Cholera Vaccine Field Trial in Rural East Pakistan[1] (First Year of Observation)

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Cholera vaccine was the first antibacterial vaccine introduced for the control of communicable disease in man. Despite widespread use of the vaccine, convincing proof of its efficacy is still not available. Accordingly, in November 1963 the Pakistan-SEATO Cholera Research Laboratory initiated a controlled field trial of cholera vaccine in rural East Pakistan. The study was designed to answer three related questions: (1) Can an injected vaccine prevent the diarrhea associated with *Vibrio cholerae*? (2) If so, for how long? and (3) Does the vaccine prevent inapparent infection among family contacts of persons with diarrhea associated with *V. cholerae*? This report deals with the experience of the first year of this field trial.

Methods

A group of 23 villages in Matlab Thana, an administrative subdivision of Comilla District about 40 miles from Dacca, was selected for study because of continued high prevalence of cholera in the recent past and location along the Gumti River, which allowed easy access by boat for case-finding and treatment. The villages are in one of East Pakistan's most densely inhabited areas; the population density exceeds 1,500 per square mile.

Local health officers had prepared a census of the population of these villages during a smallpox control program in 1961. On the basis of this census the population was estimated to be 24,000. These census lists were revised by a house-to-house survey, and each individual within each village was assigned a number in consecutive order with a letter prefix identifying the village.

The cholera vaccine selected for test was a commercial preparation containing a mixture of both an Inaba and an Ogawa strains, using the classical "Indian" strains 35A3 and 41, respectively. The vaccine was prepared by harvesting surface agar growth in 0.5 percent phenolized saline, and diluting the two components to an optical density equivalent to 8,000 million *V. cholerae* organisms per ml. The finished vaccine had a relative mouse potency of 4.40 against the Ogawa, and 32.4 against the Inaba reference vaccines of the National Institutes of Health, Bethesda, Md.[2] The vaccine contained 1.3 mg. total nitrogen per ml., 0.08 mg. trichloracetic acid-precipitable nitrogen per ml., and 0.9 mg. nitrogen per ml. precipitated by 1.5 volumes of acetone.[3] Typhoid-paratyphoid A-paratyphoid B vaccine was used as the control. The organisms were grown on agar surfaces, heat-killed, suspended in 0.5 percent phenol, and diluted by nephelometric density so that the finished vaccine contained 1,000 million typhoid and 750 million each of paratyphoid A and paratyphoid B organisms per ml. The two vaccines were distributed in identical bottles, one labeled by a red letter "A," and the other by a blue "B." The materials were coded by one observed who did not reveal the identity of the test materials throughout the field trial. The dosage schedule was 0.5 ml. for those over 12 years of age, 0.25 for those 2 to 12, and 0.1 for those under 2. After 7,027 persons had been inoculated, the dose was reduced to 0.4 ml. for those over 12 years of age because local reactions had interfered with the participation of adults.

Immunizations were performed during a 6-week period beginning on November 1, 1963. The vaccination team and their equipment proceeded along the river by houseboat. Jet injectors were used almost exclusively to perform the inoculations. Persons who had been assigned an odd number received vaccine "A," and those assigned an even number received vaccine "B." Family members who appeared at the time of vaccine administration, but who had been overlooked during the census review a few days earlier, were added to the lists and given alternately vaccine "A" or "B." All recipients were given a card, one side of which showed the following: Village, vaccine trial number, name (in Bengali), age, sex, and date of inoculation. On the reverse side of the card, a notice in Bengali requested that the study team be notified promptly in case of diarrhea, and that the patient come at

[1] This work was supported in part by Research Agreement No. 196802 between the National Institutes of Health, Bethesda, Md., U.S.A., and the Pakistan-SEATO Cholera Research Laboratory.

[2] These assays were performed by Dr. John C. Feeley, Division of Biologics Standards, National Institutes of Health.

[3] Chemical determinations were performed by Dr. John Barbaro, Department of Immuno-chemistry, Walter Reed Army Institute of Research, Washington, D.C.

once to the central field hospital for treatment if symptoms suggested cholera.

Medical care was provided by this laboratory in a facility situated in Matlab Bazar, a centrally located market community. A physician and nurse were in constant attendance at the hospital which was housed first in a small tin shed and later on a barge provided by the Government of East Pakistan. Patients were treated with intravenous fluids and tetracycline (Greenough, *et al.*, 1964).

For case finding, the 23 villages were divided into 3 groups. Surveillance within each group was under the supervision of an experienced fieldworker who had received graduate training in social sciences. Each area supervisor was assisted by seven fieldworkers and a midwife resident in each village. Families were visited twice weekly to inquire about diarrheal disease and encourage prompt use of the field hospital in the event of illness. Whenever a new episode of diarrhea occurred, details of the illness were recorded on a simple report form, and a fecal sample was collected by means of a tellurite-impregnated rectal swab which was placed in bile-peptone transport medium. Specimens were sent to the laboratory in Dacca, usually within 24 hours, for further bacteriological investigation. The laboratory procedures employed have been described previously (Monsur, 1963). Fecal specimens obtained from hospitalized patients were streaked directly on tellurite-taurocholate-gelatin agar and were also inoculated into the liquid transport medium.

Disability associated with diarrheal disease was classified as follows: No interference with usual duties, inability to perform usual duties, inability to walk, and inability to respond. Whenever persons with diarrhea showed inability to walk or respond, fecal samples were collected from vaccinated contacts and they were queried concerning diarrhea. These procedures were performed on 2 consecutive days during the first 6 months of the study, and on 4 consecutive days thereafter.

The area supervisors investigated and reported each death occurring among vaccine recipients. Whenever death was associated with acute diarrhea, fecal samples were collected from household contacts if none had been obtained from the deceased before or after death.

Results

The total population of the 23 villages was 27,639, of which 14,059 (50.9 percent) participated in the trial (table 1). An excess of males (114.8 males per 100 females) was observed, a finding consistent with previous estimates showing sex ratios in the 17 districts of East Pakistan ranging from 106.5 to 116.8 males per 100 females (Census of Pakistan, 1951). Participation was greatest (61.8 percent) among 5 to 14 year old groups. Males appeared to be less cooperative than females (47.4 percent versus 54.9 percent); this may be an artefact related to the purdah system.

Vaccine "A" was given to 7,103 persons and "B" to 6,956. The two groups did not differ by age, sex, history of prior cholera or past anticholera inoculation (table 2).

During the first 7 days after inoculation, *V. cholerae* Inaba was isolated from 6 persons with diarrhea. One had received vaccine "A" and 5 vaccine "B." These people have not been included in the analysis. The frequency of subsequent diarrheal episodes occurring in the two groups within the first year of the trial is shown in table 3. Findings are summarized by 3-month intervals according to the presence or absence of vibrios on culture, and whether the individuals were observed in the community or the hospital. Twenty-nine instances of cholera infection were seen in the first 3-month period, 13 in the second, only 1 in the third, and 12 in the last quarter of the year.

Table 1.—Participants in vaccine study by age and sex

Age group (years)	Male			Female			Total		
	Number of inhabitants	Vaccinated		Number of inhabitants	Vaccinated		Number of inhabitants	Vaccinated	
		Number	Percent		Number	Percent		Number	Percent
Less than 5	2,607	1,273	48.8	2,665	1,263	47.4	5,272	2,536	48.1
5–14	4,417	2,638	59.7	3,355	2,160	64.4	7,772	4,798	61.7
15 or over	7,731	3,088	39.9	6,837	3,632	53.1	14,568	6,720	46.1
Not recorded	11	4	.....	6	1	.....	17	5	.....
Total	14,766	7,003	47.4	12,863	7,056	54.9	27,639	14,059	50.9

TABLE 2.—Vaccine groups by age, sex, history of previous cholera and prior cholera inoculation

	Vaccine A		Vaccine B	
	Number	Percent	Number	Percent
Total	7,103	100.0	6,956	100.0
Age (years):				
Less than 5	1,288	18.1	1,248	17.9
5–14	2,414	34.0	2,384	34.3
15 or over	3,396	47.8	3,324	47.8
Unknown	5	0.1	.....	.....
MALES				
Age (years):				
Less than 5	621	17.3	652	19.0
5–14	1,342	37.5	1,296	37.9
15 or over	1,613	45.1	1,475	43.1
Unknown	4	0.1	.....	.....
Total	3,580	100.0	3,423	100.0
FEMALES				
Age (years):				
Less than 5	667	18.9	596	16.9
5–14	1,072	30.4	1,088	30.8
15 or over	1,783	50.6	1,849	52.3
Unknown	1	.....	.....	.....
Total	3,523	99.9	3,533	100.0
History of previous cholera	415	5.9	414	6.0
No history of previous cholera	6,577	94.1	6,440	94.0
Total with known history	6,992	100.0	6,854	100.0
Prior cholera immunization	5,465	78.4	5,347	78.2
No history of cholera immunization	1,508	21.6	1,488	21.8
Total with known history	6,973	100.0	6,835	100.0

The number of infections occurring in the "A" group exceeded that in the "B" group during each interval when there were enough cases to permit comparison. The frequency of diarrheal episodes from which vibrios were not isolated was similar in both groups. The age distribution of persons with vibrio-associated diarrhea is shown in table 4. Forty-eight of the 55 infected individuals were less than 15 years of age and 25 were less than 5 years of age. The overall incidence rate during the first year was 6.1 per 1,000 vaccine "A" recipients and 1.7 per 1,000 given vaccine "B." The difference between these rates is highly significant ($P = <.001$).

Only 7 asymptomatic infections were discovered among 91 inoculated family contacts. Three of the 7 had received vaccine "A" and the remaining 4, vaccine "B."

Eighty-eight deaths occurred in the study group (table 5). The total number of deaths and the proportion associated with gastrointestinal sympoms, such as "diarrhea," "loose stools," "dysentery," and "mucus and blood in stools," were similar in both vaccine groups. Rectal swabs were obtained from either patients or family contacts in 26 of the 32 deaths associated with gastrointestinal symptoms, and *V. cholerae* was isolated from only 1 of the 26. This patient (P1069) was a 5-year-old male who was given vaccine "A" on November 29 and developed typical cholera on December 9. His family refused to permit hospitalization.

DISCUSSION

While previous reports (Haffkine, 1895; Adiseshan, *et al.*, 1947) indicated that cholera inoculation reduced the incidence of the disease, the evidence was not convincing. These earlier studies were criticized (Greenwood and Yule, 1915; MacLeod, 1960) because of faulty experimental design including the lack of a suitable control group, failure to observe a strict double-blind technique, the selection of subjects during an epidemic resulting in unequal exposure to infection in the groups being compared, and inadequate diagnostic data. In this study a double-blind technique with a suitable control group was used; the two test groups were comparable in every respect measured. Intensive surveillance was maintained, and only bacteriologically confirmed instances of cholera infection were included. The riverine villages which were selected provided a typical endemic setting.

Cholera reporting in most endemic areas has been incomplete and is based largely on deaths certified by nonmedical offiicals. The present study provided an opportunity to determine accurately the frequency of diarrheal diseases associated with *V. cholerae* in a defined population. Infections were more frequent among children, as might be expected in an endemic situation.

A significantly lower incidence of diarrhea associated with *V. cholerae* was observed in the group which received vaccine "B," which proved to be the cholera vaccine. Thus, the data collected during the first year of the trial indicate that cholera vaccine can be an effective prophylactic procedure. However, the duration of effectiveness remains unknown, and surveillance is being maintained to answer this question. Data concerning the effect of inoc-

TABLE 3.—Frequency of diarrheal episodes during the first year by cultural result and place of observation

Time	December 1963 to February 1964		March to May 1964		June to August 1964		September to November 1964		December 1963 to November 1964	
Vaccine group	A	B	A	B	A	B	A	B	A	B
V. cholerae										
Culture positive: [1]										
Community	10 (3)	4 (1)	5	1	0	0	5 (4)	3 (2)	20 (7)	8 (3)
Hospital	14 (1)	1	5	2	0	1	4 (1)	0	23 (2)	4
Total	24 (4)	5 (1)	10	3	0	1	9 (5)	3 (2)	43 (9)	12 (3)
Culture negative:										
Community	708	684	1,508	1,447	2,738	2,667	2,377	2,312	7,331	7,110
Hospital	13	12	49	38	21	20	13	17	96	87
Total	721	696	1,557	1,485	2,759	2,687	2,390	2,329	7,427	7,197

[1] All isolates were Inaba serotype except those indicated in parenthesis.

TABLE 4.—Frequency of diarrhea associated with *V. cholerae* by age and vaccine groups during the first year after immunization

Age group (years)	Vaccine A		Vaccine B	
	Number	Rate [1]	Number	Rate [1]
Less than 5	19	14.8	6	4.8
5–14	18	7.5	5	2.1
15 and over	6	1.8	1	0.3
Total	43	6.1	12	1.7

[1] Per 1,000 persons.

TABLE 5.—Deaths in vaccinated persons during the first year

Time interval	Deaths associated with gastrointestinal symptoms		Total deaths	
	Vaccine A	Vaccine B	Vaccine A	Vaccine B
December 1963 to February 1964	4	2	11	7
March to May 1964	4	2	15	12
June to August 1964	2	4	5	12
September to November 1964	7	7	14	12
December 1963 to November 1964	17	15	45	43

ulation on inapparent infection are too scanty as yet to permit conclusions.

Since this study was designed to ask whether or not an injected vaccine can protect against cholera, a commercial preparation with unusually high mouse potency was used. Hence the inference cannot be made that all cholera vaccines will afford protection.

CONCLUSION

During the first year of a controlled field trial of cholera vaccine in an endemic area, the incidence rate of diarrhea associated with *V. cholerae* was 6.1 per 1,000 among those receiving a control (TAB) vaccine, and 1.7 per 1,000 among those receiving a commercial cholera vaccine of high potency. The disease was concentrated among children.

ACKNOWLEDGMENT

We wish to acknowledge the help of the following persons in the conduct of this vaccine trial: Colonel S. A. Mallick, Director of Health Services, East Pakistan; Dr. N. R. Majumdar, District Health Officer, Comilla, East Pakistan; Dr. S. H. Rizvi, Chief, Bacteriology Section, Pakistan-SEATO Cholera Research Laboratory (CRL); Dr. R. S. Gordon, Jr., former Chief, Clinical Research, CRL; Dr. W. B. Greenough III, Chief, Clinical Research, CRL; Dr. John Lindenbaum, Assistant Chief, Clinical Research, CRL; Drs. A. K. M. Jamiul Alam, Md. Matlab Ali, Md. Rafique Islam and Md. Shamsuz Zoha, Ward Physicians, CRL; S. K. Sarker and B. C. Kormakor, Nurses, CRL; S. O. Q. Haider Ali, A. Momin Chowd-

hury, Md. Shafiqul Islam and S. A. Khan, Field Supervisors, CRL; and Nuzhat Jahan, Record Systems, CRL.

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Joint Philippine-Japan-WHO Controlled Field Trials of Cholera Vaccines (Interim Report of Dec. 5, 1964)

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Since the discovery of its endemic nature and the exposing of its pandemic character in 1961, cholera El Tor has attacked one country after another. Now it is one of the major public health problems in the western Pacific region, and is fast becoming so in southeast Asia. Under these circumstances WHO, the Philippines, and Japan have undertaken studies on the effectiveness of vaccination against cholera El Tor, and upon the roles of carriers and of foodstuffs in the transmission of this disease.

Although vaccination is considered to be a practical weapon against cholera El Tor as well as classical cholera under the prevailing local situation in southeast Asia, no classical cholera or El Tor vaccine has ever been assessed for its effectiveness through controlled field trials. Such a test is essential, especially since the experience with recent typhoid vaccine studies in Yugoslavia. Taking advantage of the epidemic of cholera El Tor in the Philippines, where endemicity is low, large-scale, controlled field trials of cholera vaccines were planned in December 1963, and have been in operation since May 1964.

The objectives of the trials are: (1) to assess the effectiveness and duration of protection gained from a single dose of one of the three cholera vaccines (namely, classical cholera vaccine, classical cholera-oil adjuvant vaccine, and El Tor vaccine) in comparison with a typhoid vaccine applied as a control; (2) to observe possible side reactions from each vaccine; and (3) to try to establish reliable serological or animal tests, which would correlate well with the results of the controlled field trials.

Design of the Studies

I. *General principles*

1. *Indicators for evaluation.*—It was anticipated that the number of deaths of bacteriologically confirmed cases among the three groups immunized with the different cholera vaccines would not reveal statistically significant differences from those among the control group; and that reliable reports as to the severity of cases could not be obtained from various hospitals scattered in the trial area. Thus, it was expected that the best indicators for evaluation of the effectiveness of the three cholera vaccines should be the evidence of unequivocal bacteriologically confirmed cases and carriers.

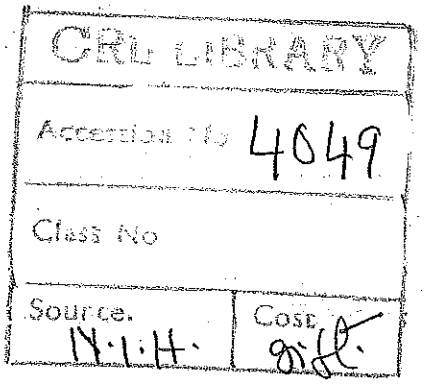
2. *Methods.*—In order to avoid bias, the principles of controlled field trials with the placebo typhoid vaccine, and the double-blind test method, labelling the three cholera and placebo vaccines with code letters (table 1), were strictly observed throughout the vaccination campaign, surveillance work, and laboratory examinations. For the same reason, the system of random allocation of vaccines to the population was adhered to throughout the vaccination campaign. The success of this program was proved by the similarity in the populations of the four vaccine groups as revealed by the provisional one percent

Proceedings of the Cholera Research Symposium

JANUARY 24–29, 1965
HONOLULU, HAWAII

Convened at the East-West Center of the University of Hawaii. Sponsored by the University of Hawaii's Pacific Biomedical Research Center and the Center for Cultural and Technical Interchange between East and West.

Funded by a contractual arrangement with the University of Hawaii, financed by the National Institutes of Health from funds made available to it by the Agency for International Development for the SEATO Cholera Research Program.



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