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STUDIES TO CONTROL ENDEMIC TYPHOID FEVER IN CHILE

ANNUAL REPORT

Contract Period 7/15/83 to 7/14/84

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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) epidemiologic study to identify risk factors and incriminate specific vehicles involved in transmission of S. typhi; 2) environmental bacteriology studies; 3) simple serologic screening test to identify chronic S. typhi carriers; 4) a simple, practical, non-surgical treatment for chronic S. typhi carriers; and 5) two large-scale trials of Ty21a attenuated S. typhi vaccine.

SUMMARY

A multi-faceted program of applied research has been undertaken intended to lead to control of endemic typhoid fever in Santiago, Chile. Information derived from these studies will be directly applicable to the control of typhoid fever in United States military personnel deployed in endemic areas. Results of studies in the major components of the program accomplished during the past contract year are summmarized below.

1) Epidemiologic Investigations

Family-based studies -

We sought to further examine factors involved in the transmission of Salmonella typhi by interviewing and culturing the household contacts of recently confirmed pediatric index cases of typhoid fever. Within the households we attempted to identify chronic typhoid carriers as well as possible concurrent cases by culturing all household members. We also questioned patients and households members about food preferences for high-risk foods (such as raw vegetables) and about consumption away from the household.

Families of 24 patients (161 contacts) were studies. Households averaged 2.4 persons per room; 19 households (79%) were connected to the sewer system, 23 (96%) had municipal water and 100% had electricity.

A chronic S. typhi carrier was identified in only one household. Apparent concurrent or secondary cases were identified in only two households, despite the large number of presumed susceptibles below 15 years of age. Eighty-six percent of the index cases ate outside the home at least once each week. These

observations suggest that the index case in most instances ingested the vehicle of transmission outside the household.

Typhoid in Infants

The paucity of notifications of typhoid fever in children below three years of age could represent an important clue in understanding the transmission of S. typhi in Santiago, implying that young children do not ingest the common vehicles of transmission consumed by older children. An alternative hypothesis is that the infants do become infected with S. typhi but that the infant host does not manifest the clinical syndrome of typhoid fever as recognized in the older child.

In order to help resolve this question we systematically performed blood cultures in 197 consecutive children less than two years of age with fever seen at two health centers in the northern administrative area (Area Norte) of Santiago during the three peak months of typhoid fever season.

Ninety-three percent of the fevers were between 38° and 39°C. Acute respiratory infections (44%), diarrhea and viral syndrome (13%) were the most common clinical diagnoses at the examination. None of the infants was severely ill and none would have had blood cultures obtained were it not for the study.

S. typhi was isolated from blood cultures of four children (2%), S. paratyphi B from two (1%) and S. paratyphi A from one (0.5%). During the same three month period, two other infants from the population served by these two health centers were admitted directly to hospital with severe typhoid fever. Thus it appears that at least two mild, unrecognized bacteremic infections occur in the community for each clinically overt recognnized case.

These data support the concept that infants become infected at a somewhat higher rate than is commonly appreciated and manifest a very mild illness not recognized as typhoid fever, albeit accompanied by bacteremia.

2) Environmental Bacteriology Studies

Our epidemiologic studies incriminated the use of untreated sewage in the irrigation of vegetables grown on the outskirts of Santiago as the primary factor responsible for transmission of S. typhi. Prior to May, 1983, 90% of the crops grown in this region were lettuce, cabbage and celery, vegetables that are difficult to wash and are eaten raw in salads in Santiago.

Despite the failure of earlier investigators to isolate S. typhi from these polluted irrigation waters, we initiated new environmental bactgeriologic studies using Moore swabs (thick wads of cotton gauze which are left in the flowing wastewater for 2-3 days allowing the gauze to act as a filter) to collect samples. Bacteriologic culturing of the Moore swabs followed earlier techniques.

Using this important modification S. typhi was repeatedly isolated from the Zanjon de la Aguada sewage canal, the Mapocho river (into which sewage drains) and from the irrigation ditches providing wastewater for cultivation of vegetables. These data prove that viable S. typhi are indeed in the waters irrigating vegetables grown in western Santiago and support the concept that this practice is largely responsible for maintaining the endemicity of typhoid fever in Santiago with its special epidemiologic features.

3) Studies with Chronic typhoid Carriers

Simple Serologic Screening Test for Carriers -

Simple, yet reliable screening tests are required to allow rapid and effective identification of chronic carriers. We evaluated a passive hemagglutination test that measures antibodies to highly purified Vi polysaccharide antigen of S. typhi as a screening test to detect carriers in Santiago, an endemic area.

A reciprocal Vi antibody titer of ≥160 was found to have a sensitivity of 75%, a specificity of >92% and a high predictive value in screening for chronic S. typhi carriers in high-risk population groups (e.g. women over 40 years in Santiago). This serologic technique is presently being uitilized in Santiago to screen foodhandlers for the presence of chronic S. typhi carriers.

A Practical Non-Surgical Treatment for Chronic Carriers

Amoxicillin is an analogue of ampicillin that is extremely well absorbed following oral dosage and is concentrated in the bile, characteristics that make it theoretically ideal for treatment of carriers. We undertook an evaluation of oral amoxicillin (2.0 gm three times daily for 28 days) plus probenecid (0.5 gm three times daily for 28 days) in the treatment of 28 chronic carriers (27 females) in Santiago, Chile. Twenty-six (93%) were able to complete the course of therapy, while two had allergic reactions shortly after onset of treatment and were removed from the study.

This oral treatment regimen successfully cured the carrier state in 15 of 26 persons (58%) who completed the course of therapy. Most of these 15 persons have been followed for at least one year and have remained culture negative. The treatment failures typically began to excrete S. typhi in stool cultures by the second month after cessation of therapy. Thirteen of the 26

carriers have had radiological evaluation of their gall bladder function; cholelithiasis, failure of the gall bladder to fill during cholecystogram, or other pathology was present in 13 of 13 carriers examined.

4) Large Scale Field Trials of Ty21a Live Oral Typhoid Vaccine

Area Norte Trial

In May and June 1982, 27,485 schoolchildren in Area Norte, Santiago, were given two doses of Ty21a vaccine in enteric-coated capsules, 32,707 got one dose of vaccine and 31,762 received placebo. Over 18 months of intensive epidemiologic surveillance, overall vaccine efficacy was 55% with two doses of vaccine but only 19% with one dose. However, the surveillance reveals three distinct periods: the first nine months, during which time two doses gave highly significant protection (67% efficacy); months 10-12, during which time no efficacy was evident; months 13-18, during which time significant protection was again encountered (86% efficacy). We hypothesize that the circulating vehicles of transmission in months 10-12 contained high inocula of S. typhi that overcome the protective effect of the vaccine. Surveillance in Area Norte is being continued.

Area Occidente Trial

A second more comprehensive field trial is underway in Area Occidente involving 150,000 schoolchildren. In this field trial children were given three doses of vaccine in one of two formulations and by one of two immunization schedules. Eighty percent of the participating children received vaccine, 20% got placebo. Vaccination took place between July and September, 1983 and surveillance is on-going.

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 reservior 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 asymptomatic chronic S. typhi.

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 resemble those of an industrialized society and would lead one to expect little typhoid fever or other enteric infections. The literacy rate in Chile exceeds 96%; the 1981 infant mortality rate was 27 per 1000 live births; measles and pertussis have been greatly reduced by immunization, and poliomyelitis has been eradicated (Table 1). Yet the incidence of typhoid fever in Chile since 1977 has exceeded 100 cases per 10^5 population. Approximately one half of all cases and the highest incidences rates occur in the capital, Santiago. The interesting 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 patterr.

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. Since 1980 the Center for Vaccine Development of the University of Maryland School of Medicine has been involved in a formal collaboration with the Ministry of Health of Chile in the project "Studies to Control Endemic Typhoid Fever in Chile." The project comprises several distinct yet mutuallly-related components involving investigations of various aspects of Salmonella typhi infection, particularly with respect to epidemiology and control.

Specific projects carried out during the past contract year include:

1.) Further epidemiologic studies (index case/family contact studies) to investigate the modes of transmission of S. typhi in Santiago, Chile.

2.) Environmental bacteriology studies of epidemiologically incriminated waters and agricultural products.

3.) Evaluation of a simple serologic screening test (Vi antibody) to assess its sensitivity, specificity and predictive value in detecting chronic S. typhi carriers in a highly endemic area, Santiago.

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

5.) Two large-scale field trials evaluating the efficacy of different formulations and immunization schedules of the promising new oral attenuated S. typhi vaccine, Ty21a.

6.) Studies of cell-mediated and humoral immune responses to oral

Ty21a vaccine to identify simple in vitro correlates of vaccine efficacy.

7.) Clinical bacteriologic studies of S. typhi.

The ultimate goal of this program is to drastically reduce the incidence of typhoid fever in Santiago to the point where it is no longer a major public health problem and can be regarded as controlled. An important benefit from this project will be the ability to apply results of the carefully measured interventions toward control of typhoid in other populations such as persons in other endemic areas, travelers and military personnel.

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

II. DESCRIPTIVE EPIDEMIOLOGY

Some background information will allow a fuller appreciation of the epidemiologic pattern of typhoid fever in Chile. Chile is a long, narrow country on the western side of the southern cone of South America, stretching more than 3000 miles from north to south. Approximately 4.5 million of Chile's 11.8 million inhabitants live in the capital, metropolitan Santiago, which is located in the center of the country in a valley between the Andes mountains and the Pacific Ocean. Santiago has a temperate Mediterranean climate with wet winters and rainless summers.

Table 2 lists the population of Santiago and all of Chile, numbers of cases typhoid fever and incidence rates for the years 1960-1981. One notes that approximately one-half of the cases of typhoid fever in any year are reported from Santiago. In the period prior to 1970 and 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 veneral disease did not go up.

Table 3 shows the seasonal distribution of cases in Santiago. It is obvious that typhoid fever is a summer disease; approximately 65% of cases occur between December 1 April 30 of each year.

The increased incidence rates for typhoid fever that began in 1977 were evident throughout most of Chile for those regions where the disease is endemic (Table 4). It is also notable that the incidence of typhoid fever drops as one goes further and further north or south from Santiago. One area of particular interest is the Lakes Region (Los Lagos) where many persons from Santiago go for vacation in summer. The daytime temperatures in this region can be quite warm in summer but there is rainfall all year long. Typhoid is very uncommon here. In 1978 we carried seroepidemiologic studies that documented the high prevalence of S. typhi H antibodies in teenagers in Santiago and the very low prevalence in teenagers in Los Lagos.

Table 5A shows the incidence rates of typhoid fever by administrative region of Santiago. It is obvious that a high incidence is reported in the affluent as well as lower socioeconomic level areas of Santiago.

In summary, an explanation of the endemicity of typhoid in Santiago must explain:

1.) High incidence in school children.

2.) Summer seasonality with the highest incidence (December to mid-March) occurring when children are on vacation from school.

3.) An apparent low incidence in children less than two years of age.

4.) Transmission despite high levels of sanitation (96% of households have bacteriologically monitored municipal water and 75% are connected to the sewer system) (1,2).

5.) The incidence of typhoid fever is very low in Los Lagos region despite in influx into that region each summer of many persons from Santiago, including presumably many carriers.

III. EPIDEMIOLOGIC INVESTIGATIONS

In the 1981 contract year we carried out a case/control study in an attempt to identify specific vehicles of transmission as well as risk factors and protective factors. This study, which involved 81 cases age 3-14 years and 81 matched controls, incriminated only one possible vehicle, flavored ices sold by street vendors (3). Another aspect of this study involved the collection of multiple coprocultures from the foodhandlers in both case and control households. Results of this culture survey revealed a chronic S. typhi carrier in only 2 of 81 (2.5%) case and 1 of 81 (1.2%) control households. This represents the most important observation to come out of the case/control study because it was the first demonstration that chronic S. typhi carriers in the household are not responsible for most cases of typhoid fever in Santiago.

A. Family Studies

During the past contract year, we sought to further examine factors involved in the transmission of S. typhi by interviewing and culturing the households of recently confirmed pediatric index

cases of typhoid fever (2). Within the households we again attempted to identify chronic typhoid carriers as well as possible concurrent cases by culturing all household members. We also questioned patients and household members about food preferences for high-rish foods such as raw vegetables and about food consumption away from the household.

Families of 24 patients were studied; the mean age of the patients was 10 years. A total of 161 persons lived in the households, excluding the index cases, of whom 30 individuals were $\leq$10 years of age.

Households had an average of 2.4 persons per room. Nineteen (79%) of the households were connected to the city sewage system, all had electricity and 23 (96%) had municipal water. The geographic distribution of the cases of shown in Fig. 1.

A chronic S. typhi carrier was identified in only one household (i.e. 1 of 161 household members, 0.62%). Apparent concurrent or secondary cases were identified in only two households, despite the large number of presumed susceptibles below 15 yrs. of age. Eighty-six percent of index cases gave a history of eating outside the household at least once each week. However, no specific vehicle of infection was identified.

These results corroborate the 1981 case/control study in demonstrating that it is uncommon to find a chronic carrier in the household of a case of typhoid fever (only 1 of 24 households had a carrier). The observation that concomitant cases could be identified in only 2 of the remaining 23 households (9%) suggests that the index case does not usually ingest the vehicle of transmission in the home but rather outside the household;

otherwise more concomitant cases would be expected.

We also found a relatively high rate of carriage of non-typhoidal Samonella and Shigella, with 11% of household members, in 54% of the households studied, infected with at least one pathogen other than S. typhi. In the absence of data from control families it is difficult to know how accurately this reflects the carriage rate of such pathogens in the general population; within the study population, however, the rates are comparable to those seen in countries such as Bangladesh (4,5). No household in the study had more than one person culture-positive for the same species of either Salmonella or Shigella, suggesting, as with S. typhi, that these pathogens were infrequently transmitted within households; one might also have expected to see more cases among young children if household contacts were important in transmission. Shigella is typically spread by direct contact. However, the lack of multiple cases within one household and the presence of several distinct serotypes suggests that in Santiago Shigella are being transmitted in the same manner as S. typhi, presumably by food vehicles.

With the relatively small size of our study we were not able to identify any specific risk factors for transmission of S. typhi, or for non-typhoidal Salmonella or Shigella. There was, however, obviously a high level of exposure to bacterial enteric pathogens within the study population, with transmission and/or exposure apparently occurring outside of the immediate household; further studies of the epidemiology of endemic typhoid fever in Santiago will need to be focused on events of transmission outside the household.

B. S. typhi Infection in Infants

Few cases of typhoid fever are reported in children less than two years of age in Santiago (Table 6). This could represent an important clue in understanding the transmission of S. typhi. Thus, it was necessary to determine whether the very low reported incidence of typhoid fever in young children represents a lack of consumption of the vehicles that transmit S. typhi to older children or whether infection occurs but the infant host manifests an atypical response that is not readily recognized clinically. To help resolve this question we systematically performed blood cultures in children less than two years of age with fever who were seen at two health centers in Santiago during the three peak months of the typhoid fever season (5a).

Rectal temperatures were recorded for all children less than two years of age who were seen at Pincoya and Consultorio Dos health centers in the northern administrative area (Area Norte) of Santiago from January through March, 1983. From all children with a temperature $\geq$38°C, 2 ml of blood were drawn for culture and inoculated into a flash containing 35ml of brain heart infusion with 0.01% sodium polyanethol sulfonate. The reason for the blood culture was explained to the parents and verbal informed consent was obtained, according to local customs. The study was discontinued in the last week of March by which time 197 consecutive children had been cultured. Cultures were incubated at 35°C for seven days and suspicious colonies were confirmed as S. typhi by standard biochemical and serological techniques. S. typhi were phage-typed at the Institute of Public Health, Santiago. A standardized medical history and physical examination was recorded

for all infants. Infants with positive cultures were recalled, reexamined and given oral chloramphenicol (50 mg/kg/day).

Of the 197 children, 50 were less than six months (no newborns), 68 were 6-11 months, 57 were 12-17 months and 22 were 18-23 months of age; 93% of the fevers recorded were between 38° and 39°C. Acute respiratory infections (44%), diarrhea (20%) and viral syndrome (13%) were the most common clinical diagnoses at the time of examination. None of the infants appeared severely ill and in no instance was enteric fever considered in the differential diagnosis; consequently, were it not for the study protocol a blood culture would not have been taken from any infant.

S. typhi was isolated from four children (2%), S. paratyphi B from 2 (1%) and S. paratyphi A from 1 (0.5%); all other blood cultures were negative. Four isolations occurred in January, one in February and two in March. Two S. typhi strains were non-typable. However, the remaining two were phage-type E1 and 46, the two most common types in Santiago.

The clinical syndrome in these infants prior to examination was mild consisting of 1-5 days of fever between 38.3° and 38.8°C (table 7). Six of the seven infants, including all four with S. typhi, presented with cough (Table 2) and one had clinical and radiologic evidence of pneumonitis. None had splenomegaly but one had minimal hepatomegaly. Upon follow-up it was found that none of the infants had completed the course of oral chloramphenicol therapy; the mothers had spontaneously discontinued the medication after one or two days because the infants appeared well. Nevertheless, in each instance the infection resolved without complications.

Most information on the age distribution of typhoid fever stems from hospital-based studies (6-13). Three major points recur in these reports:

1.) S. typhi infection is notably less common in children below two years of age (usually <10% of the cases) 2.) the clinical syndrome is often distinct from that encountered in older children and commonly includes vomiting, diarrhea, convulsions and meningismus, and respiratory signs, in additions to fever 3.) most reports state that hospitalized infants with typhoid fever are quite ill and a bacteremic infectious process (sepsis, meningitis, etc) is usually suspected. Two main hypotheses have been put forth to explain the low reported incidence of typhoid fever in young children less than two years of age.

These include:

1.) That infants and young children do not ingest the vehicles of transmission of S. typhi which are consumed by older children.

2.) That infants and young children consume contaminated vehicles of transmission but do not readily develop recognizable clinical illness because of host factors peculiar to that age group.

If the latter is correct, and infants are becoming infected but are manifesting only mild illness, one would have to seek evidence of such infections by the systematic investigations of non-hospitalized, mildly ill infants. This pilot study in Santiago, an area endemic for typhoid fever, represents the first systematic attempt to decipher this problem. The isolation of S. typhi and S. paratyphi from blood cultures of 3.6% of 197 febrile but mildly ill infants seen at health centers during the summer months demonstrates that during the peak typhoid fever season

children less than two years of age are becoming infected at a much higher rate than previously appreciated. During the same three month period two infants from the registered population served by these two National Health Service community health centers were admitted directly to hospital with severe illnesses that were confirmed by blood culture to be typhoid fever. Thus, at least two mild, unrecognized, bacteremic S. typhi infections in young children may exist for every clinically overt, confirmed case.

In the pathogenesis of typhoid fever two bacteremias occur at distinct stages. The primary bacteremia appears within hours after ingestion of the pathogen (14). Upon reaching the small intestine the S. typhi rapidly pass through the mucosa to reach the lamina propria where they elicit a chemotactic response reslulting in an influx of macrophages. Primary access to the bloodstream occurs during mucosal invasion or after drainage to mesenteric lymph nodes and entrance into the blood by way of the thoracic duct. This primary bacteremia is short-lived and clinically inapparent. Viable S. typhi persist in the reticuloendothelial system after being cleared from the blood. Following an incubation of 10-14 days, and concomitant with the onset of clinical illness, the secondary bacteremia characteristic of typhoid fever occurs. It is not clear whether the enteric fever organisms in the blood of these infants represent the fortuitous detection of primary bacteremia or whether it denotes secondary bacteremia in infants with a particularly benign form of the disease. Earlier reports noted the mildness of pathological alterations due to S. typhi; in the intestine of infants (6-9,13) compared with older children as well as the frequency of respiratory signs and symptoms (6,10).

Ashcroft (15) pondered why some less-developed areas with appalling sanitation have little typhoid fever, while other somewhat more developed countries have endemic disease with high incidences in schoolchildren and young adults. He hypothesized that in areas with the most primitive sanitation and hygiene, widespread asymptomatic or mild infection of infants and young children occurs, leading to immunity and exhaustion of susceptibles after the first few years of life. According to Ashcroft's hypothesis, one would not expect frequent infection of infants and young children in a more developed country like Chile where the epidemiologic pattern of typhoid reveals the peak reported incidence in schoolchildren and young adults. Nevertheless, our preliminary data support the concept that infants become infected at a higher rate than is commonly appreciated and manifest a very mild clinical illness (not recognized as enteric fever), albeit accompanied by demonstrable bacteremia.

C. Case to Inapparent Infection Ratio for S. typhi in an Endemic Area

Most bacterial enteric infections such as shigellosis (4,17), cholera (18), non-typhoidal salmonellosis (19), and campylobacteriosis (20) exhibit an epidemioligic pattern wherein several subclinical infections occur for each clinically apparent case. Some authors have argued that a similar "iceberg" exists for typhoid fever with several asymptomatic infections occurring for each case of typhoid fever (21). In contrast, some of the most influential authorities on typhoid fever have argued that S. typhi infection is virtually always clinically overt and that in this way S. typhi is in marked contrast to other bacterial enteropathogens

(22,23). What little information exists on this subject is largely derived from the investigation of outbreaks in non-endemic areas such as Europe and North America; little has been written on the case to inapparent infection ration in endemic areas of the less-developed world.

Seroepidemiological methods have been highly instrumental in elucidating the epidemiology of other bacterial enteric infections in endemic areas, particularly cholera (18). We applied similar methods to the study of S. typhi infection in Area Norte, Santiago, in an attempt to ascertain the existence of asymptomatic infections.

In November 1978, serum specimens were collected from 239 healthy children age 0-19 years in Area Norte. The specimens were obtained from 60 infants and young children 0-4 years in well baby clinics and in nursery schools and from schoolchildren 5-19 years of age. Sera were tested for antibody to the d flagellar (H) antigen by bacterial agglutination in tubes as previously described (24). In the seroepidemiologic screening test an H titer of 1:40 or above was considered evidence of prior S. typhi infection. (Vaccination with parenteral typhoid vaccine is not practiced in Chile.)

The reported incidence of typhoid fever for children 0-4, 5-9, 10-14 and 15-19 years of age derived from notification data were analyzed for the years 1971 to 1977 and the mean incidence was derived for each age group during this period of time (Table 8). Using this mean incidence it was possible to calculate the mean cumulative number of cases that occurred in each age group. With

this information we estimated the total number of cases that would occur in a cohort of 1000 children in their first 19 years of growing up in Area Norte (Table 8). For example, a total of 3.1 cases of typhoid fever would be expected to occur in their first four years of life, 9.7 cases from 5-9 years, 12.7 cases from 10-14 years and 7.3 cases from 15-19 years of age. Therefore, a total of 33 cases of typhoid fever would be expected to occur in a cohort of 1000 children during their first 19 years of life in Area Norte. However, our serological studies show a 25% prevalence of H antibody (at a titer $\geq$1:40) in 15-19 year olds in Area Norte (Table 8); i.e. 250 manifest serological evidence of having had infection with S. typhi. Thus, only 33 infections were clinically overt while 217 were subclinical. This gives a ratio of 6.6 subclinical infections for every case of typhoid fever in an endemic area such as Area Norte. While this exercise is based on a number of assumptions, they are all epidemiologically reasonable. This data provide the first approximation of the case to inapparent infection ratio with S. typhi in an endemic area.

IV. ENVIRONMENTAL BACTERIOLOGY STUDIES

The observation that most cases of typhoid fever are not associated with a chronic carrier in the home made us turn our attention to a closer inspection of the water and sewerage system. We learned that although three-fourths of Santiago households were connected to the sewerage system there is no treatment of sewage. Thus raw, untreated sewage enters the Mapocho River or the Zanjon de la Aguada (a large open sewer that traverses southern Santiago from east to west before emptying into the Mapocho River southwest

of the city) (Fig. 2). As the Zanjon and the Rio Mapocho reach the westernmost portion of metropolitan Santiago their fecally-polluted, untreated waters are diverted for irrigation of crops during the rainless summer season. Prior to May 1983, 90% of the crops grown in this region were lettuce, cabbage and celery, vegetables that are difficult to wash and are eaten raw in salads in Chile.

The hypothesis that raw vegetables grown with untreated waste waters represent an important vehicle of transmission successfully explains the following epidemiologic observations.

1.) The striking seasonality of typhoid; (irrigation is used in the summer when there are no rains).

2.) The low incidence in young children; (raw vegetables are not an important food item for infants and toddlers).

3.) The high incidence of typhoid fever in high socioeconomic neighborhoods of Santiago; (they eat salads in restaurants and at home).

4.) The low incidence of typhoid fever in the lakes region of Chile; (because of year round rains in this region irrigation is not used).

Prior to 1983 Chilean microbiologists carried out a series of environmental microbiology studies in attempts isolate S. typhi from waters of the Zanjon and the Mapocho River and from vegetables irrigated with untreated wastewater (25-27). While heavy coliform counts and many non-typhoidal salmonella were found, S. typhi never isolated from vegetables or from the Zanjon and was isolated only once from the Mapocho River (28). The past failure to isolate S.

typhi from the polluted waters was in conflict with our epidemiologic incrimination of this wastewater. In review of the earlier Chilean studies we concluded that the bacteriologic methods were efficient but the techniques of environmental sampling appeared suboptimal. Therefore we initiated new environmental bacteriologic studies (29) using Moore swabs (30-32) (thick wads of cotton gauze which are left in the flowing wastewater for 2-3 days allowing the gauze to act as a filter) to collect samples. The Moore swab, originally described in England in 1948 (30), is a concentrating method, that has been used successfully to locate the homes of chronic S. typhi carriers by isolating the organism from sewage effluents (30-34). Moore swabs have been extremely useful in the investigation of urban typhoid fever outbreaks (30-34) in Europe, Japan and the U.S.A. The efficacy and reliability of Moore swabs in endemic areas had not previously been assessed. Nevertheless, based on its success in finding S. typhi in sewage contaminated by carriers in industrialized areas, we decided to employ modified Moore swabs to isolate S. typhi from environmental sources in Santiago.

Microbiologic examination of rivers and irrigation canals of Santiago, Chile was carried out from January to March, 1983. The two major waterways in Santiago that carry waste water are the Mapocho River in the north and Zanjon de la Aguada canal in the south. (Fig. 2). Untreated sewage flows directly into these waters, which are used for irrigation in the agricultural districts of Maipu and Pudahuel (on the perimeter of the city). The Zanjon de la Aguada, which is heavily contaminated with industrial waste

from central section of the city, receives untreated sewage and becomes fecally polluted as it flows westward. During the final few kilometers as it approaches the agricultural areas, no further sewage is discharged in an attempt to allow a degree of self purification of the waste water.

Moore Swabs were prepared by wrapping cotton gauze, 15 cm wide by 120 cm long, around wire. The swabs were tied to nylon cord, and suspended in the flowing water of the Zanjon de la Aguada, Mapocho River, and their tributaries. Swabs were also placed directly into irrigation canals of selected farms.

After 48-72 hours, the swabs were removed and placed immediately into 500 ml of Selenite-F broth. The selenite broth was incubated at 41°C and subcultured at 24 and 48 h onto salmonella-shigella, bismuth-sulfite, and deoxycholate-citrate agars. Suspicious colonies were placed on triple-sugar-iron agar slants and confirmed as S. typhi by standard methods. S. typhi isolates were sent to the Central Public Health Laboratory, Colindale, United Kingdom for phage typing.

We placed 133 swabs into the Mapocho River (56) and Zanjon de la Aguada (77) but recovered only 93. Most lost swabs were due to passersby, who removed or cut the swab. After the first month, by camouflaging the swabs, we were able to decrease losses. Of the 48 swabs recovered from the Zanjon de la Aguada, 17 came from central city industrial areas where there is heavy chemical pollution and 31 were from agricultural areas where there is a predominance of fecal contamination. None of the 17 industrial area swabs contained S. typhi. Four of the 31 swabs from the Zanjon de la

Aguada (13%) and 4 of 45 from the Mapocho River (8.3%) contained S. typhi. Of the 76 swabs placed in agricultural areas, 8 were culture positive (11%). Five of the eight isolates were phage type E1 and 46, the two most common disease-causing types in Chile, one strain was untypable, and the other two were N and M1.

Using Moore Swabs, we were able to isolate S. typhi from irrigation water in Santiago, Chile. To our knowledge, this is the first time Moore Swabs have been used for this purpose. The sensitivity of the Moore Swab is thought to have an inverse relationship to the size of the waterway sampled (32). Thus, our isolation rate of eleven percent from these large waterways is probably an underestimate. S. typhi is fastidious, easily inhibited by coliforms, and usually present in relatively small numbers in environmental samples (33). The Moore Swab, by acting as a filter, improves the chance of isolating rare S. typhi among millions of coliforms and has been useful in isolating S. typhi from sewers during outbreaks in industrialized nations. We have now shown that it is both a practical and reliable epidemiologic tool with which to isolate S. typhi from irrigation water in endemic areas. Finding S. typhi with the same phage types as disease-causing isolates in irrigation water supports the hypothesis, based on epidemiologic observations, that contaminated vegetables in Santiago serve as important vehicles of transmission (M. Levine et al, unpublished data).

Enteric diseases can be transmitted by vegetables contaminated by polluted water (35), but a cause and effect relationship is difficult to prove. A study of kibbutzim in Israel showed that

communities that practiced waste water irrigation had a two to four times higher incidence of enteric infections (36). Although our study does not prove that S. typhi cultured from irrigation water is directly responsible for typhoid fever, its presence implies that an association likely exists between S. typhi-contaminated vegetables and infection. Recently the government of Chile has intervened to change the farming patterns and usage of contaminated irrigation water. Since May, 1983, the cultivation of certain vegetables that are eaten raw, such as lettuce and carrots has been prohibited. Other vegetables that are eaten raw are presently still premitted to be grown; these include celery and cabbage. The Department of Environmental Health of the Ministry of Health has been sending inspectors to the fields of Maipu and Pudahuel to check the crops; compliance has been excellent.

In January, 1984 Drs. M. Levine and C. Ferreccio made visits to the areas to survey the types of crops and to interview farmers. No fields were found with cultivation of lettuce; all farmers interviewed were able to produce at least one recent inspection certificate.

In January, 1984 the incidence of typhoid fever was the lowest for that peak month since 1976. The incidence has been lowered by 67%. The current rate is still high compared with many developing countries and is more than adequate for field trials of typhoid vaccines. (The current incidence rate is still much higher than Alexandria, Egypt.) Nevertheless, it is lower than the preceding five years. Our tentative, preliminary conclusion is to attribute this fall in incidence to the environmental health and agricultural

pattern interventions in Maipu and Pudahuel.

V. STUDIES WITH CHRONIC S. TYPHI CARRIERS

A. Simple, Practical, Serologic Test to Detect Chronic S. typhi Carriers

Chronic carriers represent the reservoir of S. typhi infection. Using epidemiologic techniques we previously estimated that in 1980 there existed 25,019 female and 4,575 male chronic S. typhi carriers among the 4,264,515 inhabitants of Santiago (prevalence $694/10^5$) (37).

Simple yet reliable screening tests are required to allow rapid and effective identification of chronic carriers. Shortly after the original description of the Vi antigen by Felix in the 1930s (38), he noted that chronic carriers had high titers of Vi antibody (measured by bacterial agglutination using Vi rich bacteria) and suggested that this serology might serve as a screening test to detect carriers (39). Over the next 45 years great debate occurred on this subject due to widely divergent results of various investigators. Until recently all assays were limited by the lack of purity of the antigen. However, a few years ago highly purified Vi antigen became available (40). Vi serology using this purified antigen in a passive hemagglutination (HA) test was successful in detecting chronic carriers in outbreak situations in non-endemic areas (41). Therefore we proceeded to assess the value of this serologic screening test to identify carriers in an endemic area, Chile (42). Sera were tested from the following Chilean populations:

1.) 36 bacteriologically-confirmed known chronic carriers.

2.) 29 patients of both sexes age 18 years and over with acute typhoid fever.

3.) 388 women who had confirmed typhoid fever 12-48 months earlier and who were apparently not carriers (based on three negative stool cultures).

4.) 59 healthy individuals age 16-46 years.

Of the 36 chronic carriers, 27 (75%) had Vi titers of $\geq$160 (see Table 9), whereas only 8% of the 388 non-carrier women ($p<0.001$) and 3% of 59 health subjects (who had no bacteriological screening) ($p<0.001$) had titers $\geq$160. The frequency of tiers $\geq$160 in patients with acute typhoid fever (38%) was also significantly lower than that in chronic carriers ($p<0.005$). The geometric mean titer in the chronic carriers was significantly ($p<0.001$) higher than that in any of the other groups (Table 9).

The sensitivity and specificity of each Vi antibody titer as a cut-off point in screening for chronic S. typhi carriage was determined with the 388 culture-negative women as negative controls (Fig. 3). With a Vi antibody titer of $\geq$160 taken as positive, the passive haemagglutination assay with highly purified Vi antigen had 75% sensitivity and at least 92% specificity.

The predictive value (43) of each Vi antibody titer as cut-off point in screening for chronic carriers (defined as the percentage of subjects with positive Vi serology who will be confirmed as chronic S. typhi carriers) was determined in populations with different carrier prevalence rates (Fig. 4). When we used the 388 culture-negative women with a history of confirmed typhoid fever as the negative controls, the specificity of a Vi antibody titer $\geq$160

was 92%. Thus, in Santiago the predictive value of this titer is 8% in the general adult population, 16% in women 40 years and older, and 37% in women 25 years and older with history of confirmed typhoid fever (Fig. 4A). However, the 59 healthy Chileans (who were not studied bacteriologically) may be more representative of the general population in Santiago; when they were used as the negative controls, the specificity of a Vi antibody titer $\geq$160 rose to 97% and the predictive value rose from 7% to 17% in the general adult population and from 16% to 31% in women 40 years and older (Fig. 4B).

Three to five percent of patients with typhoid fever become chronic carriers and the carrier state persists throughout life (44,45). Since man is the only natural host and reservoir of S. typhi (45), the detection of chronic carriers is essential for the control of typhoid fever. The use of bacteriological cultures for the detection of chronic S. typhi carriers is limited by expense, logistical considerations, and the fact that carriers typically have intermittent excretion of S. typhi so repeated cultures are necessary. Some reports of the non-surgical treatment of chronic S. typhi carriers have been encouraging (46,47), so the use of Vi serology to screen for carriers in selected high-risk groups in an endemic area may be justified as part of a program to control typhoid fever.

The main reason for the conflicting reports of the usefulness of Vi serology in the detection of chronic S. typhi carriers is the differing purity of the Vi antigen. The original studies with direct bacterial agglutination used an S. typhi strain rich in Vi

as antigen (39). This strain, however, also contained somatic O and flagellar H antigens so the sera had to be preabsorbed with a Vi-negative S. typhi strain. Isolation of S. typhi strain Vi 1, which was rich in Vi antigen but almost without O and H antigens, simplified direct bacterial agglutination (48). However, both the direct bacterial agglutination test and the passive haemagglutination assay with crude or partially purified Vi antigen have a high false-positive rate in the general population, especially where typhoid fever is endemic, apparently because of cross-reactivity with other antigens in these antigen preparations. The lack of specificity has been the reason for the loss of confidence in Vi serology since the late 1950s.

The development of a method to produce highly purified Vi antigen was important (40), since it provided an antigen that improved the specificity of the technologically simple passive haemagglutination assay.

The practical application of the simple passive haemagglutination assay with highly purified Vi antigen in detecting chronic S. typhi carriers in an endemic area depends not only on the sensitivity and specificity of the titer used as cut-off but also on its predictive value. Since the predictive value of each titer cut-off is greater in populations with higher chronic S. typhi carrier rates, screening high-risk groups of the population will be justifiable.

Treatment of Chronic Typhoid Carriers

When a chronic S. typhi is identified, interventions must be initiated to minimize the chance for transmission of S. typhi by

the carrier to susceptibles. Health education including counseling on personal hygiene and food preparation techniques is fundamental. Ideally, however, therapy to eradicate the chronic carrier state is desirable. The currently recognized "gold standard" of therapy involves cholecystectomy followed by several weeks of antibiotic (usually ampicillin or amoxicillin) therapy. Obviously such a therapeutic regimen involving major abdominal surgery is unsuitable as a public health intervention in endemic areas where the prevalence of carriers is high. Thus, for decades a non-surgical therapeutic regimen has been sought to successfully cure chornic S. typhi carriers.

Italian investigators (46) reported that two weeks of intravenous ampicillin (1.0 gm q 8 h) successfully cured 19 chronic S. typhi biliary carriers. However, intraveneous antibiotic therapy precludes self-administered domiciliary treatment and thus is also not practical for public health use. The advent of amoxicillin made available a superbly absorbed analog of ampicillin that provided serum levels following oral administration that were previously achievable only with parenteral administration of ampicillin. Furthermore, like ampicillin, amoxicillin is concentrated in bile. Nolan et al (47) recognized that these features of amoxicillin made it worthy of evaluation as a nonsurgical treatment for the chronic S. typhi carrier state. Nolan et al (47) treated 15 chronic S. typhi biliary carriers with oral amoxicillin (2.0 gm tid) for 28 days. Long-term cures were observed in 9 of 10 carriers who were able to complete the month of therapy.

Encouraged by these preliminary results of Nolan et al (47), we undertook to evaluate a 28 day course of oral amoxicillin (2.0 gm three times daily) plus probenecid (0.5 gm three times daily) in treatment of chronic S. typhi carriers in Santiago, Chile. Twenty-eight confirmed chronic carriers (27 female) began the course of therapy. Antibiotic and probenecid for each day of therapy were provided in small vials. Medication was taken at home or at work and the times of dosing were recorded by the patient in a small diary. Patients were visited in their homes at lease once weekly on a scheduled basis. In addition random unscheduled visits were made at least once weekly. At both scheduled and surprise visits urine specimens were collected for measurement of amoxicillin levels.

Two of the 28 patients were unable to complete the course of amoxicillin therapy because of severe allergic reactions which manifested in the first day or two of therapy. Of the remaining 26 carriers who successfully completed the 28 day course of amoxicillin and probenecid, many complained at one time or another of mild diarrhea, rash, nausea, abdominal discomfort or gastritis (Table 10). In no instance were the symptoms sufficiently severe to cause discontinuation of therapy.

The success of therapy was monitored by means of stool cultures and bile cultures (obtained by string capsule device) at monthly intervals following completion of therapy. Results are summarized in Table 11. This non-surgical, ambulatory, domiciliary oral treatment regimen resulted in long-term (>1 year) cure of 15 of the 26 carriers (58%). When failure occurred it was usually evident

within the first six weeks follwing cessation of therapy. Thirteen of the 26 carriers have had radiological evaluation of their gall bladder function; cholelithiasis, failure of the gall bladder to fill during cholecystogram, or other pathology was present in 13 of 13 carriers examined.

A cure rate of 58% with a domiciliary oral antibiotic regimen, despite the presence of gall bladder dysfunction, is encouraging news for treating an individual patient, since there is a more than even chance of cure without surgery. However, such a cure rate is too low to advocate its use in public health programs. The search will continue to identify an antibiotic regimen that will cure at least 80% of carriers, even with gallstones, without cholecystectomy.

VI. LARGE-SCALE FIELD TRIALS OF THE TY21a, LIVE ORAL TYPHOID VACCINE

The live oral typhoid vaccine, Ty21a, developed by Germanier and coworkers (49) represents a potentially major breakthough for the control of typhoid fever by immunization. In the initial clinical studies with this live attenuated Salmonella typhi oral vaccine in North American volunteers, it was shown to cause no adverse reactions and to be genetically stable and highly, protective (50).

The first field trial with Ty21a was carried out in Alexandria, Egypt where approximately 16,000 six and seven year old schoolchildren were given three doses (10^9 viable vaccine organisms per dose) within one week (51). Individual doses of lyophilized vaccine contained within small glass vials were

reconstituted on the spot and the children were vaccinated a few minutes after they chewed a tablet containing 1.0 gm of $NaHCO_3$ to neutralize gastric acid. An equal number of children ingested placebo. As shown in Table 12, in this trial the vaccine provided 96% efficacy for at least three years.

A. Area Norte Field Trial

Stimulated by highly encouraging results of the Egyptian trial, a collaborative effort was undertaken to carry out a field trial of Ty21a in Santiago, Chile to obtain new information and to evaluate the possible future use of this vaccine as a public health intervention to control endemic typhoid fever in Chile.

The collaborating institutions in the Chilean field trials include the Chilean Ministry of Health, the Center for Vaccine Development of the University of Maryland School of Medicine, the World Health Organization, the Swiss Serum and Vaccine Institute, the Pan American Health Organization, and the Walter Reed Army Institute of Research.

The goals of the first Chilean field trial include:

1.) To evaluate the efficacy of a new formulation of Ty21a (enteric-coated capsules) that is more amenable to mass vaccination since $NaHCO_3$ pretreatment is unnecessary.

2.) To investigate the efficacy of fewer (one or two) doses of vaccine.

3.) To assess the efficacy in an area of particularly high endemicity and force of infection.

In May and June, 1982, 91,954 schoolchildren of consenting parents (67% of all schoolchildren) in the northern administrative

area of Santiago (Area Norte) were given either two doses of enteric-coated vaccine (1-3 x 10^9 viable organisms per dose), one dose of vaccine and one of placebo, or two doses of placebo. In total, 27,484 children received two doses of vaccine, 32,707 got one dose and 31,762 received placebo. The remaining 45,743 children whose parents did not give permission for them to be in the trial were also kept under surveillance and considered in a separate group as contact controls.

In this large scale trial the vaccine was found to cause no significant adverse reactions. This is a stark contrast to the parenteral killed whole cell typhoid vaccines which cause notable adverse reactions in up to 25% of recipients (52-54). It was also found that the enteric-coated capsule formulation of Ty21a was highly practical and suitable for use in mass vaccination of schoolchildren.

The first year of surveillance went from July 1, 1982 to June 30, 1983. A description of the techniques of intensive epidemioligic and bacteriologic surveillance have been previously described in detail (Annual Report #2). Briefly, the major thrust of surveillance in Area Norte is through the consultorios (health centers) and the Roberto del Rio Children's Hospital which are part of the National Health Service. It is estimated that 85% of health care visits in Area Norte involve one of these National Health Service facilities. Outpatients with a clinical syndrome suspicious of typhoid fever have at least two blood cultures. Inpatients have three blood and one bone marrow cultures.

During the first year of surveillance a total of 261

culture-confirmed (blood or bone marrow) cases of typhoid fever were detected in the 137,697 schoolchildren under surveillance in Area Norte. This included 156 cases among the 91,954 children participating in the trial and 105 cases among the 45,743 contact controls.

In comparing the rate of typhoid in two dose vaccinees (113 cases/10^5 children), one dose vaccinees (174 cases/10^5) and placebo children (214 cases/10^5) during the first year of surveillance, overall vaccine efficacy was found to be 47% with two doses and 19% with one dose of vaccine.

However, these overall results are somewhat misleading since the efficacy was not equal throughout the year. In fact, during the first nine months of surveillance vaccine efficacy was 56% in the two dose group and 41% in the one dose group (Table 13) and the difference in incidence rates between two dose vaccinees and placebo recipients was highly significant (Table 13). This moderate degree of efficacy with two doses of vaccine during this period included the months of highest incidence of typhoid in Chile including December, January, February and March. Furthermore, the degree of efficacy with two doses of Ty21a during this period (67%) resembled the level of protection encountered in some field trials with the parenteral killed whole cell vaccine (55,56). It is also notable that during the peak typhoid months of December 1982 through March 1983 vaccine efficacy was evident in each month and in total only 11 cases of typhoid fever were detected in the two dose vaccine group versus 44 in the placebo group during this period.

In contrast with results of the first nine months of surveillance, beginning abruptly in April 1983, vaccine efficacy disappeared during the remaining portion of the typhoid season (April and May) (Table 13). During this period in April and May when the efficacy abruptly disappeared, the incidence of typhoid fever in the control group was high but was nevertheless lower than the period of January through March when vaccine efficacy was quite evident (66%) (Fig. 5).

In late June, 1983, after analysis of results of one year of surveillance we formed two hypotheses which could explain these observations including the sudden disappearance of vaccine efficacy.

Hypothesis 1 - That two doses of the enteric-coated formulation of Ty21a vaccine stimulate moderate vaccine efficacy but of only short duration (nine months).

Hypothesis 2 - The moderate (circa 60-70%) vaccine efficacy stimulated by two dose of Ty21a in enteric-coated formulation can be overcome when vaccinees ingest a high inoculum of S. typhi. This hypothesis proposes that in April and May of 1983 there circulated a vehicle of transmission that either had a high inoculum or was particularly adept at transmitting S. typhi through the stomach into the intestine in a viable state. Such a vehicle would not have been very widespread (which would explain why the incidence in the placebo group was not higher than in January or February, 1983) (or April of 1982). This would nevertheless explain why vaccinees who ingested the vehicle, albeit not a particularly common vehicle, would develop typhoid fever.

The volunteer studies of Hornick et al (57) clearly showed that the protective efficacy of typhoid vaccines was related to the inoculum of pathogenic S. typhi used in the challenge. High inocula were shown to overcome the protective effect of vaccines that gave significant protection against lower inocula.

In June, 1983 we concluded that results of continued surveillance would provide an answer to this question. If efficacy did not reappear in the second typhoid season we would conclude that the vaccine as used in Area Norte gave only short-term protection. However, if vaccine efficacy reappeared in the second typhoid season at the same level as in the first nine months of the first year of surveillance, we could conclude that a special epidemiologic situation (as in hypothesis number two) was operative in April and May of 1983.

Dr. Levine was in Chile during January of 1984. At this time the code was broken for the cases that occurred between July 1, 1983 and December 31, 1983. Dr. Catterine Ferreccio, the Program Coordinator, carefully checked and double-checked the computer code against the original planilla forms for each of the cases from this period. It was found that 16 cases occurred in the placebo group in this period versus only two cases in the two dose group (p=0.009) (vaccine efficacy 86%) (Table 13, Fig. 5). These results closely resemble those obtained in the same period (July 1 to December 31, 1982) of the first year of surveillance when 16 cases of typhoid occurred in the placebo group versus only four in the two dose vaccine group (vaccine efficacy 71%).

Thus, results of the first six months of the second year of surveillance clearly demonstrate that vaccine efficacy has returned and that the vaccine so far is providing a level of protection comparable to the first nine months of last year.

In summary, the following important observations have been made in the Area Norte field trial:

1.) Ty21a vaccine is extremely safe; no significant adverse reactions were encountered.

2.) The enteric-coated formulation is highly practical and ideally suited for mass immunization of schoolchildren.

3.) Two doses of Ty21a in enteric-coated capsules gives considerably more protection than a single dose of vaccine.

4.) In the first year of surveillance two doses of vaccine showed 67% efficacy in the first nine months followed by a complete lack of efficacy in the last three months of the first year.

5.) In the first six months of the second year of surveillance, vaccine efficacy reappeared with two doses giving significant protection (vaccine efficacy 86%).

6.) It is likely that in April and May 1983 a vehicle of transmission containing a high inoculum of S. typhi was responsible for the lack of efficacy encountered in that period by overwhelming the protective capacity of the vaccine.

Several critical questions remain to be answered:

1.) With what frequency does the epidemiologic situation of April and May, 1983 occur in Area Norte? If this is a rare event we can be optimistic regarding the role of Ty21a vaccine as a control measure. On the other hand, if vehicles of transmission containing

high inocula frequently in appear Santiago, to overwhelm the protective effect of the vaccine, as we hypothesize happened in April, Mary, 1983, then the utility of Ty21a as a tool of public health will be greatly diminished.

2.) How long will the efficacy induced by two doses of vaccine in Area Norte endure? Will this moderate efficacy continue for a least five years.

3.) Can a third dose of vaccine, or a different formulation, or a different immunization schedule increase the level of efficacy stimulated by Ty21a and protect against even high inocula in the field?

Continued intensive surveillance in Area Norte will provide answers to the first two critical questions. The field trial in Area Occidente and the new field trial being proposed in Area Sur will provide answers to the third question.

B. Area Occidente Field Trial

A second field trial was begun in Santiago in 1983 to try and resolve the relative importance of the major variables that may influence the efficacy of Ty21a live oral typhoid vaccine and to identify a formulation and immunization schedule that will provide high efficacy of long duration.

Between July and September of 1983, 147,127 schoolchildren of consenting parents (96% of all children in the Area) were randomized to receive one of the following:

Group 1 - Three doses of vaccine in enteric-coated capsules given within one week.

Group 2 - Three doses of vaccine with $NaHCO_3$ given within one week. The commercial formulation "Vivotif" was used which consists of two gelatin capsules each containning 0.5 of $NaHCO_3$ and one gelatin capsule containing lyophilized vaccine.

Group 3 - Three doses of enteric-coated vaccine with an interval of three weeks between the doses.

Group 4- Three doses of the "Vivotif" formulation with an interval of three weeks between the doses.

Group 5 - Three doses of placebo.

The design of this trial was intended to allow a direct comparison of two different formulations of vaccine as well as two different immunization schedules. We would like to have included the Egyptian formulation as one cell in this trial, however, that formulation was not available. These 147,127 children are presently under intensive epidemiologic surveillance. As of February 4, 1984, 61 bacteriologically confirmed cases of typhoid fever were detected in Area Occidente schoolchildren.

C. Viable Counts of Vaccine Used in Area Occidente

Irrespective of the formulation or immunization schedule (see Table 13A), each dose of vaccine given to the children in Area Occidente was supposed to contain from 1 to 3 billion viable vaccine organisms. The various vaccine preparations, identical appearing placebo capsules and the capsules of $NaHCO_3$ were shipped to Chile in glass bottles containing approximately 200 capsules. Quantitative bacteriologic cultures were carried out on multiple samples of each formulation of Ty21a vaccine at the Swiss Serum and Vaccine Institute in Berne prior to shipping the vaccine by refrigerated air freight to Chile. Upon arrival in Chile the

vaccine was maintained in a "cold-chain" and samples were sent to the Instituto de Salud Publica where further quantitative cultures were performed on July 27 and 28. Results of these bacteriological studies at the Instituto de Salud Publica documented that all of the capsules tested of both vaccines contained 1 to 4 billion viable organisms (Table 14). Throughout the period of vaccination an extraordinary amount of attention and detail was paid to maintenance of a strict cold chain. The quality of this cold-chain was verified daily throughout the days of vaccination by on-site senior supervisors who included Dr. Catterine Ferreccio and members of the Chilean Typhoid Fever Control Program staff, as well as the consultants, Drs. Robert Black, Mary Lou Clements, and Myron Levine. Because the total number of doses of vaccine available closely approximated the number of children participating in the program (i.e. there was little extra vaccine), it was necessary at the end of each day of vaccination to pool the leftover capsules of each formulation to be used on subsequent vaccination days. In the process of pooling of the capsules, the quality of the cold-chain was again verified by the supervisors. However, it was also noted that bottles containing the vaccine often contained moisture and that the capsules were often moist. Recognizing that misture conceivably could deleteriously effect the lyophilized vaccine, multiple samples of vaccine from the field were sent to Drs. Hernan Lobos and Ana Silva at the Instituto de Salud Publica for quantitative bacteriology. Capsules of both formulations of vaccine delivered to the Instituto on August 26 were tested on September 6 and 7, 13 and 21. On all days tested the capsules of enteric-coated vaccine were one and one-half logs below the minimum

acceptable level (Table 14). On September 6 and 7 the counts of the "Vivotif" formulation were still above one billion viable organism; however, by the 13th of September the counts had dropped one log (Table 14). The dimunition in the counts of viable vaccine organisms in capsules from the field in September was verified independently by the Enteric Microbiology Laboratory at the Center for Vaccine Development in Baltimore with vaccine hand-carried from Chile by Dr. Levine. Results of these studies agreed with those of Drs. Silva and Lobos in Chile (Table 14). Thus some children immunized in August and in September received capsules of either formulation of vaccine that contained less than the requisite number of viable vaccine organisms. These observations will have to be taken into account in comparing incidence rates among the various groups at the end of the first year of surveillance.

D. Serological Studies

Fortunately, serological studies were carried out in a subset of vaccinees that provide helpful data to assist us in evaluating the situation. It will be useful at this point to review the rationale for having undertaken the serological studies. In their pioneering studies in volunteers with Ty21a, Gilman et al (50) were unable to correlate protection in individuals with serum antibody responses. Nevertheless, they found that the rate of seroconversion of Salmonella typhi O antibody was significantly higher in a group of volunteers vaccinated with vaccine grown in the presence of galactose (which gives the vaccine organisms smooth lipopolysaccharide) than in a group who received vaccine grown in the absence of galactose. The former group of vaccinees were significantly protected against experimental challenge, while the latter group was not. These observations suggested that

measurement of S. typhi O antibody could be helpful in comparing populations immunized with different formulations or dosage schedules of Ty21a, even though serology was not helpful in predicting the state of protection of an individual vaccine. In 1982, concomitant with the large scale field trial of one or two doses of enteric-coated Ty21a vaccine in Area Norte, a cohort of Chilean Air Force cadets were similarly immunized. Sera from these Air Force teenagers were obtained before and 10 and 21 days after immunization and were tested for IgG antibody to S. typhi O antigen by a sensitive enzyme-linked immunosorbant assay (ELISA). Results of these serological studies are shown in Table 15 and are compared with the vaccine efficacy as observed in the Area Norte field trial. Two doses of enteric-coated vaccine stimulated significantly more seroconversions (44%) than one dose (18%) ($p<0.01$). Thus the serological results were predictive of the efficacy results in the field trial. In July 1983, concomitant with the field trial in Area Occidente, several hundred cadets in the Chilean Air Force were vaccinated with several of the regimens used in the field trial. A description of the vaccine groups and their seroconversion rates of the IgG ELISA O antibody are shown in Table 16. Several observations are notable. First, the seroconversion rate of recipients of three doses of enteric-coated vaccine within one week is at least as good (in fact slightly better) than persons immunized with three doses of the "Vivotif" formulation within one week. Second, the seroconversion rate in recipients of three doses within one week of enteric-coated vaccine this year (64%) is significantly higher than persons who received two doses within one week of enteric-coated vaccine last year (44%)

($p<0.04$). Third, the cadets vaccinated with three doses of enteric-coated vaccine with an interval of three weeks between doses had a significantly lower seroconversion rate ($p<0.002$) than those who received three doses within one week, and the rate of seroconversion (40%) closely resembled that seen in last year's recipients of two doses of enteric-coated vaccine (44%). One possible explanation for the last observation is that the third dose of vaccine administered in the long schedule involved impotent vaccine whose viable counts had dropped to unacceptable levels.

In January, 1984 we carried out another serological study in Air Force cadets that should help to elucidate differences in formulations of the vaccine, as well as in the methods of delivering $NaHCO_3$. Three groups of cadets (approx. 100 per group) were immunized as follows: Group 1 received 1.0 g $NaHCO_3$ in water followed by vaccine exactly as utilized in the Egyptian field trial. Vials containing individual doses of lyophilized vaccine were provided by the Swiss Serum and Vaccine Institute for this purpose. Group 2 ingested gelatin capsules containing 1.0 gm $NaHCO_3$ followed by vaccine in gelatin capsules ("Vivotif"). Group 3 received 1.0 gm of $NaHCO_3$ dissolved in water, followed by vaccine in a gelatin capsule. All groups ingested three doses of vaccine within one week. Sera collected before and 10 and 21 days after vaccination are being tested for 0 antibody by IgG ELISA. Results will be comapred with the previous serological data.

VII. CLINICAL BACTERIOLOGY STUDIES

A. Multiple Blood Cultures

Analysis of children hospitalized in Roberto del Rio Children's Hospital in Area Norte in the first year of the field trial showed

second and a third blood culture increased the rate of bacteriological confirmation. However, it was not clear if the increase in sensitivity was a function of the increased volume of blood cultured or of sampling at different points in time.

We decided to answer this practical question during the Area Occidente field trial. The participating consultorios were randomized to perform blood cultures by one of two methods:

1.) An initial 4 ml culture (in 40 ml brain heart infusion broth with SPS) followed 30 and 60 minutes later by a second and third culture each of 4 ml of blood.

2.) An initial 4 ml blood culture as above followed 30 minutes later by a second 8 ml blood culture (in 80 ml BHI with SPS). The number of cultures analyzed so far is too few to reveal anything other than general trends (Table 17). However, by the end of a year or two of surveillance we should have sufficient numbers to draw conclusions.

B. Antibiotic Resistance Plasmids in S. typhi

Salmonella typhi, the causative agent of typhoid fever, remains an important enteric pathogen in many parts of the world. Infection with this organism has a high morbidity and mortality rate and optimal therapy requires the use of antimicrobial agents. Despite the fact that a number of outbreaks of typhoid fever have been caused by antibiotic resistant S. typhi, such as in Mexico in the early 1970's (58) and currently in Peru (59), these organisms have in general remained surprisingly susceptible to antibiotics, particularly when one compares their resistance to that of other enteric pathogens such as the shigella and non-typhoidal salmonellae. In Chile less than 0.5% of clinical isolates of S.

typhi are resistant to chloramphenicol, despite the widespread, almost promiscuous use of chloramphenicol (which can be purchased in pharmacies across-the-counter without a prescription).

A study was initiated to examine S. typhi strains from Chile for their plasmid content, resistance to antibiotics and to characterize them in their ability to receive R factors by conjugation and for the stability of such plasmids in Chilean S. typhi strains. These studies were carried out by Dr. Barbara Murray of the University of Texas Medical School at Houston, a well-known authority on antibiotic resistance plasmids.

One hundred strains of Salmonella typhi were examined for resistance and for the presence of plasmids. None was resistant to any of seven antimicrobial agents tested and only 8 contained plasmids; the general lack of plasmids suggests that a "virulence" plasmid is not necessary for the production of invasive disease by this species. Conjugal transfer of R-factors from clinical isolates of Escherichia coli into clinical isolates of S. typhi and subsequent study of transconjugants revealed the following: 1) transfer occurred at almost the same rate into the S. typhi clinical isolates as into an E. coli K12 recipient strains; 2) the growth of S. typhi, already slow in comparison to E. coli, was further slowed by some but not all of these plasmids; and 3) 4 of 5 of the plasmids were markedly less stable in S. typhi thank in E. coli whereas the fifth was stable in all hosts. The instability of R-factors from E. coli, a likely source in nature, and perhaps the slower growth rates, may help explain why R-factors are not found more often in typhoid bacilli.

VIII. UPDATED BIBLIOGRAPHY OF PUBLISHED CONTRACT-SUPPORTED WORK:

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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 1977-1981	% 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
Araucania	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

*1977-1981 rate over 1970-1976 rate

TABLE 5A

INCIDENCE RATES OF TYPHOID FEVER BY ADMINISTRATIVE AREA, SANTIAGO, CHILE

AREA	MEAN SOCIOECONOMIC LEVEL	INCIDENCE RATE/10^5 1977	1978
SUR	LOW, MIDDLE	317.5	321.5
SUR ORIENTE	LOW, MIDDLE	117.0	113.1
OCCIDENTE	LOW, MIDDLE	140.9	177.0
NORTE	LOW, MIDDLE	125.5	220.7
CENTRAL	MIDDLE	185.3	168.6
ORIENTE	MIDDLE, UPPER MIDDLE, HIGH	118.7	161.9

Table 6

AGE-SPECIFIC INCIDENCE RATES AND CASES OF TYPHOID AND PARATYPHOID FEVER, SANTIAGO, CHILE 1977-1981

Metropolitan Santiago*			Northern Administrative Area of Santiago (Area Norte)**		
Age Group (yrs)	Mean Annual No. Cases+	Mean Annual Incidence per 10^5	Age Group (yrs)	Mean Annual No. Cases+	Mean Annual Incidence per 10^5
			<2	7	27.2
0-4	421	89.2	2-4	49	112.6
5-9	1193	272.2	5-9	149	238.3
10-14	1413	333.0	10-14	183	305.4
15-19	1465	283.4	15-19	108	173.4
20-24	728	246.7			
25-34	1023	153.3			
35-44	366	74.6			
45-54	179	50.2			
55-64	88	36.0			
≥65	79	38.5			

*Mean population 4,177,921

**Mean population 582,653

+Approximately 90% of cases in all age groups are typhoid fever, 10% are paratyphoid fever.

Table 7

CLINICAL PICTURE OF INFANTS WITH

S. TYPHI AND S. PARATYPHI BACTEREMIA**

Age (mos.)	Sex	Salmonella Isolated	Temp. upon Examination	Days of Fever	Anorexia	Vomiting	Consti-pation	Diarrhea	Cough	Hepato-megaly	Splenomegaly	Clinical Diagnosis
4	M	typhi	38.4°C	1	-	+	-	-	+	-	-	viral syndrome
9	F	typhi	38.8	1	-	-	-	-	+	-	-	viral syndrome
10	M	typhi	38.3	1	+	+	-	-	+	-	-	pneumoniti
17	F	typhi	38.3	4	+	-	-	-	+	-	-	viral syndrome
3	M	paratyphi B	38.3	5	-	+	-	+	+	+	-	bronchopne monia
8	F	paratyphi A	38.4	1	+	-	-	+	+	-	-	acute bronchitis
14	F	paratyphi B	38.4	3	+	-	+	-	-	-	-	viral syndrome

*Includes only the seven infants detected by active surveillance in this prospective study.

TABLE 8

MEAN AGE SPECIFIC INCIDENCE OF TYPHOID FEVER, NUMBER OF CASES PER FIVE YEARS LIFE EXPERIENCE AND PREVALENCE OF *SALMONELLA TYPHI* ANTIBODY BY AGE IN AREA NORTE, SANTIAGO

AGE GROUP (YEARS)	MEAN INCIDENCE PER 10^3 CHILDREN	NO. CASES PER FIVE YEARS OF LIFE EXPERIENCE IN AREA NORTE	PREVALENCE OF H ANTIBODY**
0-4	0.62	3.1	0/60 (0%)+
5-9	1.94	9.7	1/60 (1.6%)
10-14	2.53	12.7	4/43 (9.3%)
15-19	1.45	7.3	19/76 (25%)

*BASED ON NOTIFICATION DATA FROM 1971-1978.

**TITER OF 1:40 OR GREATER.

+NO. POSITIVE/NO. CHILDREN TESTED (%).

Table 9

Prevalence of Vi Antibody* in Chronic *Salmonella Typhi* Carriers, Acute Typhoid Fever and Healthy Populations in Santiago, Chile

Group	Characteristics	N	Reciprocal Geometric Mean Titer	Percent with Reciprocal Titer		
				≤40	80	≥160
Chronic *S. typhi* carriers	92% females 17-69 years	36	298	14	11	75
Acute typhoid fever patients	Both sexes 18-30 years	29	53	48	14	38
Non-carriers** with typhoid fever 1-4 years earlier	100% females 24-62 years	388	21	85	7	8
Healthy Chileans	Both sexes 16-46 years	59	16	85	12	3

*Measured by passive hemagglutination using highly purified Vi antigen.

**Negative stool (3) and bile cultures.

TABLE 10

MOST COMMON COMPLAINTS DURING 28 DAY ORAL AMOXICILLIN THERAPY OF CHRONIC TYPHOID CARRIERS*

COMPLAINT	PERCENT
DIARRHEA	42
RASH	23
ABDOMINAL DISCOMFORT	19
NAUSEA	12

*AMOXICILLIN 2.0 GM PO TID PLUS PROBENECID 0.5 GM PO TID.

TABLE 11

RESULTS OF A 28 DAY COURSE OF ORAL AMOXICILLIN AND PROBENECID IN TREATMENT OF 26 CHRONIC *S. TYPHI* BILIARY CARRIERS

OUTCOME	PERCENT
SUCCESS	58
FAILURE	42

TABLE 12

FIELD TRIAL OF EFFICACY OF THREE DOSES OF Ty21a VACCINE GIVEN WITH $NaHCO_3$ TO SIX AND SEVEN YEAR OLD SCHOOLCHILDREN IN ALEXANDRIA, EGYPT. RESULTS OF THREE YEARS OF SURVEILLANCE

Year of Observation	Confirmed Cases of Typhoid Fever	Annual Incidence per 10^5
1978-1979		
Vaccinees	0	0
Placebo	7	44
1979-1980		
Vaccinees	0	0
Placebo	8	50
1980-1981		
Vaccinees	1	6
Placebo	7	44

TABLE 13

INCIDENCE OF CONFIRMED TYPHOID FEVER IN SCHOOLCHILDREN IN AREA NORTE, SANTIAGO, FIELD TRIAL OF TY21A VACCINE (ENTERIC-COATED CAPSULES) DURING THE FIRST 18 MONTHS OF SURVEILLANCE

	Placebo		Two Dose Vaccine			One Dose Vaccine		
Months of Surveillance	No. Cases	Rate/10^5	No. Cases	Rate/10^5	Vaccine Efficacy	No. Cases	Rate/10^5	Vaccine Efficacy
1-9	53[A]	167	15[B]	55	67%	32	98	41%
10-12	15	47	16	58	0%	25	76	0%
13-18	16[C]	50	2[D]	7	86%	13	40	21%
1-18	84	265	33	120	55%	117	256	19%

A VS B $P<0.005$

C VS D $P=0.009$

Table 13A

CALENDARIO, METAS Y NECESIDADES. CAMPAÑA

VACCINE FORMULATION AND SCHEDULE	JULIO 27	AGOSTO 16	AGOSTO 17	AGOSTO 19	AGOSTO 23	SEPTIEMBRE 7
ENTERICA	1	1				1
VIVOTIF — LONG SCHEDULE	2	2				2
	← 20 días →			← 21 días →		
ENTERICA			1	1	1	
VIVOTIF — SHORT SCHEDULE			2	2	2	
PLACEBO			3	3	3	
(Aprox.) TOTAL NIÑOS A VACUNAR	40.000	40.000	60.000	60.000	60.000	40.000
TOTAL CURSOS A VACUNAR	1.680	1.680	2.520	2.520	2.520	1.680
TOTAL VACUNADORES Vac. 1 = 1 x 10 Vac. 2 = 1 x 5	252	252	336	336	336	252
SUPERVISORES (1 x 7 Vacunadores)	36	36	48	48	48	36

Table 14

A SUMMARY OF COUNTS OF VIABLE Ty21a VACCINE ORGANISMS IN ENTERIC-COATED AND GELATIN CAPSULE FORMULATIONS AT DIFFERENT POINTS IN TIME

Date Sample Vaccine Capsule were Sent to the Laboratory	Source of Sample Vaccine Capsules	Date of Quantitative Cultures	Testing Laboratory	Results of Quantitative Cultures	
				Enteric-Coated Vaccine	Vaccine in Gelatin Capsules
July 22, 1983	Unopened vaccine prior to field use	July 26 and 27, 1983	Inst. of Public Health, Chile	$1-4 \times 10^9$*	$1-4 \times 10^9$*
Aug. 26	Vaccine capsules from the field	Sept. 6 and 7	Inst. of Public Health, Chile	6×10^7	$2-3 \times 10^9$
	Vaccine capsules from the field	Sept. 13	Inst. of Public Health, Chile	5×10^7	3×10^8
	Vaccine capsules from the field	Sept. 21	Inst. of Public Health, Chile	6×10^7	2×10^8
Oct. 9	Vaccine capsules from the field	Nov. 5	Ctr. for Vaccine Development, Baltimore	2×10^7	$2-4 \times 10^7$

*No. of viable vaccine organisms per dose. Ideally each dose should contain $1-3 \times 10^9$. Below 10^9 is unacceptable.

Table 15

RATES OF SEROCONVERSION OF *SALMONELLA TYPHI* O ANTIBODY MEASURED BY IgG-ELISA IN CHILEAN AIR FORCE CADETS WHO RECEIVED Ty21a ORAL VACCINE IN ENTERIC COATED CAPSULES AND COMPARISON WITH EFFICACY IN THE AREA NORTE FIELD TRIAL

No. Vaccine Doses	No. Cadets with Seroconversions/ No. Vaccinated (%)		Vaccine Efficacy in Area Norte Field Trial after 12 Mos. of Surveillance
2	22/50 (44)	$p<0.01$	46%
1	9/50 (18)		20%
placebo	1/49 (2)		-

Table 16

SEROCONVERSION RATES OF IgG ELISA ANTIBODY TO SALMONELLA TYPHI O ANTIGEN IN CHILEAN AIR FORCE CADETS IMMUNIZED WITH TY21A VACCINE BY VARIOUS FORMULATIONS AND SCHEDULES

Vaccine Group	Seroconversion Rate (%)	Mean Net OD Pre	Post #1	Post #2
Enteric-coated Days 1,4,7	61/96 (64)	0.23 ± 0.03	0.60 ± 0.06	0.55 ± 0.05
Gelatin Capsules Days 1,4,7	50/96 (52)	0.19 ± 0.03	0.52 ± 0.05	0.47 ± 0.05
Enteric-coated Capsules Days 1,21,42	37/93 (40)	0.24 ± 0.04	0.39 ± 0.04	0.36 ± 0.04

Table 17

COMPARISON OF TWO DIFFERENT BLOOD CULTURE REGIMENS IN INCREASING THE BACTERIOLOGIC CONFIRMATION OF TYPHOID FEVER IN 61 PATIENTS BY USE OF TWO OR THREE BLOOD CULTURES

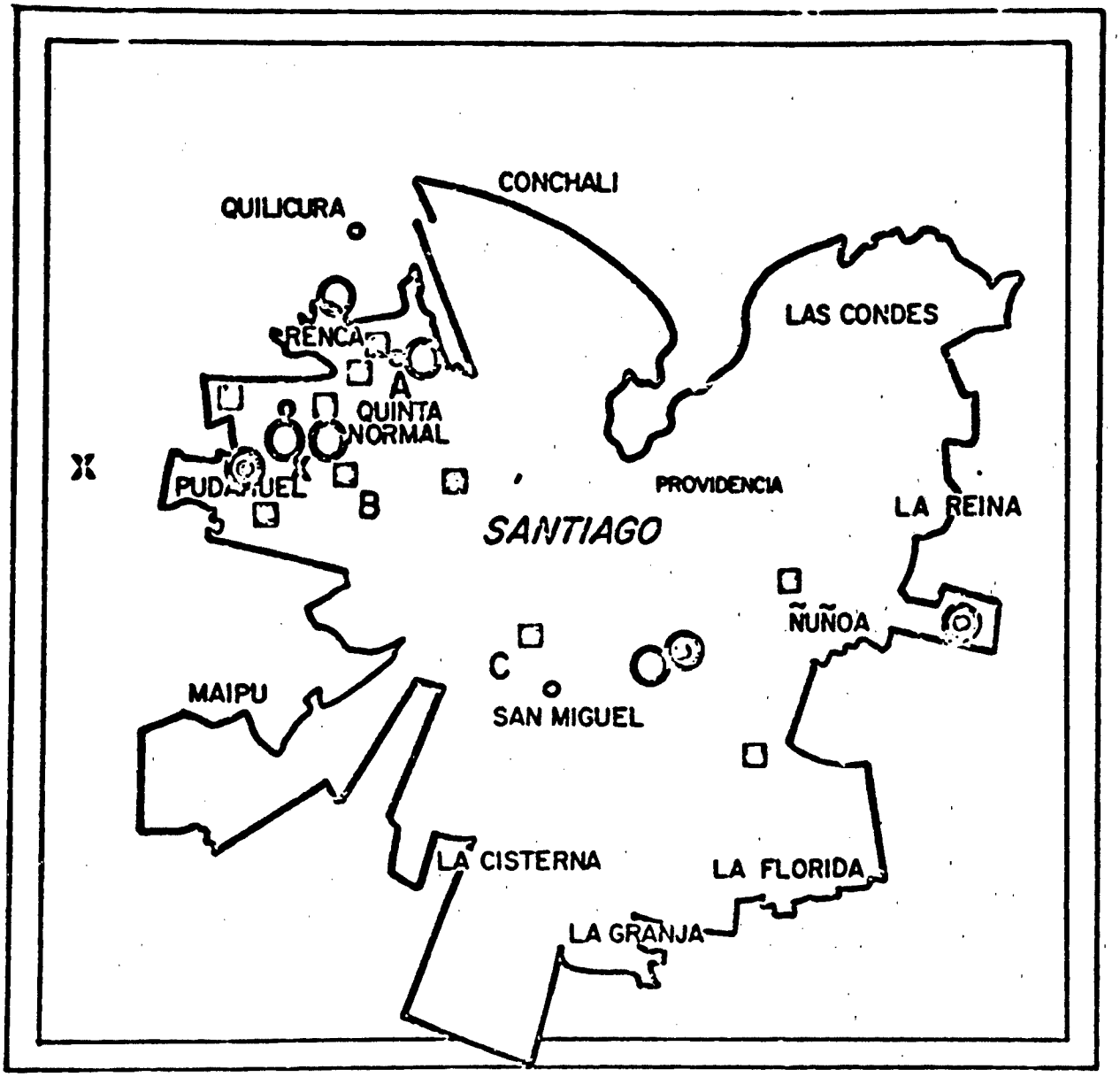
Group I

Total Patients with Positive Blood Culture	Number Positive in an Initial 4 ml Blood Culture	Number Positive Only in a Second 8 ml Blood Culture	Increase in Positivity Attributable to the Second Culture
34	27	7	18%

Group II

Total Patients with Positive Blood Culture	Number Positive in an Initial 4 ml Blood Culture	Number Positive in a Second 4 ml Blood Culture (but negative in first)	Increase in Positivity Attributable to a Second 4 ml Blood Culture	Number Positive in a Third 4 ml Blood Culture (persons whose first two cultures were negative)	Increase in Positivity Attributable to a Third 4 ml Blood Culture
27	23	3	12%	1	4%

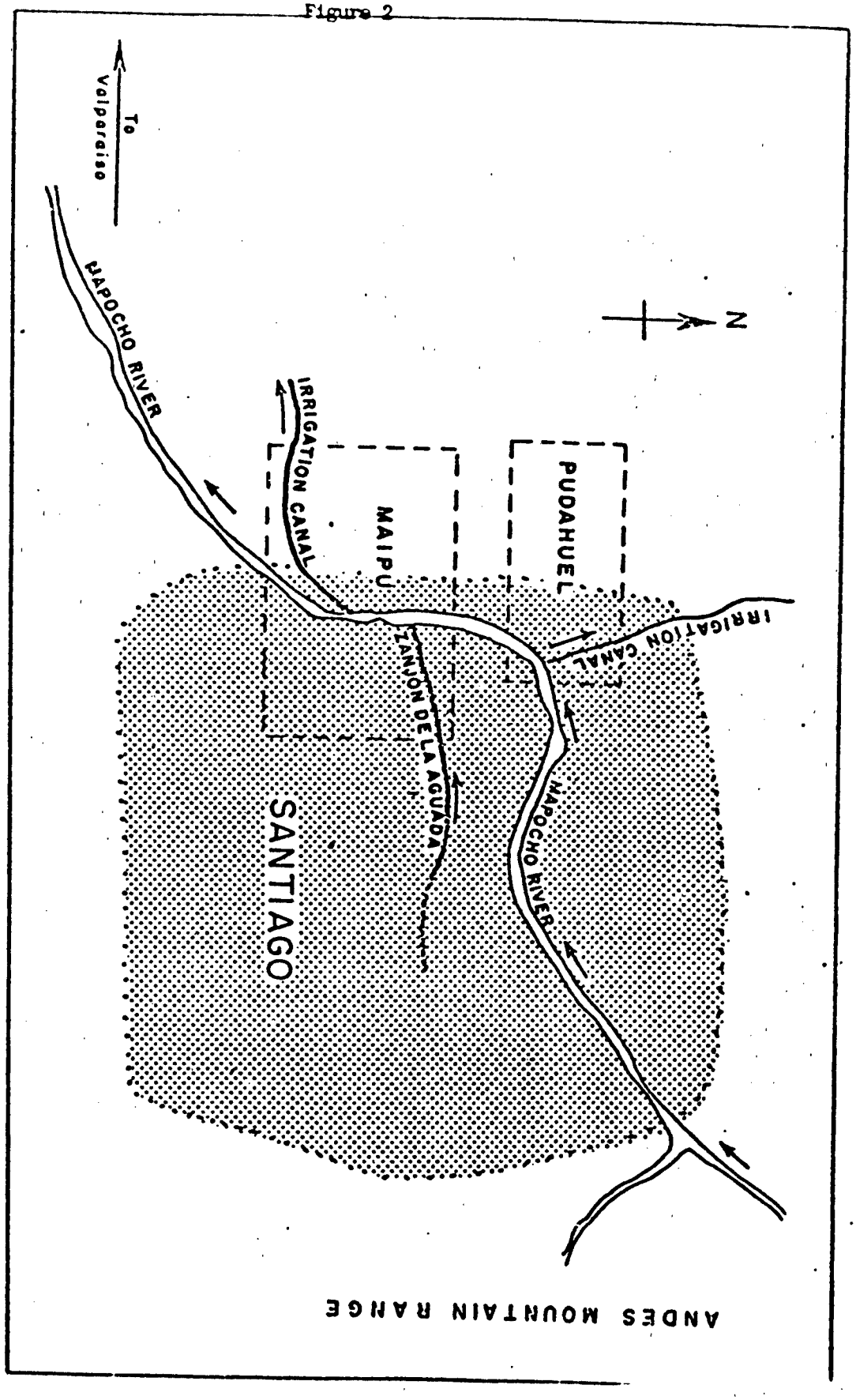
Fig.1 SANTIAGO METROPOLITAN AREA: LOCATION OF HOSPITALS AND HOUSEHOLDS STUDIED



HOSPITALS
- A HOSPITAL FELIX BULNES
- B HOSPITAL SAN JAUN de DIOS
- C INFECTIOUS DISEASE HOSPITAL

HOUSEHOLDS
- X FAMILY MEMBERS WITH TYPHOID
- O FAMILY MEMBERS WITH SHIGELLA
- o FAMILY MEMBERS WITH NON-TYPHOIDAL SALMONELLA
- □ NO OTHER CULTURE-POSITIVE FAMILY MEMBERS

Figure 2


To Valparaiso
MAPOCHO RIVER
N
IRRIGATION CANAL
MAIPU
PUDAHUEL
IRRIGATION CANAL
CANJON DE LA AGUADA
MAPOCHO RIVER
SANTIAGO
ANDES MOUNTAIN RANGE

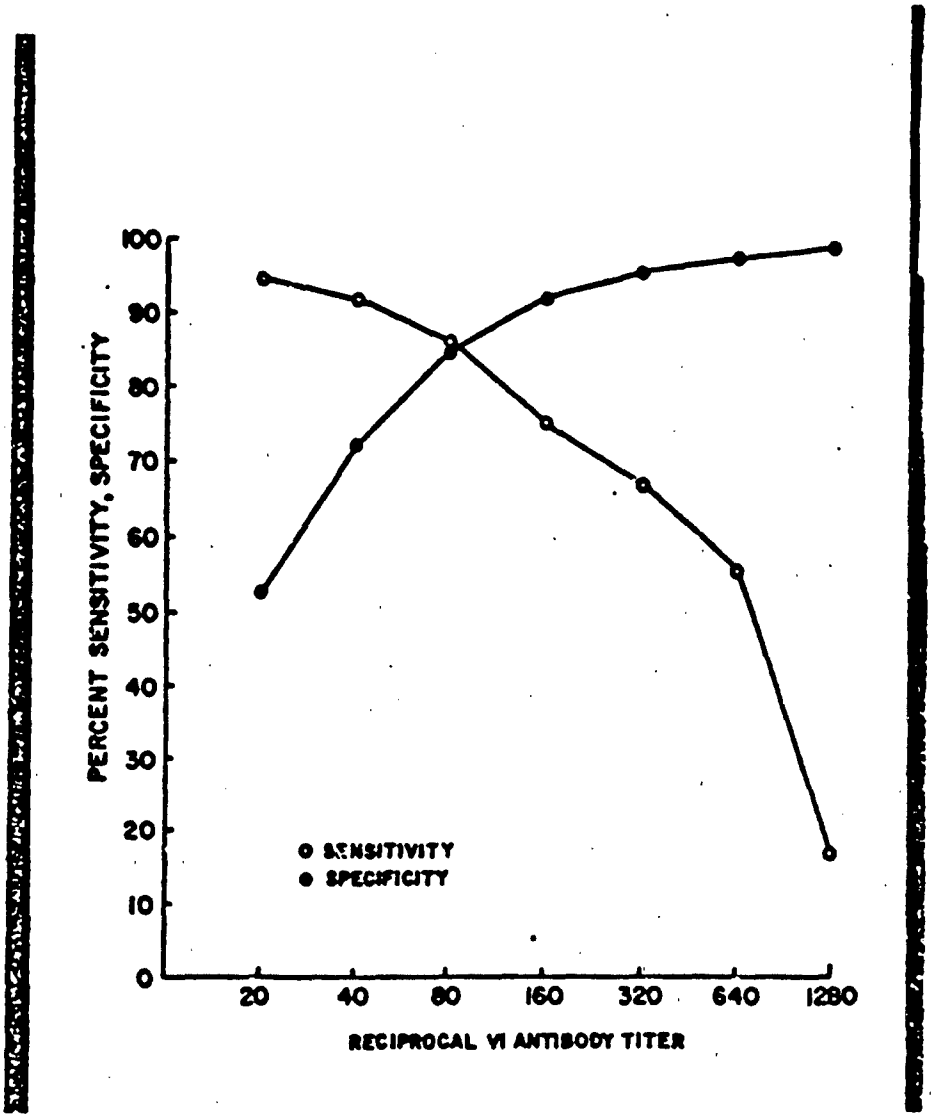


Figure 3. Sensitivity and specificity of various reciprocal Vi antibody titers as cut-off points in screening for chronic Salmonella typhi carriers, Santiago, Chile.

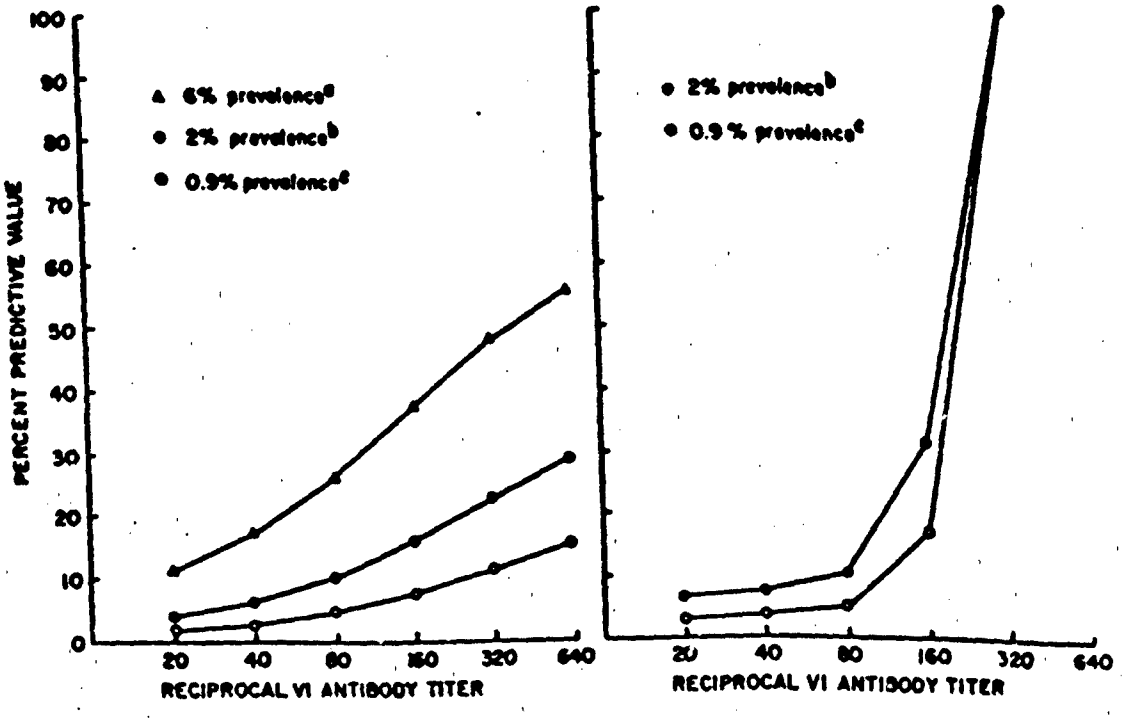


Figure 4. Predictive value of various reciprocal Vi antibody titers as cut-off points in screening for chronic Salmonella typhi carriers in populations in Santiago, Chile with different carrier prevalence rates. Fig. 2-a using culture-negative women 25 years or older with documented typhoid fever 1-4 years earlier as negative controls. Fig. 2-b: using healthy adult population, both sexes, as negative controls. Santiago, Chile.

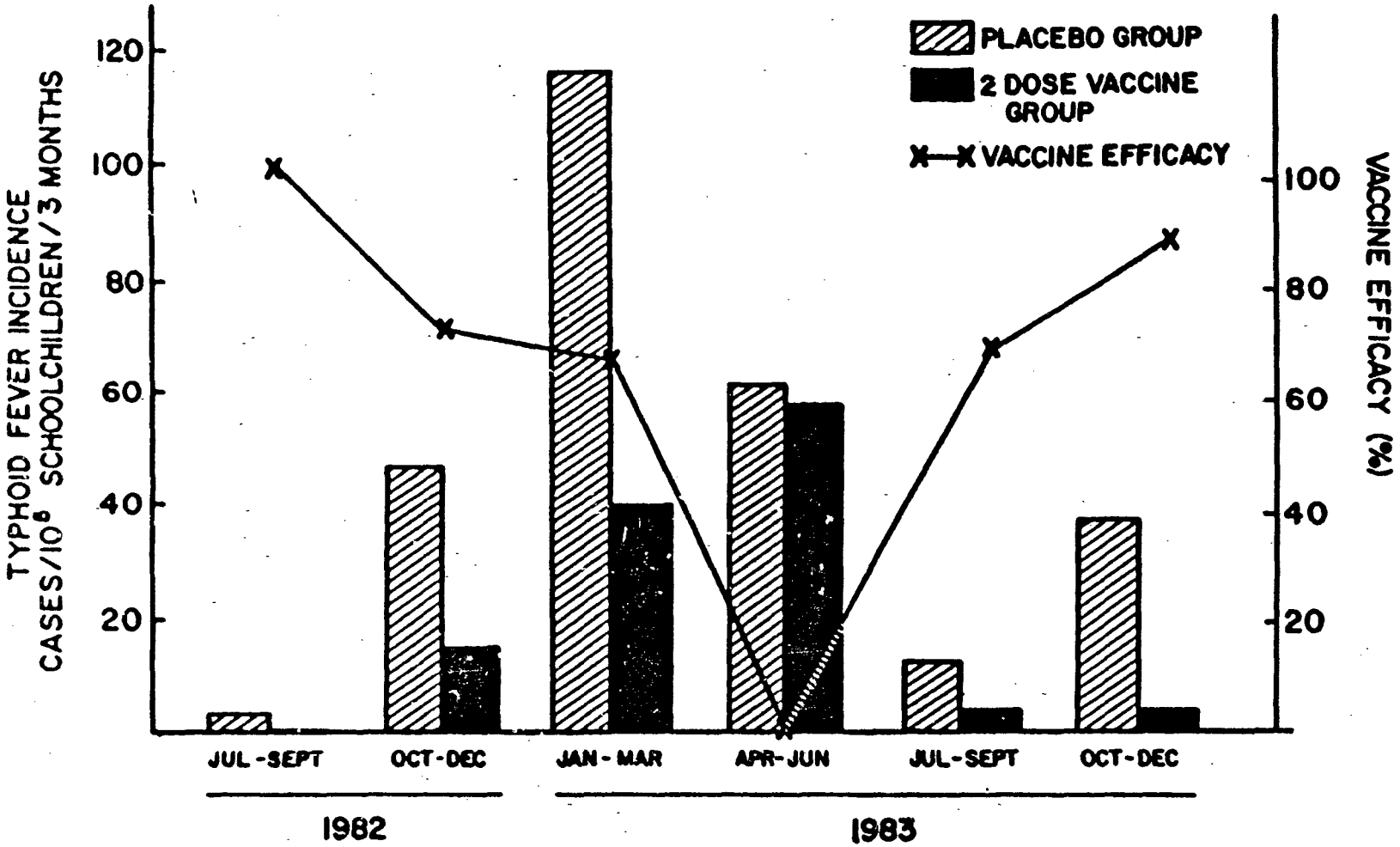
a. Women 25 years or older with documented typhoid fever 1-4 years earlier (C.F. Lanata, unpublished information).

b. Women 40 years or older (10).

c. Both sexes, 10 years or older (10).

Figure 5

INCIDENCE OF TYPHOID FEVER IN CHILEAN SCHOOLCHILDREN WHO RECEIVED TWO DOSES OF Ty21a(ENTERIC-COATED CAPSULES) OR PLACEBO DURING 18 MONTHS OF SURVEILLANCE



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