

Salmonella enterica Outbreak in a Banqueting Hall in Jerusalem: the Unseen Hand of the Epidemiological Triangle?

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ABSTRACT: **Background:** Foodborne *Salmonella enterica* outbreaks constitute both a threat to public health and an economic burden worldwide.

Objectives: To characterize the pathogen(s) involved and possible source of infection of an outbreak of acute gastroenteritis in a banqueting hall in Jerusalem.

Methods: We conducted interviews of guests and employees of the banqueting hall, and analyzed food items, samples from work surfaces and stool cultures.

Results: Of 770 persons participating in three events on 3 consecutive days at a single banqueting hall, 124 were interviewed and 75 reported symptoms. *Salmonella enterica*, serovar Enteritidis, phage type C-8, was isolated from 10 stool cultures (eight guests, one symptomatic employee and one asymptomatic employee) and a sample of a mayonnaise-based egg salad. Pulsed-field gel electrophoresis of the isolates revealed an identical pattern in the outbreak isolates, different from SE C-8 controls. A culture-positive asymptomatic employee was linked to all three events. After a closure order, allowing for cleaning of the banqueting hall, revision of food preparation procedures and staff instruction on hygiene, the banqueting hall was reopened with no subsequent outbreaks.

Conclusions: It is often difficult to pinpoint the source of infection in *S. enterica* outbreaks. Using molecular subtyping methods, a link was confirmed between patients, a food handler (presumably a carrier) and a food item – all showing an identical specific *Salmonella enterica* serovar Enteritidis. Testing asymptomatic as well as symptomatic food handlers in outbreak investigations is imperative. Pre- and post-hiring screening might be considered as preventive measures; hygiene and sanitation education are essential.

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Foodborne disease is an increasing health and economic burden the world over. Centralization and globalization of food supply, increasing microbial resistance to antibiotics and growth of susceptible immunosuppressed subpopulations are the main behavioral, environmental and biological factors responsible for the change [1,2]. The burden of diarrheal disease morbidity in industrialized countries is reflected by point prevalence rates ranging from 3.4 to 7.6% [3].

Salmonellae are divided into two species, *S. bogori* and *S. enterica*. *S. enterica*, the larger, contains over 2400 serotypes (serovars), including most serovars pathogenic to humans. Of the pathogenic serovars, *S. typhi* remains a global health problem, causing 16 million cases annually. Typhoid fever is no longer endemic to most developed countries, including Israel. When encountered, it usually occurs in travelers returning from endemic countries [4]. Other pathogenic *S. enterica* serovars cause non-typhoidal salmonellosis [5], which is the most frequently reported foodborne disease in Europe [1]. Starting in the early 1990s, *Salmonella enterica* serovar Enteritidis became the most frequently reported serotype in the European community and the United States [6]. During the year 2000, non-typhoidal salmonellosis triggered an estimated 1.3 million cases of acute gastroenteritis and was the most common cause of foodborne enteritis-related mortality in Britain. In 2004, *Salmonella* sp. resulted in an estimated 1.4 million cases of illness, 15,000 hospitalizations and 400 deaths in the U.S. [7].

The most commonly identified sources of *Salmonella* infection are food products of animal origin (contaminated meat, poultry, eggs and dairy products). Other common sources include fresh fruit and vegetables and manufactured food items like ice cream, powdered milk and snack foods [5,8]. Unlike typhoidal salmonellosis, the spread of non-typhoidal *Salmonella* infection via food items contaminated by an asymptomatic individual is infrequent [9,10].

Beginning in the late 1980s, the incidence of NTS in human beings and domestic animals rose, decreasing in the 1990s. During that time, the World Health Organization, the European Community and North American governments responded by instituting measures including improved hygiene, animal husbandry, infection control, biosecurity,

NTS = non-typhoidal salmonellosis

and consumer education [2,5,11-13]. The recent decrease in incidence may be a result of the intensified efforts [1,14]; however, it has been cautioned that "... the pattern of Salmonella infections never remains stable for long" [11].

The incidence of NTS in Israel has followed a trend similar to the pattern seen in industrialized countries, rising steadily from 1983 to peak in 1994. Although the annual incidence declined sharply from 1996 to 2004 (109.6/100,000 to 21.7/100,000 respectively), it remained higher than U.S. 2004 levels (14.7/100,000) [15-17].

Over three consecutive days in mid-August 2004, guests at a Jerusalem banqueting hall developed symptoms of acute gastroenteritis. We present the epidemiological and laboratory investigation of the outbreak and the ensuing implications.

MATERIALS AND METHODS

The epidemiological investigation included interviews, site investigations and laboratory tests. The organizers of the three events that took place on the three consecutive days in mid-August 2004 were asked to estimate the number of participants at each event. Both symptomatic and asymptomatic guests were questioned about specific foods consumed, presence of symptoms, time of onset of symptoms, and medical treatment.

Stool samples were requested from symptomatic individuals who had not received prior antibiotic treatment and from all kitchen and dining-room workers. The banqueting hall, including kitchen and dining-room facilities and work areas, were inspected and procedures were surveyed. Food items and samples from work surfaces were collected and tested.

In cases where Salmonella was isolated from food or stool, further characterization of the bacteria included the following: serotyping (Kaufman White scheme), phage typing (Sechter's scheme), antibiotic resistance testing (Kirby-Baur disk diffusion technique according to the Clinical and Laboratory Standards Institute guidelines), and pulse-field gel electrophoresis. The PFGE was performed according to the PulseNet standardized PFGE protocol for non-typhoid Salmonella (standardized molecular subtyping of foodborne bacterial pathogen by PFGE). Briefly, bacterial DNA was extracted from the outbreak-related strains, which were compared to unrelated SE strains from the national strain bank, referred to as controls; fixed in gel plugs; digested with two infrequent restriction endonucleases, XbaI and AvrII, in two separate experiments; followed by band separation using CHEFFIII PFGE. PFGE patterns were analyzed by the BioNumerics software (Applied Maths NV, Sint-Martens-Latern, Belgium) followed by UPGMA clustering analysis.

RESULTS

Of the 770 persons who participated in three events on 3 consecutive days (17th, 18th and 19th August 2004) at a banqueting hall in Jerusalem, 124 were interviewed (66 from the first event, 37 from the second and 21 from the third). Of these, 75 persons reported having symptoms (attack rate among attendees: 75/770, 9.7% and 75/124, 60.5% of persons interviewed).

The patient characteristics are presented in Table 1. The most common presenting symptoms were acute diarrhea and abdominal pain, reported by over three-quarters of the patients. Most of them reported the emergence of symptoms within less than 24 hours after participating in the event.

In general, consuming food items during any of the three events was significantly associated with having gastrointestinal symptoms. Of the guests who reported eating, 73% (73/100) became sick compared to 8.3% (2/24) of those who refrained from eating (odds ratio = 29.7, 95% confidence interval 6.1-196.9, P = 0.0001). However, an analysis of food items did not reveal a specific item significantly associated with the appearance of symptoms.

Stool samples of personnel (n=10) were tested from nine workers who were asymptomatic and one who reported acute gastrointestinal symptoms. Two samples were *Salmonella enterica* culture-positive. One was from the worker who reported acute symptoms; he became sick on the second day and did not attend work on the third day of the outbreak. The other positive sample was from an asymptomatic worker.

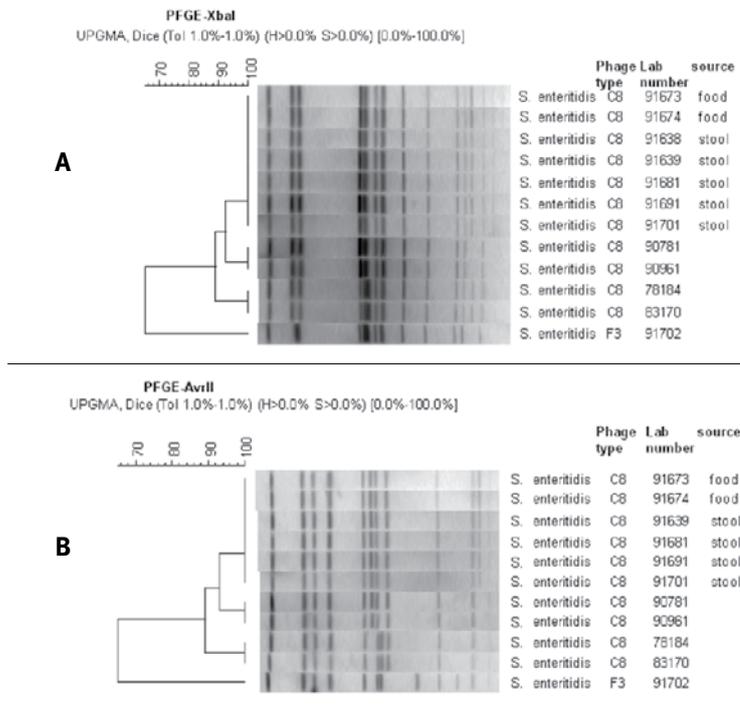
All in all, *Salmonella enterica*, serovar Enteritidis, phage type C-8 was isolated from 10 stool cultures (8 symptomatic guests, 2 employees) and from a sample of a mayonnaise-based egg salad (this sample was taken from the salad that was served on the third day). Samples of other food items (n=12) and from the kitchen work surfaces were all found to be culture-negative. Six guests reported eating the egg salad; the two employees denied

Table 1. Patient characteristics (n=75): *Salmonella enterica* outbreak

Variable	
Age (yrs, mean ± SD, range)	27.1 ± 18.2 (0.1-71)
Gender (% females)	42 (56%)
Time (hr) to symptoms (mean ± SD, range)	20.9 ± 6.2 (7.5-48)
Symptoms	
Diarrhea	61 (81.3%)
Abdominal pain	57 (76%)
Fever	42 (56%)
Nausea	27 (36%)
Vomiting	17 (22.7%)
Bloody diarrhea	2 (2.7%)

PFGE = pulse-field gel electrophoresis

Figure 1. PFGE patterns of outbreak strains and unrelated *S. enteritidis* isolates. **[A]** XbaI restriction enzyme: outbreak strains 1–7: 1, 2 = food isolates; 3, 4 = kitchen worker isolates; 5, 6, 7 = symptomatic participant isolates. Controls not connected to the outbreak 8–12: 8–11 = *S. enteritidis* strains phage type C8; 12 = *S. enteritidis* strains phage type F3. **[B]** AvrII restriction enzyme: outbreak strains 1–6: 1, 2 = food isolates; 3, 4 = kitchen worker isolates; 5, 6 = symptomatic participant isolates. Controls not connected to the outbreak 7–11: 7–10 = *S. enteritidis* strains phage type C8; 11 = *S. enteritidis* strains phage type F3.



eating that salad. PFGE analysis of the outbreak-related isolates revealed an identical pattern in these isolates, which was clearly different from the SE C-8 controls (unrelated strains). The PFGE results are presented in Figure 1.

The culture-positive asymptomatic employee was linked to all three events. He was responsible for arranging food items on the plates served to the guests; thus, all items were exposed to him. Being asymptomatic, he had not been examined or tested previously.

After administrative closure, allowing for cleaning of the banqueting hall, thorough revision of food preparation procedures and staff instruction and education on hygiene, the banqueting hall was reopened. There have been no subsequent outbreaks.

DISCUSSION

Each year millions of people around the world suffer from foodborne gastroenteritis, however data on the full extent of the global burden of unsafe food are lacking [18]. A multi-

national study of the estimated burden of diarrheal disease in Canada, Australia, Ireland and the U.S. revealed community prevalence rates of 7.6%, 6.4%, 3.4% and 7.6% respectively, during the period 2000–2002 [3].

Up to 30% of the population in industrialized countries has been estimated to suffer from foodborne disease each year [2]. The incidence of non-typhoidal salmonellosis in the U.S. in 2006 was 14.81/100,000 population, making Salmonella the most frequently identified foodborne pathogen in the U.S. Comparable rates were found in Canada, which reported 16.02/100,000 in 2004 [19], while the 25 countries of the European Union had an average incidence of 42.37/100,000 in 2004 [20], and Australia reported 41.2/100,000 for 2005 [21]. Of the 1476 cases of NTS reported in 2004 in Israel (21.7/100,000 population), 238 cases (31.7/100,000 population) were reported in the Jerusalem district [15].

The clinical features in the current outbreak [Table 1] were similar to those described by Broide et al. [22], who evaluated 295 Salmonella-positive hospitalized patients of whom the majority presented with gastrointestinal symptoms and fever.

The most common vehicles of Salmonella infection are foods of animal origin, primarily poultry and poultry products [8,11]. Continuing improvements in animal husbandry and food control have led to a decreasing incidence of infection in both humans and animals [14]. At the same time, widespread antibiotic use in commercial poultry flocks and other livestock has contributed to increasing antibiotic resistance in Israel as in other industrialized countries [16].

Disease transmission by asymptomatic food handlers is not a recently recognized phenomenon, yet it remains controversial. One hundred years ago Mary Mallon, "Typhoid Mary," was identified as the source of repeated typhoid outbreaks. She was quarantined on an island in New York harbor from 1907 to 1910 and again from 1915 until her death in 1938 to prevent her from infecting others [23].

A food handler is capable of transmitting Salmonella if acutely ill or as an asymptomatic carrier. An infected worker may not identify or report a mild episode of gastroenteritis. Chronic carriage occurs in 5% of *S. typhi* infections and in 0.2–0.6% of patients with acute non-typhoidal salmonellosis [5].

Our investigation revealed an identical PFGE pattern in samples from patients, employees and a food item. PFGE is the gold standard for molecular typing used worldwide and is the method used by PulseNet US and in Europe for molecular typing of foodborne bacterial diseases and outbreak investigations.

As no lapses in the kitchen (equipment, infrastructure) were evident and only one food item (consumed by a small number of guests) was culture-positive, we hypothesized that there was a missing link. Analysis of the employees' stool samples revealed two positive cultures: one of a symptomatic person who became ill concomitantly with the guests and was absent on the third day. The asymptomatic employee had not been

tested prior to the outbreak; hence he could not be categorically defined as an NTS carrier. However, his core function in the dining room and his presence throughout the outbreak could put him in the position of being the third, unseen, hand of the epidemiological triangle.

The role of food handlers as vehicles of NTS spread has been controversial. In 1987 Cruikshank and Humphrey [24] noted that evidence of the prevalence of *Salmonella* carriers among food handlers was insufficient. The notion that food handler-carriers were not a threat to public health was based on the absence of proof of disease transmission. Specifically, there were fewer reports of food handler NTS carriage than *S. typhi* carriage despite the fact that in the industrialized world NTS infections are far more common than typhoid fever. In 1994, Dryden et al. [9] reported on a nosocomial outbreak of gastroenteritis caused by *S. enteritidis*: 29 patients and staff members became ill over a period of 19 days on 14 wards in 2 hospitals. This outbreak was controlled only when food handlers were screened, identified as asymptomatic *S. enteritidis* carriers, and excluded from work. In a review of 23 restaurant-associated salmonellosis outbreaks in Minnesota, Medus et al. [25] emphasized the need to assess asymptomatic food workers, who may comprise a persistent reservoir of contamination, as part of the outbreak control measures. It should be noted that in point-source outbreak investigations, as few as 200 bacteria may produce NTS gastroenteritis in exposed individuals [5].

Health regulations in Israel do not require routine pre-employment or periodic testing of food handlers. The district health officer has the authority to order ad hoc testing and to prevent employees from coming to work if they are suspected of being contagious. This authority does not extend to preemptive action, but can only be exercised after an outbreak has occurred. We maintain that testing of food workers should be reconsidered, despite the fact that the planning and execution pose complex problems. Concerted action is required to cut the chain of transmission by ensuring that basic rules of hygiene are scrupulously observed, including hand-washing before and after food preparation and after using the bathroom. Protecting food workers' health is one path to achieving the imperative goal of communicable diseases prevention.

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