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REPORT #2

STUDIES TO CONTROL ENDEMIC TYPHOID FEVER IN CHILE

ANNUAL REPORT

Contract Period 1/1/82-12/31/82

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A multi-faceted program of applied research was undertaken to control endemic typhoid fever in Santiago, Chile. These studies included: (1) A Case/Control study to identify risk factors and incriminate specific vehicles involved in transmission of S. typhi. (2) Prevalence of chronic S. typhi carriers in persons with cholecystitis. (3) Simple serologic and other screening tests to identify chronic S. typhi carriers. (4) A simple, practical, non-surgical treatment for chronic S. typhi carriers. (5) An evaluation of the sensitivity and specificity of diagnosing acute typhoid fever by identifying Vi antigen in urine. (6) A field-trial of Ty21a attenuated S. typhi vaccine.

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Figure 2

SUMMARY

A multi-faceted program of applied research has been undertaken intended to lead to control of endemic typhoid fever in Santiago, Chile. Information gained from these studies will be directly applicable to control of typhoid fever in U.S. military personnel deployed to endemic areas. Previously unreported results of these studies are summarized below.

1) Analysis of Notification Data

Typhoid fever has been highly endemic in Chile for decades. In 1968, and since 1977, incidence rates have virtually doubled compared with the 1960-1981 mean annual rate. The doubling of incidence rates since 1977 is a nationwide phenomenon. Typhoid fever in Santiago exhibits notable seasonality, increased rates occurring in Chilean summer. Approximately one-half of all cases occur in metropolitan Santiago where the incidence peaks in 10-14 year old schoolchildren. Notable increases in age-specific incidence occur at 3 and 6 years of age which provides clues with respect to modes of transmission.

2) The Prevalence of Chronic S. typhi Carriers in Santiago

The availability of an accurate census and reliable data on the prevalence of biliary disease and of S. typhi carriage among persons with cholecystitis allowed an unusually precise estimate of the number of chronic S. typhi carriers. We estimate that in 1980 there existed 25,019 female and 4575 male carriers over 10 years of age in a population of 4,264,514, giving a crude prevalence of 694 carriers per 10^5 population.

3) A Simple Serologic Screening Test to Identify Chronic S. typhi Carriers

The passive hemagglutination test for Vi antibody (using highly purified Vi antigen) was found to be a simple, practical, sensitive and specific screening test to identify chronic S. typhi carriers even in an

endemic area. Three-fourths of 36 chronic carriers had reciprocal titers ≥160 versus only 8% of 388 noncarrier adult females convalescent from typhoid fever 12-48 months earlier and versus only 3% of 59 healthy Chilean adults. Only 38% of 29 patients with acute typhoid fever had Vi titers ≥160. This serologic test showed 75% sensitivity and 92% specificity in an endemic area.

4) A Practical, Non-Surgical Treatment for Chronic S. typhi Carriers

As we identify chronic carriers in the course of our project we are offering them enrollment in a non-surgical treatment study which involves a 28 day course of oral amoxicillin (2.0 gm tid) and probenicid (0.5 gm tid). So far 23 carriers have completed the course of therapy and 22 have been followed for at least one month (14 have been followed for at least six months). Ten treatment failures have occurred so far; these are usually detected within the first eight weeks of cessation of therapy. The success rate of this non-surgical therapy, 55%, is to low to justify its use as a public health tool. However, for individual patients it provides an attractive alternative to try and avoid major surgery.

5) Rapid Diagnosis of Typhoid Fever by Detecting Vi Antigen in Urine

Collaborators from the Centers for Disease Control evaluated a Staphylococcus aureus coagglutination test and an enzyme-linked immunosorbent assay (ELISA) for Vi antigenuria. Performed with currently available antisera these tests were unsatisfactory, being insufficiently sensitive and specific.

6) Large-Scale Field Trial of Ty21a Attenuated S. typhi Oral Vaccine

In March and April 1982 informed consent was solicited from the parents and guardians of 139,222 schoolchildren in Area Norte of Santiago, Chile to enter their children in the field trial evaluating Ty21a oral vaccine

in enteric-coated formulation. Parents of 92,288 children gave consent. In May and June 1982 88,163 of these children were orally vaccinated. Approximately one-third of the randomized children received two doses of vaccine, one-third received one dose of vaccine and the remainder received placebo. The controlled field-trial is being carried out in a double-blind fashion. Intensive clinical and bacteriologic surveillance has been established; as of Jan. 30, 1983, 81 cases of confirmed typhoid have been detected so far in schoolchildren of Area Norte.

7) Environmental Bacteriology Studies

Because epidemiologic observations have incriminated the use of untreated sewage for irrigation of vegetables in the Maipu section of Santiago as a potentially important source of transmission of S. typhi, environmental bacteriologic studies were begun. Multiple previous studies had shown heavy fecal contamination of the Zanjon de la Aguada (an open sewer that services southern Santiago and whose untreated sewage water is used for irrigation) but failed to isolate S. typhi. Our environmental bacteriology studies relied heavily on the use of Moore swabs, thick wads of cotton gauze that remain in the sewers for 2-3 days. During the first two weeks of sampling in January 1983, S. typhi were recovered from a sewer draining a Santiago hospital, the home of a known chronic carrier and from sewage water from two points along the Zanjon.

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I. INTRODUCTION

Typhoid fever, the acute, often debilitating, febrile illness caused by Salmonella typhi is endemic in many less-developed areas of the world. Man is the sole reservoir and host of this infection, in contrast with other salmonella serotypes which are typically zoonoses infecting domestic and herd animals. Approximately 3-5% of acute infections result in chronic gall bladder infection; such chronic S. typhi carriers help to maintain the endemicity of the disease by contamination of food and water vehicles.

Typhoid fever is highly endemic in Santiago, Chile. This is a perplexing, as well as challenging, observation since in other ways Chile is a technologically-advanced, highly literate country whose demographic and health statistics would lead one to expect little typhoid fever or other enteric infections. The literacy rate in Chile exceeds 96%, the 1981 infant mortality rate is 27 per 1000 live births, measles and pertussis have been greatly reduced by immunization, and poliomyelitis has been eradicated (Table 1). Yet the incidence rates of typhoid fever in Chile in recent years have ranged from 50 to >100 per 10^5. Approximately one half of all cases and the highest incidences rates occur in the capital, Santiago. The complexity of the epidemiologic situation responsible for maintenance of urban endemic typhoid fever in Santiago is further highlighted by the occurrence of high incidence rates in populations of the highest socioeconomic level and in a striking seasonal pattern.

In contrast, because of the relatively advanced technology and educational development of Chile and its population, there exist human resources of a level of sophistication and professional competence rarely found in other areas where typhoid fever is endemic. It is into this

challenging and fascinating area that we proceeded with our project "Studies to Control Endemic Typhoid Fever in Chile". The project comprises several distinct, yet mutually-related components including:

1) Intensive study of the descriptive epidemiology of typhoid fever in Chile (Santiago in particular) to gain insights into possible modes of transmission.

2) A case/control study to identify risk factors and to incriminate vehicles of transmission of S. typhi.

3) A study to quantify the prevalence of chronic S. typhi carriers.

4) Development of serologic and other simple screening tests to identify chronic S. typhi carriers.

5) Evaluation of a simple, practical, non-surgical treatment for chronic S. typhi carriers.

6) Evaluation of the sensitivity and specificity of the diagnosis of acute typhoid fever by detection of Vi antigen in urine.

7) A large-scale field trial of the efficacy of Ty21a attenuated S. typhi oral vaccine formulated in enteric-coated capsules.

The progress of each of these components of the project will be summarized in the ensuing pages.

II. DESCRIPTIVE EPIDEMIOLOGY OF ENDEMIC TYPHOID FEVER IN CHILE

Table 2 lists the population of Santiago and all of Chile, numbers of cases of typhoid fever and incidence rates for ten years 1960-1981. One notes that approximately one-half of the cases of typhoid fever in any year are reported from Santiago. It is clear that in the period prior to 1970 an unusually high incidence occurred in 1968. In 1977 the incidence of typhoid fever doubled and has since remained at such elevated rates. It is not clear what factors are responsible for the doubling of the notification rate for

typhoid fever since 1977. A sudden change in notifications for administrative reasons is possible but unlikely. Notifications of other reportable diseases such as meningitis, pertussis, and venereal disease did not go up. Henceforth we shall refer to the years 1977-1981 as the hyperendemic years for typhoid.

Table 3 shows the seasonal distribution of cases in Santiago. It is obvious that the cases in the hyperendemic years 1977-1981 occurred in the same seasonal pattern as typhoid fever in earlier years.

The increased incidence rates for typhoid fever that began in 1977 were evident throughout most of Chile as shown in Table 4. This observation suggests that the phenomenon was country-wide.

The age-specific incidence rates for typhoid fever in Santiago for the periods 1970-1976 and 1977-1981 are shown in Table 5. It is clear that the patterns are quite similar, so that the increased incidences since 1977 cannot be attributed to an epidemic confined to one age group. The incidence rate is relatively low in children less than five years of age. Throughout schoolage years the rates are high, peaking in the 10-14 year age group. Beyond 35 years of age the attack rates fall precipitously.

Table 6 lists the incidence rate by year of age from <1 through age 9 for children living in the northern area of Santiago (Area Norte) in the hyperendemic years 1977-1981. Very little typhoid fever is reported in the first year of life. From age 13-24 months and 25-36 months, rates are still low (38.2 and 54.4 per 10^5 children). Three year olds show a notable and marked increase in the incidence of typhoid fever (135.6 per 10^5), an increase of 149% over the rate seen in 2 year olds. The rates in four and five year olds are only slightly greater than those seen in three year olds. Again, however, in six year olds the incidence rises sharply. The rates in children 7, 8 and 9 years of age vary little from rates in six year olds.

These observations suggest that certain activities and changes in attitudes and practices (particularly regarding food) must occur in Chilean children when they reach three and six years of age which put them at significantly greater risk for development of typhoid fever than children one year younger (i.e. two and five year olds). Six year olds go to school, five year olds do not. Many children in Chile attend Jardines Infantiles (pre-school nurseries) beginning at three years of age. It is likely that these major sociological changes that occur in the lives of Chilean children at 3 and 6 years of age (Jardin Infantil or school attendance) are associated with other changes in behaviour, including food intake, that greatly modify risk of ingestion for S. typhi.

The paucity of notifications of typhoid fever in children less than three years of age could represent an important clue in understanding the epidemiology of typhoid fever in Chile. However, we must carry out studies to confirm or refute the absence of clinical S. typhi infection in these young children. This is particularly crucial since some reports[1] have suggested that in infants and toddlers the illness due to S. typhi infection is atypical and often mild and thus may be much more common than usually believed.

In January, 1983 we began a systematic study to determine whether atypical, clinically mild, S. typhi infection occurs in Chilean infants and toddlers or whether such infections are indeed rare because young children do not consume the contaminated vehicles of transmission that older children consume. A single blood culture is being collected from all children less than two years of age with fever ($\geq$38°C) attending two clinics (Pincoya and Consultorio Dos) in Area Norte. The study is in operation three days each week in each clinic. As of mid-January, 2 of 15 children cultured at Pincoya

and 0 of 12 at Consultorio Dos had positive cultures. The two positive cultures occurred in infants 9 and 10 months of age. This study will be continued at least through the end of March by which time we should have a reasonable data base from which we can formulate clearer conclusions regarding the relative frequency of S. typhi infection (or lack thereof) in young Chilean children.

III. CASE/CONTROL STUDY TO IDENTIFY RISK FACTORS AND VEHICLES OF TRANSMISSION OF S. TYPHI

Results and analysis of this component of the program were described in depth in the last annual report.

IV. A PRECISE ESTIMATION OF THE PREVALENCE OF CHRONIC S. TYPHI CARRIERS IN SANTIAGO, CHILE

In last year's annual report we summarized results of a study carried out by Dr. Conrado Ristori of the Ministry of Health, Chile. He cultured 1000 consecutive gall bladders removed from patients in Santiago undergoing cholecystectomy. S. typhi was recovered from 3.8% overall of the 1000 patients having cholecystectomy. This information and data on the age-specific prevalence of cholelithiasis in Chile were utilized to compute the prevalence of chronic S. typhi carriers in Santiago. Results of this exercise were recently published and are included as Appendix A.

V. A SIMPLE SEROLOGIC SCREENING TEST TO IDENTIFY CHRONIC S. TYPHI CARRIERS

The role that chronic carriers play in providing a reservoir of S. typhi and in serving to contaminate food and water vehicles has been discussed. Simple yet reliable screening tests are required to allow public health authorities to rapidly identify carriers and thence to offer them treatment or ensure that they do not work as foodhandlers.

Heretofore two methods were generally available to screen for carriers and another two methods were ordinarily used for confirmation. The screening tests included:

i) Culture of at least three consecutive stools.

ii) Tests for Vi antibody using partially purified Vi antigen or whole Vi-containing bacteria. [2-9]

Each of these screening tests had significant drawbacks. For example, known carriers commonly have negative stool cultures if the stool specimens are fully formed.[10-13] This is because the short chain fatty acids produced as end products of metabolism by normal anaerobic and aerobic colonic flora are highly inhibitory to salmonella; so a delayed intestinal transit time can result in suppression of the organism. In the past, the practice used to overcome this was to administer oral purgatives such as $MgSO_4$ to induce watery stools accompanied by a rapid intestinal transit.[10,13] In this way bile and upper intestinal fluid rapidly reached the colon and were excreted. Induction of bouts of diarrhea on one or more occasions was not well-appreciated by the suspect carrier!

The other method of confirming the existence of chronic gall bladder carriage was to have individuals ingest intestinal tubes leading to intubation of the duodenum or jejunum wherein bile-stained material could be collected for culture.[10,11] Prior to development of the Enterotest^R gelatin string capsule device, intestinal intubation was a procedure that was uncomfortable, time-consuming and, depending on the tube and method, required considerable clinical supervision.

Serologic screening tests for carriers based on measurement of Vi antibody were until recently highly variable and the subject of much acrimonious disagreement.[2-9] This was so because highly purified preparations of Vi antigen[14] were not yet available as a reagent.

Two significant advances have recently been described that offer great promise as screening and confirmatory tests for detection of S. typhi carriers:

i) Chau and Chan,[15] and Nolan et al.[16,17] have developed a passive hemagglutination assay (PHA) for detection of Vi serum antibodies that utilizes highly purified Vi antigen.[14] This test has been shown to be quite sensitive and specific in preliminary reports involving small numbers of individuals tested.[15-17] This PHA Vi antibody test offers great potential as a simple screening test to detect asymptomatic chronic carriers when large numbers of individuals must be screened.

ii) Gilman et al[18] have described use of the gelatin string capsule device as a simple, practical, and non-discomforting way to obtain bile-stained duodenal fluid for culture. In this way suspect chronic carriers can be confirmed by direct culture of S. typhi from proximal small intestinal fluid.

Detection of Carriers

We have undertaken studies to evaluate each of these two tests. In seeking to detect chronic S. typhi carriers, one of our project public health nurses has reviewed the records of admissions to the Hospital Enfermedades Infecciosas (the Infectious Disease Hospital) of Santiago. She has been collecting the names and addresses of women admitted with typhoid fever at least 12 months earlier who were 25 years of age or older. Letters are being sent to these women requesting that they visit our nurse at the

hospital for collection of specimens. Each woman is being asked to swallow a gelatin string capsule device to collect duodenal fluid, to provide three stool specimens for culture and to allow a blood sample to be obtained for Vi antibody tests.

Between November 1981 and December 1982 our nurse reviewed 12,728 case records to detect women age 25 years or older who were admitted 12-48 months earlier with a confirmed diagnosis of typhoid fever. Among these 12,728 cases, 1015 (8.0%) fell into the proper category. Letters on Ministry of Health stationary were sent to all 1015 women; 542 of these 1015 (53%) women were bacteriologically screened (3 stool and 1 bile culture), among whom 34 chronic carriers were detected (6.3% of the screened women). These 34 carriers had positive bile and stool cultures.

Vi Serology

Using highly purified Vi antigen kindly supplied by Dr. John Robbins of the Bureau of Biologics, Bethesda, Md., we adopted the PHA assay for Vi antibody as described by Nolan et al.[16,17]

Studies were carried out to assess the sensitivity, specificity and repeatability of the assay with respect to adapting it for use in Chile, a highly endemic area.

Studies have been completed with sera from the following populations:

i) 36 known, culture-proven, chronic S. typhi carriers.

ii) 29 Chileans with acute typhoid fever (sera drawn four to 10 days following onset).

iii) 388 Chilean females over 25 years of age who had acute typhoid fever 12-48 months earlier and were shown not to be chronic carriers.

iv) 59 healthy Chilean adult men and women living in areas of Santiago where typhoid fever is highly endemic.

Results of Vi antibody assays on these sera are shown in Tabel 7. Twenty-seven of the 36 chronic carriers (75%) had reciprocal Vi antibody titers $\geq$160. In contrast only 38% of 29 patients with acute typhoid fever manifested reciprocal tiers of 160 or above. Only 8% of 388 women who had confirmed typhoid fever 12-48 months earlier and who had negative bile and stool cultures when screened for chronic carrier status had Vi titers $\geq$160. Similarly titers $\geq$ 160 were found in only 3% of 59 healthy Chilean adults (who were not bacteriologically screened for their carrier status).

These results demonstrate that the PHA serologic test for Vi antibody is quite useful even in an endemic area like Santiago, Chile. The test was 75% sensitive and 92% specific when performed with highly purified Vi polysaccharide as antigen. These observations suggest that this serologic test can play an important role in the search for carriers in epidemiologic investigations and work-ups of outbreaks, even in endemic areas.

VI. EVALUATION OF A SIMPLE, PRACTICAL NON-SURGICAL TREATMENT FOR CHRONIC S. TYPHI CARRIERS

Once chronic S. typhi carriers are identified, one would like to be able to successfully eradicate the carrier state, thereby assuring that the individual no longer poses a risk to others as well as releasing the person from various restrictions. Until recently, medical (i.e. antibiotic) therapy alone was largely ineffective in curing the chronic S. typhi infection, particularly if gallstones were present.[19-21] Particularly disappointing in efficacy were the antibiotics chloramphenicol and trimethoprim/-sulfamethoxazole which are, in contrast, so superior in treating acute infections. The combination of cholecystectomy plus several weeks of

antibiotic therapy is highly successful in eradicating the chronic carrier state.[22] However, gall bladder surgery is neither a practical nor economical alternative and is often refused by carriers.

In 1972 Scioli et al[23] reported successful treatment of 19 chronic S. typhi carriers by medical therapy alone by use of 15 consecutive days of intravenous ampicillin (1.0 gm every eight hours) in hospitalized patients. Although intravenous ampicillin is also not a practical form of therapy, this paper established that the carrier state could be eradicated without surgery if sufficiently high concentrations of an effective antibiotic could be achieved in the gall bladder. Ampicillin is concentrated in bile and is bactericidal in vitro against S. typhi.

Amoxicillin is an analogue of ampicillin that is superbly absorbed following oral administration (giving two to three times the serum levels of a comparable dose of oral ampicillin) and is concentrated in the bile. Amoxicillin is therefore attractive as a possible oral therapy for chronic S. typhi carriers. In one report,[24] 15 carriers were treated with oral amoxicillin (2.0 gm three times daily) for 28 days. Nine of 10 patients who were able to complete therapy were cured. In five patients the dosage had to be cut in half; of these, only two were cured.

Methods and Results

We have undertaken to evaluate the efficacy of combined therapy with oral amoxicillin (2.0 gm thrice daily) and probenecid (0.5 gm thrice daily) in eradicating the chronic S. typhi carrier state. So far, 23 chronic gall bladder carriers in Santiago, Chile have completed the course of treatment; the results are summarized in Table 8. Occasional patients have experienced mild diarrhea during the first seven to ten days of therapy but not of a severity requiring cessation or alteration of treatment. None complained of

cramps, nausea or vomiting. Of the 23 patients who completed the course of therapy 22 have been followed so far for at least one month and 14 for at least six months. Of these 22 patients, 10 treatment failures were noted; in seven instances therapy failure was documented within the first or second month, while two failures were detected at four months and one at six months post-therapy. These later failure isolates are being phage-typed to compare them with the original isolates from the patients.

At present the failure rate of this non-surgical therapy is 45% (10 of 22). This failure rate is too high for this therapy to be employed as a public health tool. However, in individual patients, a one month course of therapy with amoxicillin with a 55% chance of success, thereby avoiding major surgery, must be regarded as an attractive option. Ultimately 30 patients will be entered into the study and will be followed for one year post-therapy.

VII. RAPID DIAGNOSIS OF ACUTE TYPHOID FEVER BY DETECTION OF Vi ANTIGEN IN URINE

This component of the project was carried out by Drs. David Taylor and Jeffrey Harris of the Centers for Disease Control who collaborated with clinicians and bacteriologists at Hospital Roberto del Rio (the children's hospital in Area Norte) and the Infectious Diseases Hospital. A manuscript summarizing this study is included as Appendix B.

VIII. A LARGE-SCALE FIELD TRIAL OF EFFICACY OF TY21a ATTENUATED SALMONELLA TYPHI ORAL VACCINE

This portion of the report will comprise the following sections:

1) Additional studies in U.S. volunteers to compare dosage schedules.
2) Summary of the large-scale administration of vaccine or placebo to 88,000 Chilean schoolchildren.
3) Description of the surveillance system in the field area.
4) Preliminary results of surveillance.

A. Studies of Ty21a in U.S. Volunteers

Ty21a is a stable attenuated mutant of Salmonella typhi that has proven to be a safe, protective oral vaccine. A recent field trial in Alexandria, Egypt evaluated three doses of 10^9 viable cells of this strain given in a five day period. Lyophilized vaccine preparations were reconstituted in a diluent containing phosphate salts and sucrose and drunk after neutralization of gastric acidity by one gram of $NaHCO_3$. The results of this study indicate that when given in this manner the vaccine has a 96% protection rate for at least three years.[25]

In the previous annual report we summarized preliminary studies that were carried out in college students and medical students at the University of Maryland to identify a more convenient formulation for the vaccine than was used in Alexandria and to determine the feasibility of immunizing with one or two doses. We assessed immune response by means of an enzyme-linked immunosorbent assay (ELISA) which measures serum IgG and IgM antibody to the purified lipolysaccharide of Salmonella typhi. Although circulating antibody is almost certainly not the mediator of protective immunity conferred by Ty21a

oral vaccine, such antibody is a simple and convenient measure of immune response to the vaccine; thus it was selected to compare formulations and dosages of vaccine in various groups.

Two formulations of vaccine compared include:

1) Vaccine contained in enteric-coated capsules (10^9 viable organisms per capsule).
2) Vaccine contained in a gelatin capsule (10^9 organisms); this capsule is ingested after two other gelatin capsules each containing 0.5 gm $NaHCO_3$.

In total 100 U.S. students participated in this comparison. The results are summarized in Table 9. No significant differences were noted in rates of seroconversion of O antibody in persons who ingested enteric-coated capsules versus vaccine with $NaHCO_3$, nor were significant differences encountered among those who ingested one, two or three doses of enteric-coated vaccine. Based on these observations a decision was made to employ enteric-coated capsules of Ty21a vaccine in the field trial in Chile and to compare the efficacy of one dose versus two doses versus placebo.

B. Large-scale Field Trial in Santiago, Chile - Summary of Vaccination

The northern administrative region of Santiago, Chile (Area Norte) was selected as the site to carry out the vaccine field trial. Metropolitan Santiago has a population of 4,363,026, while Area Norte contains a total population of 598,635. The general socioeconomic level in Area Norte is low-middle or low. Approximately 80% of all health care visits occur in health centers (consultorios) of the National Health Service.

In May 1981 there were 139,222 schoolchildren in Area Norte enrolled in 3655 classes within 227 schools. Since typhoid fever is largely a disease of schoolage children (incidence rates are highest in 10-14 year olds and 55-60% of cases occur in children 5-19 years of age), we proposed to vaccinate

children within schools and carry out intensive surveillance. In 1981, prior to the field trial, the incidence of confirmed (isolation of S. typhi from blood or bone marrow) cases of typhoid fever in Area Norte schoolchildren was 160 per 10^5, a rate four-fold higher than seen in the control population in the Alexandria field trial.[25]

Notices explaining the vaccine trial and requesting informed consent were sent to the parents and guardians of all 139,222 schoolchildren in Area Norte. Parents of 92,238 gave consent (66%). These children were randomized into three groups (AA, AB, BB) to receive two capsules during the immunization. The identity (vaccine or placebo) of the two preparations labelled A or B is unknown to any of the U.S. or Chilean personnel involved in the trial; the code is being maintained in secrecy in Berne, Switzerland.

In January, 1982 the Ministry of Health of Chile decided that it would be least disruptive to their on-going operations if the administration of vaccine could be carried out in two days of mass application. Accordingly, a formal agreement was signed between the Ministry of Health and the Ministry of Education identifying two dates, May 26 and June 2, 1982 when the mass administration of capsules would be carried out. It was agreed that on that day the Ministry of Health would shut down its 13 consultorios in Area Norte except for a skeleton staff to provide emergency health care. The nurses, nurses-in-training, and health auxiliaries from the 13 consultorios (totalling 225 individuals) were to be assigned to participate as "vaccinators" in the large-scale vaccination. The Ministry of Education agreed that on those dates the primary role of teachers would be to assist in vaccination. The teachers also filled out a master form (planilla) for each of their classes,

line-listing each child, his/her birthdate and whether parents had given permission to participate in the trial. These planillas were used during vaccination to mark what preparation the child received and on what date.

Capsules of A and B arrived from Switzerland and were immediately transported to the refrigerated central vaccine storage facility. Appropriate numbers of capsules were packaged for each class. Capsules were delivered to the consultorios from which vaccinators assigned to specific schools and classes collected their allotments and transported them to their schools in plastic vaccine containers with ice packs. Vaccinators also carried planillas with line lists of the children and plastic drinking cups.

In mid May a pilot vaccination was carried out in 10,000 children in a large technical/vocational school in Area Norte. This pilot vaccination proceeded without notable obstacles. Accordingly on May 26, 1982, the first scheduled mass vaccination day, 76,000 schoolchildren in 226 schools were successfully given capsules of A or B. One week later the second dose was given. Over the next 2-3 weeks the children absent on the mass vaccination days (circa 2000) were given capsules. During the mass vaccination 13 head nurses and eight senior personnel made random spot visits in the schools to assess the progress of the vaccination. In no instance were significant problems encountered. Every supervisor (MML and REB included) encountered extraordinarily smooth functioning of the vaccination process in all classrooms.

A summary of the total number of children vaccinated, with what preparation and number of doses, is shown in Table 10.

The planillas containing precise demographic information on each child as well as what vaccine was ingested were collected and the data entered into a computer. A computer print-out was obtained which contains demographic data

and whether or not the child received one or more capsules but no information of what specific preparation was received. This print-out is utilized during surveillance. The original planillas are kept locked in the Ministry of Health and will not be consulted until the end of the first season of surveillance.

C. Surveillance System

There are three main goals to our surveillance activities:

1) To increase the recognition of potential cases of typhoid fever in schoolage children.
2) To intensify the bacteriologic evaluation of suspect cases.
3) To standardize the clinical evaluation of suspect cases of typhoid fever.

Awareness of the need to maintain surveillance for cases of typhoid fever in children who attend school in Area Norte is being accomplished in several ways:

1) By weekly visits to each consultorio at which time a short discussion is held with the physician and nursing staff.
2) Weekly visits to the children's hospitals and the Infectious Disease hospital.
3) Letters sent to each private physician making him/her aware of the surveillance program and providing information on who to call if a suspect case is seen.
4) Letters and visits to private health centers in Area Norte and private laboratories that do blood cultures to explain the surveillance system.

Collection of clinical information on patients suspected of having typhoid fever has been standardized by providing a standard form (Appendix C) which the clinicians examining the patient fill out. These forms are available in all the consultorios and hospitals.

Bacteriologic surveillance has been intensified in two major ways:

1) By assuring that all suspect cases of typhoid fever seen as outpatients in consultorios have two blood cultures taken.
2) By assuring that all schoolage children admitted to the Children's Hospital in Area Norte (Hospital Roberto del Rio) have three blood cultures and a bone marrow culture.

Prior to this year blood cultures were not routinely performed on outpatients suspected of having typhoid fever seen at the consultorios. At present the 13 consultorios are provided with syringes, needles, iodine swabs and blood culture bottles containing trypticase soy broth and SPS. Blood cultures are transported daily to the bacteriology laboratory of Hospital Roberto del Rio for processing. Positive results are reported both to the consultorio and to the office of the typhoid control program.

Within Hospital Roberto del Rio patients admitted with a clinical diagnosis of typhoid fever are now routinely having three blood cultures and one bone marrow culture. This is a noticeable change from the situation that existed two years ago. At that time, although such cultures were the written hospital norm and were official policy, they were not routinely obtained.

D. Preliminary Results of Surveillance

Typhoid fever exhibits striking seasonality in Santiago, Chile, the season extending throughout the warm months of mid-December through April. (It should be noted that children are on summer holiday from mid-December through the first week of March). As of January 30, 1983, 81 cases of confirmed typhoid fever were detected in schoolchildren of Area Norte.

IX. ENVIRONMENTAL BACTERIOLOGY STUDIES

Epidemiologic investigations carried out under this project suggest that the consumption of fruits and vegetables irrigated with untreated sewage water during summer season in Santiago, Chile could represent an important mode of transmission of S. typhi. This hypothesis successfully explains the following observations:

1) Striking seasonality (irrigation is maximum in the hot season when no rain occurs; this is the typhoid season).

2) Low incidence of typhoid in young children (raw vegetables are not an important food item for infants and toddlers).

3) High incidence of typhoid fever in neighborhoods and populations of high socioeconomic level (they eat salads and fruits in restaurants as well as at home).

4) Low incidence of typhoid fever in the "Lakes" region of Chile (because of year round rains irrigation is not used in this area).

The water supply of Santiago is closely bacteriologically monitored and is potable. The vast majority of households in Santiago have flush toilets connected to a sewer system. This sewer system, however, empties into the Mapocho River without any treatment whatsoever. The largest single sewer conduit is the Zanjon de la Aguada which is an open sewer that traverses all of southern Santiago (flowing from east to west) before emptying into the Mapocho river southwest of the city. As the Zanjon traverses the southwestern portion of metropolitan Santiago (an area called Maipu) its fecally-polluted, untreated waters are diverted into irrigation ditches. This area of cultivation, Maipu, produces mainly lettuce, cabbage and celery, vegetables that are difficult to wash and are typically eaten raw in salads.

It has long been considered likely that vegetable produce from the area of Maipu is contaminated with S. typhi. Accordingly, multiple bacteriologic examinations were carried out by various Chilean microbiologists attempting to culture S. typhi from the waters of the Zanjon and the Mapocho River and from vegetables, particularly lettuce, culture in Maipu.[26-31] The waters of the Zanjon and the Mapocho River and vegetables from Maipu were all shown to be heavily contaminated with fecal coliforms. Many non-typhoidal salmonellae were isolated but S. typhi was never recovered from the Zanjon and on only one occasion was S. typni ever recovered from Mapocho River.[31] The failure to isolate S. typhi from polluted waters of the Zanjon has had far-reaching implications by giving rise in some circles to the notion that these irrigation waters are not important in transmitting S. typni. For example, one recent exercise supported by the Office of Planning[32] involved a cost-benefit analysis of establishment of sewage treatment plants for Santiago. In this analysis, typhoid fever morbidity and mortality were not included either with respect to economic consequences or potential benefits, because S. typhi had never been recovered from the waters of the Zanjon.

The past failure to isolate S. typhi from the polluted waters of the Zanjon is in conflict with our epidemiologic observations implicating consumption of vegetables irrigated with untreated sewage as a likely mode of transmission of typhoid fever. Accordingly, we decided to review the previous studies and embark on one more effort to carry out environmental bacteriology in collaboration with Drs. Ana Maria Cordano and Hernan Lobos of the Instituto de Salud Publica. From our review of the previous Chilean literature we concluded that the bacteriologic methods employed were highly appropriate,

indeed laudable. However, we felt that the environmental sampling was not optimal. Water samples removed from the Zanjon in the past were directly plated or inoculated into enrichment broth without concentration.

Our major modification then was to introduce new techniques of water sampling. The technique chosen was that of the Moore swab,[33] which is a thick wad of cotton gauze dropped into the flowing waters of a sewer and fastened by means of nylon string. Moore swabs have been used with great success in industrialized countries to detect the homes of chronic S. typhi carriers by sampling sewage effluents.[33,34] Since Moore swabs were so successful in detecting S. typhi excreted by one carrier among the sewage outfall of large numbers of non-carriers, we reasoned that this sampling method might prove useful in Chile. Consequently, Dr. Stephen Sears of the CVD went to Chile in January, 1983 to initiate these studies.

The initial sites sampled included sewers of three hospitals, homes of four known chronic S. typhi carriers and three points along the Zanjon de la Aguada. The Moore swabs were left in place for three days. S. typhi were recovered from a sewer draining Hospital Salvador, one house of a chronic carrier and from waters of two of the three sites sampled along the Zanjon. These represent the first documented isolations of S. typhi from the Zanjon. Based on these exciting positive preliminary results we will now intensify the environmental bacteriologic studies. Dr. Sears received particularly critical assistance in these studies from Dr. Julio Monreal (a sanitary engineer who accompanied the team when swabs were inserted and collected), Dr. Ana Maria Cordano (who assisted in bacteriology) and Dr. Catterina Ferreccio (our coordinating epidemiologist).

X. PERSONNEL AND LOGISTICS

The project presently employs fulltime:

1) An epidemiologist/coordinator (Dr. Catterina Ferreccio).
2) Three public health nurses.
3) Two drivers.
4) A secretary.

During the typhoid fever season we have also employed for several months:

1) Two laboratory auxiliaries to assist in handling the vast quantity of bacteriological work (blood cultures, marrow cultures, etc.).
2) Two nurse auxiliaries to assist in clinical aspects.

One public health nurse from the Ministry of Health also works part-time on the typhoid fever project throughout the year.

During typhoid fever season the full-time staff spend 80-100% of their time on surveillance activities related to the vaccine field trial. Maintenance of intensive surveillance is time-consuming since 13 Consultorios must be visited at least weekly, Hospital Roberto del Rio daily, the Infectious Diseases Hospital every other day, and three other hospitals must be visited at least once weekly. The Consultorios must be kept supplied with surveillance forms, blood culture materials etc. Information on positive blood cultures must be promptly transmitted to physicians and nurses at the Consultorios to assist in management of the patients.

The project has at its disposal one vehicle belonging to the project and one more assigned by the Ministry of Health. During peak typhoid fever season the vehicles are in constant use for surveillance-related activities. During this high season there is definite need for one additional vehicle and driver.

XI. CONTRACT RELATED PUBLICATIONS

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Table 1

INFANT MORTALITY RATE AND INCIDENCE OF CERTAIN IMMUNIZABLE COMMUNICABLE DISEASES IN CHILE 1964-1980

	Measles		Pertussis		Poliomyelitis		Infant Mortality Rate
Year	Cases	Incidence	Cases	Incidence	Cases	Incidence	
1964	35,941	428.3*	5,279	62.9*	363	4.3*	105.3†
1969	9,538	99.7	2,905	30.4	64	0.7	78.7
1975	8,413	82.1	2,550	24.9	2	0.0	55.4
1980	3,844	34.0	2,795	25.2	0	0	31.8

*Rate per 100,000

†Rate per 1,000 live births

Table 2

POPULATION SIZE, NUMBER OF CASES OF TYPHOID FEVER AND MORBIDITY RATES FOR TYPHOID FEVER IN CHILE AND METROPOLITAN SANTIAGO 1960 - 1981

	CHILE			SANTIAGO		
Year	Population	No. of Cases	Rate per 10^5	Population	No. of Cases	Rate per 10^5
1960	7,585,350	4548	59.6	2,439,093	2078	85.2
1961	7,770,270	4618	59.2	2,530,593	2401	94.9
1962	7,955,190	3873	47.9	2,622,094	2034	77.6
1963	8,140,110	4185	50.9	2,713,595	2158	79.5
1964	8,325,030	4732	56.0	2,805,096	2731	97.4
1965	8,509,950	5598	64.8	2,896,596	2754	95.1
1966	8,681,671	4576	51.5	2,984,350	2688	90.1
1967	8,853,393	4536	49.8	3,072,103	2747	89.4
1968	9,025,115	7091	75.8	3,159,857	4590	145.3
1969	9,196,837	5358	46.0	3,247,610	3463	106.6
1970	9,368,558	5344	57.0	3,334,936	3408	102.2
1971	9,545,449	4784	50.1	3,425,061	3007	87.8
1972	9,722,341	4527	46.6	3,514,220	2640	75.12
1973	9,899,231	3688	37.3	3,603,465	1865	51.8
1974	10,076,123	4655	46.2	3,693,767	2424	65.6
1975	10,253,014	6110	59.6	3,786,016	3500	92.4
1976	10,454,387	6180	59.1	3,879,626	3545	91.4
1977	10,655,757	11,533	108.2	3,974,437	7070	177.9
1978	10,857,128	13,114	120.8	4,070,293	8334	204.8
1979	11,058,498	10,760	97.3	4,167,000	6358	152.6
1980	11,259,871	10,872	96.6	4,264,518	6827	160.1
1981	11,477,150	10,789	94.0	4,363,026	6936	159.0

Table 3

MEAN NUMBER OF TYPHOID FEVER CASES BY MONTH

IN SANTIAGO, CHILE, 1970 - 1976 AND 1977 - 1981

Month	Mean No. of Cases 1970-1976	% of Total	Mean No. of Cases 1970-1976	% of Total
Jan.	421	14.1	948	13.3
Feb.	403	13.5	917	12.9
March	415	13.9	1015	14.3
April	400	13.4	867	12.2
May	302	10.1	605	8.5
June	183	6.1	529	7.4
July	103	3.5	282	4.0
Aug.	76	2.5	124	1.7
Sept.	62	2.1	162	2.3
Oct.	109	3.7	215	3.0
Nov.	167	5.6	601	8.5
Dec.	340	11.4	841	11.8

Table 4

INCIDENCE RATES OF TYPHOID FEVER IN CHILE

BY REGION, 1970-1976 AND 1977-1981

Region	1970-1976 Mean Pop'n	1970-1976 Rate	1977-81 Mean Pop'n	1977-81 Rate	Percent Rise over 1970-1976 Rate
Chile	9,872,660	50.8*	10,920,125	103.4	104
Tarapaca	200,782	39.3	233,543	81.0	106
Antonfagosta	279,930	33.5	308,454	52.7	57
Atacama	172,423	23.5	194,856	36.2	54
Coquimbo	376,871	51.4	410,836	123.6	140
Valparaiso	1,084,437	29.0	1,209,677	60.5	109
Santiago	3,605,299	80.9	4,167,855	170.9	111
O'Higgins	531,786	86.9	561,867	106.4	22
Maule	663,875	39.5	700,129	66.1	67
Bio Bio	1,355,981	24.6	1,455,678	59.0	140
Araucani..	643,877	20.3	653,817	53.3	163
Los Lagos	804,820	13.3	854,213	21.3	61
Aysen	54,804	39.3	62,206	55.3	41
Magallares	97,774	74.6	106,992	31.7	-58

*Cases per 100,000

Table 5

AGE-SPECIFIC INCIDENCE RATES AND CASES OF TYPHOID FEVER, SANTIAGO, CHILE 1970-1976 AND 1977-1981.

	1970 - 1976			1977 - 1981			
Age Group	Mean No. Cases	% of Total	Mean Incidence per 10^5	Mean No. Cases	% of Total	Mean Incidence per 10^5	Fold Increase
0-4	170	5.9	38.7	421	6.4	89.2	2.3
5-9	524	18.3	126.5	1193	17.1	272.2	2.2
10-14	624	21.8	152.2	1413	20.3	333.0	2.2
15-19	497	17.3	135.5	1465	21.0	283.4	2.1
20-24	421	14.7	126.4	728	10.5	246.7	2.0
25-34	407	14.2	72.0	1023	14.7	153.3	2.1
35-44	134	4.7	33.6	366	5.3	74.6	2.2
45-54	52	1.8	17.4	179	2.6	50.2	2.9
55-64	23	0.8	10.8	86	1.2	36.0	3.3
$\geq$65	9	0.3	5.2	79	1.1	38.5	7.4

Table 6

INCIDENCE RATES OF TYPHOID FEVER BY AGE,

AREA NORTE 1970-1981 AND 1977-1981

Age	1970-1981	Percent Increase in Rate over Preceding Year of Age	1977-1981	Percent Increase in Rate over Preceding Year of Age
<1	14.9 *		16.1	
1	32.9	121	38.2	137.3
2	40.8	24	54.4	42.4
3	90.8	123	135.6	149.2
4	96.9	6.7	144.9	6.9
5	120.3	24	165.0	13.9
6	173.0	43	248.1	50.3
7	155.9	11	247.8	0
8	199.6	28	264.0	6.5
9	203.1	1.7	269.2	2.0

* Cases per 100,000

Table 7

PREVALENCE OF Vi ANTIBODY* IN CHRONIC SALMONELLA TYPHI CARRIERS, ACUTE TYPHOID FEVER, AND HEALTH POPULATIONS IN CHILE

Group	Characteristics	N	Geometric Mean Titer	Percent with Reciprocal Titer: ≤80	Percent with Reciprocal Titer: ≥160
Chronic S. typhi carriers	female ≥25 yrs.	36	296	25	75
			p<0.01		
Acute typhoid fever patients	Both sexes, mostly age 10-30 yrs.	29	53	62	38
		p<0.001			p<0.0000
Non-carriers** who had acute typhoid fever 12/14 months earlier	female ≥25 yrs.	388	21	92	8
Healthy Chilean adults	Both sexes ≥25 yrs.	59	16	97	3

*Measured by passive hemagglutination using highly purified Vi antigen.

**Negative stool (3) and bile cultures.

Table 8

PROGRESS REPORT OF TREATMENT OF CHRONIC BILIARY CARRIERS OF *SALMONELLA TYPHI* WITH A 28 DAY COURSE OF ORAL AMOXICILLIN (2gm TID) AND PROBENECID (0.5gm TID)

Carrier	Date of Completing Therapy	Gallstones	Outcome of Treatment*	
			Success	Failure
1	Sept. 1981	+	+	
2	Dec. 1981	NT†		+(6)**
3	Jan. 1982	NT	+	
4	April 1982	NT	+	
5	April 1982	NT	+	
6	April 1982	+		+(4)
7	May 1982	+		+(1)
8	May 1982	+		+(4)
9	July 1982	NT	+	
10	July 1982	NT		+(1)
11	July 1982	NT	+	
12	August 1982	+		+(1)
13	August 1982	NT	+	
14	August 1982	NT	+	
15	Sept. 1982	+		+(2)
16	Sept. 1982	+		+(1)
17	Sept. 1982	+	+	
18	Oct. 1982	NT		
19	Oct. 1982	+		+(2)
20	Nov. 1982	NT	+	
21	Nov. 1982	NT	+	
22	Nov. 1982	+		+(1)
23	Dec. 1982	NT	results pending	

*Based on monthly bacteriologic examinations of stool and bile post-therapy for up to 12 months.

†Not tested

**() Months post-therapy when *S. typhi* was first detected.

Table 9

NUMBER AND PERCENTAGE OF U.S. ADULTS HAVING A SEROCONVERSION IN IgM OR IgG ELISA SALMONELLA TYPHI O ANTIBODY AND MEAN RISE IN NET OPTICAL DENSITY IN CHILDREN WITH SEROCONVERSIONS AFTER INGESTION OF ONE, TWO, OR THREE 10^9 ORGANISM DOSES OF Ty21a S. TYPHI VACCINE IN TWO DIFFERENT FORMULATIONS

Dose and Formulation	No. Adults Vaccinated	IgM Seroconversions No. (%)	IgM Seroconversions Mean (SEM) Rise in Net Optical Density*	IgG Seroconversions No. (%)	IgG Seroconversions Mean (SEM) Rise in Net Optical Density	Total No. (%) with Seroconversions
One dose						
Enteric coated	36	4 (11)	0.26 (0.06)	5 (14)	0.26 (0.05)	7 (19)
Gelatin/$NaHCO_3$†	44	9.(20)	0.31 (0.06)	9 (20)	0.49 (0.09)	14 (32)
Two dose						
Enteric coated	30	6 (20)	0.34 (0.08)	2 (7)	0.28 (0.05)	7 (23)
Three dose						
Enteric coated	16	3 (19)	0.22 (0.03)	4 (25)	0.26 (0.09)	7 (44)
Gelatin/$NaHCO_3$	15	3 (20)	0.41 (0.16)	3 (20)	0.44 (0.15)	4 (27)

*Net optical density at 400 nm is a direct measure of ELISA S. typhi O antibody

†Gelatin capsule containing vaccine taken after 2 capsules with 0.4 g $NaHCO_3$ each

Table 8

PROGRESS REPORT OF TREATMENT OF CHRONIC BILIARY CARRIERS OF *SALMONELLA TYPHI* WITH A 28 DAY COURSE OF ORAL AMOXICILLIN (2gm TID) AND PROBENECID (0.5gm TID)

Carrier	Date of Completing Therapy	Gallstones	Outcome of Treatment*	
			Success	Failure
1	Sept. 1981	+	+	
2	Dec. 1981	NT†		+(6)**
3	Jan. 1982	NT	+	
4	April 1982	NT	+	
5	April 1982	NT	+	
6	April 1982	+		+(4)
7	May 1982	+		+(1)
8	May 1982	+		+(4)
9	July 1982	NT	+	
10	July 1982	NT		+(1)
11	July 1982	NT	+	
12	August 1982	+		+(1)
13	August 1982	NT	+	
14	August 1982	NT	+	
15	Sept. 1982	+		+(2)
16	Sept. 1982	+		+(1)
17	Sept. 1982	+	+	
18	Oct. 1982	NT		
19	Oct. 1982	+		+(2)
20	Nov. 1982	NT	+	
21	Nov. 1982	NT	+	
22	Nov. 1982	+		+(1)
23	Dec. 1982	NT	results pending	

*Based on monthly bacteriologic examinations of stool and bile post-therapy for up to 12 months.

†Not tested

**() Months post-therapy when *S. typhi* was first detected.

Table 10

ESTIMATED NUMBER OF SCHOOLCHILDREN VACCINATED WITH ONE OR TWO DOSES OF SALMONELLA TYPHI Ty21a ORAL VACCINE OR PLACEBO (DOUBLE BLIND), AREA NORTE, SANTIAGO, CHILE, MAY-JUNE 1982

Consultorio	No. of Schools	No. of Classes	No. of Students	No. of Students Accepting	No. of Students Vaccinated	Type of Vaccine Received				
						AA	AB	BB	A	B
Maruri	43	716	25,816	19,053	18,738	5,901	5,720	5,766	736	615
Independencia	32	605	21,882	13,855	13,439	4,319	4,383	4,425	146	166
Lucas Sierra	36	550	19,389	12,599	12,106	3,814	4,022	4,010	243	17
Quinta Buion	21	313	11,066	6,466	5,945	1,924	1,902	1,910	122	87
Lo Aranguiz	7	115	4,535	2,536	2,271	719	727	720	50	55
El Cortijo	9	161	6,010	3,603	3,688	1,249	1,161	1,158	64	56
Valdivieso	16	355	15,314	10,221	9,440	3,123	3,018	3,193	54	52
Lampa	5	50	1,797	1,258	1,150	406	376	330	25	13
Batuco	6	55	1,693	807	800	212	227	229	72	60
Colina	16	177	5,835	3,776	3,678	1,090	1,122	1,104	193	169
Til Til	13	113	2,500	1,673	1,684	606	432	481	98	67
E. Gonel	8	128	4,291	2,890	2,721	925	871	840	33	52
Pincoya	11	213	8,414	4,148	3,935	1,248	1,241	1,261	116	69
Complejo OH (Maruri)	4	104	10,680	9,355	8,568	2,598	2,491	2,473	551	455
Total	227	3,655	139,222	92,238	88,163	28,134	27,693	27,900	2,503	1,933

Appendix A

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Precise Estimation of the Numbers of Chronic Carriers of *Salmonella typhi* in Santiago, Chile, an Endemic Area

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As part of a program to control endemic typhoid fever in Santiago, Chile, an assessment was made of the magnitude of the reservoir of chronic carriers of *Salmonella typhi*. The availability of an accurate census and reliable data on the prevalence of biliary disease and of *S. typhi* carriage among persons with cholecystitis allowed an unusually precise estimate of the number of carriers. In 1980 there existed 25,019 female and 4,575 male carriers in a population of 4,264,514, yielding a crude prevalence of 694 carriers per 10^5 population. Because of the magnitude of this human reservoir, which includes many females of <40 years of age, it is recommended that a typhoid control program include the identification of carriers followed by health education and therapeutic interventions.

The human population is the reservoir as well as the natural host for *Salmonella typhi*. In general, ~2%–5% of all individuals who develop clinical or subclinical infection with *S. typhi* become chronic gallbladder carriers and thereby serve to maintain endemicity of the disease [1–6]. The propensity to become a chronic carrier after acute infection increases with age and is greater in women [1, 2, 5, 7], observations which are in keeping with the epidemiology of cholelithiasis [8–11].

Typhoid fever is highly endemic in Santiago, Chile, despite the widespread availability of potable water, the sewered sanitation, and the effective control of most other communicable diseases [12, 13]. Chile also has one of the highest prevalences of cholelithiasis in the world [10, 11, 14]. This combination of a high incidence of typhoid fever and a high prevalence of gallbladder disease probably results in a high prevalence of chronic carriers. Continued contamination of vehicles of transmission by these carriers maintains the endemic cycle and interferes with effective control of typhoid fever.

As part of a program to control endemic typhoid fever in Santiago, we estimated the number of chronic *S. typhi* carriers. The availability in Santiago of a reliable census, coupled with a large necropsy survey of the prevalence of cholelithiasis and quantitative data on the frequency of *S. typhi* carriage among persons with cholecystic disease, provided an opportunity to assess the magnitude of the human reservoir of infection with a precision heretofore not possible.

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Materials and Methods

The sizes of the male and female populations of Santiago were obtained from official census data [15]. The prevalence of persons with gallbladder disease in each decennial age group over 10 years of age was obtained from 1,967 autopsies performed at the Medico-Legal Institute, Santiago [10]; in the vast majority of instances, these autopsies were performed on persons who died as a result of motor vehicle accidents or other trauma [10]. The percentage of persons in each age group who had gallbladder disease was multiplied by the number of persons of that age in the general population to estimate the number with gallbladder disease.

The prevalence of chronic infection of the gallbladder with *S. typhi* among persons with biliary disease in 1980 is known from a recent study of persons undergoing cholecystectomy in seven ma-

Table 1. Estimate of the number of chronic carriers of *Salmonella typhi* in Santiago, Chile, in 1980 based on the prevalence of gallbladder disease in the population and the prevalence of chronic infection with *S. typhi* in persons with cholelithiasis.

Characteristic	Age group (years)								
	10–19	20–29	30–39	40–49	50–59	60–69	70–79	≥80	Total
Female	443,408	407, 190	323,987	221,209	169,221	113,581	59,238	21,714	1,759,548
Cholelithiasis (%)*	9.7	23.4	43.1	51.7	60.0	69.2	69.2	55.5	. . .
Cholelithiasis†	43,010	95,282	139,638	114,365	101,533	78,598	40,993	12,051	620,675
S. typhi carrier‡	1,720	3,811	5,586	4,575	4,061	3,144	1,640	482	25,019
Carriers per 10^5 population	388	940	1,724	2,068	2,400	2,768	2,768	2,220	. . .
Male	438,665	373,895	294,595	193,984	139,772	82,883	39,221	9,536	1,572,551
Cholelithiasis (%)*	0	4.5	13.4	16.7	19.8	24.7	43.5	40.0	. . .
Cholelithiasis†	0	16,825	39,476	32,395	27,675	20,472	17,061	3,814	157,718
S. typhi carrier‡	0	488	1,145	939	803	594	495	111	4,575
Carriers per 10^5 population	0	131	389	484	575	717	1,262	1,164	. . .

NOTE. Data are no. of persons except where percentages are indicated.

* Percentages based on 1,967 persons studied at autopsy at the Medico-Legal Institute, Santiago.

† Estimate computed by multiplying the no. of persons in each age group of the general population by the percentage of persons in that age group who were found to have cholelithiasis at autopsy.

‡ Estimate based on the observation that 4% of females and 2.9% of males with gallbladder disease in Santiago have chronic *S. typhi* bile infection [16].

jor hospitals in Santiago [16]. Cultures of bile and gallbladder were made at the time of surgery from 1,000 consecutive patients of all ages. The mean prevalence of *S. typhi* carriage was 4.0% among the 796 female patients, and there was little variation by age; the mean prevalence of *S. typhi* among the 204 male patients was 2.9%. The mean prevalence of *S. typhi* infection for each sex was multiplied by the calculated number of persons with gallbladder disease within each age group to derive the number of chronic carriers.

Results

A summary of the population of Santiago in 1980 by age and sex, the number of persons with gallbladder disease, and the calculated number of *S. typhi* carriers is shown in table 1. In total, 25,019 female and 4,575 male chronic carriers of *S. typhi* over 10 years of age were calculated to exist among the population of 4,264,514 in greater Santiago; the overall prevalence was 694 carriers per 10^5 population. The prevalence of chronic carriers increased with age; among women over 40 years of age, 2.1%–2.8% (that is, 2,068–2,768 per 10^5 women) were computed to be chronic *S. typhi* carriers.

Discussion

Typhoid fever is highly endemic in Chile, (the annual incidence since 1975 has ranged from 59 to 121 cases per 10^5 population), particularly in Santiago where peak incidence rates occur in older schoolchildren and young adults. This has been somewhat enigmatic to epidemiologists, since based on other demographic, socioeconomic, and health indicators, Chile is a fairly developed country. The relationship between biliary disease and chronic *S. typhi* carriage has been recognized for many decades [1–7]. Chile also has one of the highest prevalences of gallbladder disease in the world [10, 11, 14], and gallbladder disease appears among young female Chileans [10, 11]. We therefore surmised that there must exist a particularly high prevalence of chronic carriers in Santiago who serve as reservoirs and disseminators of *S. typhi* and who help maintain a high level of endemicity of typhoid fever.

The present report provides the most precise estimation ever made of the number and prevalence of chronic carriers of *S. typhi* in an endemic area. This precision was possible because of the existence of accurate data providing the age-specific prevalence of cholelithiasis [10, 11] and the frequency of chronic biliary infection with *S. typhi*

among persons with gallbladder disease [16]. The few previous attempts at estimating the number of chronic carriers of *S. typhi* in other geographic areas were rough estimates which, with one exception, did not take into account the relationship between the age of the patient at the time of acute infection and the development of the chronic carrier state [7, 17, 18].

The large number of chronic carriers of *S. typhi* (29,594 persons) and the high crude prevalence rate (694 carriers per 10^5 population) calculated for Santiago demonstrate the notable magnitude of the human reservoir of *S. typhi*. Furthermore, the existence of many carriers younger than 30 years of age implies that a significant reservoir will be present for many decades to come.

The outstanding efficacy of Ty 21a attenuated *S. typhi* oral vaccine in preventing acute typhoid fever in Alexandria, Egypt, has generated considerable hope that mass application of this vaccine in endemic areas may greatly diminish the incidence of typhoid fever and can serve as the keystone of typhoid fever control programs [19]. Nevertheless, the identification of chronic *S. typhi* carriers followed by health education, counseling, and treatment should also be considered critical components of a typhoid fever control program. In this context it would be particularly beneficial to identify young carriers (<40 years of age) who will play a role for many decades in disseminating *S. typhi*. Simple serologic [20] and bacteriologic [21] methods have become available to screen for *S. typhi* biliary carriers. Similarly, preliminary experience suggests that there now exists an effective, nonsurgical, domiciliary therapy to eradicate chronic *S. typhi* gallbladder infection [22]; the therapy involves a 28-day course of oral amoxicillin and probenecid [22] (C.L., unpublished observations). Identification, supervision, and treatment of chronic carriers should be part of a typhoid fever control program.

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Appendix B

Detection of urinary Vi antigen as a diagnostic test for typhoid Fever

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ABSTRACT

Since Vi antigen is nearly unique to Salmonella typhi, it has been thought that detection of the antigen may be a useful method to diagnose acute typhoid. The slide coagglutination and enzyme linked immunosorbent methods (ELISA) have recently been suggested as ways to detect small quantities of Vi antigen in urine. We compared the results of these 2 methods in Santiago, Chile among patients with acute typhoid, paratyphoid, other febrile illnesses and afebrile control subjects. Using a cutoff value that maximally separated typhoid patients from controls, the ELISA method was positive in 62.4% of 141 culture-proven cases of typhoid when 13.2% of 159 afebrile control subjects were positive, but the ELISA test was falsely positive in 64.7% of patients with culture-proven paratyphoid A or B and 47.1% of 21 patients with other non-typhoidal febrile illnesses. The coagglutination test was positive in 34% of typhoid patients, 14% of afebrile control subjects, and 46% of febrile control subjects. We conclude that these tests when performed with the currently available Vi antibodies are of little value for the diagnosis of typhoid.

INTRODUCTION

Microbiologic methods for the diagnosis of typhoid are frequently not available in rural areas of developing countries where typhoid is a major problem. When microbiologic methods are available, identification of S. typhi from blood or stool often takes several days and can be falsely negative if antimicrobials have been previously administered. Currently used immunologic methods to diagnose typhoid are of limited value. The most widely used immunologic assay is the Widal test for serum antibodies to O and H antigens. In areas of the world where typhoid is endemic the test has been found to be sensitive but non-specific. For example, Levine et al. have demonstrated O antibodies to be elevated in 90% of typhoid patients in Mexico, but O and H antibodies were elevated in 30 and 75%, respectively, of healthy Peruvians (4). Antibodies to the capsular Vi antigen of S. typhi are more specific for typhoid but appear too late in the course of illness to affect decisions on therapy (8).

Attempts to improve immunologic methods have focused on Vi antigen detection since this antigen is nearly unique to S. typhi and would presumably be present early in the course of infection. Rockhill et al. have reported a slide coagglutination test to detect soluble S. typhi Vi, D, and d antigens in the urine of Indonesian patients with typhoid. In a group of 61 culture confirmed patients and 46 well control subjects the test was 97% sensitive and 83% specific (7). To improve specificity Barrett et al. used an enzyme-linked immunosorbent assay (ELISA) to detect urinary Vi antigen (1). ELISA provided

an increase in both sensitivity and specificity over the slide coaglutination method in laboratory tests with pure antigen and in a limited field trial performed in our laboratory.

Typhoid is endemic in many areas of South America. In Santiago, Chile, the annual incidence of typhoid is 90 per 100,000 inhabitants. Over 50% of the cases occur during the summer months (December-March) and approximately 65% of the cases occur among persons 5-19 years of age (6). We compared the diagnostic capabilities of the coagglutination and ELISA tests in Santiago because of the particularly high incidence of typhoid, the availability of a well-equipped laboratory, and the current interest in typhoid there in typhoid stimulated by an ongoing oral typhoid vaccine trial.

METHODS

Case selection: Blood, stool, and occasionally marrow cultures are obtained from patients seen at a large municipal pediatric hospital (Roberto del Rio Hospital) or at an adult infectious diseases hospital (Infectious Diseases Hospital) in Santiago, Chile when the diagnosis of typhoid or paratyphoid fever is considered. We (DNT or JRH) collected a urine specimen (15 ml) from patients with suspect typhoid seen at these 2 hospitals during January and February 1982. Patients with suspected typhoid were interviewed and their hospital records reviewed to ascertain age, sex, hospital, date of admission, date of onset of fever, typhoid history, typhoid vaccination history, symptoms, physical findings, and antimicrobial treatment. A case of typhoid or paratyphoid was defined as the isolation of S. typhi or paratyphi from blood, stool, or bone marrow from a patient clinically suspected of having acute typhoid.

Control subject selection: Afebrile patients admitted to surgical wards of Roberto del Rio hospital or to a general medical hospital (Trudeau Hospital) adjacent to the Infectious Diseases Hospital, during January and February 1982 served as afebrile control subjects. These patients were usually admitted for elective surgery and had no history of recent infections. The reason for admission, history of previous typhoid, and typhoid vaccination history were recorded. Patients who were admitted to the pediatric or the adult infectious diseases hospital who had a clearly diagnosed, non-typhoidal febrile illness served as febrile control subjects.

Specimen handling: The urine specimens were collected from all inpatients during the first 2 days after admission and from outpatients at the time blood cultures were obtained. The urine was collected in 15 ml plastic tubes, refrigerated at 5 C overnight, and tested for the presence of Vi antigen the next day.

Microbiologic methods: Five ml of whole blood were inoculated into 50 ml of brain heart infusion broth at the pediatric hospital and 2 ml of whole blood were inoculated into 15 ml of trypticase-salt-glucose broth at the infectious diseases hospital. Stool specimens were inoculated onto a solid agar medium (S-S or MacConkey). S. typhi and paratyphi were identified according to standard methods (2).

Immunologic methods: Serum specimens obtained at admission from patients with febrile illnesses were processed at each hospital for O and H antigens (Widal test) using standard methods. Vi antibody titers were determined in 24 paired acute and convalescent sera from typhoid patients obtained during the course of the study by an indirect hemagglutination method (5).

The slide coagglutination test was done by the method of Rockhill et al (7), with Cowan I Staphlococcus aureus cells coated with anti-Citrobacter Vi rabbit sera (1). Control cells were coated with normal rabbit antiserum that did not cross react with Salmonella group or Vi antigens. Urine specimens were tested with anti-Vi coated and control staphylococci on the same slide. Agglutination was read after 1 minute of slide agitation. A positive test was defined as agglutination with the anti-Vi coated cells but not with the control cells. A negative test was defined as no agglutination with either anti-Vi coated or control sera coated cells. Any other combination was defined as uninterpretable.

For the ELISA we used the inner 60 wells of 96 well polyvinyl microtiter plates (Dynatech Laboratories, Inc., Alexandria, VA), and ran each specimen in duplicate. One-half of the wells were coated with burro anti-Vi sera and one-half were coated with normal horse serum (which cross-reacts with burro serum) as described previously (1). The absorbance of the contents of each well was read 30 minutes after the addition of phosphatase substrate (Sigma 104) at 410 nm using a through the plate photometer (Dynatech Laboratories, Inc.). The ELISA value was calculated as the ratio of the average absorbance in the Vi coated wells to the average absorbance in the control serum wells, rounded to the nearest tenth. The test was standardized using serial dilutions (100 nanograms to 100 picograms) of a highly purified Citrobacter Vi antigen preparation.

Statistical methods: Discriminant analysis was performed to determine the ELISA value that maximally separates the typhoid cases from the afebrile controls. A classification function was then generated that determined the number of cases and controls that could be correctly categorized. Multiple

regression analysis was used to examine the relationship between the ELISA scores of the typhoid cases and the afebrile controls controlling for age group, hospital, prior history of typhoid, or typhoid vaccination. Linear logistic regression was performed to investigate the relationship between the coagglutination test result and case status controlling for age group, hospital, prior history of typhoid, or typhoid vaccination.

RESULTS

There were 188 culture proven cases of typhoid and 43 cases of paratyphoid (table 1). S. typhi was isolated from blood in 81%, from stool alone in 16%, and from marrow alone in 3% of cases. S. typhi was isolated from 42% of persons suspected of having typhoid. Sixty-three percent of typhoid cases occurred in males and 72% of cases occurred in patients aged 10-29 years (table 2). The mean age of typhoid or paratyphoid case patients was significantly lower than the mean age of afebrile controls ($p<0.001$, t-test).

S. paratyphi-B was isolated from 32 of the 43 paratyphoid cases. S. paratyphi-B was isolated from the blood in 20 (63%) patients and from the stool alone in 12 (37%) patients. S. paratyphi-A accounted for the remaining 11 cases. In 10 patients the organism was isolated from the blood and in 1 it was isolated from the stool. Sixty-seven percent of paratyphoid cases occurred in males and 80% occurred in persons 10-29 years old (table 2).

In Chile a 100 picograms/ml preparation of Citrobacter Vi antigen gave a mean ELISA value of 2.0, 1 ng/ml gave a mean value of 6.7, and 10 ng/ml gave a near maximum mean value of 8.3. The test results for cases and controls appear in table 3. Cases had significantly higher ELISA scores than did

controls ($p<0.0001$, t-test). The discriminant analysis using ELISA scores to predict typhoid cases and afebrile controls yielded an F statistic that was significant [$F(1, 298) = 49.8$, $p<0.0001$]. In the classification function, a person with an ELISA score of 1.9 or below (rounded to the nearest tenth) is classified as an afebrile control and one with a score of 2.0 or greater is classified as a typhoid case. This function correctly classified 62.4% of the cases and 86.8% of the afebrile controls. The effect of changing the cut-off point for a positive ELISA value on the true and false positivity rate is shown in figure 1.

Multiple regression analysis investigating the relationship between ELISA and case status, age, hospital, prior history of typhoid, and typhoid vaccination yielded a significant F statistic [$F(5, 164) = 5.28$, $p<0.01$]. Controlling for the above variables, cases had significantly higher ELISA scores than did controls [$F(1, 168) = 7.99$, $p<0.01$].

Slide coagglutination could detect 100 ng of the Citrobacter Vi antigen standard. The slide coagglutination test was positive in 34% of typhoid, 48% of paratyphoid, 46% of febrile control-subjects, and 14% of afebrile control-patients, but frequently the test result was uninterpretable, largely because of agglutination in control cells (table 3). Typhoid cases were more likely than controls to have a positive coagglutination test when controlling for previous history of typhoid, hospital, and age ($p<0.05$, linear logistic regression). A weak correlation between the ELISA and coagglutination test scores of typhoid cases and afebrile controls was found ($r=0.23$, $p<0.01$).

Seventy-seven percent of typhoid cases and 44% of paratyphoid cases had an O antibody titer of $\geq$1:40 or an H antibody titer of $\geq$1:80. Titers were not obtained from afebrile control subjects. Antibodies to Vi antigen were

detected in the convalescent sera of 11 (46%) of 24 patients with culture confirmed typhoid. The mean Vi antibody titer was 1:80 (range 1:10-1:320). In all 24 sera tested, H and O antibody titers were elevated, and ELISA and coagglutination test results did not differ by the presence of Vi antibody. S. typhi isolated from patients in Chile all agglutinated in Vi specific antisera and S. paratyphi-A and B isolates did not.

DISCUSSION

Based on their ability to detect purified Citrobacter Vi antigen, we found that the coagglutination and ELISA tests were equally sensitive in Chile and in our laboratory in the U.S. However, in Chile detection of Vi antigen in urine by either test was not a useful way to diagnose acute typhoid because of low sensitivity and specificity and a large number of uninterpretable test results. The ELISA, which was 100 times more sensitive than the coagglutination test in detecting the Vi antigen standard, was moderately useful in distinguishing typhoid cases from afebrile controls (specificity 87%, sensitivity 62%), but was not able to distinguish typhoid cases from cases of paratyphoid or febrile controls.

The high percentage of paratyphoid illnesses with positive ELISA values was unexpected since S. paratyphoid-A and B do not possess Vi antigen; and suggests that our Vi antibody reagents contain antibodies which cross-react with paratyphoid organisms. S. typhi, paratyphi-A and paratyphi-B share the O-12 antigen, but the Citrobacter freundii 5396/38, from which the Vi antigen used to produce the rabbit antisera is obtained, does not, and the Citrobacter Vi antiserum does not agglutinate S. paratyphi-A or B organisms. However, the

ELISA and coagglutination test are considerably more sensitive than slide agglutination and might detect cross-reactions that are not detectable in the agglutination of whole organisms.

Both tests were difficult to interpret because of positive reactions in normal serum coated cells and wells, which frequently occurred in the presence of highly positive samples. This and positive reactions in febrile controls suggests that there is something in febrile patients that is cross-reacting in these test systems. Rockhill noted a similar phenomenon and suggested that urinary leucocytes could cause non-specific agglutination which could be eliminated by centrifugation of the urine specimens before testing. We were unable to improve our results by this procedure.

In Chile, Vi antibodies were detected in less than half of the convalescent sera tested. Since Vi antigen was present in almost all strains of S. typhi isolated in Chile, perhaps there were qualitative or quantitative differences in the Vi antigen produced by the typhoid strains in Chile that might explain the lower sensitivity of the coagglutination test in Chile than in Indonesia. It is still not clear that Vi antigens from Citrobacter or other Vi-positive organisms are immunologically identical with Vi antigen(s) from S. typhi (3), nor is it clear how Vi might be metabolized by the kidney before it appears in the urine. The non-specificity of the currently available Vi antibodies and our poor understanding of Vi antigens suggest that there is a need for more specific, possibly monoclonal, antibodies directed to S. typhi Vi and possibly at metabolites of Vi. Our results suggest that Vi antigen tests are worth refining because test results, although non-specific, did not appear to be affected by factors which have made other tests difficult to interpret such as prior history of typhoid infection or vaccination.

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Table 1

Patients with typhoid or paratyphoid and control subjects, by hospital, Santiago, Chile, January-February 1982

	Culture proven		Febrile Controls	Afebrile Controls
	Typhoid	Paratyphoid		
Pediatric				
inpatient	28	3	4	41
outpatient	12	7	0	22
Adult inpatient	148	33	29	98
Total	188	43	33	161

Table 2
Sex and age distribution of patients and control subjects
Santiago, Chile, January-February 1982

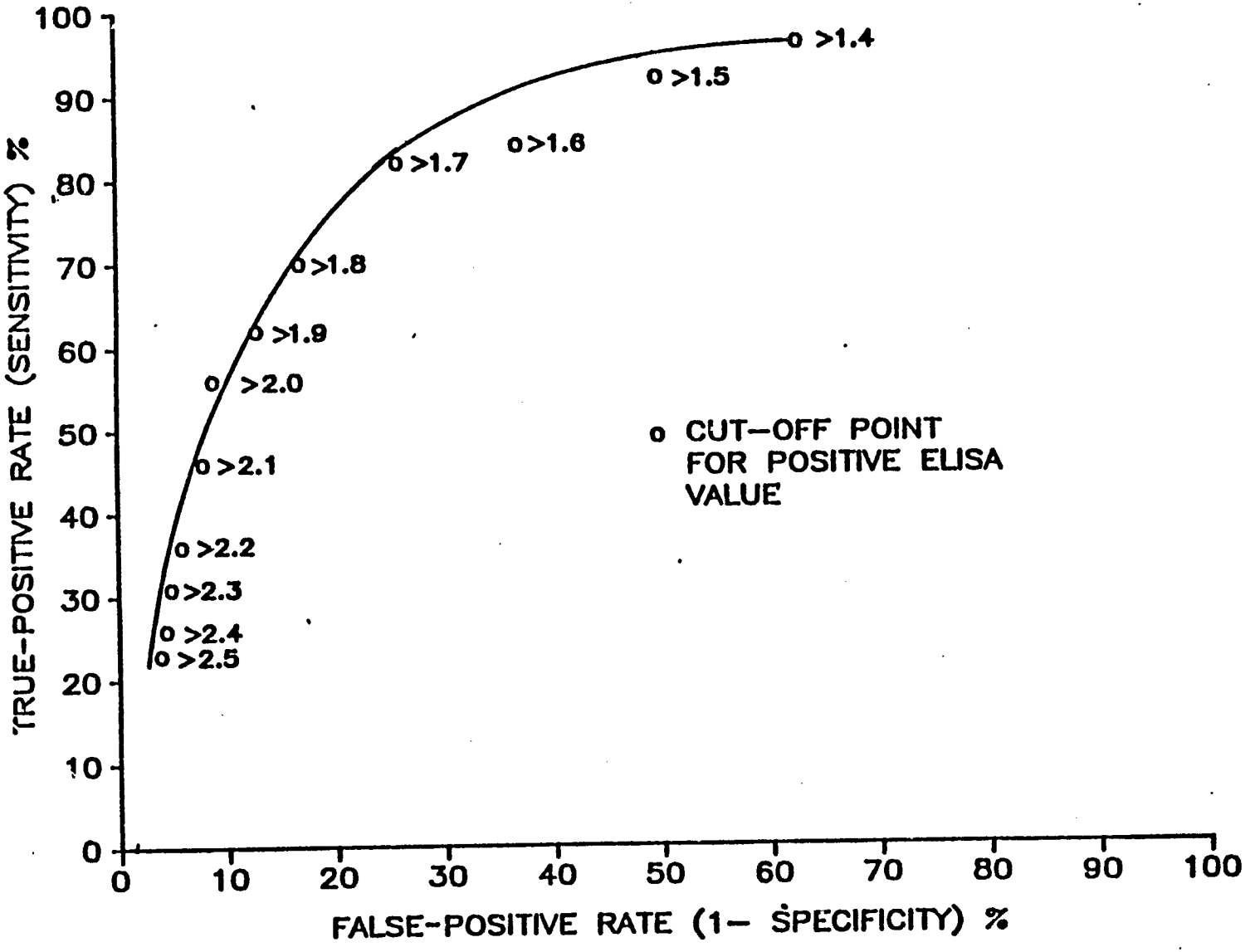
	Culture proven		Febrile Controls	Afebrile Controls
	Typhoid N=188	Paratyphoid N=43	N=33	N=161
	%	%	%	%
Males	63	67	50	55
Age group (yrs)				
0-4	3	2	6	2
5-9	6	12	9	18
10-19	38	39	34	24
20-29	34	41	19	5
30-39	12	2	13	12
40-49	7	2	7	11
50-59	1	0	9	10
≥60	0	0	3	18
age unknown	8	2	12	1
Mean age	21.6	18.7	25.4	32.7

Table 3
A comparison of 3 diagnostic tests for acute typhoid
Santiago, Chile, January-February 1982

	Culture proven		Febrile	Afebrile
	Typhoid	Paratyphoid	Controls	Controls
	N=141	N=34	N=21	N=159
ELISA value				
mean	2.34	2.09	2.08	1.65
SD	1.05	0.47	0.52	0.62
95% range:				
low	1.50	1.28	1.50	1.20
high	4.37	2.90	3.35	2.40
value >1.9*	62.4%	64.7%	47.6%	13.2%
COAGGLUTINATION				
	N=145	N=33	N=24	N=158
Positive	34%	48%	46%	14%
Negative	45%	33%	33%	45%
uninterpret.	21%	18%	21%	41%
WIDAL				
Reciprocal Ab titer				
	N=174	N=43	N=4	0
O≥40	78%	52%	25%	nt
O≥80	72%	33%	0%	nt
O≥160	35%	12%	0%	nt
H≥40	78%	55%	25%	nt
H≥80	72%	33%	0%	nt
H≥160	35%	9%	0%	nt
O≥40 or H≥80	77%	44%	25%	nt
O≥160 or H≥160	41%	12%	0%	nt

* values rounded to nearest tenth
nt=not tested

URINARY Vi DETECTION BY ELISA AT VARIOUS CUT-OFF POINTS, SANTIAGO, CHILE, JANUARY-FEBRUARY 1982



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FIEBRE TIFOIDEA

Appendix C

CODIGO Nº _____

CONSULTORIO _____

Nº FICHA _____

NOMBRE: _____

FECHA NAC._____ EDAD _____ SEXO: F [ ] M [ ]

VACUNA: SI [ ] NO [ ]

REFERIDO POR: EPIDEMIOLOGIA [ ]; LABORATORIO [ ]; MEDICO PRIVADO [ ]

DIAGNOSTICO: CONFIRMADO [ ] SOSPECHOSO [ ]

HOSPITALIZACION : SI [ ] NO [ ]

HOSPITAL : _____ Nº OBSERVACION _____

RESULTADO EXAMENES

TIPO		FECHA	RESULTADO
MIELOCULTIVO		_____	_____
		_____	_____
HEMOCULTIVO		_____	_____
		_____	_____
		_____	_____
COPROCULTIVO		_____	_____
		_____	_____
AGLUTINACION :	E.O.	_____	_____
	E.H.	_____	_____
	E.O.	_____	_____
	E.H.	_____	_____
OTROS		_____	_____

OBSERVACIONES: _____

Nº FICHA _____

Appendix C

INVESTIGACION VACUNACION ANTTITIFICA ORAL (*)

I. NOMBRE: _____ EDAD _____ FECHA NAC. _____

II. DIAGNOSTICO PRESUNTIVO:

☐ FIEBRE TIFOIDEA

III. ANTECEDENTES PREVIOS AL EXAMEN:

FIEBRE SI ☐ NO ☐ DURACION (**) _____

CONSTIPACIÓN SI ☐ NO ☐

DIARREA SI ☐ NO ☐

DOLOR ABDOMINAL SI ☐ NO ☐

CEFALEA SI ☐ NO ☐

IV. SINTOMAS AL EXAMEN

TEMPERATURA SI ☐ C° _____ NO ☐

HEPATOMEGALIA SI ☐ NO ☐

ESPLENOMEGALIA SI ☐ NO ☐

METEORISMO SI ☐ NO ☐

ROSEOLAS SI ☐ NO ☐

V. OBSERVACIONES: _____

_____

_____

VI. EXAMENES DE LABORATORIO: _____

- HEMOCULTIVO: FECHA DE TOMA DE MUESTRA _____ RESULTADO _____

- OTROS _____

VII. DIAGNOSTICO CLINICO _____

_____

NOMBRE DEL MEDICO _____
(optativo)

(**) Hasta la fecha de esta consulta.