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Research Note

An Outbreak of *Salmonella enterica* Serotype Litchfield Infection in Australia Linked to Consumption of Contaminated Papaya

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ABSTRACT

An outbreak of 26 cases of *Salmonella* Litchfield infection occurred in the states of Western Australia and Queensland between October 2006 and January 2007. A case-control study was conducted with 12 cases and 24 controls, and a significant association was found between illness and consumption of papaya (odds ratio, 32.8; 95% confidence interval, 2.71 to 883.5). Papaya samples were collected from 26 stores in Western Australia, and 9 of 38 samples were contaminated with *Salmonella* Litchfield. These samples had pulsed-field gel electrophoresis patterns and multilocus variable-number tandem-repeat analysis profiles indistinguishable from the outbreak strain. Three farms in Western Australia supplied the contaminated papaya, and two of these farms were inspected. *Salmonella* Litchfield was not detected in papaya samples, fungal sprays, or water samples from the farms; however, at one farm other serotypes of *Salmonella* were detected in untreated river water that was used for washing papaya. Only treated potable water should be used for washing fresh produce that is to be eaten raw.

In Australia, cases of *Salmonella* infection are reported to state and territory health departments, who in turn report these infections to the National Notifiable Diseases Surveillance System (NNDSS). The Australian government funds and coordinates OzFoodNet, a network of foodborne disease epidemiologists based in each of Australia's eight states and territories (jurisdictions). OzFoodNet maintains a national database of all reported foodborne disease and gastroenteritis outbreaks and routinely reviews *Salmonella* notification data in NNDSS to detect potential clusters or outbreaks of *Salmonella* infection. In November 2006, a routine review of *Salmonella* notifications in NNDSS revealed that notification rates for *Salmonella* Litchfield were higher than expected in the states of Western Australia and Queensland. Between 5 and 16 November, four cases of *Salmonella* Litchfield infection were reported in Western Australia, which was above expected based on the historical average notification rate of five cases per year. Between 26 October and 8 November, Queensland authorities were notified of six cases of *Salmonella* Litchfield infection, also above the expected number based on the average annual notification rate of 12 cases per year.

The *Salmonella* Litchfield serotype has been detected previously in other countries *(6)* and in Australia *(2)*. Nonhuman isolates of *Salmonella* Litchfield were found from 2002 to 2005 in four Australian states and included isolates from cats, cows, cucumber, dogs, tree nuts, and millet *(2)*. Review of OzFoodNet outbreak data from 2001 to 2005 revealed two outbreaks due to *Salmonella* Litchfield, one suspected to be associated with cucumbers *(3)* and one suspected to be waterborne *(10)*.

We began a multijurisdictional outbreak investigation in November 2006 to determine the source of the outbreak and to implement control measures.

MATERIALS AND METHODS

Epidemiological investigation. A case was defined as a person with *Salmonella* Litchfield infection who was reported to the Western Australia or Queensland health departments between 26 October 2006 and 16 January 2007. The pulsed-field gel electrophoresis (PFGE) profile of the infection isolate had to be indistinguishable from the outbreak strain. A case series analysis was conducted using hypothesis-generating interviews from the first seven cases who met the case definition criteria. The hypothesis-generating questionnaire contained questions about the illness, food consumption in the 7 days before illness, and other likely sources of infection such as contact with animals or other infected people and overseas travel. Based on the results of these questionnaires, hypotheses for a case-control study were developed. The case-control study was unmatched, with two controls interviewed for each case; controls were selected by progressively dialing telephone numbers that were one digit higher or lower than the case's telephone number. The person in the household with the next birthday after the interview date was interviewed. People

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were excluded from being controls when they had been ill with gastroenteritis in the 4 weeks before the interview or had traveled outside Australia in the 7 days before the interview. Odds ratios (ORs), Cornfield 95% confidence intervals (CIs), and chi-square or Fisher's exact *P* values were calculated using Epi Info 2002, revision 2 (Centers for Disease Control and Prevention, Atlanta, GA).

Environmental investigation. On 1 December 2006, 38 papaya samples were collected from 26 stores in Western Australia. These stores included branches of three major retail chains and four specialty fruit stores. Half of the samples were whole papaya, and the other half were papaya that had been cut in half in the store and wrapped in plastic wrap.

Two farms identified by traceback were inspected on 8 and 13 December 2006. Samples of papaya samples, water, fungicide, and spray oil were collected from the farms.

Laboratory investigation. Each whole papaya was rinsed in 250 ml of buffered peptone water (BPW; Merck, Darmstadt, Germany), and the rinse water was incubated at 37°C overnight. A 25-g sample of the flesh, skin, and seeds of the whole papaya was mixed with 250 ml of BPW and incubated at 37°C overnight. The plastic wrap covering the cut half-fruit was aseptically removed, placed into 250 ml of BPW, and incubated overnight. A 25-g sample of the skin, flesh, and seeds of the half-fruit was mixed with 250 ml of BPW and incubated at 37°C overnight. All preenrichment broths were tested for the presence of *Salmonella* by the DuPont Qualicon BAX system and culture method AS 5013.10-2004 *(4).* Aliquots were transferred to the selective enrichment broths: Muller-Kaufmann tetrathionate broth with novobiocin (Merck) and Rappaport-Vassiliadis (RV; Oxoid, Basingstoke, UK). Cultures were incubated overnight and subcultured onto xylose lysine desoxycholate agar (XLD; Oxoid) and bismuth sulfite agar (BSA; Oxoid) plates. Suspected *Salmonella* colonies were inoculated into a composite medium slant that detected fermentation of glucose, lactose, sucrose, sorbose, mannitol, and dulcitol, production of hydrogen sulfide, and splitting of urea *(8).* *Salmonella* isolates were then serotyped as described below.

Nine samples of rinse waters and fungicide solutions were tested for *Salmonella* using a validated variation of the Australian Standard method AS 4276.14-1995 *(1).* Samples were filtered through 0.45-μm-pore-size cellulose acetate filter membranes (Pall Gelman, Sigma), and the membranes were placed into 100 ml of BPW broth and incubated overnight at 37°C as a preenrichment procedure. Aliquots of 0.1 ml were transferred to RV enrichment broth and incubated at 42°C and into single strength strontium chloride B broth (Merck) *(7)* and incubated at 37°C. Both cultures were incubated for 48 h. After incubation, each broth was subcultured onto BSA and XLD plates that were incubated overnight at 37°C. Suspected *Salmonella* colonies were inoculated into the composite medium slant *(8)* and then serotyped as described below.

Fecal specimens from patients with suspected *Salmonella* infection were directly cultured onto XLD and deoxycholate citrate agar (DCA; Oxoid) plates. Strontium chloride B broth also was inoculated with a small amount of feces and incubated at 42°C for 18 to 24 h, and aliquots were then subcultured onto BSA and DCA plates. All plates were incubated at 35°C for 18 to 24 h. All negative BSA plates were incubated for a further 18 to 24 h to confirm the negative results (no metallic sheen, which is indicative of salmonellae).

Suspected colonies were inoculated into the composite medium slant *(8)* to test for substrate utilization. Slopes with typical *Salmonella* reactions were subsequently screened for O (somatic) and H (flagellar) antigens using polyvalent antisera (Remel, Lenexa, KS). Serotyping was completed by testing the suspect organism against polyvalent and individual (monovalent) *Salmonella* antisera (Serobact, Oxoid).

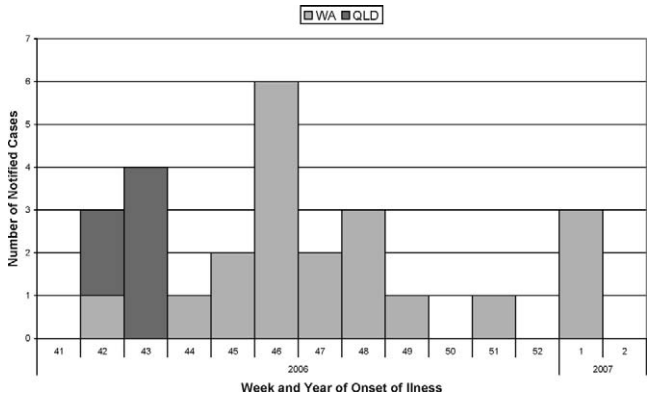

FIGURE 1. *Date of onset of illness for cases of infection with the outbreak strain of* Salmonella *Litchfield.*

Salmonella Litchfield isolates were typed by PFGE and multilocus variable-number tandem-repeat analysis (MLVA). PFGE typing was performed using the PulseNet method *(12)* after digestion with the enzymes *Xba*I and *Avr*II. PFGE was performed with a CHEF DRIII (Bio-Rad, Hercules, CA) using switch times of 2 to 64 s and 6 V/cm for 19 h at 14°C. MLVA was conducted using three of the five primer sets, STTR3, STTR5, and STTR9, from the MLVA typing method described by Lindstedt et al. *(9).* PCR products were sized by capillary electrophoresis on an ABI-310 genetic analyzer (Applied Biosystems, Foster City, CA).

RESULTS

Epidemiological investigation. Twenty-six people met the case criteria, 17 females and 9 males, with an age range of 8 months to 86 years, and a median age of 34 years. Twenty cases were from Western Australia, and six cases were from Queensland. The dates of onset of illness for the cases are shown in Figure 1.

The case series analysis revealed that the most commonly consumed foods in the 7 days before illness were papaya (6 of 7 cases, 86% of the time), banana (3 cases, 43%), red apples (3 cases, 43%), lettuce (3 cases, 43%), and eggs (3 cases, 43%). Because the papaya consumption was higher than expected based on previous *Salmonella* case interviews conducted in Western Australia in the previous year (none of 40 previous cases had consumed papaya), a case-control study was conducted to investigate the hypothesis that papaya consumption was associated with illness.

The case-control study was conducted with 12 cases (9 from Western Australia and 3 from Queensland) and 24 controls (18 from Western Australia and 6 from Queensland). The case-control study was stopped after these 36 cases had been interviewed because an interim analysis revealed a significant association between papaya consumption and illness (OR, 32.2; 95% CI, 2.71 to 883; $P = 0.0006$).

Environmental investigation. Papayas collected from stores on 1 December 2006 were contaminated with *Salmonella.* Of the 38 papayas collected, 9 were contaminated

TABLE 1. *Type and origin of papaya samples contaminated with* Salmonella *Litchfield*

Sample no.	Farm no.	Type of sample	Contamination[a]
1	1	Half of a papaya	Surface
2	2	Half of a papaya	Surface + flesh
3	3	Whole papaya	Surface + flesh
4	3	Half of a papaya	Surface
5	3	Whole papaya	Surface + flesh
6	3	Whole papaya	Surface
7	3	Half of a papaya	Surface
8	Not recorded	Whole papaya	Surface
9	Not recorded	Whole papaya	Surface

[a] *Salmonella* was isolated from surface rinsate or flesh (mixture of skin, flesh, and seeds).

with *Salmonella* Litchfield. The contaminated papayas were five whole papayas and four half-papayas (cut in half by store personnel) (Table 1). For six of the papayas, the contamination was found only on the surface, and for the other three papayas contamination was both on the surface and in a sample made from a mixture of flesh, skin, and seeds (Table 1). The nine contaminated papayas were from different branches of two major supermarket chains and from three fruit shops. Traceback revealed that the fruit came from three farms in one growing region in northern Western Australia; for two contaminated papaya samples the farm was not identified. Two farms were inspected, and samples were collected. Salmonellae were not detected in papaya and water samples collected from one farm. Three serotypes of *Salmonella* were detected in water samples from the other farm, including samples of water used to wash the papayas (Table 2). These serotypes were *Salmonella* Chester, *Salmonella* Eastborne, and *Salmonella* Poona.

The inspections revealed that the two farms were using similar growing and production practices. They were irrigating via trickle irrigation. Papayas were picked from trees by hand and transported to the processing sheds in small trucks. Papayas were then washed in a bath containing untreated water piped from a nearby river. A fungicide and low concentrations of chlorine (5 ml/100 liters at one farm and 118 ml of chlorine per month at the other farm) were added to the wash water. The papayas were then air dried, placed in individual foam covers, and transported to Perth (the major city in Western Australia) for distribution. The farms used different transport companies to transport the fruit to Perth. Papayas were picked by seasonal workers, who worked at only one farm at a time according to reports. Farms did not report a major pest problem, and baits were used to control rats and mice. Fruit bats had been a problem earlier that year, in May and June, but were not a problem at the time of the outbreak. Farms were producing other fruits and vegetables at the time of the outbreak, including bananas, pumpkins, and mangoes.

Investigators in Queensland were informed that papayas from Western Australia were not being distributed to Queensland at the time of the outbreak; however, bananas from this growing region in Western Australia were being distributed to Queensland.

TABLE 2. Salmonella *isolation results from samples collected from two farms that supplied contaminated papaya*

Farm no.	Type of sample	*Salmonella* isolation results
1	Unwashed papaya	Not detected
	Unwashed papaya	Not detected
	Washed papaya	Not detected
	Washed papaya	Not detected
	Papaya wash water	Not detected
	Water from a hose	Not detected
	Water from a storage tank	Not detected
2	Water from a hose	Not detected
	Water from the river	*Salmonella* Chester
	Papaya wash water	*Salmonella* Eastbourne
	Water from a basin for hand washing	*Salmonella* Poona
	Fungicide	Not detected

Laboratory investigation. Initial PFGE analysis of 17 *Salmonella* Litchfield outbreak isolates with the enzymes *Xba*I and *Avr*II indicated that *Avr*II was more discriminatory, so further analysis was carried out with only *Avr*II. The analysis included 6 outbreak clinical isolates from Queensland, 20 outbreak isolates from Western Australia, 9 isolates from outbreak papaya samples, 12 historical clinical isolates from Queensland, 2 historical clinical isolates from Western Australia, and 2 historical environmental isolates from Queensland, one from macadamia nuts and one from soil (Fig. 2). PFGE profiles of outbreak isolates were indistinguishable and distinct from historical Western Australia and Queensland clinical and environmental isolates (Fig. 2). MLVA analysis included 6 outbreak clinical isolates from Queensland, 16 outbreak isolates from Western Australia, and 14 historical clinical isolates from Queensland that were not associated with the outbreak. Polymorphism was found in the PCR products for only one of the variable number tandem repeats, STTR5. Allele sizes ranged from 240 to 330 bp. All of the Queensland and Western Australia outbreak isolates, and only these isolates, had the 240-bp allele (Table 3).

DISCUSSION

We report the first Australian *Salmonella* outbreak associated with the consumption of papaya. The multijurisdictional outbreak investigation coordinated by OzFoodNet identified a strong epidemiological association between *Salmonella* Litchfield infection and the consumption of contaminated papaya in the Australian states of Western Australia and Queensland in late 2006 and early 2007. This link to contaminated papaya was established through an initial case series analysis, a case-control study to test the association with illness, and detection of *Salmonella* Litchfield on papaya samples collected from stores. Two different genetic typing methods (PFGE and MLVA) revealed that isolates from the outbreak cases from both Western Australia and Queensland and from the papayas had indistinguishable genetic profiles, which were distinct from those of historical isolates.

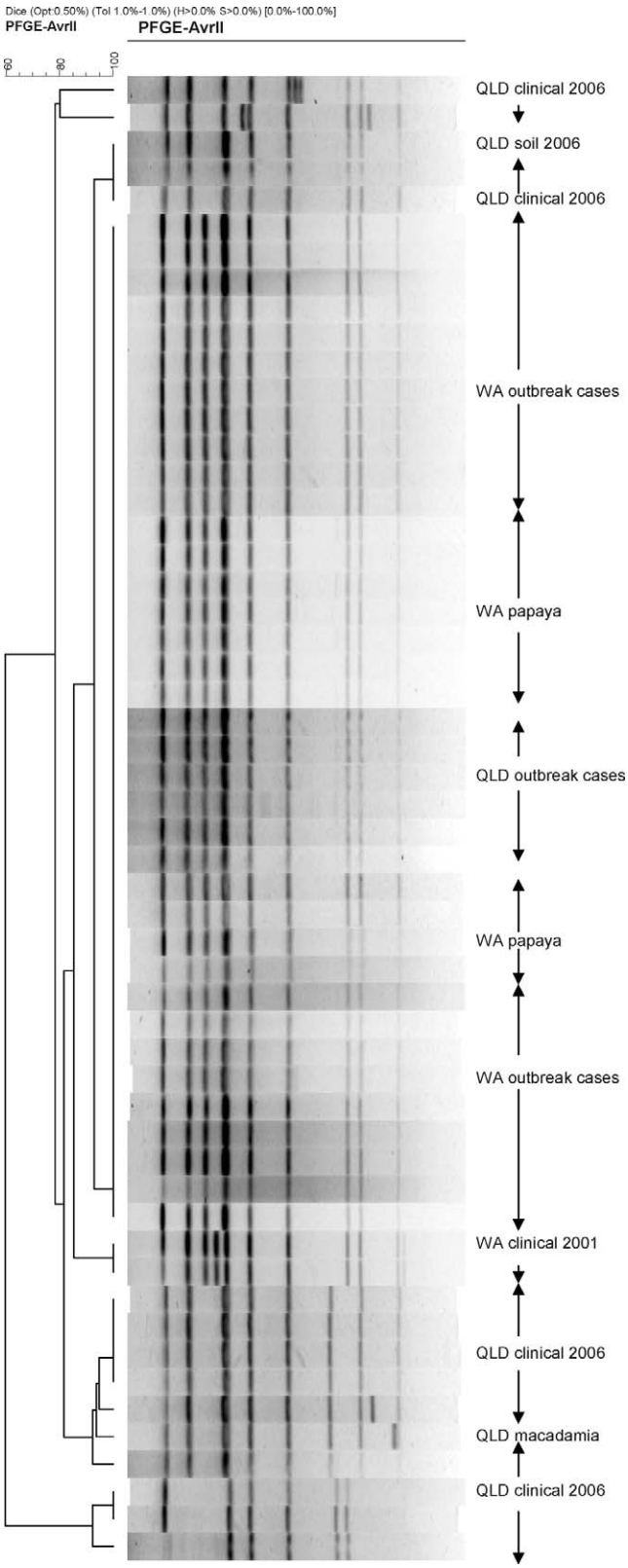

FIGURE 2. *Dendrogram of* Salmonella *Litchfield PFGE results from cases, papayas, and historical clinical and environmental samples.*

TABLE 3. *MLVA profiles, sample types, and collection dates for nonoutbreak and outbreak isolates of* Salmonella *Litchfield*

State[a]	Sample type	No. of isolates	Collection dates (2006)	STTR5 alleles (bp)[b]
Nonoutbreak isolates				
QLD	Human clinical	1	12 Jan	252
QLD	Human clinical	3	16 Jan, 7 Feb, 12 Sep	258
QLD	Human clinical	6	19 Jan, 23 Feb, 27 Feb, 5 Mar, 6 Apr	264
QLD	Human clinical	1	10 Feb	318
QLD	Human clinical	1	20 Mar	246
QLD	Human clinical	1	21 Apr	330
QLD	Human clinical	1	22 Sep	294
QLD	Soil	1	9 Mar	258
QLD	Nuts	1	8 Jun	258
Outbreak isolates				
QLD	Human clinical	6	13–29 Oct	240
WA	Human clinical	11	28 Oct–27 Nov	240
WA	Papaya	5	1 Dec	240

[a] QLD, Queensland; WA, Western Australia.
[b] All isolates had an STTR3 allele of 136 bp and an STTR9 allele of 153 bp.

The contaminated papayas were produced in one growing region in northern Western Australia. Although environmental investigations were carried out at two of three farms that had supplied contaminated papayas, *Salmonella* Litchfield was not detected in papaya or environmental samples collected at the farms. However, other *Salmonella* serotypes were detected in water samples collected at one of the farms. The most likely source of the *Salmonella* Litchfield contamination is the untreated river water that was used to wash the fruit with fungicide before the papayas were packaged and transported to distribution centers. Chlorine was added to the water in the wash tubs but not at levels that would provide disinfection. The U.S. Department of Agriculture (USDA) has recommended that water used for fruit processing should be filtered, flocculated, and chlorinated to a concentration of 200 ppm and should be checked for microbial contamination on a regular basis *(14).*

To our knowledge, this is the first report of a *Salmonella* outbreak associated with papaya; however, a similar outbreak in the United States in 1999 was associated with mangoes *(13).* Both mangoes and papayas are tree fruits with similar processing procedures on the farm. Mangoes in the U.S. outbreak were produced in Brazil and were dipped in water from a nearby canal as part of postharvest processing to treat for fruit fly. Testing revealed that this water was contaminated with *Escherichia coli* and *Salmonella.* Following this outbreak, the USDA Animal and Plant Health Inspection Service recommended that all mango producers who export to the United States ensure that the processing water is filtered and adequately chlorinated *(14).* Despite these recommendations, another *Salmonella* outbreak associated with mangoes occurred in the United States in 2001 *(5).* This outbreak was associated with mangoes from a farm in Peru. Separate investigations revealed that mango growers in Peru also were dipping fruit in untreated water as part of a fruit fly control program, and

again this water was thought to be the source of the *Salmonella* contamination.

In the investigation described here, papayas were contaminated with *Salmonella* Litchfield as revealed by analysis of both surface rinsates and a mixture of skin, flesh, and seeds. In a previous study *(11),* researchers determined that salmonellae could become internalized into mangoes during postharvest processing, particularly in the stem region. In that study, mangoes were dipped in water contaminated with *Salmonella* Enteritidis, and internalization occurred in both ripe and immature mangoes. In the Australian papaya-associated outbreak, salmonellae may have become internalized into papayas when they were dipped in water, which would have been at a temperature similar to that described for mangoes.

One anomaly with this investigation was that Queensland cases appeared to be linked to the outbreak through genetic typing results, but the investigators were informed by a primary industry spokesperson that papaya from Western Australia was not being distributed to Queensland at the time of the outbreak. We believe that a batch of Western Australia papayas may have been unofficially transported to Queensland along with bananas that were being transported to Queensland at the time of this outbreak.

The outbreak investigation described here revealed that tree-grown fruit can become contaminated with salmonellae, which can then result in human infection. These findings support those of previous outbreak investigations that have linked contamination to postharvest processing of fruit using water that had not been adequately treated to remove pathogenic organisms.

Following the papaya-associated outbreak, farms in this growing region were advised to develop and implement a sanitizing treatment as part of their on-farm production process. Standards for the production of fresh produce should be developed in Australia and should include a requirement that fresh produce that is to be eaten raw should be washed only with water that meets drinking water quality standards.

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