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Detection of *Salmonella typhi* Carriers in Food Handlers by Vi Serology in Lima, Peru[1]

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♦♦♦ INDEXED

The work described here sought to assess the merits of using an indirect hemagglutination test employing highly purified Vi antigen to screen a high-risk population for chronic* S. typhi *carriers in Lima, Peru. A total of 1,931 female food handlers over 30 years old were enrolled in the study. Indirect hemagglutination tests performed on these subjects' sera, taking a titer of 1:40 or more as positive, yielded 29 positive results. Subsequent bacteriologic testing performed on 26 of these 29 subjects identified four (15%) as* S. typhi *carriers. The procedure had a sensitivity of 79%, indicating that the prevalence of* S. typhi *carriers among the population studied was on the order of 262 per 100,000. It appears that Vi serology employing highly purified Vi antigen offers a practical and cost-effective way of screening for* S. typhi *carriers in both endemic and nonendemic typhoid fever areas.

Typhoid fever persists as a serious public health problem in many developing countries. Only humans are affected, and they are the natural reservoir of *Salmonella typhi*, the disease agent (*1*). It is acknowledged that between 2% and 5% of those who develop typhoid fever become chronic *S. typhi* carriers with gallbladder infections that may persist for life (*2*). In general, the prevalence of chronic *S. typhi* is greater among women, increases with age, and relates to the presence of chronic calculous cholecystitis (*3*). It has been estimated that the prevalence of chronic *S. typhi* carriers in endemic areas may range from 500 to 700 per 100,000 inhabitants (*4*).

Theoretically, detecting chronic *S. typhi* carriers by screening an unselected population provides a potentially effective means of controlling typhoid fever. However, the cost of taking serial stool cultures to isolate *S. typhi* has restricted the use of this traditional method in public health programs.

In 1930 Felix (*5, 6*) described *S. typhi* Vi antigen and the association between high levels of serum antibodies directed against this antigen and the chronic carrier state. Between 1940 and 1960 this gave rise to Vi serology using a strain of *S. typhi* rich in Vi antigen in a direct agglutination assay to detect chronic carriers (*7*). This test subsequently fell into disuse because it lacked adequate sensitivity and specificity (*8*).

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In 1972 Wong and Feeley (*9*) described a method for preparing highly purified Vi antigen, and this was used by Nolan et al. (*10*) in a passive hemagglutination assay to detect chronic *S. typhi* carriers. This new method demonstrated high sensitivity (100%), high specificity (97%), and efficacy in detecting chronic carriers in Arkansas, an area not endemic for typhoid fever in the United States (*11*).

Subsequently, Lanata et al. (*12*) showed that this method (using a titer of 1:160 or more to indicate a positive result) had a sensitivity of 75% and a specificity of 92% in screening for *S. typhi* carriers in Santiago, Chile, an endemic typhoid fever area. On the basis of these results in Santiago, it was estimated that indirect hemagglutination using highly purified Vi antigen should have a positive predictive value (defined as the percentage of true positive *S. typhi* carriers among those positive by Vi serology—*13*) of 8% to 17% if applied to the general population (*12*). Its real value would depend on the prevalence of chronic *S. typhi* carriers in the population to be screened.

With a view to further assessing the new test's predictive value in screening for *S. typhi* carriers and evaluating its use in public health programs directed at eradicating chronic *S. typhi* infections in high-risk populations, we decided to apply Vi serology as part of a control program dealing with female food handlers in Lima, Peru, an area endemic for typhoid fever (*14*).

MATERIALS AND METHODS

Study Population

At the time of our study, a sanitary control program for food handlers was being developed in Lima municipalities. This program included issuance of health cards valid for one year upon certification that the food handler had a negative chest radiography and was serologically negative for syphilis. Coordination was arranged with the Municipality of the District of San Martín de Porras in Lima to obtain 0.2–0.3 cc of serum from each test subject, together with a card stating the subject's name, address, and age, and the date the specimen was taken. We decided to include only female food handlers over 30 years of age, since we considered them at especially high risk of being chronic *S. typhi* carriers (*3*).

Vi Serology

Serologic specimens were kept frozen at −20°C until the time of their analysis. The indirect hemagglutination technique was used, together with highly purified Vi antigen prepared from a strain of *Citrobacter freundii* following the techniques of Wong and Feeley (*9*) and Nolan et al. (*10*). Vi antigen was provided by Dr. J. B. Robbins at the Division of Bacterial Products, U.S. National Center for Drugs and Biologics.

The serologic samples were pretreated with sheep erythrocytes not sensitized with Vi antigen in order to absorb antibodies against these erythrocytes. Other sheep erythrocytes treated with glutaraldehyde were then sensitized with Vi antigen at a concentration of 10 μg/ml (*15*). Serial dilutions were made of the serum samples, and each of these was added to similar volumes of erythrocytes that had and had not been sensitized with Vi antigen. Hemagglutination was read after two hours of incubation at room temperature and again after 18 hours of incubation at 4°C. The titer recorded was the last dilution showing positive hemagglutination. Positive and negative controls were run in each test.

To establish the assay in Lima, we made a preliminary evaluation of serologic samples from three other groups of

subjects. The first group consisted of 19 chronic *S. typhi* carriers with documented histories of excreting *S. typhi* in bile and/or stools for more than one year, who participated in another study at the Alexander von Humboldt Institute of Tropical Medicine (AVHITM). The second group included 135 people with prior clinical pictures of documented typhoid fever who were on record as not having excreted *S. typhi* for periods ranging from one to six years. And the third group consisted of 72 healthy adult women who had applied for health cards at the Cayetano Heredia University Hospital, and whose records showed one bile culture and two stool cultures negative for *S. typhi*.

As Table 1 shows, if a titer ≥ 1:40 was accepted as positive, the test was found to have a sensitivity of 79% among the chronic carriers, a specificity of 99% among former typhoid fever patients, and a specificity of 100% among 72 members of the healthy adult population. Accordingly, it was decided to employ this titer in screening the food handlers.

Bacteriology

Food handlers with titers of 1:40 or more were contacted and asked to come to the AVHITM for bacteriologic *S. typhi* screening after written informed consent was granted. Bile cultures were obtained by means of an encapsuled string device (*16*) after four hours of fasting on the day of each handler's visit. Only strings impregnated with duodenal contents (as indicated by the color of the bile and a pH of 6 or greater) were cultured. One stool culture obtained by a rectal swab was done on the day of the visit. In addition, each food handler was given two sterile swabs and instructed to take two stool specimens, each on a different day, place each in Cary-Blair transport medium (*17*), and deliver it to the AVHITM laboratory within 24 hours of the time it was procured.

The stool specimens and duodenal fluids were inoculated into a Selenite-F culture broth and onto Salmonella-Shigella and MacConkey agars. *S. typhi* was identified by means of established biochemical and serologic techniques (*18*). Those persons found to be carrying *S. typhi* were enrolled as participants in a subsequent AVHITM follow-up project where a therapeutic test employing oral antibiotics was carried out.

RESULTS

Regarding the food handler survey, over a period of 15 months (July 1985–September 1986) 1,931 serum samples were collected from female food handlers over 30 years of age. As indicated in Table 2, only 29 (1.5%) were found to be positive, yielding titers ≥ 1:40 for antibodies to Vi antigen. Twenty-six of these 29 serologically positive women were successfully contacted for microbiologic screen-

Table 1. Results of preliminary tests conducted in July 1985 on sera from chronic *S. typhi* carriers, former *S. typhi* carriers, and healthy adults in Lima, Peru.

Group of study subjects	No. of subjects	Sera yielding positive results (Vi antibody titers ≥ 1:40) No.	(%)
Chronic *S. typhi* carriers	19	15	(79)
Formerly infected people not excreting *S. typhi* for 1–6 years	135	1	(1)
Apparently healthy health card applicants	72	0	(0)

Table 2. Results obtained with indirect hemagglutination using highly purified Vi antigen to test sera obtained in July 1985–September 1986 from 1,931 female food handlers over 30 years of age in Lima, Peru.

No. of women screened	1,931
No. positive (antibody titer ≥ 1:40)	29 (1.5%)
No. contacted and cultured	26
No. of *S. typhi* excretors bacteriologically confirmed among the 26 contacted and cultured	4
Predictive value of Vi serology at titer ≥ 1:40 (4/26)	15%
Estimated prevalence of *S. typhi* carriers among female food handlers over 30 years of age in Lima	262/100,000
Cost of Vi serology per person screened	US$0.30

ing. This screening showed four to be *S. typhi* excretors, establishing a positive predictive value for the test on the order of 15%.

As our preliminary work had indicated that the test's sensitivity for chronic carriers in Lima was 79%, detection of four chronic carriers among the 1,931 female food handlers over 30 years old suggests a prevalence of *S. typhi* carriers among such food handlers in the Lima area that is on the order of 262 per 100,000. It was also possible to test 21 (53%) of 40 women with titers having positive values below 1:40, but no other carriers of *S. typhi* were detected. Two of the women with low titers were found to be excreting *Salmonella paratyphi A*.

The cost of this Vi serology in Lima, including personnel costs and the bacteriologic testing of those women with titers ≥ 1:40, was US$0.30 per person screened. In contrast, traditional tests using three serial stool samples from each woman screened would have cost approximately US$1.72 per person, or nearly six times as much.

DISCUSSION

Use of Vi serology became very popular in the 1940s. In some British industries, such as those related to the treatment of water and foods, negative Vi serology was required in order to obtain work (*7*). However, with more extensive use it was observed that the test was negative in approximately 20% of the chronic *S. typhi* carriers and positive in up to 20% of the normal population with negative *S. typhi* cultures, particularly in areas where typhoid fever was endemic (*8*). Consequently, the technique was abandoned. The traditional method of carrying out serial stool cultures to isolate *S. typhi* was never employed on a large scale as a method for screening chronic carriers, mainly because of its high cost.

The discovery that other enterobacteria possessed Vi antigen immunologically identical to that of *S. typhi* led to the development of tests using crude or partially purified antigens in passive hemagglutination. These methods, although of greater sensitivity than the direct bacterial agglutination test as developed by Felix (*6*), continued to show low specificity (*19, 20*).

Development of the indirect hemagglutination method using highly purified Vi antigen demonstrated that its sensitivity and specificity in areas in which typhoid fever was not endemic was very high (*10, 11*). This was also true when the test was evaluated in Santiago, Chile, a typhoid fever endemic area, and suggested the usefulness of reintroducing screening of chronic carriers of *S. typhi* through the use of Vi serology (*12*).

The results of our work in Lima, an-

other area endemic for typhoid fever, support the Santiago findings. They indicate that the positive predictive value of the test in screening *S. typhi* carriers within a high-risk population (female food handlers over 30 years of age) is approximately 15%.

Regarding affordability, instead of culturing 1,931 women with three serial stool cultures (the traditional method) to detect five carriers of *S. typhi* at a cost of US$1.72 per person screened, the tested Vi serology method (followed by bacteriologic screening of women who had a positive anti-Vi titer) detected four *S. typhi* carriers among these women at a cost of US$0.30 per person screened. These results demonstrate that this method of screening for *S. typhi* carriers among high-risk populations in typhoid fever endemic areas could be affordable as a public health program.

The need to use a lower titer (1:40) in Lima than the 1:160 titer employed in Santiago arose from the fact that this titer needs to be selected on the basis of local data at the time the technique is implemented. However, in order to assess the possibility that the differences involved might be due to changes occurring in the Vi antigen or in the technique employed, a group of 25 sera from the Lima study were sent to the laboratories of the Center for Vaccine Development at the University of Maryland, U.S.A., together with samples of the Vi antigen used in Lima. These sera were evaluated in parallel, using a Vi antigen maintained at the University of Maryland (the same used to evaluate the Santiago sera) and the Vi antigen from Lima. All the sera were found to be comparable, and none of the titers obtained in Maryland varied from one another by more than one dilution, with the sole exception of one Maryland titer that varied by more than two dilutions from the titer obtained with the same serum in Lima. This result suggests that regional differences may exist in the prevalence of anti-Vi antibodies among chronic *S. typhi* carriers.

CONCLUSIONS

The Vi serology method described affords a practical and cost-effective way of screening for chronic *S. typhi* carriers in nonendemic as well as endemic areas.

For the time being the method can be used to detect carriers so that appropriate sanitary measures can be taken. When effective treatment other than surgical treatment (*21, 22*) becomes available at low cost for chronic carriers, screening to detect chronic carriers will be fully justified as a measure for controlling typhoid fever.

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