<i>P</i> value†
Place of residence				
Sydney West AHS	5/12 (42)	11/21 (52 %)	0·6 (0·2–2·7)	0·55‡
Sydney South West AHS	7/12 (58)	10/21 (48 %)		
Sex				
Male	2/12 (17)	6/21 (29)	0·5 (0·04–3·7)	0·68
Female	10/12 (83)	15/21 (71)		
Age (years)				
Mean age (range)	26·8 (2–51)	15·8 (4–74)		0·37§
Median age	29·5	17		
Food consumption in February				
Lamb	7/10 (70)	13/21 (62)	1·4 (0·2–11·0)	1·00
Cooked lambs' liver	6/12 (50)	0/21 (0)	Incalculable	0·00083
Raw lambs' liver	5/12 (42)	0/21 (0)	Incalculable	0·0033
Any lambs' liver	8/12 (67)	0/21 (0)	Incalculable	0·000036
Beef	4/10 (40)	13/21 (62)	0·4 (0·07–2·4)	0·44
Mince	6/10 (60)	14/21 (67)	0·8 (0·1–4·9)	1·00
Sliced meats	4/10 (40)	5/21 (24)	2·1 (0·3–14·0)	0·41
Sausages	5/11 (45)	4/21 (19)	3·5 (0·5–23·9)	0·21
Chicken	9/9 (100)	19/21 (90)	Incalculable	1·00
Fresh fish	7/9 (78)	1/21 (5)	70·0 (4·2–3299·2)	0·00013

AHS, Area Health Service.

\* OR, Odds ratio; CI, confidence interval (exact CI when *P* value calculated with Fisher's exact test).

† All *P* values calculated with Fisher's exact test (two-sided), unless otherwise specified.

‡ *P* value calculated with  $\chi^2$  test (if all cells expected counts > 5).

§ Wilcoxon Rank Sum test (two-sided).

re-interviewed, two who had suffered from gastroenteritis whilst overseas, one secondary case and another case who was overseas at the time of interview.

To obtain controls, we contacted 167 households. Of these, 83 (50 %) refused to participate or were unable to answer the call, and 47 (28 %) were unable to be contacted. Of the remaining 37 (22 %) potential controls, 16 (43 %) people did not meet the inclusion criteria (15 were not of Lebanese descent and one reported gastroenteritis between 15 January 2005 and the interview date).

Twelve cases and 21 controls were included in the case-control study. The 12 cases included three clusters of two people from single households: two sisters, a mother and her daughter, and a mother and her son. None were secondary cases. None of the controls lived in the same household.

When food consumed by cases in the week before onset was compared with food consumed by controls in a typical week in February there were no significant differences among cases and controls by place of residence, sex, age, and reported consumption of

lamb, beef, mince, sliced meats, sausages and chicken (Table). However, cases were significantly more likely to report eating raw, cooked or any lambs' liver, and fish.

Eight (67 %) cases reported eating lambs' liver in the week before onset. Of these, five became ill within less than 48 h of consuming lambs' liver (three cases could not remember the exact exposure date). Five (42 %) cases reported eating raw liver. Three cases reported buying the liver from butcher A, and two from butcher B. A sixth case reported possibly buying liver from butcher A. Another two cases could only remember the suburb where the butcher was which did not correspond with the location of either butchers A or B.

Seven cases reported eating fish in the week before their onset of illness. Of these, four had also eaten lambs' liver. Among those participants who ate fish, there was no significant association between eating lambs' liver and illness (OR incalculable, *P* = 1·0). Three cases who ate fish did not report eating lambs' liver. Among those participants who did not eat lambs' liver, there was a strong association between

eating fish and illness (OR 60.0, 95% CI 2.9–1236.6,  $P=0.0067$ ). Of the seven cases, three had caught the fish themselves or by another family member, and four had bought the fish from three different fish shops. Only one control reported having eaten fish. Among all participants there was a strong correlation between eating fish and liver (OR 18, 95% CI 1.2–933.4,  $P=0.017$ ).

One case, a child who became ill on 15 February 2005, who was not of Lebanese descent, was not apparently linked to the outbreak. However, on interview the case's mother reported that the case's father worked at abattoir X and regularly brought home lambs' meat for human and canine consumption. The case's mother reported that the child had probably fed raw lamb to the family's pet dogs, but had not eaten liver herself.

### Environmental investigation

The inspections of butchers A and B on 14 and 15 March 2005 did not identify evidence of cross-contamination between lambs' liver and other meats in current practices. The livers arrived in bags and were displayed in a separate tray avoiding direct contact with other meats. Samples of available lambs' liver from both butchers were negative for salmonella as were the environmental swabs taken from the meat slicer and the cutting board at butcher A. Butchers A and B sourced their liver from abattoir X. Records from abattoir X showed between 2500 and 3000 sheep were slaughtered daily in January 2005. Quantities of lambs' liver in the order of hundreds of kilograms per day were distributed to over 90 shops and wholesalers in NSW during that period.

An audit of the abattoir coincidentally done on 10 February 2005 assessed the hygiene and sanitation practices, slaughter and processing, structural integrity of the buildings, and operation of the business against the Hazard Analysis and Critical Control Points (HACCP) programme. Procedures such as cleaning of surfaces in the offal room, washing and trimming of liver from lamb carcasses, handling and chilling of liver post-cutting, and movement of liver from the offal room were found to be adequate and conformed to the HACCP programme. Microbial testing and practices in the offal area were reviewed by the abattoir once the outbreak was identified. Four swab samples collected from the offal room (viscera trays, viscera chutes, viscera tubs, table surfaces) in

the second week of April 2005 were negative for *Salmonella* species.

### DISCUSSION

The most likely cause of this outbreak was consumption of contaminated lambs' liver or foods cross-contaminated from contaminated lambs' liver during preparation. That the outbreak was associated with lambs' liver is supported by the strong association of illness with consumption of lambs' liver, the trace-back to a single abattoir and the unrelated ill child. Although we found that eating fresh fish was also associated with illness it is less likely that fish was responsible for this outbreak because consumption of fish and lambs' liver was highly correlated, the fish came from divergent sources and we could not find a plausible explanation as to how eating fish could account for the outbreak.

Limited published literature suggests that salmonella in sheep is not very common. A study of 1117 sheep carcasses from 20 Australian abattoirs over winter and summer in 2004 failed to detect any salmonella [27]. Another study conducted in Great Britain examining the prevalence of faecal carriage of salmonella in healthy sheep at the time of slaughter showed a very low prevalence (0.1%) compared to pigs (23.0%) [28]. An incidence rate of 1.5% of salmonella in lamb carcasses (24 h after slaughter) was found in a study from the United States [29]. While environmental swabs from the butchers and abattoir were negative for *Salmonella* sp., the swabs may not reflect conditions at the time of the outbreak, and the small number of swabs may not have been representative of conditions throughout the premises.

There are several limitations in this study. Numbers of cases and controls are small and we included cases on whom hypothesis-generating interviews were done in the case-control study. Some of the cases and controls spoke only Lebanese. We tried to minimize the language and cultural barriers by using an Arabic-speaking interviewer for cases who did not speak or had difficulty in speaking English. The recruitment of controls also had its limitations in regard to the small number of potential controls contacted who were eligible for inclusion and completed the interview. Whether our small number of controls is truly representative of the Lebanese community in Sydney is unknown. The relatively long time lag between the time of illness and interview of cases for food exposure, may have led to recall difficulties. This was



mainly due to the time between specimen collection and the availability of the results of salmonella phage-typing. In addition, we did not collect information on how the lambs' liver eaten by cases was prepared, or practices that may have contributed to cross-contamination with other foods during preparation. Cases and controls were asked detailed questions about what they ate during their exposure periods, rather than their normal eating habits. We do not believe that the later information would have provided additional insights as to the causality of this outbreak.

Little is known about salmonellosis associated with lambs' liver. Only two studies showing an association between offal and salmonellosis were identified, where food-handling error [23] and processing [24] resulted in cross-contamination of cooked with raw product. Both these studies confirm that undercooked or raw offal can pose a risk for salmonellosis. It is likely that the cases who ate cooked liver in our study became ill from either undercooked liver or through cross-contamination during a food-handling error.

Eating raw or undercooked meats has been associated with *Salmonella* infection in several studies [7–9, 14, 20]. This pattern of eating continues to be an ongoing public health risk. Greater awareness among the public, especially ethnic groups, who favour raw or undercooked meat or offal, may help reduce this risk.

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## DECLARATION OF INTEREST

None.

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