

Evaluation of a New Assay for Vi Antibody in Chronic Carriers of *Salmonella typhi*

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The assay for serum antibody to the *Salmonella typhi* capsular polysaccharide (Vi) antigen has recently been revised because of the availability of a purified, highly polymerized Vi antigen. We compared this revised Vi antibody assay to the traditional one for potential usefulness in the surveillance of chronic enteric carriers of *S. typhi*. The purified Vi antigen of *Citrobacter freundii* was incorporated into a passive hemagglutination assay for serum Vi antibody; the standard Vi antibody assay was also a hemagglutination assay that employed as the Vi antigen a crude extract of *Citrobacter* (Ballerup O group 29). As determined by the revised assay, Vi antibody was found in the sera of 22 (71%) of 31 current typhoid carriers, none of 6 resolved carriers, and none of 22 control subjects. According to the traditional assay, Vi antibody was present in 23 of those current carriers (74%), 1 of the resolved carriers (17%), and 4 of the control subjects (18%). The rate of false-positive Vi antibody tests among resolved carriers and control subjects was less with the revised assay ($P < 0.05$). Successful antimicrobial therapy resulted in a reversion to seronegativity within 1 year in 8 of 10 Vi-positive carriers according to the revised assay, but in only 3 of 11 according to the standard assay ($P < 0.05$). During a 2-year period of observation, 15 (94%) of 16 current typhoid carriers had at least one positive purified Vi antibody test; among 12 of those patients with Vi titers of 1:40 or greater, 9 (75%) were continuously Vi positive. Thus, the revised Vi antibody assay is more specific and no less sensitive than the standard assay for the condition of current enteric carriage of *S. typhi*. This serological test could be of value in the surveillance of typhoid carriers by public health agencies.

The asymptomatic enteric carrier of *Salmonella typhi* is a key link in the epidemiology of typhoid fever. The most recent statistics from the United States indicate that over 30% of cases can be traced directly to such a carrier (16). The cornerstone of surveillance of typhoid carriers by public health agencies is the periodic culture of feces. Nevertheless, it is often difficult to obtain fecal specimens for this important test. Many typhoid carriers are elderly, poorly educated, and fail to understand the implications of their condition. In addition, most individuals have a natural aversion to handling their own feces. Consequently, typhoid carriers are frequently uncooperative when asked to submit fecal specimens for culture (18).

An alternative means of following typhoid carriers would be desirable. A serological test, serum antibody to the Vi capsular polysaccharide of *S. typhi*, has traditionally been associated with the persistence of *S. typhi* in the body after acute typhoid fever (2). However, previous studies showed that this test possessed neither the specificity nor the sensitivity for the condition

of chronic typhoid carriage to have broad clinical usefulness (1, 3, 9, 14).

The Vi antigen of *S. typhi* and that of *Citrobacter freundii*, which is antigenically identical to the former (19), have recently been purified in a highly polymerized form during a search for an improved typhoid vaccine (20, 21). In the process, the assay for serum Vi antibody was revised to employ this purified antigen, in contrast to the crude antigen preparations that were available for Vi antibody testing by earlier investigators. We decided to reevaluate serum Vi antibody as determined by this enhanced assay for its usefulness in the surveillance of typhoid carriers. The question was specifically addressed by correlating Vi serology with fecal excretion of *S. typhi* in a prospective fashion. The Vi antibody response after successful and unsuccessful antimicrobial treatment of typhoid carriers was also evaluated.

MATERIALS AND METHODS

Patients and controls. Over a 2.5-year period from January 1975 to June 1977, 37 individuals

throughout Arkansas that were followed by the Arkansas Department of Health, Little Rock, Ark., because of chronic, asymptomatic fecal excretion of *S. typhi* were requested to submit serum specimens for the determination of typhoid Vi, O, and H antibodies at the time annual surveillance stool cultures were performed.

For purposes of this study, a patient was classified as a current typhoid carrier if *S. typhi* was isolated from the stool specimen obtained with the test serum specimen. Patients with negative stool cultures at that time were cultured at least two additional times within 1 year. Those with positive stool cultures upon retesting were also declared to be current carriers. If a patient who had previously excreted *S. typhi* was persistently stool culture negative during that period, he was classified as a resolved carrier.

Serum and stool specimens were also obtained from 22 unhospitalized patients matched with respect to age, sex, and race to the group of typhoid carriers. Control subjects were selected from patients who attended several outpatient clinics held by the Little Rock City Department of Health. None had a history of previous typhoid fever or typhoid vaccination.

At the time that the test specimens of feces and sera were collected from the 37 typhoid carriers, all those who could be classified as current carriers and were younger than 80 years of age, free from serious or debilitating underlying illness, and without a history of allergy to penicillin were offered a course of antimicrobial therapy in an effort to eradicate *S. typhi* from the feces. A total of 16 patients completed the treatment study, the results of which have been published (12). Those patients received a 28-day course of amoxicillin, 2 g orally three times a day. At intervals of 3, 6, and 12 months after the termination of antimicrobial therapy, stool cultures were done, and serum specimens were obtained to define the Vi serological response to treatment.

A total of 15 current typhoid carriers who declined participation in the treatment study or did not meet the criteria for antimicrobial therapy, 5 carriers who were not cured by antibiotics, and 6 resolved carriers were enrolled in a prospective study that sought to correlate the results of stool cultures with Vi antibody status. Those participating in this study were asked to submit simultaneous stool and serum specimens every 6 to 12 months for a period of 2 years.

Bacteriological methods. Fecal specimens were collected in 0.5% phosphate-buffered glycerin (pH 7.2) and sent via mail or courier to the Laboratory Division, Arkansas Department of Health. The specimens were then subjected to routine handling for the isolation of enteric pathogens (7), including streaking on bismuth sulfite, SS, and MacConkey agars (Difco Laboratories, Detroit, Mich.) and inoculation into Selenite broth (Difco). *S. typhi* was identified by standard biochemical and serological reactions (7).

Serological methods. Blood was collected in a glass tube, allowed to clot, and sent via mail or courier to the Laboratory Division, Arkansas Department of Health. Sera were separated by centrifugation, portioned, and stored at -20°C . Every 3 months, serum specimens were sent on Dry Ice to the Center for Disease Control for serological assays.

The Vi antigen of a *C. freundii* strain traditionally called *Citrobacter* 5396/38 was purified as previously described (20). Briefly, the process involved the following three steps: (i) the removal of nucleic acids and proteins from acetone-killed and dried bacterial cells by treatment with deoxyribonuclease, ribonuclease, and pronase; (ii) concentration of the Vi polysaccharide by extraction with 60% ethanol; and (iii) separation of the Vi polysaccharide from residual somatic antigens by precipitation with 0.1% hexadecyltrimethylammonium bromide. In double-gel diffusion studies with antiserum to *Citrobacter* 5396/38 and *S. typhi* strain Ty-2, this purified Vi antigen gave one sharp, narrow precipitin band (20).

The crude Vi antigen was prepared by incubating *Citrobacter* (Ballerup O group 29) on infusion agar overnight. The bacteria were harvested in 0.15 M NaCl and steamed for 1 h. After cooling, the suspension of killed bacteria was centrifuged, and the supernatant fluid containing the Vi antigen was decanted and stored at -20°C .

A passive hemagglutination assay was used to measure serum antibodies to the crude and purified Vi antigens (17). Sheep erythrocytes were sensitized with the crude Vi antigen in a 1:400 dilution of the NaCl extract and with the purified Vi antigen at a concentration of 10 $\mu\text{g}/\text{ml}$. Sera were added to suspensions of sensitized and unsensitized erythrocytes in doubled dilutions from 1:10 to 1:2,560. A hemagglutination titer of less than 1:10 was considered to be negative. Serum antibodies to the O and H antigens of *S. typhi* were determined by a standard tube agglutination method (7).

RESULTS

A total of 22 (71%) of 31 current enteric carriers of *S. typhi* had antibody to the purified Vi antigen (purified Vi antibody) in their sera (Table 1); 23 (74%) of them possessed antibody to the crude Vi antigen (crude Vi antibody). In 21 patients, the titers of purified and crude Vi antibody agreed within one tube dilution. Five patients had titers of purified Vi antibody ex-

TABLE 1. *Titers of serum antibodies to crude and purified Vi antigens among 37 typhoid carriers*

Vi antigen ^a	No. of patients with reciprocal Vi titer of:			
	<10	10-40	80-320	640-2,560
Current carriers (31)				
Purified	9	8	7	7
Crude	8	12	8	3
Resolved carriers (6)				
Purified	6			
Crude	5	1		

^a Type of antigen used to sensitize erythrocytes in the passive hemagglutination assay. Numbers in parentheses represent the total numbers of current and resolved carriers.

ceeding those of the crude antibody by more than one tube dilution; in the remaining five patients, the opposite pattern was observed, with the crude Vi antibody titers exceeding those of the purified Vi antibody by more than one tube dilution. There was no correlation between the titers of purified or crude Vi antibody and age, sex, or length of carriage of *S. typhi*. Typhoid H agglutinins were present in the sera of four current carriers, whereas only two carriers possessed O agglutinins.

One of six resolved typhoid carriers had a low titer of crude Vi antibody (Table 1). That patient also had a typhoid H antibody titer of 1:20. No resolved carrier had purified Vi or typhoid O antibody.

In the control group, all 22 stool cultures were negative for *S. typhi*, and all sera were negative for typhoid O and H agglutinins. Likewise, all were negative for purified Vi antibody, but four of them (18%) contained crude Vi antibody in titers ranging from 1:20 to 1:80. Thus, the overall false-positive rates of the two Vi tests among resolved carriers and controls were 0% (0 of 28) for the purified Vi test and 18% (5 of 28) for the crude Vi test ($P < 0.05$; χ^2 analysis) (11). Serum specimens were periodically tested in random fashion in a hemagglutination assay that employed the purified Vi antigen of *S. typhi* (21) as the sensitizing agent. Antibody titers obtained in these assays agreed within one tube dilution with those in the assay with the *Citrobacter* Vi antigen in over 95% of specimens.

The purified Vi antibody titer reverted to negative within 1 year of successful antimicrobial therapy in all eight carriers who had an initial titer of 1:640 or less (Fig. 1). During that same period of time, the crude Vi antibody titer declined in most patients but became negative in only 3 of 11 patients in whom it was originally positive ($P < 0.05$; Fisher exact test) (11). Among five carriers who failed to be cured by antimicrobial therapy, both types of Vi antibody, if initially positive, remained positive with a minor change in titer.

A total of 26 current carriers were initially enrolled in the prospective study that sought to correlate stool culture results and purified Vi antibody; 18 submitted at least three paired specimens of feces and sera during the 2-year period and were considered to be evaluable. Among the remainder, one patient who submitted specimens only at the beginning and end of the 2-year period was also included because she had been a constant excretor of *S. typhi* for 14 years and was undoubtedly a current carrier.

Among the 12 current typhoid carriers who had at least one purified Vi titer of 1:40 during the prospective study, 9 (75%) had all serum

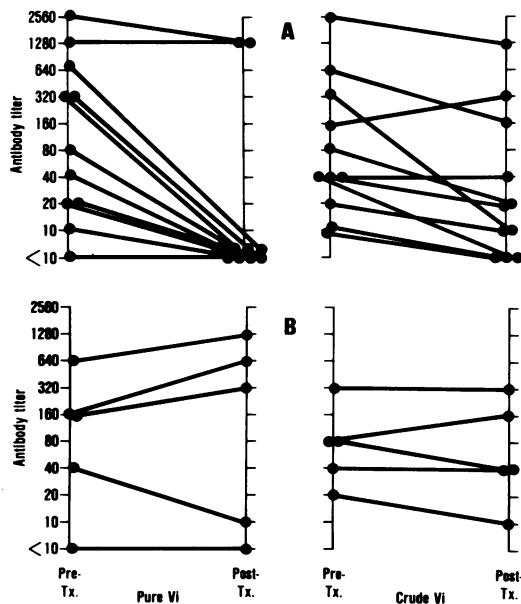


FIG. 1. Vi antibody response to antimicrobial treatment of typhoid carriers. (A) Response of 11 carriers successfully treated; (B) response of 5 carriers unsuccessfully treated. Pre-Tx., Pretreatment; Post-Tx. 1 year posttreatment. Pure Vi, Antibody to a purified Vi antigen; crude Vi, antibody to a crude Vi antigen.

specimens positive for purified Vi antibody (Table 2). None of the four whose highest purified Vi titers were less than 1:40 were persistently Vi positive. Altogether, 15 of 16 current carriers had at least one positive Vi antibody test during the period of observation. As all three resolved carriers were continuously Vi antibody negative, the predicted value (8) of the purified Vi antibody test in the diagnosis of current typhoid carriage during this 2-year period was 95%.

DISCUSSION

The surface antigen of *S. typhi* designated Vi is a linear homopolymer of *N*-acetyl, *O*-acetyl-polygalacturonic acid. This acidic polysaccharide, which lies exterior to the bacterial cell wall, is thus analogous in location and biochemical composition to the *Escherichia coli* K antigens (15) and probably serves a purpose similar to those and other polysaccharide capsular or envelope antigens, namely, acting as a deterrent to host defense systems such as complement-mediated bacterial lysis (13) and phagocytosis (10). Antibodies to the Vi antigen appear in serum during the course of convalescence from typhoid fever in approximately 80% of cases (4). Likewise, typhoid vaccination evokes a Vi antibody response in 85% of patients.

TABLE 2. Comparison of stool cultures and the purified Vi antibody test in the surveillance of typhoid carriers during a 2-year period

Patient, age, sex ^a	No. of paired stool and serum specimens	No. of stool specimens positive for <i>S. typhi</i>	No. of positive Vi antibody tests ^b	Reciprocal Vi titer range
Current carriers				
1, 62, M	4	4	1	<10-40
2, 76, F	2	2	1	<10-10
3, 68, F	3	3	0	<10
4, 72, F	3	3	3	640-1,280
5, 65, F	5	3	3	<10-1,280
6, 76, F	3	2	1	<10-10
7, 84, M	3	3	3	320-640
8, 75, F	3	3	3	640-2,560
9, 84, F	3	2	3	320-640
10, 66, M	3	3	1	<10-10
11, 71, F	3	2	2	<10-640
12, 82, F	3	2	3	80-320
13, 79, M	3	3	3	40-160
14, 57, F	3	2	3	640-1,280
15, 67, F	3	3	3	160-320
16, 52, F	3	3	3	80-640
Resolved carriers				
17, 46, M	3	0	0	<10
18, 75, F	3	0	0	<10
19, 82, F	3	0	0	<10

^a M, Male; F, female.

^b Titers \geq 1:10 were positive.

For years it has been appreciated that most chronic carriers of *S. typhi* have serum Vi agglutinins. Numerous attempts have been made to apply the Vi serological test to the detection of asymptomatic typhoid carriers, but results have not been encouraging (1, 3, 5, 9, 14). The present study shows that the Vi antibody test with a purified, highly polymerized Vi antigen of *C. freundii* has a greater diagnostic potential for the condition of current typhoid carriage than the traditional Vi antibody test with a crude Vi antigen. The primary advantage of the newer test lies in its increased specificity. The purified Vi test was negative in all resolved carriers and control subjects, whereas the crude antibody test had a false-negative rate of 17% in those groups. Moreover, the study on antimicrobial therapy of typhoid carriers revealed that the purified Vi antibody reverted to negative with bacteriological cure more frequently than did the crude Vi antibody during a 1-year period of observation. This latter finding strengthens the argument that the purified Vi antibody test is more specific for the condition of current typhoid carriage; it also implies that this test may have a role in the evaluation of therapy for typhoid carriers.

Results of the prospective study comparing

stool excretion of *S. typhi* and Vi serology suggest that the latter may supplement and thereby reduce the need for frequent stool cultures in the surveillance of typhoid carriers. The purified Vi antibody test should be particularly valuable in current typhoid carriers who have a Vi titer of 1:40 or greater as 75% of such patients in the present study were continuously seropositive during a 2-year period of observation. In carriers such as these, it should be possible to reduce the frequency of stool cultures and to perform Vi serology at intervening intervals. Reversion from Vi seropositivity to Vi seronegativity on two successive specimens should prompt an evaluation for a spontaneous cessation of typhoid carriage, a phenomenon that is well described (6).

Vi serology could also be applied to the detection of an asymptomatic typhoid carrier during the evaluation of a sporadic case of typhoid fever. In Arkansas, over 75% of cases of typhoid fever are associated with an undiagnosed enteric carrier (unpublished observations). Such an evaluation may involve culturing the stool specimens of as many as 10 individuals who are intimate contacts of the patient with typhoid fever. A simple screening procedure that is sufficiently sensitive and specific could be very useful in such evaluations, which consume a great deal of time and effort on the part of public health agencies. The main limitation of the crude Vi test in this circumstance is its lack of specificity; up to 50% of certain control populations were found to be Vi positive by this method (3, 4, 9). Our results on a small group of matched control subjects suggest that in this geographic region, the purified Vi antibody test is more specific for the condition of current typhoid carriage than is the crude Vi antibody test. Consequently, the former is currently being investigated prospectively in the screening for new typhoid carriers.

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LITERATURE CITED

- Anderson, K. 1970. Vi hemagglutination test for the identification of typhoid carriers. *Guy's Hosp. Rep.* 119: 111-117.
- Bhatnagar, S. S. 1944. Prognostic value of laboratory investigations in typhoid fever. *Br. Med. J.* 1:417-420.
- Bokkenheuser, V., P. Smity, and N. Richardson. 1964. A challenge to the validity of the Vi test for the detection of chronic typhoid carriers. *Am. J. Public Health* 54:1501-1503.
- Brodie, J. 1977. Antibodies and the Aberdeen typhoid outbreak of 1964. I. The Widal reaction. *J. Hyg.* 79: 161-180.
- Chau, P. V., and A. C. H. Chan. 1976. Modified Vi tests in the screening of typhoid carriers. *J. Hyg.* 77:97-104.
- Christie, A. B. 1974. Typhoid and paratyphoid fevers, p.

- 55-130. *In Infectious diseases: epidemiology and clinical practice*, 2nd ed. Churchill Livingstone, London.
7. Ewing, W. H., and W. J. Martin. 1974. *Enterobacteriaceae*, p. 204-221. *In* E. H. Lennette, E. H. Spaulding, and J. P. Truant (ed.), *American Society for Microbiology*, Washington, D.C.
 8. Feinstein, A. R. 1975. Clinical biostatistics. XXXI. On the sensitivity, specificity, and discrimination of diagnostic tests. *Clin. Pharmacol. Ther.* 17:104-116.
 9. Forrest, C. R., R. N. Matthews, M. S. Robertson, and W. D. Hanley. 1967. Vi reaction in Hong Kong. *Br. Med. J.* 2:472-475.
 10. Howard, C. J., and A. A. Glynn. 1971. The virulence for mice of strains of *Escherichia coli* related to the effects of K antigens on their resistance to phagocytosis and killing by complement. *Immunology* 20:767-777.
 11. Ipsen, J., and P. A. Feigl. 1970. Bancroft's introduction to biostatistics, 2nd ed., p. 77-82. Harper & Row, Publishers, New York.
 12. Nolan, C. M., and P. C. White, Jr. 1978. Treatment of typhoid carriers with amoxicillin. Correlates of successful therapy. *J. Am. Med. Assoc.* 239:2352-2354.
 13. Osawa, E., and L. H. Muschel. 1964. The bactericidal actions of O and Vi antibodies against *Salmonella typhosa*. *J. Immunol.* 92:281-185.
 14. Public Health Laboratory Service Working Party. 1961. The detection of the typhoid carrier state. *J. Hyg.* 59:231-239.
 15. Robbins, J. B., G. H. McCracken, Jr., E. C. Gotschlich, F. Ørskov, I. Ørskov, and L. A. Hanson. 1974. *Escherichia coli* K1 capsular polysaccharide associated with neonatal meningitis. *N. Engl. J. Med.* 290:1216-1220.
 16. Ryder, R. W., and P. A. Blake. 1979. Typhoid fever in the United States, 1975 and 1976. *J. Infect. Dis.* 139:124-126.
 17. Schubert, J. H., P. R. Edwards, and C. H. Ramsey. 1959. Detection of typhoid carriers by agglutination tests. *J. Bacteriol.* 77:648-654.
 18. Sharp, J. C. M. 1966. Chronic enteric carriers: management of personal problems. *Br. Med. J.* 2:551-555.
 19. Whiteside, R. E., and E. E. Baker. 1959. The Vi antigens of the enterobacteriaceae. II. Immunologic and biologic properties. *J. Immunol.* 83:687-696.
 20. Wong, K. H., and J. C. Feeley. 1972. Isolation of Vi antigen and a simple method for its measurement. *Appl. Microbiol.* 24:628-633.
 21. Wong, K. H., J. C. Feeley, R. S. Northrup, and M. E. Forlines. 1974. Vi antigen from *Salmonella typhosa* and immunity against typhoid fever. I. Isolation and immunologic properties in animals. *Infect. Immun.* 9:348-353.