

Identification of a Carrier by Using Vi Enzyme-Linked Immunosorbent Assay Serology in an Outbreak of Typhoid Fever on an Indian Reservation

N. CARY ENGLEBERG,^{1,2*} TIMOTHY J. BARRETT,² HELAINE FISHER,³ BRUCE PORTER,⁴ ELISA HURTADO,¹ AND JAMES M. HUGHES²

Phoenix and Sells Areas, Indian Health Service, Phoenix, Arizona 85016¹; Division of Bacterial Diseases, Center for Infectious Diseases, Centers for Disease Control, Atlanta, Georgia 30333^{2}; Bureau of Disease Prevention and Epidemiology, Arizona State Department of Health Services, Phoenix, Arizona 85007³; and Pima County Health Department, Tucson, Arizona 85701⁴*

Received 8 June 1983/Accepted 7 September 1983

In May 1981 an outbreak of typhoid fever occurred in a small village on a southwestern United States Indian reservation. Five of the six culture-proven cases, but only 2 of 15 community, age-matched controls, had eaten food prepared for a party held in the village on 20 April (chi-square = 4.3; $P < 0.05$). Food histories obtained from 16 persons who ate food at the party suggested that chicken with chili ($P = 0.03$) and potato salad ($P = 0.09$) were possible vehicles. Eleven adults who attended the party, 5 of whom helped prepare an implicated food, were studied with one or more stool cultures and serum for Vi antibody by using enzyme-linked immunosorbent assay (ELISA) and hemagglutination techniques. All initial stool cultures were negative for *Salmonella typhi*; however, one subject, a 70-year-old female foodhandler, had a Vi antibody titer of 1:320 by ELISA. Subsequent cultures from this subject were positive for *S. typhi*. ELISA for Vi antibody directed the investigators to a single individual as the most probable carrier source and obviated the need for multiple fecal cultures from the other potential carriers identified by the epidemiological investigation.

Chronic carriers of *Salmonella typhi* have been implicated as probable sources for most outbreaks of typhoid fever in the United States and for about one third of sporadic U.S.-acquired cases (8). Since most of these cases are associated with a previously unrecognized carrier, it is usually necessary to screen several individuals suspected on epidemiological grounds to identify the probable source. As a screening test, stool culturing for *S. typhi* presents two difficulties: (i) subjects are often uncooperative about providing fecal specimens (10), and (ii) approximately 25% of single specimens from known carriers are negative (9). The use of Vi serology as a screening tool was first suggested over 40 years ago (4), but until recently, none of the proposed assays for Vi antibody have possessed sufficient specificity to be broadly useful (2, 4). In 1980, Nolan et al. (5, 6) markedly improved the specificity of their hemagglutination assay by using a purified, highly polymerized form of Vi antigen from *Citrobacter freundii*.

In this report, we describe a small outbreak of typhoid fever which occurred on a southwestern United States Indian reservation in May 1981. A recently developed enzyme-linked immunosorbent assay (ELISA) method using purified Vi antigen was used to identify the carrier who was the possible source (1).

MATERIALS AND METHODS

During the 2-week period from 5 to 19 May 1980, six patients admitted to the reservation hospital with febrile illnesses had *S. typhi* isolated from blood or stool or both. All six patients lived in a nearby village of fewer than 500 inhabitants. A review of hospital patients seen during the same period for either fever or diarrhea failed to uncover additional suspected cases from other communities. The hospital laboratory had reported no other isolates of *S. typhi* in the previous 5 years.

To identify a possible common source for these infections, we conducted a door-to-door census of the entire village and selected up to four age-matched (± 3 years) controls for each of the six culture-proven cases. We excluded household contacts of cases and any persons who gave a history of fever or abdominal pain or both lasting 7 days or more during the month of May. A questionnaire was administered to all subjects which focused on four possible sources of contaminat-

* Present address: Infectious Diseases Service, Department of Medicine, University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, TX 78284.

TABLE 1. Food-specific attack rates among individuals who ate at the party on 20 April

Food item ^a	Ate			Did not eat			P value ^b
	No. ill	Total	% ill	No. ill	Total	% ill	
Chicken with chili	6	10	60%	0	6	0%	0.03
Potato salad	6	11	55%	0	5	0%	0.09

^a Other foods and drinks served included beans, tortillas, menudo, rice punch, cake, and water. None of these items were associated with illness ($P > 0.1$).
^b Fisher's exact test, two-tailed.

ed food: traveling food vendors, the village snack bar, and two widely attended events in April, a party (20 April) and a dance (26 April).

Because five of the six case-patients had attended the party on 20 April, we also obtained food histories from these patients and from other persons known to have attended the party. Household contacts of cases were excluded. For purposes of the food-specific analysis, we considered an individual "ill" if he/she had an illness with fever and abdominal pain lasting a week or more after the party. We considered an individual "well" if he/she had no unexplained fever or abdominal pain after the party. Individuals satisfying neither definition were excluded.

One or more fecal and serum specimens were obtained from persons suspected of being asymptomatic carriers and processed for *S. typhi* by using standard procedures (3). Sera were frozen at -30°C and shipped on dry ice to the Enteric Bacteriology Laboratory, Center for Infectious Disease, Atlanta, Ga., where they were tested for Vi antibody by ELISA (1) and hemagglutination (5) methods and for O and H antibodies by using standard methods (3, 12).

RESULTS

In five of six culture-proven cases, but in only two of 15 controls, did the individuals attend the party on 20 April (chi-square = 4.3, $P < 0.05$; calculated using the method of Pike and Morrow [7]). Assuming that 20 April was the date of exposure, we determined that the median incubation period for the five case-patients who attended the party was 13 days. In addition to the five culture-proven cases, 11 other persons who also ate at the party were interviewed; 10 experienced no illness, whereas 1 experienced an illness compatible with typhoid fever by our definition.

Two foods were associated with illness and were eaten by all ill persons: chicken with chili ($P = 0.03$) and potato salad ($P = 0.09$) (Table 1). Test results of fecal and serum specimens from the five well individuals who participated in the food preparation and from six other adult family members of patients who attended the party are shown in Table 2. O and H antibody titers were negative for all of these specimens.

A 70-year-old woman who was visiting the village at the time of the party prepared rice punch and assisted with the preparation of the chicken with chili but took no part in preparing the potato salad. Her first stool culture was negative, but her Vi antibody titer of 1:320 identified her as a possible carrier. Additional stool cultures from this woman were positive for *S. typhi*. To evaluate the possibility that she and others with suggestive Vi antibody may have acquired asymptomatic or mild infection at the party, we determined the O and H antibody titers in all sera. All sera were collected 6 to 8 weeks after the time of exposure; we expected that any titer increases associated with infections from the common source would still be present after this short period (11). All sera from asymptomatic individuals, including the carrier, had reciprocal O and H antibody titers of ≤ 40 and ≤ 80 , respectively; serum from the only person who fulfilled our definition of "ill" but was never cultured had reciprocal O and H antibody titers of 320 and 20, respectively.

One of the six case-patients, an 8-year-old boy, denied attending the party, eating food prepared for the party, or having any direct contact with the carrier. We suspect that this patient may have acquired a secondary infection from his 19-year-old sibling, who attended the party, was one of the five other case-patients, and whose condition required assistance and close personal contact.

DISCUSSION

By epidemiological methods, we determined the time and place of common exposure and the probable vehicles of foodborne transmission in

TABLE 2. Laboratory results for 11 adult household contacts of typhoid fever patients, May 1981

Contact no.	Stool culture			Vi antibody reciprocal titer ^a	
	1st	2nd	3rd	ELISA	Hemagglutination
1 ^b	—			<20	10
2 ^b	—			40	<10
3 ^b	—			20	80
4 ^b				<20	<10
5 ^b	—	+		320	40
6	—			20	20
7	—	—	—	40	10
8				<20	<10
9	—			<20	<10
10	—			<20	<10
11	—	—	—	80	40

^a Reciprocal titers of ≥ 80 and ≥ 40 were considered positive for the ELISA and hemagglutination assays, respectively.

^b Persons who prepared food for the April 20 party.

this small typhoid outbreak. However, because the foods associated with illness were prepared by several persons, the identification of a particular food vehicle offered no additional clues in the search for a carrier. The initial stool cultures from the food handlers were all negative; however, one individual (the 70-year-old woman) was distinguished from the others by her strikingly elevated Vi antibody ELISA titer, and repeat cultures from this individual were positive for *S. typhi*. Vi antibody titers measured by the hemagglutination method did not distinguish the serum of the woman from the others tested. Although negative serological tests do not prove that recent infection has not occurred, we suggest that the combination of elevated Vi and negative O and H antibody titers in the serum of the woman constitutes strong supportive evidence that the *S. typhi* carrier state preceded, rather than followed, the party.

Vi serology proved to be of value in identifying the source of this outbreak, and should be considered by health departments trying to identify typhoid carriers. The recent adaptation of the technique to the ELISA method provides a test that may be more attractive than the hemagglutination test to laboratories which are already doing other ELISA tests.

ACKNOWLEDGMENTS

We thank David Osterman, Brad Lawrence, David Logan, Dorothy Shuff, and Julie Wilder for their technical contributions and Toni Toya and Dorothy Anderson for their assistance in the preparation of the manuscript.

LITERATURE CITED

1. Barrett, T. J., P. A. Blake, S. L. Brown, K. Hoffman, J. Mateu Llor, and J. C. Feeley. 1983. Enzyme-linked immunosorbent assay for detection of human antibodies to *Salmonella typhi* Vi antigen. *J. Clin. Microbiol.* 17:625-627.
2. Bokkenheuser, V., P. Smit, and N. Richardson. 1964. A challenge to the validity of the Vi test for the detection of chronic typhoid carriers. *Am. J. Public Health* 54:1507-1513.
3. Edwards, P. R., and W. H. Ewing. 1972. Identification of Enterobacteriaceae, 3rd ed. Burgess Publishing Co., Minneapolis.
4. Felix, A., K. S. Krikorian, and R. Reitler. 1935. The occurrence of typhoid bacilli containing Vi antigen in cases of typhoid fever and of Vi antibody in their sera. *J. Hyg.* 35:421-427.
5. Nolan, C. M., J. C. Feeley, P. C. White, Jr., E. A. Hamble, S. L. Brown, and K. H. Wong. 1980. Evaluation of a new assay for Vi antibody in chronic carriers of *Salmonella typhi*. *J. Clin. Microbiol.* 12:22-26.
6. Nolan, C. M., P. C. White, Jr., J. C. Feeley, S. L. Brown, E. A. Hamble, and K. H. Wong. 1981. Vi serology in the detection of typhoid carriers. *Lancet* 1:583-585.
7. Pike, M. C., and R. H. Morrow. 1970. Statistical analysis of patient-control studies in epidemiology: factor under investigation on all-or-none variable. *Br. J. Prev. Soc. Med.* 24:42-44.
8. Ryder, R. W., and P. A. Blake. 1979. Typhoid fever in the United States, 1975 and 1976. *J. Infect. Dis.* 139:124-126.
9. Schubert, J. H., P. R. Edwards, and C. H. Ramsey. 1959. Detection of typhoid carriers by agglutination tests. *J. Bacteriol.* 77:648-654.
10. Sharp, J. C. M. 1966. Chronic enteric carriers: management of personal problems. *Br. Med. J.* 2:551-555.
11. Weber, D. M. 1976. The diarrheal diseases and food-borne illnesses, p. 171-182. In G. W. Hunter, J. C. Schwarzwelder, and D. F. Clyde (ed.), *Tropical medicine*, 5th ed. The W. B. Saunders Co., Philadelphia.
12. Widal, F. 1896. Serodiagnostics de la fièvre typhoïde. *Bull. Mem. Soc. Med. Hop. Paris* 13:561-565.