

A Shipyard Outbreak of Salmonellosis Traced to Contaminated Fruits and Vegetables

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Abstract

A large outbreak of food poisoning occurred in September 1996 and involved at least 116 workers at a shipyard in Jurong. *Salmonella weltevreden* was isolated from the stool specimens of 24 hospitalised cases and three food handlers, giving a total of 27 bacteriologically confirmed cases (25 symptomatic and two asymptomatic) in this outbreak. Based on a case-control analysis, a number of food items consumed on 23 and 24 September 1996 were implicated in the outbreak, viz. a vegetable dish ($P < 0.01$), watermelon juice ($P < 0.01$) and cut watermelon ($P < 0.001$), papaya ($P < 0.001$) and pineapple ($P < 0.05$). The median incubation period was 38 hours and the median duration of illness was two days. Four samples of cut fruits obtained from a food stall at the canteen were found to be positive for *Salmonella weltevreden* (The antibiogram of all the strains isolated from food samples and stool cultures was similar, consistent with a common source of infection.) Extensive investigation suggested that contamination may have occurred through unauthorised use of industrial water for washing the fresh produce. Early recognition of the outbreak and prompt implementation of control measures prevented further spread of infection.

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Introduction

Non-typhoidal salmonellosis, a form of bacterial food poisoning, has emerged as an important cause of food-borne illness in many developed countries, with substantial increases in its occurrence over the past decade.¹⁻³ The causative *Salmonella* bacteria are gram-negative bacilli that can infect the gastrointestinal tract of both humans and animals. Human salmonellosis commonly present as an acute enterocolitis, with sudden onset of headache, abdominal pain, anorexia, diarrhoea, nausea and vomiting usually 12 to 36 hours after consumption of contaminated food.⁴ Fever is almost always present and dehydration may be severe. The bacteria could localise in body tissues, especially in patients with cancer or other debilitating conditions, and produce abscesses or cause complications such as pyelonephritis, cholecystitis, endocarditis and pneumonia.

We report herein the investigation of a large common source food-borne outbreak of salmonellosis at a shipyard traced to the consumption of fruits and vegetables which were probably contaminated with industrial water.

The Shipyard

The shipyard is located at a Jurong industrial estate in the western part of Singapore. In addition to being served by potable water and modern sanitation, it was supplied with industrial water which was used for flushing toilets and general washing of the premises. The shipyard employed 1244 workers who were engaged in ship building, servicing and repair. About 60% of the employees were foreign workers, mainly from India and China. There was a staff canteen with three food stalls selling Chinese food, Malay food, and drinks and cut fruits. The food items were cooked in batches in three separate kitchens which were all well equipped with modern sanitary and refrigeration facilities. Meals were consumed by the workers in a common area of the canteen furnished with tables and benches.

The Outbreak

On 25 September 1996, the Quarantine and Epidemiology Department, Ministry of the Environment, was alerted by a shipyard doctor of a sudden increase in the number of gastroenteritis from an average weekly figure

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of 3 to 5 cases to over 50 cases that day. The initial cases had presented with vomiting, diarrhoea, abdominal pain and fever early in the morning at his clinic within the shipyard premises. Some of them were suffering from dehydration and had to be hospitalised. We immediately initiated epidemiological investigations to determine the source of the infection, and its mode of transmission. Prevention and control measures were simultaneously implemented to prevent further transmission of infection.

Materials and Methods

All cases admitted to the hospital were interviewed on their detailed food history. Active case surveillance was conducted in the shipyard to look for other mild and unreported cases. We defined a case of food poisoning as any person who had consumed food or water within the shipyard between 23 and 25 September 1996 and subsequently developed two or more of the following symptoms: nausea, vomiting, diarrhoea, abdominal pain and fever. All supervisors were asked to refer their workers with health complaints to the factory clinic or a nearby private clinic for medical examination.

The canteen and other areas in the shipyard were thoroughly inspected. The environmental sanitation in the premises was stepped up, and the canteen cleaned and disinfected. The food handlers were reminded to observe strict personal and food hygiene. All the 18 cooks and their assistants in the canteen were referred to the Communicable Disease Centre, Tan Tock Seng Hospital. No food remnants from meals consumed on 23 and 24 September 1996 were available from the food stalls but similar food samples were obtained on 25 September 1996 for bacteriological examination at the Food and Water Laboratory, Singapore General Hospital. Water samples from a potable water tap and three coolers in the shipyard were also sent for laboratory testing.

When it was observed that the management had shut off the potable water supply to the water coolers, cross-contamination between potable and industrial water supplies was suspected. The building plans and water piping systems were carefully scrutinised.

A case-control study was carried out to determine the vehicle of transmission. Unmatched controls were selected from non-affected workers in the shipyard at almost three times the number of cases studied to ensure adequate statistical power. Differences in the food-specific attack rates were compared by the Chi-square test, and odds ratios with 95% confidence intervals derived. To control the effect of confounders, we used the Mantel-Haenszel procedure to evaluate the odds ratios of stratified data. A difference was considered statistically significant if it was likely to occur by chance no more often than one in twenty.

Stools collected from hospitalised patients and impli-

cated food handlers were inoculated onto MacConkey and Salmonella-shigella agar plates, as well as into selenite F broths. The selenite F broths were subcultured onto MacConkey and Salmonella-shigella agar plates after incubation in air for 18 hours at 35°C. The agar plates were read after incubation in air at 35°C for 24 hours, and suspicious looking colonies were subjected to identification by biochemical tests. All food samples were homogenised aseptically and then inoculated into lactose broths. After 24 hours of incubation in air at 37°C, the lactose broths were inoculated into tetrathionate and Rappaport-Vassiliadis broths. The tetrathionate broths were incubated at 37°C and the Rappaport-Vassiliadis broths at 42°C. Both were incubated in air and subcultured onto Xylose-lysine-desoxycholate and Hektoen enteric agar plates after 24 hours and 48 hours. The agar plates were read after incubation at 37°C in air for 24 hours, and suspicious looking colonies identified by biochemical reactions.

Organisms that gave biochemical reactions typical of *Salmonella* species were serotyped by means of agglutination with specific antisera to the somatic, flagella and Vi antigens using the Kauffmann-White classification. Antibiotic susceptibility testing using the Kirby-Bauer method of disc diffusion⁵ was performed for bacterial strains and interpreted according to the guidelines of the National Committee for Clinical Laboratory Standards.⁶

Results

During the period 23 to 27 September 1996, a total of 116 workers at the shipyard sought medical attention from their factory doctor or nearby medical practitioners and fulfilled the criteria in our case definition of food poisoning (Fig. 1). The overall attack rate was 9.3%. The clinical symptoms were fever (100%), abdominal cramps (93%), diarrhoea (88%), headache (88%), general weakness (68%), nausea (50%) and vomiting (33%). Thirty-two (28%) were found to be seriously dehydrated and required inpatient treatment. Two of them were admitted to the Singapore General Hospital while the rest were hospitalised at the Communicable Disease Centre. All of them recovered following symptomatic treatment. The duration of illness ranged from 1 to 3 days, with a median of two days. The age-sex distribution of those affected and their attack rates are shown in Table I.

Analysis of the food-specific attack rates based on 60 different food items served at the staff canteen during the outbreak period implicated five food items consumed on 23 and 24 September 1996 (Table II). They were watermelon juice ($P < 0.01$), cut watermelon ($P < 0.001$), papaya ($P < 0.001$) and pineapple ($P < 0.05$) served by the drinks and fruits stall, and vegetables in oyster sauce ($P < 0.01$) served by the Chinese food stall.

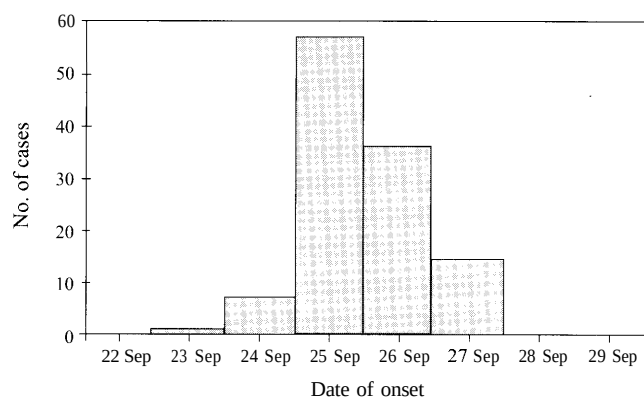


Fig. 1. Onset of symptoms of 116 reported cases of *Salmonella* food poisoning, 23 to 29 September 1996.

TABLE I: AGE-SEX DISTRIBUTION AND ATTACK RATES OF 116 REPORTED CASES OF *SALMONELLA* FOOD POISONING AT A SHIPYARD

Age	Males			Females		
	No. at risk	No. affected	Attack rate	No. at risk	No. affected	Attack rate
15-24	116	10	8.6%	14	2	14.3%
25-34	520	64	12.3%	36	4	11.1%
35-44	311	20	6.4%	19	0	0%
45-54	174	14	8.0%	7	0	0%
55-64	46	2	4.3%	1	0	0%
Total	1167	110	9.4%	77	6	7.8%

Consumption of unboiled water from the water cooler/tap was not significantly associated with illness. Stratified analyses showed that the affected workers were still more likely than the others to have taken the cut fruits even after controlling for the consumption of the vegeta-

bles (Mantel-Haenszel weighted odds ratio = 10.9; 95% confidence interval = 4.6 to 26.1; $P < 10^{-6}$). The incubation period based on the interval between time of consumption of the implicated food item and onset of illness ranged from 11 to 75 hours, with a median of 38 hours.

Salmonella weltevreden was isolated from the stool cultures of 24 cases who were hospitalised at the Communicable Disease Centre, and three of the implicated food handlers (two asymptomatic). The antigenic formula of the strains were 0 antigens "3,10" and H antigens "r" (phase 1) and "z6" (phase 2). All the strains isolated showed the same antibiotic sensitivity pattern, and were susceptible to ampicillin, chloramphenicol, ceftriaxone, trimethoprim-sulfamethoxazole, and ciprofloxacin.

Of the 13 food items submitted to the laboratory for bacteriological testing, four samples of cut watermelon, pineapple, papaya and honeydew melon were found to be positive for *Salmonella weltevreden*. The antibiotic susceptibility pattern of all the strains isolated from the food samples was similar to that of the stool specimens. Samples of potable water taken from the factory premises yielded no bacterial growth.

We did not find any seepage or backflow that could have resulted in cross-contamination between the industrial and potable water supply systems although some plumbing works had taken place in early September 1996. The piping system for each type of water supply was totally separate, and no disruptions to the potable water supply were reported in the period prior to the outbreak. However, it was noted that some of the industrial water supply taps were connected with long rubber hoses for washing purposes but were not labelled as industrial water.

TABLE II: ANALYSIS OF FOOD-SPECIFIC ATTACK RATES IN AN OUTBREAK OF *SALMONELLA* FOOD POISONING AT A SHIPYARD

Food item*	Ate			Did not eat			Odds ratio**	P value
	Ill	Not Ill	Ill (%)	Ill	Not Ill	Ill (%)		
23 September 1996								
Watermelon juice	5	2	71.4	37	117	24.0	7.9 (1.3-44.2)	<0.01
Cut watermelon	21	18	53.8	21	101	17.2	5.6 (2.4-13.3)	<0.001
Cut pineapple	8	6	57.1	34	113	23.1	4.4 (1.3-15.7)	<0.05
Vegetables in oyster sauce	18	19	48.6	24	100	19.4	4.0 (1.7- 9.3)	<0.01
24 September 1996								
Watermelon juice	6	2	75.0	36	117	23.5	9.8 (1.7-47.5)	<0.01
Cut watermelon	23	13	63.9	19	106	15.2	9.9 (4.0-25.0)	<0.001
Cut papaya	10	5	66.7	32	114	21.9	7.1 (2.0-26.1)	<0.001
Vegetables in oyster sauce	16	20	44.4	26	99	20.8	3.1 (1.3- 7.2)	<0.01

* A list of 55 other food items not implicated in the outbreak were excluded from the table.

** 95% confidence intervals provided in parenthesis.

Discussion

Non-typhoidal salmonellosis is an important food-borne zoonotic disease in Singapore.^{7,8} About 40 serotypes are encountered annually. Multiple antibiotic-resistant strains caused three successive epidemics of gastroenteritis in young children, viz. *Salmonella typhimurium* in 1971 to 1977, *Salmonella oranienburg* in 1978 to 1981 and *Salmonella blockley* in 1983 to 1984.⁹⁻¹³ *Salmonella typhimurium* continued to be the prominent non-typhoidal salmonella serotype isolated from 1972 to 1993 until it was replaced by *Salmonella enteritidis* in 1994 and 1995. *Salmonella* food poisoning outbreaks have been associated with the consumption of a variety of food items such as “nasi lemak” (coconut rice),¹⁴ mutton curry,¹⁵ luncheon pork¹⁶ and “roti jala” (Malay pancake).¹⁷ Although the main contributing factor was cross-contamination between raw meat and cooked food, *Salmonella* bacteria were rarely isolated from cooked and ready-to-serve food items.¹⁸

In this outbreak, cut fruits and vegetables were implicated. *Salmonella weltevreden* was isolated from the stool cultures of the hospitalised cases as well as the cut fruits. The antibiotic sensitivity pattern of the strains isolated from the cases was similar to that of the implicated food and consistent with a common source of infection. Unfortunately, at the time of the investigations, strain typing by pulsed field gel electrophoresis was not available at the laboratory. The use of at least two different types of molecular strain typing would have confirmed the presence of a single strain causing the outbreak.

The implicated fresh produce may have become contaminated at the farms, or during transportation and handling prior to delivery to the shipyard canteen. Alternatively, they could have become contaminated during the process of washing and slicing at the canteen. The fact that no cases were reported in other parts of Singapore excluded the possibility of the food items having been contaminated from outside sources. Moreover, these food items were served by two different food stalls which had acquired their fresh produce independently from unrelated distributors. Hence, all evidence pointed to contamination having occurred within the shipyard.

The exact mechanism leading to the bacterial contamination in the factory premises remained unclear. Lapses in hygiene were observed at the food stalls, but an unusual feature of this outbreak was that the implicated food items had been prepared in two separate kitchen areas over two days. We excluded the possibility of the three implicated food handlers being the source of infection, as they did not directly prepare the fruits or vegetables. They were probably infected during the outbreak. We also excluded the possibility of cross-contamination between the industrial and potable water supply at the shipyard. Our case-control analysis showed no significant association with drinking potable water, and sam-

ples from three coolers and a tap yielded no bacterial growth. One important observation, however, was that a number of industrial water taps near the canteen had not been labelled. As they were connected with long rubber hoses, it was possible that one or more of the assistants had used the water for washing the fresh produce. Hence, unauthorised use of the industrial water supply constituted the most likely explanation.

Industrial water is derived from sewage effluent that has been treated and provides an inexpensive source of water for factory use. The Jurong Industrial Water Works is the only plant in Singapore producing about 65,000m³ of industrial water a day for its consumers in the Jurong area. Its source of water is from the Ulu Pandan Sewage Treatment Works. In the process of treating industrial water to an acceptable quality, liquid chlorine is added to eradicate the presence of faecal organisms. However, industrial water must be used within the day because of possible microbial proliferation when the chlorine residue breaks down with exposure. The risks associated with the accidental ingestion of treated sewage effluent have been established.^{19,20} A study of eleven kibbutzim (cooperative agriculture settlements) in Israel found that summer enteric disease rates were higher during the years when they switched from sprinkler irrigation with non-effluent water sources to partially treated stabilisation pond effluent irrigation sources.²¹ An outbreak of gastroenteritis involving ten employees at a factory in Jurong on 22 March 1978 was traced to an illegal connection in which potable water had been used to supplement the industrial water supply to cool the industrial machinery.²² Backflow resulted in the contamination of their drinking water supply.

An increasing number of food poisoning outbreaks associated with contaminated fruits and vegetables have been reported in recent years, including outbreaks of salmonellosis traced to watermelon.²³ Recent work showed that very few watermelons carried *Salmonella* on their rind, but as they are grown resting on the soil, the risk of contamination is always present.²⁴ Under normal circumstances, the external surfaces prevented entry of bacteria but the flesh could become contaminated during the slicing process either from the knife or from the rind of the melon. The flesh of watermelons provide a good medium for bacterial growth, especially at room temperature.²⁵ Other reported microbial agents and their vehicles of transmission included *Salmonella bovis/morbidicans* in alfalfa sprouts,²⁶ *Salmonella heidelberg* in tomato salad,²⁷ *Shigella sonnei* in lettuces,²⁸ *Shigella flexneri* in salad,²⁹ *Citrobacter freundii* in parsley,³⁰ *Escherichia coli* 0157:H7 in salad,³¹ small round structured virus in salad,³² and *Cyclospora cayatenensis* in raspberries.³³ Fresh produce that are consumed raw or partially cooked have been implicated in a significant proportion of the reported food-borne outbreaks because they are

especially vulnerable to problems of contamination.³⁴⁻³⁶

When we conveyed the findings of our epidemiological investigation to the shipyard management, prominent notices were promptly put up in the factory premises and the industrial water taps labelled to warn the workers and food handlers not to use the water for drinking, bathing or washing of fruits and vegetables. This episode has shown that use of industrial water from treated sewage effluent should be carefully monitored in the factory premises, and that early recognition of any outbreak with prompt implementation of control measures is essential to prevent further transmission of infection.

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