A statewide outbreak of *Salmonella* Bovismorbificans phage type 32 infection in Queensland

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Abstract

Between 30 May and 1 June 2001, 10 cases of *Salmonella* Bovismorbificans infection were reported to Public Health Services, Queensland Health. Investigations included enhanced surveillance, case interviews, a matched case control study, environmental audit and microbiological testing of faecal isolates (phage typing) and implicated food products. Forty-one cases of *S.* Bovismorbificans infection were detected, 36 cases were phage type 32. A matched case control study identified that illness was associated with consumption of food from 15 outlets of a fast food chain, Company A (matched odds ratio [MOR] 17.5, 95% CI 2.0 –657.3, p = 0.004) and consumption of a particular product, Product X (MOR undefined, p < 0.001) in the week before onset of illness. Manufacturers of Product X ingredients were audited. Deficiencies were identified in equipment cleansing at the salad mixture processing plant (Manufacturer M). A swab of food residue behind the cutting wheel rim of the lettuce shredder was positive for *S.* Bovismorbificans phage type 32. This appears to be the first reported Australian outbreak of salmonellosis associated with a lettuce product. The investigations suggest that inadequate maintenance of cutting equipment to prepare lettuce ingredients for Product X by Manufacturer M was a key factor in this statewide outbreak. The statewide nature of this outbreak demonstrates the role of timely serovar identification of *Salmonella* isolates by a reference laboratory as an aid to outbreak identification, and the importance of adherence to appropriate food safety procedures in the manufacture and preparation of mass produced food items for the public. *Commun Dis Intell* 2002;26:568–573.

Keywords: Salmonella *Bovismorbificans; outbreak; fast food; lettuce*

Introduction

On 30 May 2001, the State Public Health Microbiology Laboratory reported 7 cases of *Salmonella* Bovismorbificans infection to the Foodborne Disease Epidemiologist, Queensland Health. A further 3 cases were reported in the next 48 hours. The 10 cases had occurred across several public health jurisdictions within Queensland. All 10 reported cases had a faecal collection date within a seven-day period. Previously, an average of 3 cases per month of *S.* Bovismorbificans infection were reported in Queensland between 1998 and 2000, with 8 cases reported in 2001 prior to 30 May. This paper describes the subsequent identification, investigation and control of a statewide outbreak of *S.* Bovismorbificans phage type 32 infection in Queensland during May to June 2001.

Methods

A statewide outbreak control team was formed with the aim of determining the cause of these infections and preventing further cases. All other Australian states and territories were notified of this outbreak and asked to review notifications of *S.* Bovismorbificans in their jurisdictions.

Selected hospitals, laboratories and clinicians were informed of the outbreak and were requested to collect faecal samples from all suspected cases of food poisoning. All *Salmonella* isolates were forwarded to the Public Health Microbiology Laboratory, Queensland Health Scientific Services for serovar identification. Initial cases of *S.* Bovismorbificans infection were interviewed by the Public Health Unit staff using a standardised questionnaire

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that sought information on environmental and occupational exposures and diet in the week before onset of illness.

Based on these interviews, a case control study was conducted using a telephone administered questionnaire that sought details on items consumed from fast food outlets in the week before onset of illness in the cases and the corresponding period in matched controls. A case was defined as any person notified to Queensland Health from 30 May 2001 with a *Salmonella* Bovismorbificans phage type 32 infection. Controls were obtained by asking the general practitioner of the case to nominate three patients matched by age group who had attended the practitioner within the last month and who did not have symptoms of gastrointestinal disease during that time. The case control study ceased on 17 June following publication of a media article on this date that described a *Salmonella* outbreak possibly linked to a fast-food chain.

Data were entered and analysed using Epi Info version 6.04d software.[1] Unmatched and matched odds ratios with 95 per cent confidence intervals and statistical significance tests were calculated to determine any associations between an exposure and illness. Analyses were performed using all cases including those who had been previously interviewed during the hypothesis-generating interviews. Further analyses were conducted using only those cases who had not previously been interviewed during the hypothesis-generating phase of the investigation (prospective cases only).

Manufacturers of implicated food products were audited. Food retention samples and environmental swabs collected from food suppliers and raw food products collected from farms, were submitted to the State Public Health Microbiology Laboratory. All *S.* Bovismorbificans isolates were forwarded to the Institute of Medical and Veterinary Science, Adelaide for phage typing.

Results

National surveillance data confirmed that the majority of cases of *S.* Bovismorbificans phage type 32 infections reported during the first 7 months of 2001 occurred in Queensland (Figure 1, Joan Powling, National Enteric Pathogens Surveillance Scheme Co-ordinator, Microbiological Diagnostic Unit, University of Melbourne, personal communication). No cases occurred outside Queensland during the period of the outbreak. By 30 July 2001, 41 cases of *S.* Bovismorbificans infection were notified, 36 of which were phage type 32. Thirty-two (89%) of these cases were interviewed. The median age of these cases was 22.5 years (range 1–72 years) and the M:F ratio was 1:1. Reported symptoms included diarrhoea (94%), abdominal cramps (91%) and vomiting (34%). Fourteen (44%) cases reported bloody stools. Dates of illness onset are described in Figure 2. The median time interval between onset of patient symptoms and receipt of notification was 7 days (range 3–22 days). Six (19 %) of the interviewed phage type 32 cases were hospitalised. There were no secondary household cases detected.

Figure 1. Reports of *Salmonella* Bovismorbificans phage type 32 to the National Enteric Pathogens Surveillance Scheme, 1997 to July 2001

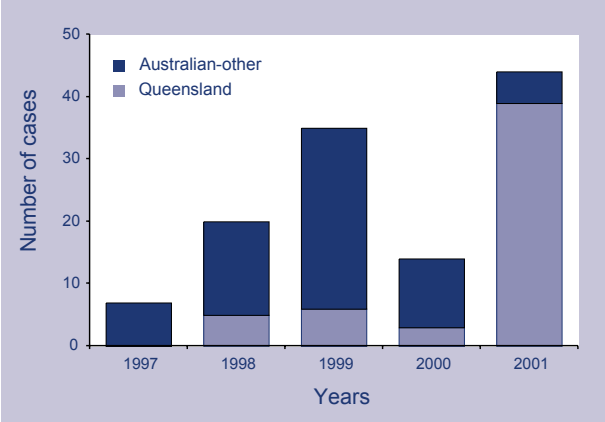
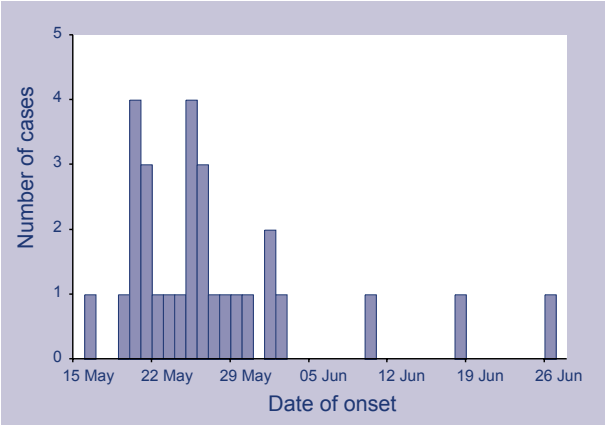


Figure 2. Notifications of *Salmonella* Bovismorbificans phage type 32, Queensland, by date of onset (n = 29)



Twenty cases (63%) reported having eaten at one of 15 outlets of a fast food chain (Company A) around the State in the week preceding their onset of illness. Most (95%) of these cases reported consuming Company A products between 13 and 30 May 2001, with one case consuming Company A products on 25 and 26 June 2001. Fourteen (70%) of these 20 cases reported consuming a particular product (Product X).

Twenty cases and 44 controls were included in the case control study. Among these 20 cases, the median age was 21.5 years (range 1–43 years), 50 per cent were aged between 20 and 39 years, and 55 per cent were female. Cases were more likely than controls to have eaten at a Company A outlet (MOR 17.5, 95% CI 2.0 –657.3, p = 0.004) and to have consumed Product X (MOR undefined, p < 0.001) in the week before their illness (Table 1). Findings were similar for cases that had not been previously interviewed with cases more likely to have eaten at a Company A outlet in the week before their illness (MOR 11.0, 95% CI 1.1 to 440.0, P = 0.05) and to have consumed Product X (MOR undefined, P < 0.001) (Table 2). There was no significant association between illness and consumption of items from any of eight other fast food chains in the week prior to onset of illness.

Table 1. Analysis of case control study of all *Salmonella* Bovismorbificans phage type 32 cases

Company/ product	Proportion exposed				Crude odds ratio	95% CI*	Matched odds ratio	95% CI	P value†
	Cases (n=20)		Controls (n=44)						
	n.	%	n	%					
Company A	14	70	13	30	5.6	1.5–21.2	17.5	2.0–657.3	0.004
Product U	0	0	2	5	0.0	0.0–9.6	?	–	0.90
Product V	0	0	1	2	0.0	0.0–42.8	?	–	0.72
Product W	0	0	2	5	0.0	0.0–10.1	?	–	0.80
Product X	11	55	0	0	?‡	9.5–?	?	–	<0.001
Product Y	2	10	0	0	?	0.4–?	?	–	0.10
Product Z	0	0	1	2	0.0	0.0–40.5	?	–	0.72
Company B	2	10	11	25	0.3	0.4–1.9	0.3	0.0–1.9	0.33
Company C	2	10	6	14	0.7	0.1–4.6	0.6	0.1–4.2	0.89
Company D	0	0	0	0	–	–	–	–	–
Company E	0	0	3	7	0.0	0.0–5.2	?	–	0.60
Company F	1	5	7	16	0.3	0.0–2.6	0.3	0.0–2.4	0.35
Company G	3	15	4	10	1.7	0.3–10.8	1.4	0.1–12.3	0.87
Company H	0	0	5	11	0.0	0.0–2.6	?	–	0.30
Company I	3	15	1	2	7.6	0.6–207.5	5.5	0.4–275.6	0.30

* 95% confidence interval

† Mantel Haenszel summary chi^2 test

‡ Odds ratios could not be calculated due to zero cell values

Table 2. Analysis of case control study of prospective *Salmonella* Bovismorbificans phage type 32 cases only

Company/ product	Proportion exposed				Crude odds ratio	95% CI*	Matched odds ratio	95% CI	P value†
	Cases (n=14)		Controls (n=33)						
	n.	%	n	%					
Company A	10	71	12	36	4.4	0.9–21.8	11.0	1.1–440.0	0.05
Product U	0	0	2	6	0.0	0.0–10.5	?	–	0.90
Product V	0	0	1	3	0.0	0.0–47.9	?	–	0.72
Product W	0	0	2	6	0.0	0.0–11.4	?	–	0.80
Product X	8	57	0	0	?	6.7–?	?	–	<0.001
Product Y	2	14	0	0	?	0.5–?	?	–	0.10
Product Z	0	0	1	3	0.0	0.0–44.3	?	–	0.72
Company B	2	14	9	28	0.4	0.1–2.7	0.4	0.0–2.9	0.56
Company C	1	7	4	12	0.6	0.0–6.5	0.6	0.0–5.9	1.00
Company D	0	0	0	0	–	–	–	–	–
Company E	0	0	2	6	0.0	0.0–10.5	?	–	0.90
Company F	1	7	3	9	0.8	0.0–10.0	0.9	0.0–18.2	0.61
Company G	2	14	3	9	1.6	0.2–14.6	1.4	0.1–12.3	0.87
Company H	0	0	4	12	0.0	0.0–3.8	?	–	0.42
Company I	2	14	0	0	?	0.5–?	?	–	0.21

Inspection of food safety standards at individual outlets identified no concerns. An audit of the manufacturer (Manufacturer M) of the salad mixture component used in Product X identified deficiencies in cleaning the equipment used for shredding the lettuce component of the salad mixture. A swab of food residue obtained from behind the cutting wheel rim of the lettuce shredder on 21 June was positive for *S.* Bovismorbificans phage type 32. Other environmental swabs, retention samples and source samples of Product X ingredients were negative for *Salmonella*. Epidemiological findings prompted Company A to obtain alternative supplies of salad mixture for their Product X on 20 June.

Discussion

Salmonella Bovismorbificans is a relatively common serovar with approximately 100 cases reported annually in Australia.[2,3,4] There have been 9 outbreaks of *S.* Bovismorbificans infection recognised in Australia since 1989, associated with phage types 7, 13, 14, 21, 23, and 24 (Milka Karna-Marelj, Salmonella Reference Laboratory, Institute of Veterinary and Medical Science, personal communication). Outbreaks of *S.* Bovismorbificans in Sweden and Finland were associated with Australian alfalfa sprout seed.[5] *Salmonella* Bovismorbificans phage type 32 has been reported in Australia only recently, with a national annual average of 19 cases between 1997 and 2000. It has been isolated from a variety of non-human sources, including ruminant animals and dogs.[6]

This outbreak of *S.* Bovismorbificans phage type 32 infection was strongly associated with eating food from Company A outlets and the consumption of Product X. The size of this outbreak is likely to be much larger than the 36 cases that were notified to Queensland Health. A recent population survey of diarrhoeal illness in Queensland found that 2.6 per cent of adults with acute diarrhoea during the preceding month had a faecal specimen submitted for pathology testing.[7]

All but one of the 20 cases of *S.* Bovismorbificans phage type 32 who indicated they had eaten Company A food had consumed it during a two and a half week period in May. Company A implemented a contingency plan on 20 June to obtain salad mixture for Product X from an interstate food manufacturer. The one case with an onset of 26 June may still be related to this outbreak on the basis of a prolonged incubation period and the case's reported regular consumption of Product X salad mixture. There were no cases notified to Queensland Health with an onset date after 26 June 2001. This supports the epidemiological evidence that Product X was the likely vehicle of transmission in this outbreak.

Based on the lack of cases appearing in other states or territories and the salad mixture being the only Product X component that was specific to Queensland, the outbreak control team suspected the salad mixture as a likely source of contamination for Product X. The manufacturer of the salad mixture (Manufacturer M) used a single lettuce shredder to prepare lettuce solely for the salad mixture for Product X. The detection of *S.* Bovismorbificans phage type 32 in food residue behind the rim of the julienne knife wheel of the shredder, supports the epidemiological evidence as to the likely vehicle of transmission in this outbreak. This evidence indicates that contaminated lettuce was the likely source of this pathogen and that the salad ingredient was responsible for the outbreak.

Six cases who ate at a Company A outlet had not consumed Product X. Four cases specified Product Y and 2 cases specified Product W. Company A confirmed that the salad mixture was also used as an ingredient of Product Y. It is difficult to explain the source of infection for the 2 cases who consumed Product W. Poor recall, cross-contamination of products, or sources other than Company A products are possible explanations.

Twelve (38%) cases of *S.* Bovismorbificans 32 infection had not eaten at Company A outlets in the week before their infection. A proportion of all cases during an outbreak will not identify the suspected exposure. Prolonged incubation periods, difficulties with recall of exposure history, or alternative unrecognised exposures may explain this finding.

The median time between onset of symptoms and notification to Queensland Health was 7 days. Cases and controls were interviewed about the fast food they had consumed in a seven-day period some 2 weeks before their interview. Consequently, recall bias is unlikely to have significantly influenced the outcome of the case control study. Questions were asked about food consumption from nine major fast food chains. There was no attempt to lead the cases or controls to choose a particular chain. The questions also specifically asked about all items on the menu thus offering the same non-leading information to both cases and controls. Interviewers were trained prior to implementation of the questionnaire and it is unlikely that they led the interviewees to their answers. Controls were obtained from GPs who were unaware of the hypothesis being tested and consequently selection bias should have minimal impact on these findings.

The similar demographic profile between all 32 cases investigated and the cases enrolled in the case control study, suggests that cases used in the case control study were representative of all notified cases. In addition, using retrospective cases in the case control study did not greatly bias the study results as shown by the similar findings when data were analysed using prospective cases only.

The environmental audit of Manufacturer M found that the cutting parts of the lettuce shredder were not being disassembled for daily cleaning as recommended in the instruction manual for the shredder. Trace back investigations did not reveal a source for the contamination of the lettuce and the manner by which the lettuce shredder became contaminated is unknown.

This appears to be the first reported outbreak in Australia of salmonellosis associated with a lettuce product. It demonstrates the importance of timely serovar identification of *Salmonella* isolates by a reference laboratory as an aid to outbreak identification. It illustrates the potential risk to public health created by the trend for

production of fresh food to be concentrated with larger food businesses, which can rapidly distribute the food to diverse geographic locations. Consequently, contamination of a single product may result in a major outbreak of foodborne illness because of the quantity of food produced and consumed.[8] Such outbreaks may be difficult to identify and investigate. This outbreak also contains important lessons for manufacturers of fresh food products about adherence to instructions for the cleaning of food preparation equipment.

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