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Restaurant-Associated Outbreak of Typhoid Fever in Maryland: Identification of Carrier Facilitated by Measurement of Serum Vi Antibodies

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Ten cases of typhoid fever occurred between 24 August and 1 September 1986 in the vicinity of Silver Spring, Md. Shrimp salad served in a fast-food restaurant was implicated as the source of infection. Stool cultures were obtained from 104 employees, and serum Vi antibodies were assayed in 97 of the employees. *Salmonella typhi* was isolated from stool cultures of an 18-year-old asymptomatic female employee, who was a food handler. A high level of Vi antibodies (79.0 μg/ml), measured by radioimmunoassay, was found in her serum. She had emigrated from an endemic area at the age of 14 years and had visited that endemic area 2 years previously. The causal relation between the carrier and the 10 cases of typhoid fever was confirmed by a common bacteriophage type, denoted "degraded Vi resembling O," in the *S. typhi* isolates. This phage type is rare in the western hemisphere but common in the endemic area from which the carrier had emigrated. The high level of Vi antibody in the asymptomatic carrier, in contrast to the lower levels in the convalescent- and postimmunization-phase sera, facilitated the identification of the source infection in this outbreak. This radioimmunoassay offers a rapid and standardized method for identifying carriers of *S. typhi*.

Typhoid fever is one of the enteric fevers caused by salmonellae. The etiologic agent, *Salmonella typhi*, is distinguished from other salmonellae by its metabolic characteristics, its capsular polysaccharide (Vi antigen), its inability to colonize or cause a similar disease in other animal species, and its ability to establish a chronic asymptomatic infection in the gall bladder in about 3% of individuals after an acute infection. Patients who have an established infection in their gall bladder may excrete *S. typhi* for years (13).

Restaurant-associated outbreaks of typhoid fever, caused by contamination of food by infected handlers, occur rarely in the United States (4–6, 9, 17, 19, 23, 26–28). Identification of asymptomatic carriers as the source of an outbreak usually requires multiple cultures from personnel for several weeks (8). In only two of six food-borne outbreaks reported in the United States was the source of the *S. typhi* identified (4–6, 9, 17, 19, 28).

The Vi antigen is both a virulence factor and a protective antigen of *S. typhi* (1, 16). Its repeating unit is an unusual monosaccharide in bacteria (1, 16). After his discovery of the Vi antigen in 1934, Felix devised a serological assay in which agglutination of *S. typhi* was used to identify carriers (10). The sensitivity and accuracy of this bacterial agglutination assay were challenged (2). Later, hemagglutination of erythrocytes coated with purified Vi was reported to be a more reliable assay (15, 18). Reports of this and other methods confirmed the value of measuring Vi antibodies for identifying carriers (7, 8, 14, 20, 22, 24). These assays however, did not allow results to be compared between laboratories. A radioimmunoassay in which a standard serum calibrated by precipitin analysis and defined references was used to control variability was developed to measure Vi antibodies (25). With this radioimmunoassay, an asymptomatic carrier, among about 100 employees in a fast-food restaurant, was quickly identified during an outbreak of 10 cases of typhoid fever.

MATERIALS AND METHODS

Case finding. The public was informed of this outbreak by the news media. Persons with unexplained fever, especially those who had eaten shrimp salad from restaurant A, were advised to notify their physician or local health department. A walk-in clinic was set up at a health center in Silver Spring, Md., and all who registered were given a standard questionnaire and stool culture kits. Individuals with fever were advised to submit three stool specimens for culture. Household members or others who had intimate relations with the patients were screened for typhoid fever.

Investigation of restaurant A and its employees. During July through September, the restaurant employed 118 individuals, of whom 104 were still working at the time of the investigation. These employees were requested to complete standard questionnaires for demographic data, including their length of employment, type of work, travel, eating and food-handling histories, and clinical symptoms. They were also requested to submit three stool specimens collected at 24-h intervals. Blood samples were obtained on a voluntary basis for determination of Vi antibodies. An unannounced inspection was conducted on 12 September when the preliminary data suggested that restaurant A was a source of *S. typhi*.

Laboratory investigation. Stools were examined for *S. typhi* by the Rockville Branch Laboratory in Montgomery County, Md. The presence of the Vi on these isolates was confirmed by the antiserum agar technique (21). Bacteriophage typing was performed by the Regional Laboratory of the Pennsylvania Department of Health, Harrisburg (11).

Sera from newly discovered and treated carriers, from

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TABLE 1. Epidemiologic, clinical, and serologic data of patients with typhoid fever during a food-borne outbreak in Maryland in 1986

Case no.	Age (yr)	Sex[a]	County of residence	Date (mo/day)			*S. typhi*		μg of Vi antibody/ml of serum
				Onset of symptoms	Admitted to hospital	Specimen taken	Blood	Stool	
1	21	F	Montgomery	8/26	8/27	8/27	+	+	0.42
2	23	F	Montgomery	8/27	9/6	9/7	+	−	0.48
3	36	F	Baltimore	9/1	9/8	9/8	+	−	NA[b]
4	30	F	Prince George's	8/28	9/2	9/3	+	−	NA
5	5	M	Montgomery	8/24	9/16	9/20	−	+	NA
6	33	M	Fairfax	8/24	9/1	9/2	ND[c]	+	NA
7	31	F	Montgomery	8/30	9/2	9/24	−	+	0.93
8	42	F	Prince George's	8/30	9/22	9/22	+	+	0.57
9	24	F	Montgomery	8/25	9/23	9/23	+	−	NA
10	23	F	Montgomery	8/25		10/1	ND	+	0.71

[a] F, Female; M, male.
[b] NA, Blood sample not available.
[c] ND, Not done.

U.S. Army recruits immunized with typhoid vaccine, and from healthy adults were kindly supplied by Sam Formal, John Boslego, Charles M. Nolan, Myron M. Levine, and Michel Cadoz. Serum Vi antibodies were measured by radioimmunoassay (25).

RESULTS

On 5 September 1986, typhoid fever (case 1) was reported in a 21-year-old resident of Montgomery County. The onset of symptoms was on 26 August. Initial investigation revealed no source of infection. On 11 September, two additional cases were reported. Patient 2 (onset on 27 August) was a 23-year-old female resident of Montgomery County, and patient 3 (onset, 1 September) was a 36-year-old female from Baltimore County. Two of these three patients worked in the same office building, and the other worked in a nearby building in Silver Spring. The geographical clustering of the three cases prompted us to contact all hospitals in the Baltimore-Washington area. Patient 4 (onset, 25 August) worked in the same building as the first two patients and was hospitalized on 2 September. The common exposure was that all four patients had eaten shrimp salad in a fast-food restaurant (restaurant A) in Silver Spring.

Six more cases were reported. Two cases, 5 and 6, were reported by hospitals in Washington, D.C., and Northern Virginia. Two patients, 7 and 9, were identified by the walk-in clinic. Case 8 was reported by an infection control nurse in a hospital in Prince George's County, Md., and case 10 was identified by the screening of the employees of restaurant A. All patients but one were hospitalized for an average of 12 days. All 10 patients had fever and chills (average duration of fever was 11 days). Two patients had intestinal hemorrhage. *S. typhi* was recovered from blood cultures in six patients. Four cases were confirmed by stool culture alone. The antibiotic susceptibility patterns were available for seven cases; all of these *S. typhi* were susceptible to chloramphenicol, and all but one were resistant to ampicillin. All 10 isolates had the phage type "degraded Vi resembling O." None of 28 household contacts of the 10 patients had a positive stool culture. Data for the 10 cases are summarized in Table 1.

Only restaurant A was reported by all the patients as a source of food. Nine of the patients had eaten shrimp salad 8 to 22 days before the onset of symptoms. Patient 5, a 5-year-old boy, had eaten shrimp salad in restaurant A 1 day before the onset of his symptoms. This patient had eaten frequently in restaurant A during the month before his illness, but his father was unable to remember what the child had eaten during these times.

Of the 118 persons who had worked in restaurant A during July, August, and September, 107 (90.7%) returned the questionnaires. Stool specimens were obtained from 104 employees (83 submitted three or more, 4 submitted two, and 9 submitted one). Two of the 104 employees had *S. typhi* isolated from three consecutive specimens. Only one, patient 10, reported an illness compatible with typhoid fever. The other denied a history of typhoid fever. This asymptomatic carrier had emigrated to the United States from an endemic area 4.5 years previously. Two years ago, she returned to that endemic area for a visit of 2 weeks. *S. typhi* from the carrier also had the phage type "degraded Vi resembling O." The strain from the carrier was susceptible in vitro to ampicillin and chloramphenicol. The carrier gave no history of gall bladder disease.

Transmission investigation. Restaurant A was inspected according to schedule on 22 August. Deficiencies were cited, including some food maintained at ambient temperature. Another inspection, prompted by the outbreak, was conducted on 12 September. At that time it was found that food items, including ingredients of salads, were maintained at room temperature. About 150 salads, prepared daily, were stored in a refrigerator up to 6 h.

The shrimp salad was prepared from frozen, cooked, and peeled shrimp packed in 5-lb (ca. 2.27-kg) bags imported directly from the supplier in Norway. A portion of shrimps was taken from a bag, thawed in cold water, drained, and put on the top of tossed salad in a container. The tossed salad was delivered in a bag ready for use. The shrimp, chef, and garden salads and bags of shrimps were cultured and found to be negative for *S. typhi*.

Control measures. Restaurant A was closed on 16 September when it was suspected to be the source of the outbreak. Exposed foods were discarded, and the facility was sanitized and inspected. It was opened after all the workers had been replaced. Employees who had worked during August and September were required to have three successive negative stool cultures collected not less than 24 h apart to be rehired. The carrier was removed from work. Employees were instructed to follow proper food-handling techniques and to practice good personal hygiene. No additional cases occurred after institution of these measures.

TABLE 2. Serum Vi antibodies in the asymptomatic carrier, patients with typhoid fever during acute phase, and healthy employees: comparison with other carriers, healthy adults, and U.S. Army recruits immunized with typhoid vaccine[a]

Origin of serum	n	Vi antibodies (μg/ml) (geometric mean [range])
This outbreak		
Restaurant A employees	97	1.61 (0.03–8.21)
Typhoid fever patients	5	0.72 (0.30–0.97)
Carrier	1[b]	79.0
Other sera		
Carriers	8	67.0 (19.0–221)
Treated carriers[c]	3[b]	1.35 (0.91–8.71)
Healthy adults	49	0.09 (0.03–1.45)
U.S. Army recruits		
Preimmunization	55	0.08 (0.01–0.51)
Postimmunization	55	0.30 (0.02–5.44)

[a] The difference between the Vi antibody levels of the nine carriers, including the one in restaurant A, and those of each of the other groups of individuals was significant ($P < 0.001$) by Fisher's exact test.
[b] Geometric mean not calculable.
[c] Negative stool cultures for at least 2 years.

Serum Vi antibodies. The levels of Vi antibodies in sera from the carrier, patients, and healthy employees of the restaurant and in other sera are listed in Tables 1 and 2. Blood samples were obtained from 97 employees; 44 (45.4%) were citizens of the United States, and 53 (54.7%) were citizens of 24 other countries. The asymptomatic carrier had a level of 79.0 μg/ml. She was treated with sulfamethoxazole-trimethoprim for 1 week and then ampicillin for 1 month. Three stool specimens taken in March were negative for *S. typhi*, and her Vi antibodies had declined to 47.0 μg/ml. Three subsequent stool cultures were negative, and the patient was discharged from follow-up. Five employees (two U.S. citizens and three noncitizens) had Vi antibody levels ranging from 2.05 to 8.3 μg/ml; the remainder had levels of less than 1.0 μg/ml. Five of the patients with typhoid fever whose blood was available for assay had levels of Vi antibodies of less than 1.0 μg/ml. None of the patients had been immunized with typhoid vaccine.

The level of Vi antibodies from 31 carriers in the United States, France, and Chile ranged from 19.0 to 141 μg/ml. Three treated carriers, with negative stool cultures for at least 2 years, had levels of 7.8, 1.8, and 0.40 μg/ml. The postimmunization geometric mean Vi antibody level of U.S. Army recruits was 0.26 μg/ml (range, 0.02 to 5.49 μg/ml). The difference between the Vi antibody levels of the nine carriers, including the carrier from restaurant A, and those of the other groups of individuals, including the U.S. Army recruits immunized with typhoid vaccine, was significant ($P < 0.001$).

DISCUSSION

Typhoid is not often suspected as a cause of unexplained fever in the United States. Identification of the source of infection during outbreaks by stool cultures only may require multiple specimens taken over several weeks because of the intermittency of *S. typhi* excretion in many carriers (13, 17, 23, 26). The radioimmunoassay, however, can be completed in 2 days. The levels of Vi antibodies in healthy individuals, in young adults immunized with typhoid vaccine, or in patients with typhoid fever are considerably less than those in carriers. The high levels of Vi antibodies in carriers are probably caused by the prolonged stimulation (the carrier probably contracted her infection while she was in an endemic area 2 years before this outbreak). Based upon these limited, although significant, data, we suggest that a level of ≧15 μg of Vi antibodies per ml could serve to identify carriers.

The value of measuring Vi antibodies in countries with endemic typhoid fever has been established (7, 14, 15, 18, 20, 22, 24). Vi antibody levels in healthy individuals in these countries are higher than those in the United States, probably due to the prevalence of patients convalescent from typhoid fever (7, 14, 15, 18, 20, 22, 24). Vi antibody levels in carriers in underdeveloped nations may be depressed due to less than optimal nutrition and health status. Differences of Vi antibody levels between carriers and patients in these countries, therefore, may not be as great as those observed in this study (14). The radioimmunoassay is simple, rapid, and quantitative and should both provide definition of the carrier state and enable comparisons between laboratories.

Phage typing provided additional data that the source of the *S. typhi* in the outbreak was the carrier. *S. typhi* from the patients and the carrier had the phage type with the unusual designation "degraded Vi resembling O," which is common in the endemic area from which the carrier had emigrated but rarely detected in the western hemisphere (3, 11, 12). *S. typhi* strains from the carrier and from one patient were susceptible to ampicillin; the remaining strains were resistant to this antibiotic. This finding may be explained by a mixed culture of ampicillin-susceptible and ampicillin-resistant *S. typhi* in the gall bladder of the carrier. Unfortunately, we cannot test this possibility at this time. The carrier had worked in restaurant A for 18 months, and it is probable that she harbored *S. typhi* during this period; yet transmission of *S. typhi* occurred only in August when she handled shrimp salad. Several food items, including the shrimp salad, were found to be kept at room temperature on two occasions. Transmission of *S. typhi* was probably potentiated by poor personal hygiene and inadequate food handling.

Immigrants or travelers from countries where typhoid fever is endemic can be asymptomatic carriers. Many are employed as food handlers. It is likely, therefore, that outbreaks of food-borne typhoid fever in the United States will continue to occur. Rapid identification of carriers by assay of serum Vi antibodies could assist in the control of such outbreaks.

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