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A Controlled Field Trial of the Effectiveness of Cholera and Cholera El Tor Vaccines in the Philippines *

Preliminary Report

PHILIPPINES CHOLERA COMMITTEE [1]

In a controlled field trial on some 584 000 people in an endemic cholera El Tor area in the Philippines, it was demonstrated that cholera vaccines gave moderate protection of short duration. Injection of a single dose of vaccine prepared from either Vibrio cholerae *or* Vibrio El Tor *gave over 50 % protection for the first two months. The immunity conferred by the* V. cholerae *vaccine rapidly declined after three to four months. The* V. El Tor *vaccine gave protection for six months, but its effectiveness declined. An oil-adjuvant vaccine prepared from* V. cholerae *conferred an increasing degree of protection of long duration, but, owing to severe vaccination reactions, its use could not be recommended.*

INTRODUCTION

Since Ferran (1885) introduced the first cholera vaccine 80 years ago, no evaluation of it has been made in a strictly controlled field trial. Collaborative studies on the efficacy of cholera vaccine were initiated in 1964 following an agreement between the Government of the Philippines, the Government of Japan, and the World Health Organization. The controlled field study in the Philippines was carried out in an effort to obtain new information on the efficacy of vaccines prepared from *Vibrio cholerae* and *Vibrio El Tor* against infection with cholera El Tor.

Oil-adjuvant vaccine developed by H. Ogonuki and produced in Japan, and El Tor vaccine and classical cholera vaccine produced in the Philippines by methods in current use were studied in a controlled trial on 584 026 persons. Funds, equipment and personnel for the study were provided by the two governments and WHO.

OBJECTIVES OF THE TRIAL

The objectives of the trials were:

(*a*) to assess the protective power of vaccines against clinical attacks of cholera due to *V. El Tor*;

(*b*) to assess the protective power of the vaccines against asymptomatic infection with *V. El Tor* and its carrier stage;

(*c*) to assess the degree and duration of protection (if any) and the effectiveness of each vaccine; and

(*d*) to study the reaction of immunized people to each vaccine.

The study was organized according to the principles of strictly controlled field trials (Hill, 1951).

The effectiveness of each vaccine was evaluated by comparing the morbidity and carrier rates due to cholera El Tor among those vaccinated with each of the cholera vaccines and those vaccinated with a control vaccine antigenically unrelated to *V. cholerae* or *V. El Tor*.

The group vaccinated with cholera vaccines and the group vaccinated with the control vaccine were

* This study was supported by grants from WHO, the Ministry of Health, Japan, and the Government of the Philippines. The studies were planned by the Joint Philippines-Japan-WHO Cholera Committee and executed by the Philippines Cholera Committee with the assistance of field staff from the Philippines, Japan and WHO.

[1] The members of the Philippines Cholera Committee were: Dr J. C. Azurin, Director of Quarantine, Manila (over-all co-ordinator of the study), Dr A. Cruz, Director of Health Services, Manila (project director of the vaccine field studies), Dr J. Dizon, Chief, Diseases Intelligence Center, Manila (director of epidemiological studies), and Dr T. Pesigan, Director, Bureau of Research and Laboratories, Manila (supervisor of the production of vaccines).

similar in all respects—namely, age, sex, occupation, economic status and hygienic standards. This similarity was achieved by a random allocation of vaccines to the population.

The cholera vaccines used were demonstrated to be safe, and they met the potency tests described by the WHO Study Group on Requirements for Yellow Fever Vaccine and Requirements for Cholera Vaccine (1959).

In order to avoid bias on the part of the observers and the observed, the cholera vaccines and the control vaccine were indicated by code letters. Cases of cholera were thoroughly examined bacteriologically to confirm the diagnosis.

In order to satisfy ethical principles and to ensure public acceptance and co-operation, efforts were made to provide maximum possible health care for the population in the trial area.

The above and other relevant principles were applied when the protocol for the study was prepared.

STUDY AREA AND POPULATION

Negros Occidental province covers an area of some 7740 km² and consists of 28 municipalities and three cities. The study area is shown in the accompanying figure.

The 1960 census showed that Negros Occidental had a population of 1 332 323, of whom 682 808 were males and 649 515 females. The inhabitants are engaged mainly in the sugar and fishing industries.

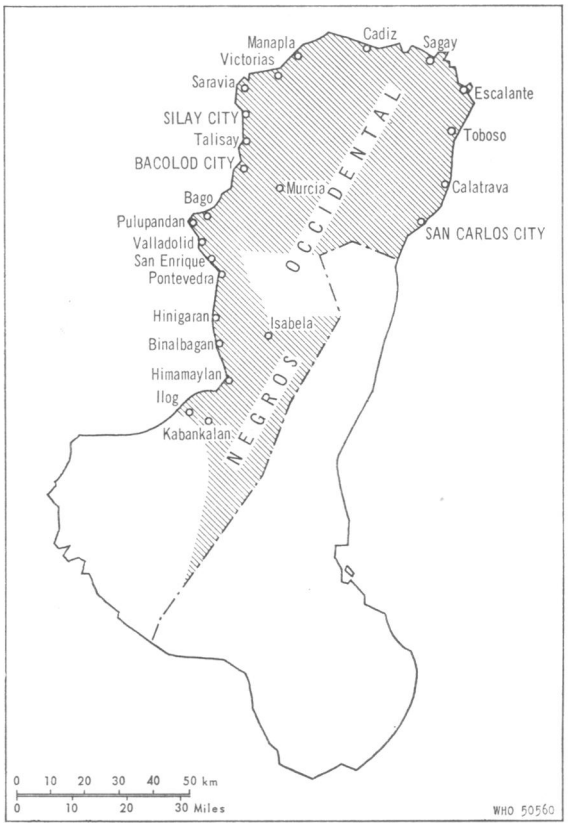
Roughly 160 000 people depend for their water supply on drilled wells. The rest of the population (63%) obtain their drinking water from shallow wells.

No town has a sewerage system. Excreta disposal is achieved by means of 4500 septic tanks and 105 000 pit privies. The rest of the population use various unhygienic methods of disposal.

All municipalities have rural health units. There are 276 health workers in the entire province, including health officers, public health nurses, midwives, and sanitary inspectors.

The expected over-all incidence of cholera in the area was estimated at 0.5 per 1000 of the population. It was therefore decided that at least 520 000 voluntary vaccinees should be included in the trial in order to obtain statistically significant results.

NEGROS OCCIDENTAL, SHOWING THE AREA OF THE FIELD TRIAL (SHADED) AND THE PRINCIPAL TOWNS COVERED IN THE TRIAL [a]



[a] Two districts of the province were excluded from the field trial and have therefore been left unshaded.

VACCINES

The following vaccines were used in the controlled field trial:

(*a*) Cholera vaccine prepared in the Philippines according to the method adopted by the Bureau of Research and Laboratories in Manila. This vaccine was made from *V. cholerae*, strains Inaba 35A-3 and Ogawa 41, and met the requirements established by the WHO Study Group (1959).

(*b*) Cholera El Tor fluid vaccine prepared in the same laboratory from lyophilized cultures of *V. El Tor*, strains Ogawa 1418 and Inaba 6973.

(*c*) Cholera oil-adjuvant vaccine prepared according to the method developed by H. Ogonuki of the

Chiba Serum Institute, Japan, using true *Vibrio cholerae*, strains Inaba 35A-3 and Ogawa 41.

(*d*) Monovalent typhoid vaccine prepared by the Bureau of Research and Laboratories, Manila, which was used as a control.

Full details of vaccines *a*, *b* and *c* are given in the Annexes.

The vaccines were administered in a single dose, according to the following scale:

(*a*) Cholera and cholera El Tor vaccines:

0-4 years of age	0.25 ml
5-9 years of age	0.5 ml
10 years and over	1.0 ml.

(*b*) The cholera oil-adjuvant vaccine:

0-4 years of age	0.05 ml
5-9 years of age	0.1 ml
10 years and over	0.2 ml.

Each millilitre of the vaccines contained 8×10^9 organisms. Vaccines were bottled in 50-ml containers marked with appropriate code letters. All the vaccinations were given subcutaneously in the upper arm.

MASS VACCINATION CAMPAIGN

In anticipation of the rise in the incidence of cholera, intensive preparation for the mass vaccination campaign was started during the first week of May. Members of the vaccination teams—vaccinators, recorders and supervisors—were recruited and trained. Instructions for field teams were distributed and used as a guide in the training. Each vaccination team was composed of one recorder and one vaccinator. The recorder filled in the cholera immunization cards and the random allocation cards. The vaccinator administered the vaccines indicated by the recorder.

The campaign started in mid-May 1964. The study area was divided into three sectors. A field supervisor was assigned to each sector, each supervisor having in his charge 54 teams. A total of 162 teams were sent to the field.

The vaccination campaign was conducted in public places such as markets and schools, in places of work such as sugar mills and plantations, and on a house-to-house basis. Vaccination was, however, administered only to volunteers. Random allocation of vaccines to volunteers was achieved by a random ordering of vaccine code letters, strictly adhered to.

The vaccination campaign lasted for about six weeks and was terminated on 3 July 1964. Records show that 584 026 vaccinees took part.

COMPARISON OF VACCINATED AND CONTROL GROUPS

The comparability of the four groups was achieved by strict application of the random allocation list of vaccine code letters. The groups were therefore similar in size and structure, containing the same distribution of age, sex, occupation, previous history of cholera and typhoid, and history of vaccination. They were also similar in other respects, such as the numbers of vaccinees with the same environment, conditions of work, and exposure to the disease. It may therefore be assumed that the susceptibility to the disease and the risk of infection were the same in all the groups.

To compare the four groups, an analysis was made of a 1% sample of the immunization cards—a total of 5840 cards. The analysis is presented in Appendix Tables 1-6, which show that the four vaccinated groups were similar.

REACTIONS TO VACCINATION

Reactions to inoculation were observed in 1000 individuals selected at random. About 250 persons in each vaccine group were observed. Examinations were made on the first, second and third days after vaccination. The reactions manifested were erythema, swelling, pain, induration, fever and a feeling of weakness. The vaccinees were equally affected by the different vaccines, except that erythema and induration were more pronounced in those given the oil-adjuvant vaccine.

After the first week, it was noted that many of the vaccinees reported to health centres for treatment of abscesses or ulcers due to vaccination. A rapid survey of the study area revealed that many vaccinees developed hard masses at the site of vaccination. These were of various sizes ranging from 1 cm to 8 cm in diameter. It was further observed that during the succeeding months some of these hard masses showed signs of fluctuation and, if not treated, later developed into ulcers. Fluctuating masses were aspirated by means of a syringe. Smears and cultures of abscesses were found to be bacteriologically negative. The hard masses often subsided after aspiration or disappeared completely, but sometimes, even after repeated aspirations, they developed into ulcers that healed slowly. Vaccinees who exhibited persistent hard masses over

a period of time were requested to report to the headquarters in Bacolod for excision of the mass. To date, 1755 vaccinees have shown severe reactions ranging from a hard mass to an abscess or ulcer.

The vaccination reactions analysed in Appendix Tables 7, 8, 9 and 10 were observed beginning one week after the start of the vaccination campaign. The reactions included abscesses, ulcers, ulcers with proud flesh, and hard masses. These 1755 severe reactions were evenly distributed over the study area. Calatrava, Escalante, Ilog, Kabankalan and San Carlos City had, however, comparatively low rates of reaction, for which there was no apparent reason.

From Appendix Table 8 it may be seen that a very high percentage (96.1%) of the total number of severe reactions were caused by the oil-adjuvant vaccine. The cholera vaccine accounted for only 0.8% of the total number of severe reactions, the El Tor vaccine 1.7%, and the control vaccine 1.4%. Of the 146 000 persons given oil-adjuvant vaccine, 1687 developed severe reactions—a ratio of 1:86.

Appendix Table 9 shows that all the occupational groups were affected. The differences in reaction rate among these groups are not significant.

Appendix Table 10 shows how the different age-groups were affected. As in the previous tables, 96% of all the severe reactions were found among those given oil-adjuvant vaccine.

It may be relevant to mention that individuals of mixed Filipino ancestry (such as the Chinese) were among those who suffered severe reactions.

SURVEILLANCE

Immediately after the vaccination campaign, intensive surveillance was carried out to detect cholera El Tor cases among the immunized groups. Surveillance was performed by three epidemiologists, eight nurse supervisors and 26 epidemiological aides (trained midwives). In addition, assistance was given by 263 personnel of local health units within the study area.

Diarrhoeal patients reporting to rural health units, to local private physicians, to barrio (village) leaders, to pharmaceutical stores and to schools constituted suspected cases. This number was supplemented by diarrhoeal cases revealed by a house-to-house inquiry. Rectal swabs on these diarrhoeal cases were performed (once), and the material was inoculated into alkaline peptone water. These specimens were taken to the local laboratory in Bacolod for bacteriological examination. The cases that were found to be positive were recorded as confirmed, and their contacts were examined. All household contacts of confirmed cases were subjected to rectal swabbing. Appropriate records were made of all cases and carriers.

RESULTS OF THE FIELD TRIAL

The aggregate number of cholera El Tor cases occurring during the whole 26-week period of surveillance is given in Table 1. All these cases were confirmed bacteriologically. A total of 325 cases occurred within the study group. Their distribution and the attack rate are shown in the table. Statistically significant differences were noted between the morbidity rates in the three vaccine groups and between the morbidity rate in any of the vaccine groups and that in the control group. From the table it may be seen that the protective effect [1] of the vaccines was a follows: classical cholera vaccine, 26%; *Vibrio El Tor* vaccine, 42%; oil-adjuvant vaccine, 56%. These percentages, however, give only a rough indication of the protective effect of the vaccines, since the sample size was not large enough for a precise estimate.

TABLE 1

CASES OF CHOLERA EL TOR AMONG VACCINATED GROUPS, 7 JUNE TO 5 DECEMBER 1964

Vaccine	Number immunized [a]	Number of cases	Attack rate per 100 000
Cholera	146 000	87	59.6
El Tor	146 000	68	46.6
Oil-adjuvant	146 000	52	35.6
Control	146 000	118	80.8
Total	584 000	325	55.7

[a] Preliminary numbers.

The monthly distribution of confirmed cases in each vaccine group is shown in Table 2. Table 3 shows that, from June to September inclusive, a period of about four months, there was little appreciable difference in the protective value of the three

[1] The effectiveness of the vaccines is expressed as a percentage reduction in the incidence rate in immunized groups when compared with the control group.

TABLE 2
MONTHLY DISTRIBUTION OF CHOLERA CASES, 7 JUNE—5 DECEMBER 1964

Month	Cholera vaccine group	El Tor vaccine group	Oil-adjuvant vaccine group	Control vaccine group	Total
7-30 June	1	0	0	0	1
1-31 July	8	4	14	23	49
1-31 August	9	11	8	24	52
1-30 September	16	14	12	18	60
1-31 October	24	21	5	23	73
1-30 November	26	17	11	26	80
1-5 December	3	1	2	4	10
Total	87	68	52	118	325

cholera vaccines. Compared with the control, the vaccines had a protective capacity of about 50%. Il the next two months of the period there were significant changes, which are indicated by a comparison of the protective value of the vaccines in the June-September and October-December periods.

In the period October to December, the protective capacity of the classical *V. cholerae* vaccine was completely dissipated while that of the El Tor vaccine was retained at only a 26% level. Oil-adjuvant vaccine, on the other hand, increased its protective potential to 66%.

Preliminary data from a further follow-up study show that by the end of March 1965, nine months after immunization, the protective value of the El Tor vaccine had also disappeared, while that of the oil-adjuvant vaccine was still 50%.

As shown in Table 4, 52% of the cases occurred in the age-group below 10 years of age. In the latter part of the period (October-December), as in Table 3, the occurrence of cases in the El-Tor-vaccine group was comparable to that in the control group, but the oil-adjuvant vaccine continued to show a high protective value.

Tables 5 and 6 show the number of confirmed cases occurring in the vaccinated groups in relation to the time interval from vaccination to onset of illness. In Table 5 it is shown that in 25 out of 325 cases the subject died. Of these deaths, nine each occurred in the cholera-vaccine and control-vaccine

TABLE 3
DISTRIBUTION OF CHOLERA CASES IN THE PERIODS JUNE-SEPTEMBER, OCTOBER-DECEMBER 1964 AND DECEMBER 1964 TO MARCH 1965

	7 June to 30 September		1 October to 5 December		6 December to 31 March [a]	
	Number of cases	Protective value (%)	Number of cases	Protective value (%)	Number of cases	Protective value (%)
Cholera vaccine group	34	48	53	0	34	0
El Tor vaccine group	29	55	39	26	35	0
Oil-adjuvant vaccine group	34	48	18	66	18	50
Control vaccine group	65	0	53	0	36	0

[a] Preliminary data obtained after this report was prepared for publication.

TABLE 4. MONTHLY DISTRIBUTION OF CASES IN EACH VACCINE GROUP[a] ANALYSED BY AGE, 7 JUNE TO 5 DECEMBER 1964

Age-group	June			July			August			September			October			November			December			Total number of cases
	ch	ET	o-a	ch	ET	o-a	c	ch	ET	o-a	c	ch	ET	o-a	c	ch	ET	o-a	c	ch	ET	o-a	c	
0-1				1	1	1		1	1			1		2		1	1	1		1	1			14
1-4				1	2		7	5	4	2	5	4	4	4	7	1	7	1	4	6	7	3	7			1	2	87
5-9				3		6	3	1		1	8	3	2	2	2	7	4	1	4	6	4	2	6	1	1	1		68
10-14						1	2		3			1	2			1	2		2	2		1						17
15-24						1	2	2		1	4		1		2	2	1		1	1	1		3	1				24
25-34	1			1		4	5	1		1	1	3		1	2	3	2		4	1	3	1	3			1		38
35-44					1	1	1			2	1	2	3	1	2	5		1	3	4	1	2	3					33
45-54				2			1	1			2	2			1	1	2	1	1	3	1	1	1					20
55-64							1	1			2		1		1		1		4			1				1		14
65 and over							1				1		1		1		1			2			2					10
Total	1			8	4	14	23	9	11	8	24	16	14	12	18	24	21	5	23	26	17	11	26	3	1	2	4	325

[a] Vaccines: ch = cholera; ET = El Tor; o-a = oil-adjuvant; c = control.

groups, five in the El-Tor-vaccine group and two in the oil-adjuvant-vaccine group. These two tables demonstrate the protective capacities of the vaccines.

By arbitrarily grouping the number of confirmed cases into 60-day periods reckoned from the date of vaccination, the figures in Table 7 are obtained. It can be seen here that the cholera and El Tor vaccines conferred better protection than the oil-adjuvant vaccine for the first 60 days. During the next 60 days, the protection given by the cholera and El Tor vaccines decreased, while the oil-adjuvant vaccine maintained and showed a tendency to increase its protective capacity. At the end of six months, the oil-adjuvant vaccine conferred 67% protection and the El Tor vaccine 25%. The cholera vaccine gave no protection at all. At the end of nine months, accordint to the latest preliminary data presented in Table 3, the protective effect of the oil-adjuvant vaccine was retained at the 50% level, while the El Tor vaccine no longer gave protection.

Table 8 shows that there are many more cases of cholera in the younger age-groups (under 1 to 9 years old) than in the older age-groups.

Table 9 shows that the relative incidence of cholera is very much higher in the younger age-groups, the highest incidence of all occurring in the age-group 1-4 years. During the period of adolescence, there is relatively less incidence, and in the age-group 45 years and above the incidence is very low.

Table 10 shows that both sexes are more or less equally affected by the cholera El Tor infection. Of the total number of cases, 48% occurred among males and 52% among females.

The extent and intensity of surveillance can be gleaned from Table 11, in view of the fact that about 33% of the cases were discovered at home in a house-to-house search. The table also shows (though unintentionally) the ratio between hospital and home cases.

Diarrhoea was observed in the 300 confirmed cases, and the duration of the diarrhoea in the various vaccine groups is shown in Table 12. The 25 fatal cases were excluded from these figures. The duration of diarrhoea is not significantly altered by vaccination.

An analysis of symptoms of the 325 cases in the different vaccine and control groups in Table 13 shows that the course of cholera El Tor disease is not significantly altered by vaccination. In all cases there was diarrhoea. Vomiting was observed in two-thirds of the cases; other predominant symp-

TABLE 5

CONFIRMED CASES IN EACH VACCINE GROUP ANALYSED ACCORDING TO THE TIME INTERVAL FROM VACCINATION TO ONSET OF ILLNESS 7 JUNE TO 5 DECEMBER 1964

Time from vaccination to illness (days)	Number of cholera cases in each vaccine group [a]				Total
	Cholera vaccine group	El Tor vaccine group	Oil-adjuvant vaccine group	Control vaccine group	
0-7	0	0	0	0	0
8-15	1	0	0	3	4
16-22	5	1	2	2	10
23-30	0	2	3	7 (1)	12 (1)
31-45	2	3	9	8	22
46-60	5	7	4	15 (1)	31 (1)
61-75	6	7	4	8	25
76-90	7 (1)	4	6 (1)	13	30 (2)
91-105	9 (1)	7	6	13 (1)	35 (2)
106-120	8	7 (1)	3	9	27 (1)
121-135	10 (1)	6 (1)	1	4 (1)	21 (3)
136-150	9	7 (2)	6	12 (1)	34 (3)
151-165	12 (5)	13 (1)	2	14 (2)	41 (8)
166-180	10 (1)	2	4 (1)	9 (2)	25 (4)
181-195	3	2	2	1	8
Total	87 (9)	68 (5)	52 (2)	118 (9)	325 (25)

[a] The numbers in parentheses indicate the cases that terminated in death.

toms were abdominal pain, cramps, aphonia and cyanosis.

The cholera carrier rate by vaccine group (Table 14) shows that the cholera vaccines do not increase the carrier rates. The carrier rates in cholera El Tor vaccines are slightly lower than in the control. The over-all carrier rate per cholera vaccine of 8.2% is lower than the carrier rate of 12.4% in the control group.

The carrier rate of 6.4% in the cholera-vaccine group, when compared with the 12.4% carrier rate in the control group, gives a probability of 0.02, and is therefore statistically significant. However, owing to the small number of carriers at this time, conclusions in this regard must be deferred.

The relative attack rate in towns and cities is shown in Table 15. In June, which is the beginning of the rainy season, cholera El Tor cases began to appear in some parts of the province. From June to September, the incidence of cases was higher in the towns south of Bacolod. Bacolod City itself had a steady number of cases.

Following the seasonal pattern of the past few years, cases in San Carlos City and in the northern towns of the province occurred after the season of prevalence. Calatrava, Toboso and Victorias were free of cases until October 1964, except for one case in Toboso in August.

The incidence in the coastal areas and the adjacent plains was higher than in the interior.

DISCUSSION

The results of this controlled field trial show that the classical cholera vaccine used in this study may confer about 50% protection for a period of about three months against cholera El Tor infection. The cholera El Tor vaccine, on the other hand, confers a

TABLE 6

CUMULATIVE DISTRIBUTION OF CASES ACCORDING TO THE TIME INTERVAL FROM VACCINATION TO ONSET OF ILLNESS, 7 JUNE TO 5 DECEMBER 1964

Time from vaccination to illness (days)	Number of cholera cases in each vaccine group			
	Cholera vaccine group	El Tor vaccine group	Oil-adjuvant vaccine group	Control vaccine group
0-7	0	0	0	0
8-15	1	0	0	3
16-22	6	1	2	5
23-30	6	3	5	12
31-45	8	6	14	20
46-60	13	13	18	35
61-75	19	20	22	43
76-90	26	24	28	56
91-105	35	31	34	69
106-120	43	38	37	78
121-135	53	44	38	82
136-150	62	51	44	94
151-165	74	64	46	108
166-180	84	66	50	117
181-195	87	68	52	118

comparatively longer protection. The oil-adjuvant vaccine showed remarkable results in terms of protective value. It showed high effectiveness of long duration. At the end of the nine-month period of observation, it had a protective value of about 50%.

The oil-adjuvant vaccine could be the vaccine of choice except for the fact that 96% of individuals exhibiting severe reactions to vaccination belonged to this group. The reactions commonly observed were abscesses, ulcers and hard masses. The abscesses and ulcers healed slowly. The hard masses were found to be non-malignant after biopsy. The cause

TABLE 7

EFFECTIVENESS[a] OF THE VACCINES DURING THREE PERIODS OF TWO MONTHS EACH

Period (days)	Cholera vaccine group		El Tor vaccine group		Oil-adjuvant vaccine group		Control vaccine group	
	No. of cases	Effectiveness (%)	No. of cases	Effectiveness (%)	No. of cases	Effectiveness (%)	No. of cases	Effectiveness (%)
8-60	13	63	13	63	18	49	35	0
61-120	30	30	25	42	19	56	43	0
121-195	44	0	30	25	15	67	40	0
Total cases and average effectiveness	87	26	68	42	52	56	118	0

[a] The effectiveness of the vaccines is expressed as the percentage reduction in the incidence rate in immunized groups whe compared with the control group.

TABLE 8

CONFIRMED CHOLERA CASES ACCORDING TO AGE, 7 JUNE TO 5 DECEMBER 1964

Age-group	Cholera vaccine group	El Tor vaccine group	Oil-adjuvant vaccine group	Control vaccine group	Total
Under 1	6	3	4	1	14
1-4	20	24	11	32	87
5-9	21	11	13	23	68
10-14	4	7	2	4	17
15-24	4	5	3	12	24
25-34	9	6	7	16	38
35-44	11	5	7	10	33
45-54	9	3	2	6	20
55-64	1	2	2	9	14
65 and over	2	2	1	5	10
Total	87	68	52	118	325

TABLE 9

CHOLERA CASES ANALYSED ACCORDING TO AGE STRUCTURE OF THE VACCINE GROUPS, 7 JUNE TO 5 DECEMBER 1964

Age-group (years)	Cholera vaccine group		El Tor vaccine group		Oil-adjuvant vaccine group		Control vaccine group	
	Percentage of total number in vaccine group	Percentage of total cholera cases in vaccine group	Percentage of total number in vaccine group	Percentage of total cholera cases in vaccine group	Percentage of total number in vaccine group	Percentage of total cholera cases in vaccine group	Percentage of total number in vaccine group	Percentage of total cholera cases in vaccine group
0.5-1	1.4	6.8	1.1	4.4	1.2	7.7	1.1	0.8
1-4	15.1	22.7	16.9	34.8	14.8	21.2	15.7	26.9
5-9	21.7	23.9	21.4	17.4	19.7	25.0	22.9	19.3
10-14	16.4	4.6	16.4	10.1	16.4	3.8	14.5	3.4
15-24	18.1	4.6	17.6	7.2	18.2	5.8	17.8	10.1
25-34	10.4	10.2	11.1	8.7	12.0	13.5	12.9	13.4
35-44	6.9	13.6	7.2	7.2	8.1	13.5	7.0	9.2
45-54	5.7	10.2	5.4	4.4	6.1	3.8	4.9	5.0
55-64	2.8	1.1	2.0	2.9	1.9	3.8	1.8	7.6
65 and over	1.3	2.3	0.9	2.9	1.5	1.9	1.4	4.2
Total	99.8	100.0	100.0	100.0	99.9	100.0	100.0	99.9

TABLE 10

CONFIRMED CHOLERA CASES BY SEX
7 JUNE TO 5 DECEMBER 1964

Sex	Cholera vaccine group	El Tor vaccine group	Oil-adjuvant vaccine group	Control vaccine group	Total
Male	43	31	23	61	158
Female	44	37	29	57	167
Total	87	68	52	118	325

TABLE 11

CONFIRMED CHOLERA CASES BY PLACE OF CONFINEMENT
7 JUNE TO 5 DECEMBER 1964

Place of confinement	Cholera vaccine group	El Tor vaccine group	Oil-adjuvant vaccine group	Control vaccine group	Total
Hospital	52	42	35	86	215
Home	35	26	17	32	110
Total	87	68	52	118	325

TABLE 12

DURATION OF DIARRHOEA IN CONFIRMED CHOLERA CASES IN THE FOUR VACCINE GROUPS (DEATHS EXCLUDED), 7 JUNE TO 5 DECEMBER 1964

Duration of diarrhoea (days)	Cases in cholera vaccine group	Cases in El Tor vaccine group	Cases in oil-adjuvant vaccine group	Cases in control vaccine group	Total
1	20	16	14	24	74
2	25	11	12	19	67
3	13	21	11	23	68
4	6	8	6	17	37
5	6	1	3	12	22
6	3	1	2	4	10
7	2	1	0	5	8
8	1	3	1	1	6
9	0	1	0	1	2
10	0	0	0	1	1
More than 10	2	0	1	2	5
Total	78	63	50	109	300

TABLE 13
CLINICAL SYMPTOMS AMONG CONFIRMED CASES IN THE FOUR VACCINE GROUPS (DEATHS EXCLUDED)
7 JUNE TO 5 DECEMBER 1964

Symptom	Cases in cholera vaccine group	Cases in El Tor vaccine group	Cases in oil-adjuvant vaccine group	Cases in control vaccine group
Diarrhoea	87	68	52	118
Vomiting	52	40	30	75
Abdominal pain	20	14	16	43
Cramps	26	16	15	39
Aphonia	19	11	15	40
Cyanosis	24	22	16	25
Anuria	4	2	3	4
Tympanism	0	1	0	0
Fever	0	0	0	0
Number of cases	78	63	50	109

of the severe reactions is now being investigated, and it is hoped that a remedy may be found to render the vaccine reaction-free.

Cholera vaccination does not change the course of the disease once the infection manifests itself in an individual.

While it is claimed that vaccines may increase the cholera carrier rate in the general population, it can be seen from the results of this trial that this is not so. In fact, the carrier rate in the vaccinated group was somewhat lower than that in the control group.

The effectiveness of routine cholera vaccines used in this and in some other trials seems to be still low (B. L. Taneja—personal communication) and of short duration, and further study directed to the possible improvement of these vaccines is indicated.

It is unfortunate that the most effective vaccine cannot be recommended for use because of its severe side-effects. Another vaccine that gave relatively good protection against *V. cholerae* infection in a recent field study done elsewhere has also produced serious reactions that make it unsuitable for routine use (Oseasohn et al., 1965).

Further laboratory and field investigations to improve and assess the value of different types of cholera vaccine are required if the control of cholera is to be effected through immunization programmes.

TABLE 14
CHOLERA CARRIER RATES IN THE FOUR VACCINE GROUPS
7 JUNE TO 5 DECEMBER 1964

	Cholera vaccine group	El Tor vaccine group	Oil-adjuvant vaccine group	Control vaccine group	Total
Number of carriers	15	22	16	27	80
Number of contacts	235	197	211	217	860
Carrier rates [a]	6.4 %	11.2 %	7.6 %	12.4 %	9.3 %

[a] Average carrier rate for cholera vaccine groups $= \frac{53}{643} = 8.2\,\%$.

TABLE 15

GEOGRAPHICAL DISTRIBUTION OF CHOLERA CASES OVER A PERIOD OF SIX MONTHS, 7 JUNE TO 5 DECEMBER 1964

Sector	Town or city	Population	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total cases	Incidence per 1000
I	San Carlos	139 000			3	13	16	76	1	109	0.78
	Calatrava	76 000					6	9		15	0.20
	Toboso	42 000			1		14	21		36	0.86
II	Escalante	69 000		1	1		28	36		66	0.96
	Sagay	82 000		3	6	5	36	27	5	82	1.00
	Cadiz	102 000		6	5	3	1	5	1	21	0.21
III	Manapla	54 000					2	4	2	8	0.15
	Victorias	40 000				2	2	6		10	0.25
	Saravia	37 000		2	1	3	7	10	8	31	0.84
	Silay City	70 000			8	9	9	16	3	45	0.64
IV	Talisay	54 000		3	15	7	4	21	6	56	1.04
	Bacolod City	138 000	10	37	46	34	25	41	28	221	1.60
	Murcia	27 000		1	6	2		13	3	25	0.93
V	Bago	68 000	1	7	15	25	14	13	2	77	1.13
	Pulupandan	18 000		4	6	8	8	3		29	1.61
	Valladolid	17 000		22	8	11	2			43	2.53
	San Enrique	12 000		2	3	4	6	1		16	1.33
	Pontevedra	26 000		2	2	2	1	2		9	0.35
	Hinigaran	43 000	4	31	6	14	14	4		73	1.70
VI	Binalbagan	36 000		9	6	8	1	2		26	0.72
	Isabela	34 000	1	2	4	9	6	3		25	0.74
	Himamaylan	49 000	1	15	9	18	11	2		56	1.14
	Kabankalan	68 000		21	7	6	10	1		45	0.66
	Ilog	28 000		5	4	3	6	2		20	0.71
	Total	1 329 000	17	173	162	186	229	318	59	1144	0.86

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The Philippines Cholera Committee and the Joint Philippines-Japan-WHO Cholera Committee are deeply grateful to the late Governor Gatuslao and to the people of Negros Occidental for the support and co-operation extended to the vaccination teams, epidemiologists, and other workers. They are indebted to the Secretary of Health of the Philippines, Dr Manuel Cuenco, the Under-Secretary of Health, Dr Rodolfo T. Caños, His Excellency Hiroshi Kanda, Minister of Health of Japan, and their staffs, and to WHO.

This undertaking is an example of what can be accomplished when countries work together in harmony and understanding. It is fitting to commend the many field and laboratory workers whose dedication and devotion to their work have made this study possible.

RÉSUMÉ

Organisée en collaboration par le Gouvernement des Philippines, le Gouvernement du Japon et l'OMS, une vaste enquête, destinée à évaluer l'efficacité respective de différents vaccins anticholériques a débuté en 1964, dans une région des Philippines où l'incidence du choléra atteint 0,5 par 1000 habitants. Les premiers résultats viennent d'être publiés.

Les vaccins utilisés ont été les suivants: *a*) un vaccin anticholérique classique, préparé avec *Vibrio cholerae*; *b*) un vaccin liquide préparé avec *V. El Tor ; c*) un vaccin

à base de *V. cholerae* avec adjuvant huileux; *d*) un vaccin antityphoïdique monovalent, servant de préparation témoin. Ils ont été administrés à dose unique, par voie sous-cutanée, à 584 000 volontaires répartis en quatre groupes, chaque groupe recevant l'une des quatre préparations.

Pendant la période de surveillance succédant aux vaccinations, les cas de choléra provoqués par *V. El Tor* ont été diagnostiqués et confirmés par les méthodes bactériologiques.

On a constaté des différences statistiquement significatives (de l'ordre de 50%), durant les trois premiers mois suivant les vaccinations, entre les taux de morbidité par choléra El Tor des groupes vaccinés contre le choléra et du groupe témoin vacciné contre la fièvre typhoïde. Le pouvoir protecteur, au cours des premiers six mois d'observation, a été évalué à 26%, 42% et 56% respectivement pour le vaccin classique, le vaccin El Tor et le vaccin avec adjuvant huileux.

La protection conférée par le vaccin avec adjuvant huileux s'est maintenue au cours des six mois et a même augmenté pour atteindre près de 70% à l'issue de cette période. Quant au pouvoir protecteur du vaccin El Tor, élevé au début, il s'affaiblit progressivement pour n'être plus, à la fin de la période de surveillance, que de 25%. Enfin, dans le cas du vaccin anticholérique classique, la protection a été moins effective et de durée inférieure: elle était inexistante à l'issue des six premiers mois d'observation.

Ces différents vaccins anticholériques n'ont pas témoigné d'une réelle efficacité en cas de choléra El Tor avéré; ils ont, en revanche, déterminé une certaine réduction du nombre des porteurs de germes asymptomatiques. Le vaccin avec adjuvant huileux, dont l'efficacité et la durée d'action ont été les plus intéressantes, a malheureusement provoqué des réactions post-vaccinales intenses qui contre-indiquent son utilisation pour les vaccinations de masse.

En annexe à ce travail, figure un exposé détaillé des méthodes employées pour la préparation des vaccins et des épreuves de laboratoire auxquelles ils ont été soumis.

La surveillance de la population vaccinée est toujours en cours.

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Annex 1

PREPARATION OF CHOLERA AND EL TOR VACCINES

Vibrio strains

The cholera strains used are Inaba 35A-3 and Ogawa 41. The El Tor strains used are El Tor Inaba 6973 and El Tor Ogawa 1418. The method of preparation of each vaccine is exactly the same.

Production of vibrios

A tube of lyophilized vibrios is opened and the contents inoculated into broth and incubated for 24 hours at 37°C. They are then plated in meat infusion agar (pH 8.0) and incubated again at 37°C for 24 hours. Typical smooth colonies are fished and transferred to nutrient agar slant (pH 8.0) and incubated for another 24 hours at 37°C. Purity tests and characterization of the strain are carried out at this point and, when satisfactory, seed-culture bottles containing meat extract agar (pH 8.0) are inoculated with suspension from the agar slant and incubated for 24 hours at 37°C. These seed cultures are then used for seeding culture bottles for mass production. The agar slants are used for seed only up to four subcultures from the original lyophilized material. After this, a new tube is opened. The strains have the following characteristics:

Morphological properties. Smears prepared from agar slant before inoculating seed cultures and stained by means of Gram's staining method show that the organisms are Gram-negative and in the form of slightly curved rods, uniform in size and shape.

Cultural properties. When inoculated into nutrient agar medium (pH 8.0) without bile salts and incubated for 24 hours at 37°C, the strains produce translucent smooth colonies.

Biochemical properties. When inoculated into peptone water media with different sugars, both strains ferment mannose and saccharose with production of acid and no gas. Neither strain ferments arabinose.

Haemolytic properties. Both strains are non-haemolytic to 5% sheep's erythrocytes.

Serological properties. Both strains are agglutinable by cholera O group I antiserum. Inaba 35A is agglutinable with specific Inaba antiserum, and the Ogawa 41 is agglutinable with specific Ogawa antiserum. Suspensions of the strains are not agglutinable by rough typing antiserum.

Stability in suspension. Suspensions of both strains are stable for more than 5 hours when incubated at 37°C.

Pathogenic properties. Suspensions of both strains (containing 4×10^9 organisms) from a 5-hour culture incubated at 37°C, when injected intraperitoneally, are capable of killing two guinea-pigs weighing 300 g each within 72 hours.

Culture medium

Meat extract agar (pH 8.0) is used for mass production. It has the following formula:

Peptone	1.0%	or	800 g
Sodium chloride . . .	0.5%	or	400 g
Beef extract	0.3%	or	240 g
Agar	3.0%	or	2400 g
Distilled water			80 litres

The agar and other ingredients are dissolved in the autoclave and filtered through several layers of gauze and cotton and dispensed in 100-ml Pyrex Roux bottles. The bottles are sterilized in the autoclave at 20 lbf/in² (1.4 kgf/cm²) for 30 minutes. The pH of the medium is 8.0.

The seed cultures from 12 Roux bottles are pooled into 24 litres of normal salt solution. The production bottles are inoculated with 50 ml of the seed suspension by a gravity method using a closed system to prevent contamination. The bottles are incubated in an inverted position, that is with the agar layer uppermost.

Production precautions

Protocols are prepared for each product.

The apparatus, equipment and materials are prepared with the utmost care and cleanliness. All glassware is sterilized.

Orderliness is maintained at all times and in all stages of production, and the bottles are always carefully marked.

The precautions against contamination include routine disinfection of workrooms with benzalkonium chloride before and after use. Employees suffering from infectious diseases are not allowed in the workrooms. Visitors are not admitted to the sterile rooms.

Animals used for test purposes are healthy and bred in a separate area and provided with a balanced diet. Dead animals are burned.

Control of the monovalent stage

Production cultures are incubated at 37°C for 24 hours before harvesting. For harvesting, the culture bottles are turned the right way up and shaken gently to wash down the growth on the agar. The liquid in the culture bottles is then drawn off aseptically with a closed harvesting system into the collecting bottle until all the production cultures have been used.

Before starting the harvest, each flask is scrutinized for any abnormal appearance and sign of contamination; suspicious bottles are discarded. Samples taken from monovalent bulk are examined for morphological and cultural properties as described above.

The harvested cholera vibrios are killed by immersing the bottles in a water-bath at 56°C for one hour, after which phenol is added at a final concentration of 0.5%. To control the killing procedure, samples are taken before and after killing and agglutinated with the International Reference Specific Antiserum, using the tube method. The agglutination titre both before and after killing is 1/1600 Inaba, 1/1600 Ogawa.

A test is carried out on the smooth to rough variation, both strains being non-agglutinable to rough serum. Both strains show a stable suspension after 5 hours at 37°C.

A sterility test is done in nutrient agar (pH 6.8 and 7.8) and in aerobic and anaerobic broth.

When prepared in the manner described, the concentration of vibrios, measured against the International Opacity Reference Preparation, should be: Inaba strain, 24×10^9 organisms/ml; Ogawa strain, 35×10^9 organisms/ml.

The monovalent preparations of both strains are stored at a temperature between 5°C and 8°C until use.

Divalent bulk product

The divalent bulk product is made by mixing 24 642 ml of monovalent bulk Inaba containing 24×10^9 organisms/ml with 16 909 ml of monovalent bulk Ogawa containing 35×10^9 organisms/ml and diluting to make a total volume of 148 litres with a final content of 4×10^9 organisms/ml of Inaba and 4×10^9 organisms/ml of Ogawa.

The divalent bulk is stored in sterile 50-ml vials, rubber-capped and aluminium-sealed. The filling is done in a separate sterile room reserved for this purpose.

Control tests on the final product

The tests carried out on the final product consist of:

(*a*) an identity test, in which there is agglutination in both Ogawa and Inaba antisera;

(*b*) a sterility test, in which 36 sample vials are tested in nutrient agar (pH 6.8 and 7.8) and in aerobic and anaerobic broth;

(*c*) a safety test for abnormal toxicity, in which three white mice weighing 15-20 g are injected intraperitoneally with 0.5 ml of the vaccine from the final container and observed for 7 days;

(*d*) a safety test for abnormal toxicity of the phenol, in which three white mice weighing 15-20 g are injected subcutaneously with 0.5 ml of the vaccine from the final container (the tremor and spasm observed lasting an average time of 12 minutes);

(*e*) a potency test according to the method of Pittman and Feeley, using the International Reference Preparation;

(*f*) a test to ascertain that the hydrogen-ion concentration of the final vaccine is 7.3; and

(*g*) a test of the homogeneity of the suspension, in which all the containers are examined against a strong light after shaking.

Annex 2

PREPARATION OF OIL-ADJUVANT VACCINE

The cholera oil-adjuvant vaccine of the type used in the controlled field trial in the Philippines is a water-in-oil emulsion prepared by mixing equal parts of cholera vaccine and a mineral oil of low viscosity containing an emulsifying agent.

Preparation of the aqueous phase of the vaccine

The seed strains of *Vibrio cholerae* used are Inaba 35A-3 and Ogawa 41. They are preserved by lyophilization. Their biological properties meet the requirements specified by the WHO Study Group on Requirements for Yellow Fever Vaccine and Requirements for Cholera Vaccine (1959).

The seed strains are cultivated in heart infusion agar for 5 hours at 37°C and are inoculated onto

brain-heart infusion agar for a further 5 hours at 37°C. The condensation water found in the culture bottle is decanted, and the growth is harvested by washing the surface of the medium with physiological saline.

Control of the harvested growth is effected by taking a sample from each batch of harvest to test for purity, smooth to rough variation, suspension stability, and sterility, as defined by the WHO Study Group mentioned above.

Suspensions of the harvested vibrios are killed by adding a 0.2% solution of formalin. After being stored overnight in a refrigerator at 4°C, the suspensions are centrifuged to remove free formaldehyde. The supernatant is decanted and the sediment resuspended in phosphate buffer saline (M/75, pH 7.2) containing 1 part in 10 000 of merthiolate as a preservative.

The vibrio concentration is determined from the formalin-treated suspensions, which are kept for 24 hours at 4°C. The concentration is measured by comparing the opacity of the suspension with that of the International Opacity Reference Preparation.

Aqueous solutions of the monovalent bulk products are prepared separately. The concentration of the monovalent suspensions is adjusted to 100×10^9 vibrios per ml. Equal parts of the monovalent bulk products of both types are then combined to give a concentration of 5×10^9 vibrios per ml.

The resulting aqueous phase of the vaccine is subjected to the tests described by the WHO Study Group mentioned above (Annex 2, section 5).

Mineral oil and emulsifying agent

The purest form of light liquid petroleum obtainable is used for the mineral oil (US National Formulary, tenth edition). In the field-trial vaccine, the petrolatum used was Drakeol 6-VR Q 126 manufactured by the Pennsylvania Refining Company, Butler, Pa., USA. The mineral oil is stored in airtight bottles and kept separate from the emulsifying agent.

The emulsifying agent is the purest form of mannide mono-oleate obtainable. For the field-trial vaccine, a preparation called "Specially-treated Arlacel A" manufactured by the Atlas Powder Company, USA, was used.

The mineral oil and the emulsifying agent are mixed in a ratio of 9 : 1 respectively, and the mixture is then sterilized by passage through a Seitz S3 bacteria-retaining filter.

Mixing of ingredients

A water-in-oil emulsion is made by mixing equal parts of the aqueous phase of the vaccine and the mixture of mineral oil and emulsifying agent. The mixing is carried out in a Waring blender, and air is excluded during the process.

The stability of the emulsion is ascertained by centrifuging the mixture at 200 rev/min for 10 minutes. The excess oil that separates from the emulsion phase does not exceed 10% of the total volume. There is no more than a trace of free aqueous phase present after centrifugation. The emulsified vaccine is capable of being delivered through a 23-gauge needle with manual pressure.

Filling and storage

Single doses are prepared in individual containers ready for immediate injection. Vaccines for children and babies are better prepared separately with reduced concentrations of vibrios. However, 15 ml of the vaccine is put in a 50-ml vial so that no difference is observed during dispensing, according to the principle of double-blind experiments.

The vaccine is stored at a temperature between 2°C and 5°C.

Control tests on final products

Sterility tests and safety tests are carried out on the oil-adjuvant vaccine as described by the WHO Study Group on Requirements for Yellow Fever Vaccine and Requirements for Cholera Vaccine (1959), Annex 2.

Comparative mouse-protection tests are performed, using a fivefold dilution of the vaccine. In these tests the oil-adjuvant vaccine is compared with the International Reference Preparation of cholera vaccine, as indicated by the WHO Study Group. Challenge inoculations are carried out two, four and eight weeks after immunization.

A maternal immunity test is performed on infant rabbits. Oil-adjuvant vaccine prepared from a fivefold dilution of the aqueous phase is injected subcutaneously into five adult female rabbits, which are mated between one and two weeks after immunization. The same procedures are carried out with control vaccines. Ten or fifteen days after delivery, the litters are challenged with minimal intra-intestinal lethal doses of virulent vibrios, the infant rabbits being under light ether narcosis. The challenge strains are 5-hour cultures in heart infusion broth of Inaba 35A-3 and Ogawa 41 (true cholera) and Inaba 6973 and Ogawa 1418 (El Tor cholera).

APPENDIX TABLE 1

DISTRIBUTION OF PERSONS IN THE FOUR VACCINE GROUPS ACCORDING TO LOCALITY, ON THE BASIS OF A 1% SAMPLE OF THE IMMUNIZED POPULATION

City or municipality	Cholera vaccine		El Tor vaccine		Oil-adjuvant vaccine		Control vaccine		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
Bago	78	5.36	102	6.89	96	6.69	118	8.04	394	6.75
Binalbagan	42	2.89	46	3.11	38	2.65	41	2.79	167	2.86
Cadiz	94	6.46	99	6.68	103	7.17	96	6.54	392	6.71
Escalante	88	6.05	72	4.86	77	5.36	68	4.63	305	5.22
Hinigaran	44	3.02	70	4.73	53	3.69	61	4.16	228	3.90
Manapla	39	2.68	35	2.36	33	2.30	45	3.07	152	2.60
Murcia	44	3.02	31	2.09	42	2.92	30	2.04	147	2.52
Pontevedra	28	1.92	47	3.17	45	3.13	35	2.38	155	2.65
Pulupandan	26	1.79	31	2.09	24	1.67	32	2.18	113	1.93
Sagay	129	8.87	108	7.29	93	6.48	102	6.95	432	7.40
San Enrique	18	1.24	15	1.01	14	0.97	21	1.43	68	1.16
Saravia	45	3.09	46	3.11	52	3.62	42	2.86	185	3.17
Talisay	69	4.74	62	4.19	81	5.64	61	4.16	273	4.67
Valladolid	23	1.58	20	1.35	25	1.74	24	1.63	92	1.58
Victorias	47	3.23	53	3.58	51	3.55	61	4.16	212	3.63
Bacolod	227	15.60	247	16.68	218	15.18	243	16.55	935	16.01
Silay	81	5.57	85	5.74	86	5.99	77	5.25	329	5.63
Toboso	27	1.86	35	2.36	28	1.95	30	2.04	120	2.05
Calatrava	60	4.12	52	3.51	48	3.34	65	4.43	225	3.85
San Carlos	95	6.53	84	5.67	74	5.15	90	6.13	343	5.87
Himamaylan	50	3.44	49	3.31	70	4.87	41	2.79	210	3.60
Kabankalan	42	2.89	38	2.57	38	2.65	31	2.11	149	2.55
Ilog	15	1.03	18	1.22	11	0.77	15	1.02	59	1.01
Isabela	44	3.02	36	2.43	36	2.51	39	2.66	155	2.65
Total	1 455	100.00	1 481	100.00	1 436	100.00	1 468	100.00	5 840	99.97

APPENDIX TABLE 2

DISTRIBUTION OF PERSONS IN THE FOUR VACCINE GROUPS ACCORDING TO OCCUPATION, ON THE BASIS OF A 1% SAMPLE OF THE IMMUNIZED POPULATION

Occupation	Cholera vaccine		El Tor vaccine		Oil-adjuvant vaccine		Control vaccine		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
Administrative, executive	3	0.2	1	0.1	2	0.1	1	0.1	7	0.1
Professional, technical	11	0.7	114	0.8	11	0.8	13	0.9	49	0.8
Clerical, sales	33	2.3	38	2.6	39	2.7	39	2.7	149	2.6
Subordinate technical	261	17.9	256	17.3	280	19.5	281	19.1	1 078	18.5
Service occupations	241	16.6	229	15.5	256	17.8	229	15.6	955	16.3
Manual workers	906	62.3	943	63.7	848	59.1	905	61.6	3 602	61.7
Total	1 455	100.0	1 481	100.0	1 436	100.0	1 468	100.0	5 840	100.0

APPENDIX TABLE 3

DISTRIBUTION OF PERSONS IN THE FOUR VACCINE GROUPS ACCORDING TO AGE, ON THE BASIS OF A 1% SAMPLE OF THE IMMUNIZED POPULATION

Age (years)	Cholera vaccine		El Tor vaccine		Oil-adjuvant vaccine		Control vaccine		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
Less than 1	21	1.44	16	1.08	17	1.18	16	1.09	70	1.20
1	46	3.16	58	3.92	43	2.99	54	3.68	201	3.44
2	52	3.57	53	3.58	40	2.79	59	4.02	204	3.49
3	66	4.54	68	4.59	66	4.60	55	3.75	255	4.37
4	56	3.85	71	4.79	63	4.39	62	4.22	252	4.32
5-9	315	21.65	317	21.40	283	19.71	336	22.89	1 251	21.42
10-14	239	16.43	243	16.41	236	16.43	213	14.51	931	15.94
15-19	136	9.35	146	9.86	146	10.17	134	9.13	562	9.62
20-24	128	8.80	115	7.77	115	8.01	128	8.72	486	8.32
25-29	76	5.22	90	6.08	91	6.34	119	8.11	376	6.44
30-34	76	5.22	74	5.00	82	5.71	70	4.77	302	5.17
35-39	49	3.37	52	3.51	67	4.67	61	4.16	229	3.92
40-44	52	3.57	55	3.71	50	3.48	42	2.86	199	3.41
45-49	43	2.96	36	2.43	50	3.48	35	2.38	164	2.81
50-54	40	2.75	44	2.97	38	2.65	37	2.52	159	2.72
55-59	18	1.24	15	1.01	14	0.97	16	1.09	63	1.08
60-64	23	1.58	14	0.94	14	0.97	10	0.68	61	1.04
65-69	4	0.27	2	0.14	7	0.49	7	0.48	20	0.34
70 and over	15	1.03	12	0.81	14	0.97	14	0.95	55	0.94
Total	1 455	100.00	1 481	100.00	1 436	100.00	1 468	100.01	5 840	99.99

APPENDIX TABLE 4

DISTRIBUTION OF PERSONS IN THE FOUR VACCINE GROUPS ACCORDING TO AGE AND SEX, ON THE BASIS OF A 1% SAMPLE OF THE IMMUNIZED POPULATION

Age (years)	Males								Females							
	Cholera vaccine		El Tor vaccine		Oil-adjuvant vaccine		Control vaccine		Cholera vaccine		El Tor vaccine		Oil-adjuvant vaccine		Control vaccine	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
0-4	115	15.88	134	17.54	113	15.83	111	15.61	126	17.24	132	18.41	118	16.30	135	17.83
5-9	153	21.13	167	21.86	143	20.03	150	21.10	162	22.16	150	20.92	140	19.34	186	24.57
10-14	108	14.92	131	17.15	112	15.69	104	14.63	131	17.92	112	15.62	124	17.13	109	14.40
15-19	68	9.39	75	9.82	64	8.96	64	9.00	68	9.30	71	9.90	82	11.33	70	9.25
20-24	81	11.19	52	6.81	63	8.82	63	8.86	47	6.43	63	8.79	52	7.18	65	8.59
25-29	42	5.80	42	5.50	48	6.72	61	8.58	34	4.65	48	6.69	43	5.94	58	7.66
30-34	29	4.01	41	5.37	44	6.16	29	4.08	47	6.43	33	4.60	38	5.25	41	5.42
35-39	27	3.73	29	3.80	34	4.76	29	4.08	22	3.01	23	3.21	33	4.56	32	4.23
40-44	20	2.76	27	3.53	26	3.64	23	3.23	32	4.38	28	3.91	24	3.31	19	2.51
45-49	21	2.90	14	1.83	26	3.64	22	3.09	22	3.01	22	3.07	24	3.31	13	1.72
50-54	22	3.04	27	3.53	18	2.52	23	3.23	18	2.46	17	2.37	20	2.76	14	1.85
55-59	12	1.66	10	1.31	7	0.98	11	1.55	6	0.82	5	0.70	7	0.97	5	0.66
60-64	16	2.21	8	1.05	6	0.84	6	0.84	7	0.96	6	0.84	8	1.10	4	0.53
65-69	0	0	2	0.26	4	0.56	6	0.84	4	0.55	0	0	3	0.41	1	0.13
70 and over	10	1.38	5	0.65	6	0.84	9	1.27	5	0.68	7	0.98	8	1.10	5	0.66
Total	724	100.00	764	100.01	714	99.99	711	99.99	731	100.00	717	100.01	724	99.99	757	100.01

APPENDIX TABLE 5

PREVIOUS HISTORY OF INFECTIOUS DISEASES AMONG VACCINEES, BASED ON A 1% SAMPLE OF THE IMMUNIZED POPULATION

Vaccine administered	Number in sample	Previous history of infectious diseases							
		Cholera				Typhoid			
		Positive		Negative		Positive		Negative	
		No.	%	No.	%	No.	%	No.	%
Cholera	1 455	6	0.4	1 449	99.6	9	0.6	1 446	99.4
El Tor	1 481	9	0.6	1 472	99.4	5	0.3	1 476	99.6
Oil-adjuvant	1 436	9	0.6	1 427	99.4	7	0.5	1 429	99.5
Control	1 468	10	0.7	1 458	99.3	8	0.5	1 460	99.5
Total and average percentage	5 840	34	0.6	5 806	99.4	29	0.5	5 811	99.5

APPENDIX TABLE 6

PREVIOUS HISTORY OF VACCINATION AMONG VACCINEES, BASED ON A 1% SAMPLE OF THE IMMUNIZED POPULATION

Vaccine administered during field trial	Number in sample	Previously vaccinated against cholera				Previously vaccinated against typhoid			
		Positive		Negative		Positive		Negative	
		No.	%	No.	%	No.	%	No.	%
Cholera	1 455	1 156	79.5	299	20.5	984	67.6	471	32.4
El Tor	1 481	1 192	80.5	289	19.5	970	65.5	511	34.5
Oil-adjuvant	1 436	1 173	81.7	263	18.3	948	66.0	488	34.0
Control	1 468	1 177	80.2	291	19.8	971	66.1	497	33.9
Total and average percentage	5 840	4 698	80.4	1142	19.6	3 873	66.3	1 967	33.7

APPENDIX TABLE 7

NUMBER AND RATE OF VACCINATION REACTIONS ACCORDING TO LOCALITY, JUNE-NOVEMBER 1964

Municipality or city	Number immunized	Reactions											
		Total		Abscess		Ulcer		Ulcer with proud flesh		Tumour		Hard mass	
		No.	Rate per 10 000	No.	Rate per 10 000	No.	Rate per 10 000	No.	Rate per 10 000	No.	Rate per 10 000	No.	Rate per 10 000
Bago	39 414	95	24.1	19	4.8	9	2.3	2	0.5	0	0	65	16.5
Binalbagan	16 765	41	24.5	13	7.8	4	2.4	1	0.6	1	0.6	22	13.1
Cadiz	39 167	168	42.9	86	22.0	37	9.4	5	1.3	1	0.3	39	10.0
Calatrava	22 492	1	0.4	0	0	1	0.4	0	0	0	0	0	0
Escalante	30 479	20	6.6	2	0.7	6	2.0	2	0.7	0	0	10	3.3
Himamaylan	20 991	38	18.1	7	3.3	6	2.9	3	1.4	0	0	22	10.5
Hinigaran	22 864	37	16.2	17	7.4	9	3.9	2	0.9	0	0	9	3.9
Ilog	5 949	5	8.4	2	3.4	1	1.7	0	0	0	0	2	3.4
Isabela	15 433	23	14.9	9	5.8	1	0.6	0	0	0	0	13	8.4
Kabankalan	14 912	10	6.7	3	2.0	5	3.4	0	0	0	0	2	1.3
Manapla	15 144	64	42.3	35	23.1	5	3.3	0	0	0	0	24	15.8
Murcia	14 718	91	61.8	12	8.2	23	15.6	4	2.7	0	0	52	35.3
Pontevedra	15 453	21	13.6	7	4.5	8	5.2	0	0	0	0	6	3.9
Pulupandan	11 313	22	19.4	15	13.3	2	1.8	2	1.8	0	0	3	2.7
Sagay	43 224	140	32.4	53	12.3	19	4.4	4	0.9	0	0	64	14.8
San Enrique	6 856	19	27.7	1	1.5	8	11.7	4	5.8	1	1.5	5	7.3
Saravia	18 466	39	21.1	15	8.1	12	6.5	6	3.2	2	1.1	4	2.2
Talisay	27 270	163	59.8	40	14.7	39	14.3	15	5.5	0	0	69	25.3
Toboso	12 024	82	68.2	20	16.6	5	4.2	0	0	0	0	57	47.4
Valladolid	9 194	48	52.2	9	9.8	14	15.2	0	0	1	1.1	24	26.1
Victorias	21 208	45	21.2	25	11.8	10	4.7	1	0.5	0	0	9	4.2
Bacolod	93 561	401	42.9	135	14.4	116	12.4	40	4.3	4	0.4	106	11.3
San Carlos	34 258	18	5.3	7	2.0	7	2.0	1	0.3	0	0	3	0.9
Silay	32 871	164	49.9	45	13.7	20	6.1	14	4.3	0	0	85	25.9
Total and average rate	584 026	1 755	30.1	577	9.9	367	6.3	106	1.8	10	0.2	695	11.9

APPENDIX TABLE 8

VACCINATION REACTIONS AMONG PERSONS IN THE FOUR VACCINE GROUPS ACCORDING TO LOCALITY, JUNE-NOVEMBER 1964

Municipality or city	Number of reactions	Number and percentage of persons in following vaccine groups showing vaccination reaction in given localities							
		Cholera vaccine		El Tor vaccine		Oil-adjuvant vaccine		Control vaccine	
		No.	%	No.	%	No.	%	No.	%
Bago	95	0	0	3	3.2	90	94.7	2	2.1
Binalbagan	41	0	0	2	4.9	39	95.1	0	0
Cadiz	168	1	0.6	3	1.8	162	96.4	2	1.2
Calatrava	1	0	0	0	0	1	100.0	0	0
Escalante	20	0	0	0	0	20	100.0	0	0
Himamaylan	38	0	0	0	0	38	100.0	0	0
Hinigaran	37	0	0	0	0	36	97.3	1	2.7
Ilog	5	0	0	0	0	5	100.0	0	0
Isabela	23	2	8.7	1	4.3	19	82.6	1	4.3
Kabankalan	10	0	0	0	0	9	90.0	1	10.0
Manapla	64	2	3.1	0	0	61	95.3	1	1.6
Murcia	91	1	1.1	0	0	89	97.8	1	1.1
Pontevedra	21	0	0	0	0	21	100.0	0	0
Pulupandan	22	0	0	1	4.5	20	90.9	1	4.5
Sagay	140	2	1.4	2	1.4	135	96.4	1	0.7
San Enrique	19	0	0	3	15.8	15	78.9	1	5.3
Saravia	39	0	0	0	0	37	94.9	2	5.1
Talisay	163	1	0.6	2	1.2	160	98.2	0	0
Toboso	82	0	0	1	1.2	81	98.8	0	0
Valladolid	48	1	2.1	1	2.1	46	95.8	0	0
Victorias	45	0	0	0	0	45	100.0	0	0
Bacolod	401	3	0.7	6	1.5	384	95.8	8	2.0
San Carlos	18	1	5.6	2	11.1	15	83.3	0	0
Silay	164	0	0	2	1.2	159	97.0	3	1.8
Total and average percentage	1 755	14	0.8	29	1.7	1 687	96.1	25	1.4

APPENDIX TABLE 9

VACCINATION REACTIONS AMONG PERSONS IN THE FOUR VACCINE GROUPS ACCORDING TO OCCUPATION

Occupation	Total number of reactions	Number and percentage of persons showing reaction							
		Cholera vaccine group		El Tor vaccine group		Oil-adjuvant vaccine group		Control vaccine group	
		No.	%	No.	%	No.	%	No.	%
Administrative, executive	4	0	0	0	0	4	100.0	0	0
Professional, technical	24	0	0	0	0	24	100.0	0	0
Clerical, sales	61	0	0	0	0	60	98.4	1	1.6
Subordinate technical	368	4	1.1	4	1.1	358	97.3	2	0.5
Service occupations	274	0	0	5	1.8	266	97.1	3	1.1
Manual workers	1 024	10	1.0	20	2.0	975	95.2	19	1.9
Total and average percentage	1 755	14	0.8	29	1.7	1 687	96.1	25	1.4

APPENDIX TABLE 10

VACCINATION REACTIONS ANALYSED BY AGE, 7 JUNE TO 5 DECEMBER 1964

Age-group (years)	Number of reactions	Number and percentage of persons showing reaction							
		Cholera vaccine group		El Tor vaccine group		Oil-adjuvant vaccine group		Control vaccine group	
		No.	%	No.	%	No.	%	No.	%
Less than 1	15	1	6.7	0	0	13	86.7	1	6.7
1	62	0	0	0	0	61	98.4	1	1.6
2	53	1	1.9	2	3.8	50	94.3	0	0
3	37	1	2.7	1	2.7	34	91.9	1	2.7
4	43	0	0	2	4.7	39	90.7	2	4.7
5-9	283	3	1.1	7	2.5	268	94.7	5	1.8
10-14	409	3	0.7	8	2.0	392	95.8	6	1.5
15-19	127	2	1.6	3	2.4	119	93.7	3	2.4
20-24	131	2	1.5	1	0.8	126	96.2	2	1.5
25-29	138	0	0	1	0.7	136	98.6	1	0.7
30-34	110	0	0	0	0	110	100.0	0	0
35-39	80	0	0	1	1.2	77	96.3	2	2.5
40-44	77	0	0	1	1.3	76	98.7	0	0
45-49	70	1	1.4	0	0	69	98.6	0	0
50-54	58	0	0	0	0	58	100.0	0	0
55-59	25	0	0	0	0	24	96.0	1	4.0
60-64	22	0	0	1	4.5	21	95.5	0	0
65-69	5	0	0	0	0	5	100.0	0	0
70-74	8	0	0	1	12.5	7	87.5	0	0
75-79	2	0	0	0	0	2	100.0	0	0
80 and over	0	0	0	0	0	0	0	0	0
Total and average percentage	1 755	14	0.8	29	1.7	1 687	96.1	25	1.4