

# OUTBREAKS OF *SALMONELLA* TYPHIMURIUM PHAGE TYPE 197 OF MULTIPLE GENOTYPES LINKED TO AN EGG PRODUCER

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## Abstract

This paper describes outbreaks of *Salmonella* Typhimurium phage type 197 (STm197) linked to eggs from the farm of a single egg producer. Epidemiological and microbiological investigations (genotyping by multiple locus variable number tandem repeats analysis [MLVA]) identified outbreaks of STm197 with the same or closely-related MLVA profiles in a series of restaurants across Brisbane over 2 months. Environmental health investigations revealed that these restaurants were supplied with eggs from the same egg producer and that cross-contamination may have contributed to the outbreak. Environmental swabs taken from restaurant kitchens and the farm of the egg producer identified a number of salmonellas including STm197, many with MLVA profiles matching or closely related to the human strains from outbreak cases. A case-to-case comparison study showed a significant association between illness with 1 MLVA type and attending a restaurant during the 5 days before onset of illness (odds ratio [OR] 8.1, 95% confidence interval [CI] 1.8, 35.4). MLVA has become a valuable tool for *S. Typhimurium* surveillance and outbreak investigation. This outbreak further justifies the Commonwealth Government's decision to develop a draft national primary production and processing standard for eggs and egg products to address food safety risks posed by cracked and dirty eggs. *Commun Dis Intell* 2009;33(4):419–425.

**Keywords:** eggs, *Salmonella* Typhimurium phage type 197, outbreak, genotyping, epidemiology, environmental health

## Introduction

In Australia, *Salmonella* Typhimurium (STm) is the most commonly notified serovar<sup>1</sup> of salmonellas causing gastrointestinal disease. For epidemiological purposes, isolates of STm have been differentiated by phage typing with more than 80 different phage types associated with infections in humans<sup>2</sup> in Australia. *S. Typhimurium* phage type 197 (STm197) was first reported in humans in Queensland in 2000. During 2003 to 2006, STm197 emerged as one of the 10 most commonly noti-

fied salmonellas in the State<sup>3</sup> with an average of 119 cases notified annually (personal communication, OzFoodNet Queensland).

Between 2002 and 2005 there were 4 reported STm197 outbreaks in Queensland.<sup>3–5</sup> Two of these outbreaks occurred in restaurants and one at a private residence with no food vehicle or source identified. The 4th was related to consumption of bakery products sourced from a manufacturer who used cracked and dirty eggs. Multiple small producers supplied the eggs and no trace-back was possible. Eggs have previously been implicated in STm outbreaks overseas,<sup>6–10</sup> throughout Australia<sup>11–14</sup> and in Queensland.<sup>15</sup>

Further rapid differentiation of salmonella isolates is often required in outbreak situations to identify additional cases and for source tracking. A variety of genotypic methods have been used to subtype STm including plasmid profiling, ribotyping, amplified fragment length polymorphism and pulsed-field gel electrophoresis.<sup>16</sup> A recently developed DNA fingerprinting technique, multiple locus variable number tandem repeats analysis (MLVA) has become very useful for this purpose. This polymerase chain reaction (PCR)-based technique, described by Lindstedt et al has been shown to have good discriminatory power between strains of STm.<sup>17</sup> Strain characterisation is based on differences in amplified DNA fragments at various loci in the salmonella genome, due to varying numbers of short-sequenced DNA tandem repeats (VNTR) at these sites.

In December 2006, contaminated eggs were the suspected source of a STm197 outbreak associated with 3 separate functions at a Brisbane restaurant (Restaurant A). Environmental sampling from the farm of the egg producer identified a matching STm197 genotype to the restaurant cases as well as several closely related MLVA genotypes. Surveillance in the following months identified further cases of STm197 with the same genotypic profiles. Case investigations led to the identification of outbreaks in 4 other restaurants (B1, B2, C, D).

This paper describes how MLVA typing, in combination with environmental and epidemiological investigations effectively linked this series of STm197 outbreaks of multiple related genotypes to

several restaurants in Brisbane and identified contaminated eggs from a single egg producer as the source of infection.

## Methods

### Microbiology

All human and environmental isolates of STm197 were typed by MLVA at the Public Health Microbiology Laboratory, Queensland Health. Five primer pairs were used to amplify the 5 VNTR targets and PCR products were sized by capillary electrophoresis. Fragment sizes were assigned a numerical code based on the coding system of Lindstedt et al,<sup>17</sup> e.g. 2-6-20-14-2 (corresponding to fragment sizes 171-330-312-369-489 for VNTRs STTR 9, STTR5, STTR6, STTR10 and STTR3). MLVA profiles which differ in size by one or 2 repeats at 1 locus are considered to be closely related and isolates with such closely related profiles should be viewed as possibly part of the same outbreak if epidemiological evidence is supportive.<sup>17</sup>

All STm isolates were sent to the Microbiological Diagnostic Unit in Melbourne for phage typing.

### Epidemiology

Notified cases of STm infection with closely related MLVA types were interviewed to obtain information on clinical presentation, food histories (including dining venues) for the 5 days prior to onset of illness, as well as other potential risk factors such as travel, exposure to other ill persons, activities associated with water, and domestic and wild animal exposure.

In the initial outbreak, retrospective cohort studies of patrons who attended two of the 3 functions at Restaurant A were conducted. A case was defined as any person who developed diarrhoea, vomiting and/or stomach cramps within 3 days after attending the restaurant. Both well and ill attendees were interviewed using a standard questionnaire, which included details of the food items consumed at the restaurant.

As more STm cases with closely-related MLVA profiles were identified, associations with other restaurants became evident. A case-to-case comparison study was undertaken to attempt to obtain epidemiological evidence demonstrating an association between illness and consumption of eggs.<sup>18-20</sup>

A case was defined as a person with STm 197 infection with the MLVA profile 2-6-20-14-2 notified after 27 January 2007 and residing in the Brisbane Statistical Division. The comparison group was randomly selected from all non-STm197 infections notified during the same period and residing in the

Brisbane Statistical Division. They were frequency matched to cases by age group: 0-4 years, 5-19 years and 20+ years. Statistical analysis as an unmatched case-control study was conducted using Epi Info® version 3.3.2.<sup>21</sup>

### Environmental health

Restaurants identified following case investigation were inspected by Environmental Health Officers to assess compliance with the *Food Act 2006* and the Food Safety Standards. This also included environmental sampling. The egg producer provided a list of all restaurants that were directly supplied with eggs by the producer.

Restaurant A was reinspected in early January 2007 and samples of chicken and eggs were collected and a trace-back of their origin was conducted. Information about the egg and poultry producers was obtained from Safe Food Production Queensland (SFPQ). SFPQ is the Queensland arm of the network of food safety regulatory agencies across Australia responsible for safety and suitability of food for human or animal consumption from the primary production sector (meat, dairy, eggs, seafood, and plant products).

Inspections at the farm of the egg producer took place on 19 January and 19 February 2007. A variety of samples were taken and submitted to QH Public Health Laboratory for microbiological testing (Table 1).

## Results

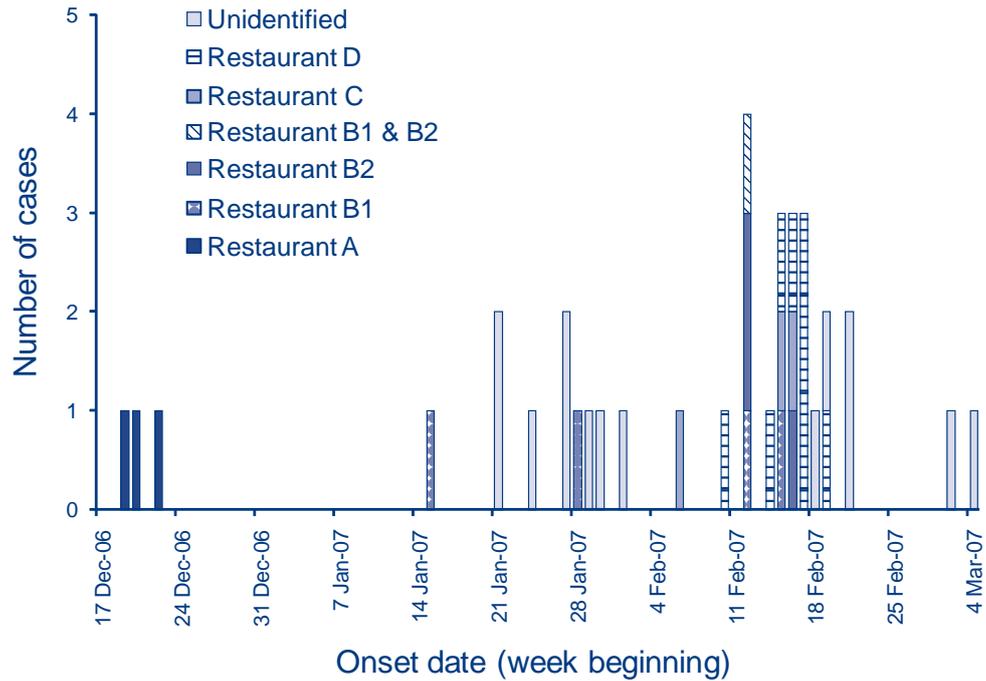
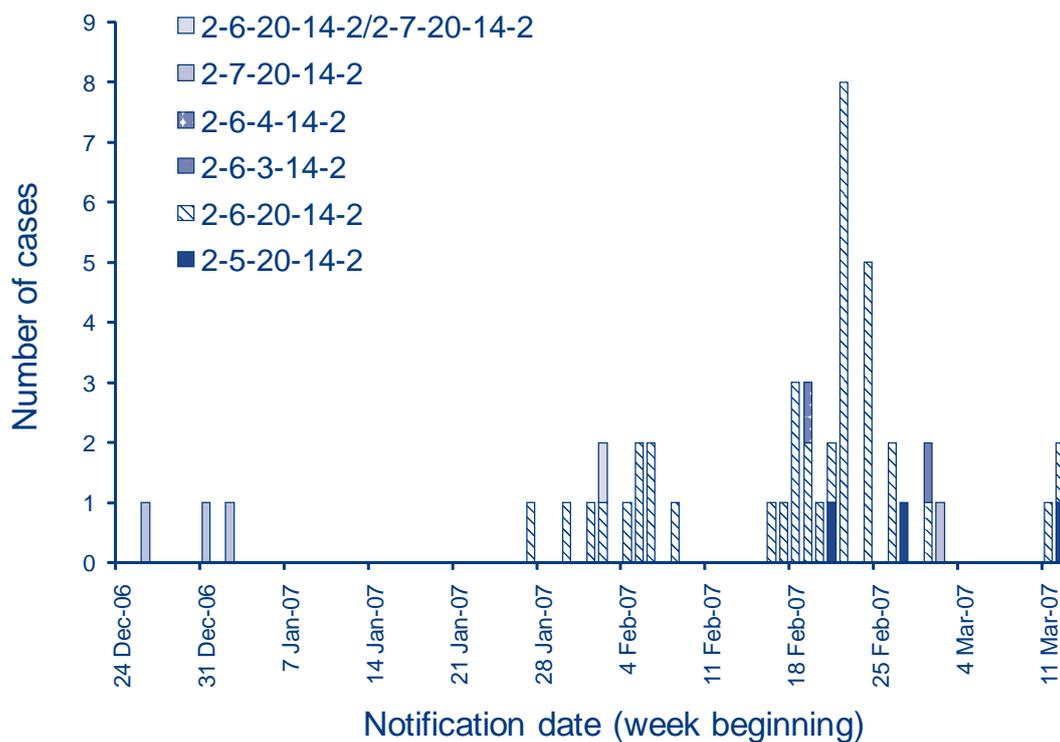
### Microbiology

In December 2006, there were 3 laboratory-confirmed cases of STm infection related to Restaurant A (one from each function) (Figure 1). STm was also isolated from a tea-towel obtained as part of environmental sampling at the restaurant. All these isolates had the MLVA profile (2-7-20-14-2) (Figure 2) and were subsequently phage typed as STm197.

Between 28 January and 4 March 2007, there were a further 19 cases of STm197 associated with a further 4 restaurants and 14 cases for which no restaurant could be identified (Figure 1). The isolates displayed 6 different closely related genotypes (Figure 2), with only two having the same genotype as the outbreak strain from Restaurant A. One case had 2 MLVA profiles identified in their stool specimen.

Many of the environmental samples taken from the farm of the egg producer were positive for STm197 (Table 1). MLVA profiles included the strain from the cases from Restaurant A (MLVA 2-7-20-14-2 from sawdust) as well as 2 closely related strains

Figure 1: Onset dates of cases in relation to eating at specific restaurants

Figure 2: Cases of implicated *Salmonella* Typhimurium infection, by notification date and multiple locus variable number tandem repeats analysis profile

The legend refers to the multiple locus variable number tandem repeats analysis profiles.

(MLVA 2-6-20-14-2 from sawdust and drag swabs; MLVA 2-6-3-14-2 from sawdust, drag swabs and boot covers), which were detected in later cases. In addition, there were other serovars of *Salmonella* identified including Singapore, Tennessee and Zanzibar (Table 1).

### Epidemiology

In total, 45 people attended the 3 functions held in mid-December 2006 at Restaurant A. Of these, 29 were included in the retrospective cohort studies, with 16 meeting the case definition. No specific food vehicle of transmission was identified in these studies.

From late January 2007, detailed investigation of cases yielding STm isolates with MLVA profiles identical or closely related to strains identified from the outbreak or the farm was carried out. By mid March 2007, there were 44 further cases of these STm197 infections notified to QH. Thirty-six of these cases had MLVA 2-6-20-14-2, which was the major profile identified in isolates from the egg producer. There were 4 other strains among the remaining 8 cases with MLVA profiles that were closely related.

Thirty-three of the 44 cases (76%) were interviewed. Eleven cases refused or were not contactable. The median age of interviewed cases was 21 years (range < 1–54 years); 14 were male and 19 were female (male:female ratio 1:1.4). Dates of onset of illness for the 33 interviewed cases were between 15 January and 4 March 2007 (Figure 2) with 8 cases hospitalised.

Hypothesis-generating interviews identified outbreaks from another 4 restaurants (Restaurants B1, B2, C and D) in Brisbane (Figure 1), involving a total

of 23 persons (19 laboratory-confirmed cases and 4 epidemiologically-linked cases). Two of the restaurants (B1 and B2) were in the same retail chain.

For the case-to-case comparison study, there were 25 cases and 22 comparisons recruited. Comparisons had a range of *Salmonella* serotypes including non-STm197 (n = 6), Virchow (3), Stanley (2), Birkenhead (2), Saintpaul (1), Reading (1), Infantis (1), Give (1), Chester (1), Chailey (1), Bovismorbificans (1), Aberdeen (1), and Muenchen (1).

Analysis of case-case comparison data showed cases were significantly more likely to have attended a restaurant during the 5 days before the onset of their illness compared with comparisons for the same period (OR = 8.1, 95% CI 1.8, 35.4,  $P = 0.003$ ). Similarly, cases were significantly more likely to have eaten at a restaurant that was supplied eggs by the egg producer, compared with comparisons for the same 5 day period (OR undefined,  $P < 0.001$ ) (Table 2).

### Environmental health

Environmental investigations revealed that all restaurants with identified outbreaks were supplied with eggs from the same egg producer. Trace-back of the restaurant's eggs found the egg producer had recently been investigated by SFPQ for selling cracked and dirty eggs.

Restaurant A was temporarily closed down by the local authority because of identified breaches of the *Food Act 2006*, which included issues with temperature control of food items and with cross-contamination, including problems with food storage, maintenance and cleaning, and staff practices.

**Table 1: Environmental samples taken from implicated egg farm (both visits)**

Collection date	Description	Pathogen detected / Number of samples	Pathogen(s)	MLVA profiles
19/1/2007	Drag swab	5/5	<i>S. Typhimurium</i> 197	2-6-3-14-2 2-6-20-14-2
19/1/2007	Sawdust	5/5	<i>S. Typhimurium</i> 197 <i>S. Tennessee</i>	2-6-3-14-2 2-6-20-14-2 2-7-20-14-2
19/1/2007	Boot covers	4/5	<i>S. Typhimurium</i> 197 <i>S. Zanzibar</i>	2-6-3-14-2 2-6-20-14-2
19/1/2007	Feed	1/1	<i>S. Singapore</i>	
19/1/2007	Faecal matter	2/4	<i>S. Typhimurium</i> 197	2-6-20-14-2
19/1/2007	Sorting machine swab	0/1	–	–
19/2/2007	Water supply	2/2	<i>S. Typhimurium</i> 197	2-6-20-14-2
19/2/2007	Eggs	1/1	<i>S. Typhimurium</i> 197	2-6-20-14-2

**Table 2: Odds ratios (OR) and 95% confidence intervals (CI) for STM 197 infection related to environmental exposures for cases and controls who ate outside the home**

Exposure 5 days prior to illness	Cases exposed		Controls exposed		OR	95% CI	P value
	n	%	n	%			
Attended any restaurant	22/25	88.0	10/21	48.0	8.1	1.8, 35.4	0.003
Restaurant (sit down)	20/24	83.3	6/21	28.6	12.5	2.98, 52.3	<0.001
Attended restaurant supplied by egg farm	15/24	62.5	0/21	0.0	Undefined	–	<0.001
Ate a meal outside of home	24/25	96.0	15/22	68.0	11.2	1.3, 100	0.015
Bakery	4/24	16.7	1/22	4.5	4.2	0.4, 40.9	0.20
Fast food or takeaway restaurant	14/22	64.0	10/22	46.0	2.1	0.6, 7	0.23
Café	7/24	29.2	4/22	18.2	1.9	0.4, 7.5	0.40
Hotel or pub	4/24	16.7	2/22	9.1	2.0	0.33, 12.2	0.38
Travel	0/17	0.0	0/22	0.0			
Hospitalised	7/25	28.0	5/22	22.7	1.3	0.35, 4.97	0.68
Any other family members sick	0/25	0.0	0/22	0.0			

There were also previous similar breaches of the Act identified by the local council with a cycle of enforcement followed by rectification.

Faecally-contaminated and cracked eggs were identified on the premises of Restaurant B1 on 15 February and environmental swabs (hand wash basin, chopping board, preparation bench and a display unit lid) from that restaurant identified 5 isolates of STm197 (MLVA 2-6-4-14-2), a profile found in only 1 case but closely related to the profiles of isolates from other cases. Samples from the other 3 restaurants were all negative.

At the time of the 2nd environmental health inspection of the farm of the egg producer (19 February 2007), the producer agreed to withdraw their eggs from sale. Attempts to identify the retailers and restaurants supplied by the egg producer were hindered by the producer's practice of distributing eggs from other producers co-mingled with their own stock. There was inadequate differentiation in the product traceability records to confirm the source of the eggs supplied to each restaurant as the producer's own stock or co-mingled stock from other producers. There were no further restaurant-associated cases detected with onset dates after 19 February 2007. There were no further outbreak strain cases notified to Queensland Health with an onset date after 4 March 2007.

Because of problems identified during audits of the establishment by SFPQ (including the supply of cracked and dirty eggs), SFPQ issued the egg producer with a Compliance Notice that all their eggs had to be washed and handled by another approved egg producer and were not to return to the

farm before being sold in Queensland. This was to prevent possible co-mingling of these eggs with eggs supplied by other producers.

## Discussion

The source of this outbreak was identified by a combination of work by public health laboratory staff, epidemiologists and environmental health investigators including those outside the health sector. MLVA of STm has become a valuable tool for surveillance and outbreak investigation. Its value arises from its ability to rapidly produce a genotype profile for isolates and to differentiate within phage types. In this outbreak, the MLVA technique was able to differentiate STm197 from other phage types, and was also able to distinguish the closely related outbreak strains from non-outbreak STm197. In Queensland, at least 32 different MLVA profiles of STm197 have been identified. Closely related profiles may reflect genetic drift in the genome of the bacteria, however the coding system used by Lindstedt et al<sup>17</sup> may not always reflect these affiliations. There is now a move in Australia to use a coding system developed in New South Wales that can more easily identify closely related MLVA profiles.

In early January 2007, epidemiology related the first 3 cases with STm infection of the same MLVA type (2-7-20-14-2) to Restaurant A, though retrospective cohort studies conducted from those who attended separate functions in mid-December identified no specific food vehicle. However, environmental health investigation did identify food hygiene issues, which facilitated the cross-contamination of food and this was supported by the microbiological findings.

Detailed investigation of cases with related MLVA profiles following the inspection at the egg pro-

ducer's farm helped identify further outbreaks in restaurants. Environmental health investigation of these restaurants also identified food hygiene and potential cross-contamination issues. The cracked and faecally-contaminated eggs found at Restaurant B1 then provided further support for the link to the egg producer and the results of environmental swabs taken from the same restaurant found MLVA profiles closely linked to those found at the egg producer's farm.

In retrospect, an earlier Queensland outbreak of STm197 in 2006 may also be linked to this egg producer. Another restaurant in south Brisbane with identified cross-contamination issues was found to be the source of this previous outbreak. Again, no specific food vehicle was identified. The MLVA profile of this earlier outbreak was closely related (2-5-20-14-2) to the current one. There were 3 notifications of this MLVA profile identified in the outbreaks discussed. Records could not confirm whether this restaurant had received product from the egg producer.

The case-to-case comparison study also provided supporting evidence with a significant association between cases of STm infection with MLVA profile 2-6-20-14-2 (the most common profile found by surveillance of cases of STm infection with MLVA profiles found at the farm) and attending any restaurant or attending a restaurant supplied by the egg producer. Case-to-case comparison studies should reduce selection and recall bias.<sup>19-21</sup> However, some potential limitations with this study can be considered:

- Though reduced, selection bias may have occurred because not all cases could be contacted or consented to participate in the analytical study.
- As comparisons were not more precisely geographically matched to cases (other than the Brisbane Statistical Division), they may not have had the same chance of eating at a restaurant supplied by the egg producer.
- There may have been misclassification of exposure based on the producer's practice of co-mingling eggs from other producers and on-selling to restaurants. This may lead to an over-estimate of the measure of association.
- There was potential for misclassification of the cases and comparisons as other serovars of *Salmonella* were also identified at the farm of the egg producer. Though none of the comparisons had serovars that were identified from the farm there is still a possibility that some of these comparisons may still have acquired their infection from the egg producer. This would reduce the measure of association towards the null.
- Comparisons may have eaten at restaurants supplied by the egg producer. However, it would still not be known if the eggs were from the implicated farm because other producers' eggs were co-mingled.

The cross-contamination problems and breaches of the Food Safety Standards in restaurants played a contributory role in this outbreak where once again, cracked and dirty eggs were found to be the culprit. Outbreaks of STm197 in Queensland in previous years have been related to restaurants<sup>4,5</sup> and to a bakery<sup>18</sup> where the manufacturer used cracked and dirty eggs. The national Food Standards Code prohibits the sale of cracked and dirty eggs *for retail or catering*. However, this Standard does not appear to have adequately protected public health as it allowed the sale of potentially contaminated, cracked and dirty eggs to restaurants and bakeries where food handling practices may allow cross-contamination of food products and food contact surfaces. The evidence from this outbreak illustrates that this does occur.

SFPQ introduced the Queensland Egg Food Safety Scheme in July 2005 to manage these identified hazards posed by cracked and dirty eggs. This scheme also covers unpasteurised egg pulp. Under this Egg Food Safety Scheme individual eggs are required to be stamped to identify their source, to ensure product traceability and facilitate foodborne illness investigation. The egg producer had identified compliance issues and SFPQ were taking steps to ensure compliance. However, experience in Tasmania<sup>11</sup> and of other jurisdictions<sup>12-14,22</sup> justifies the need for a national standard to address the food safety risks posed by cracked and dirty eggs (and unpasteurised egg pulp supply) and to tackle inadequate traceability issues at a national level. Food Standards Australia and New Zealand are addressing this issue with a proposed national primary production and processing standard for eggs and egg products, which is currently in the late stages of development.

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