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Report of the 1966–67 Cholera Vaccine Field Trial in Rural East Pakistan *

2. Results of the Serological Surveys in the Study Population — The Relationship of Case Rate to Antibody Titre and an Estimate of the Inapparent Infection Rate with *Vibrio cholerae*

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The 1966–67 cholera vaccine field trials in East Pakistan tested 1- and 2-dose schedules of a commercial cholera vaccine in 40 000 children aged 3 months to 14 years. Random-sample serological surveys, made prior to the inoculations and 3 months and 6 months after the inoculations, demonstrated that there was a rise in the vibriocidal titres of the vaccinated children during the first 3 months after inoculation and a subsequent fall by the end of the second 3 months. The antibody response to 2 doses of cholera vaccine was better than the response to a single dose in children under 5 years of age. In children aged 5–14 years, the antibody response was similar for both inoculation schedules. Since the majority of the older children had vibriocidal antibodies before inoculation, the data suggest that the single dose acted as a booster, and this effect was not enhanced by a second inoculation.

Serological studies of the hospitalized cholera patients indicated that the majority had low vibriocidal titres on admission to hospital. By comparing the distribution of admission titres of the hospitalized patients with the distribution of titres found in the population survey, it was possible to demonstrate a progressive reduction in the cholera case rate for the population with high levels of vibriocidal antibody.

The sample surveys from the control population revealed a rise in titre following the peak of the cholera season, and a fall 3 months later. The data suggest that the rate of infection with V. cholerae *for the 10 000 children in the control group during the cholera season may have been as high as 27 %, while the clinical case rate was only 0.26 %.*

In the fall of 1965, a serological survey employing single-bleeding random samples was conducted among the several population groups that had participated in the 1963–64 and 1964–65 cholera vaccine field trials undertaken by the Pakistan–SEATO Cholera Research Laboratory (PSCRL) (Mosley et al., 1968b, 1968c). This survey revealed that the prevalence of vibriocidal antibodies increased rapidly with increasing age. More than half of the children in the 5–14-years age-group were found to have significant titres. Furthermore, the titres among the vaccinated children were con-

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siderably higher than among the controls. These findings suggested that vibriocidal antibody might be a measure of cholera immunity, both naturally acquired and vaccine-conferred.

If such observations can be substantiated in repeated studies, the vibriocidal antibody test may become a most useful tool, not only for sero-epidemiological field investigations but also for the evaluation and standardization of cholera vaccines. Accordingly, during the 1966–67 cholera vaccine field trial (Mosley et al., 1969), more extended serological studies were undertaken.

Three random-sample serum surveys were conducted before, during and after a moderately severe epidemic. These surveys provided the basis for following the antibody response to the 1- and 2-dose schedules used and for evaluating further the relationship of the antibody titre to the effectiveness of the vaccine. In addition, it was possible to obtain an estimate of the inapparent infection rate in the control group and to measure the rise and fall of naturally acquired immunity during and following an epidemic.

METHODS

The details of the design of the 1966–67 field trial are presented in the first paper of this series (Mosley et al., 1969). Basically, the trial included approximately 40 000 children, aged 3 months to 14 years. They were randomly assigned to 3 vaccine groups. *Group OO*, the control group, received 2 doses of tetanus and diphtheria toxoids. *Group XO* received 1 dose of a commercial cholera vaccine, followed by 1 dose of tetanus and diphtheria toxoids. *Group XX* received 2 doses of cholera vaccine. The interval between injections was approximately 30 days. The vaccines were assigned so that group XX was twice as large as groups OO and XO.

Serological surveys were taken from this population in September 1966, prior to the vaccination programme in January 1967, during the peak of the cholera season, and in May 1967, at the end of the cholera season. The sampling technique made use of the census books that had been prepared for the vaccine trial population. The sample for the September survey was drawn by selecting every fiftieth family listed in the census books, beginning with a random number. The samples for the January and May surveys were obtained by selecting every thirty-fifth family listed in the census books, beginning with a different random number each time. Thus, a different series of families was included in each cross-sectional sample of the population. Efforts were made to obtain a blood specimen from all children under the age of 15 years in each family sampled.

Fingertip blood specimens were collected in a calibrated 0.05 ml capillary tube [1] and immediately diluted 1 : 10 into 0.45 ml of sterile saline in a screw-capped vial and stored in a portable ice-box. These were identified only by the census number of the individual and the date of collection. The specimens were transported daily on ice to the central laboratory in Dacca where the cells were separated from the diluted blood and the specimens were frozen until titrations were performed. The specimens from each of the sample surveys were titrated within 2 months after collection. Approximately 60 specimens were titrated daily in the order in which they were received from the field. For comparison between surveys, a standard reference serum was titrated each day.

A venous blood specimen was obtained at the time of admission from all hospitalized cholera patients who were residents of the field trial area. These specimens were packed in wet ice and sent to the Dacca laboratory, where the cells were separated and the serum was frozen until titrations were performed.

The vibriocidal antibody titres against the Inaba and Ogawa organisms were determined by the microtechnique described by Benenson et al. (1968). The specimens were tested in doubling dilutions from 1 : 20 to 1 : 2560. The titres reported here represent the serum dilutions prior to the addition of antigen. Only the titres against Inaba organisms are reported in this paper although comparable results were obtained with Ogawa organisms. Geometric mean titres were calculated after assigning a titre of 1 : 10 to titres less than 1 : 20.

RESULTS

Results of the serological surveys

The time relationships of the serological surveys and of the vaccine programme to the cholera season are shown in the figure in the first paper of this series (Mosley et al., 1969). The first survey was conducted in September 1966, just prior to the vaccine programme. Vaccination proceeded from 20 September to 15 November 1966, and was completed just as the cholera season began. The second survey was made at the end of January 1967, when the peak of the

[1] Microcaps, Drummond Scientific Co., Broomall, Pa. USA.

TABLE 1

NUMBER OF PERSONS IN THE STUDY POPULATION AND NUMBER SAMPLED IN THE SEROLOGICAL SURVEYS OF SEPTEMBER 1966, JANUARY 1967 AND MAY 1967 BY VACCINE GROUP AND AGE

Vaccine group	Age-group (years)	No. of persons in study population	Number sampled		
			September 1966	January 1967	May 1967
OO	0–4	3 793	61	94	96
	5–14	6 130	94	122	170
Total		9 923	155	216	266
XO	0–4	3 818	66	83	97
	5–14	6 202	89	124	136
Total		10 020	155	207	233
XX [a]	0–4	7 636	135	170	199
	5–14	12 283	188	241	292
Total		19 919	323	411	491
Grand total		39 862	633	834	990

[a] This group was designed to be twice as large as the other two.

cholera season had just passed and approximately 3 months after the vaccine programme. The third survey, in May 1967, was at the end of the cholera season and approximately 6 months after the vaccine programme.

Table 1 shows the number of children in the study population who received 2 injections of the assigned vaccines, and the number of samples obtained from each vaccine group in the 3 serological surveys. Samples from children who did not receive both assigned injections were excluded from this analysis. Altogether, 633 blood specimens were collected in the September 1966 survey, 834 in the January 1967 survey and 990 in the May 1967 survey. The samples in each survey represented from 1.5% to 2.5% of the children in the study population. As Table 1 illustrates, the 3 vaccine groups were equally represented proportionately in each of the serological surveys.

Table 2 presents the distribution of vibriocidal titres found in the September survey by age and vaccine group. Over-all, only 35.7% of the children in the study population had titres of 1 : 20 or greater, but the proportion increased with age; only 8% of children under 5 years of age had titres of 1 : 20 or greater as compared with 55.3% of children aged 5 to 14 years. The distribution of antibody titres and the geometric mean titres for each of the 3 vaccine groups were comparable prior to the vaccination programme. Thus, the results for the total population survey in September 1966 will be taken as a base line for comparison with the surveys of January and May 1967.

Table 3 presents the results of the survey taken in January 1967 by age and vaccine group. This revealed a marked shift in antibody titre to higher levels in the groups that received cholera vaccine. A single dose of cholera vaccine resulted in titres of 1 : 20 or greater in 89.9% of the children with a rise in mean titre slightly more than 4-fold—from 1 : 16 in September 1966 to 1 : 74 in January 1967. The over-all response was only slightly better in children receiving 2 doses of cholera vaccine: 94.9% had titres of 1 : 20 or greater with the mean titre rising 5-fold from 1 : 17 to 1 : 88 by January 1967. An examination of the vibriocidal response by age

TABLE 2

DISTRIBUTION OF VIBRIOCIDAL TITRES AGAINST INABA ORGANISMS BY AGE AND VACCINE GROUP IN SEPTEMBER 1966

A. Distribution of titres by age

Age-group (years)	Reciprocal titre									Total	GMT [a]	Percentage of titres 1 : 20 or greater
	<20	20	40	80	160	320	640	1280	>2560			
0–4	241	11	4	4	1	1				262	11	8.0
5–14	166	74	75	33	14	4	3	1	1	371	23	55.3
Total	407	85	79	37	15	5	3	1	1	633	17	35.7

B. Distribution of titres by vaccine group

Vaccine group	Reciprocal titre									Total	GMT [a]	Percentage of titres 1 : 20 or greater
	<20	20	40	80	160	320	640	1280	>2560			
OO	104	22	15	8	3	1	2			155	16	32.9
XO	93	28	18	11	2	3				155	17	40.0
XX	210	35	46	18	10	1	1	1	1	323	17	35.0

[a] Geometric mean titre (reciprocal). A mean value of 10 was considered for the group <20.

TABLE 3

DISTRIBUTION OF VIBRIOCIDAL TITRES AGAINST INABA ORGANISMS BY VACCINE GROUP AND AGE IN JANUARY 1967

Age-group (years)	Reciprocal titre									Total	GMT [a]	Percentage of titres 1 : 20 or greater
	<20	20	40	80	160	320	640	1280	>2560			
Vaccine group OO												
0–4	54	13	14	5	3	2		1	2	94	21	42.6
5-14	26	33	26	22	11	2	2			122	34	78.7
Total	80	46	40	27	14	4	2	1	2	216	28	63.0
Vaccine group XO												
0–4	18	17	20	14	6	6	2			83	40	78.3
5–14	3	7	21	33	31	18	8	2	1	124	112	97.6
Total	21	24	41	47	37	24	10	2	1	207	74	89.9
Vaccine group XX												
0–4	17	22	51	41	21	11	4	3		170	58	90.0
5-14	4	8	41	83	50	26	17	10	2	241	118	98.3
Total	21	30	92	124	71	37	21	13	2	411	88	94.9

[a] Geometric mean titre (reciprocal).

reveals that in children under 5 years of age, 2 doses of cholera vaccine resulted in a significantly better mean antibody titre (1 : 59) than 1 dose (1 : 40). Children aged 5 to 14 years, however, responded much better to a single dose of cholera vaccine than did younger children. A single dose resulted in titres of 1 : 20 or greater in 97.6% of the recipients with a mean titre of 1 : 111 by January 1967. Two doses did not produce an improved antibody response in this age-group.

Significantly higher titres were also found in the control population in the January survey. Over-all, 63% of vaccine group OO had titres of 1 : 20 or greater in January 1967, compared with only 35.7% in September 1966. This change was most striking in children under 5 years of age, where only 8% had titres of 1 : 20 or greater in September 1966 compared with 42.6% in January 1967.

Table 4 presents the results of the May 1967 survey by age and vaccine group. This survey revealed an approximately 2-fold fall in the mean titres of the 2 vaccinated groups from the January 1967 levels. In vaccine group XO, the mean titre fell from 1 : 74 in January to 1 : 35 in May. In group XX, the fall was from 1 : 88 to 1 : 48 over the same time period. A significant fall was also seen in the control group, so that the titres in May 1967 were only slightly higher than in September 1966.

Fig. 1, illustrating the distribution of vibriocidal titres by vaccine group, summarizes the results of the 3 serological surveys. In vaccine group OO, the rise in the distribution of titres from September 1966 to January 1967 and the fall by May 1967 is evident, particularly in the children under 5 years of age. By examining the distribution of titres in vaccine groups XO and XX, it is evident that 2 doses of cholera vaccine resulted in a better response than 1 dose in children under 5 years of age; in children aged 5–14 years, the effect of 1 and 2 doses of vaccine on the distribution of antibody titres was similar. The fall in titres for both vaccine groups XO and XX by May is apparent.

Relationship of case rate to antibody titre

Since the serological surveys revealed a marked fall in the titres of the population from January 1967

TABLE 4

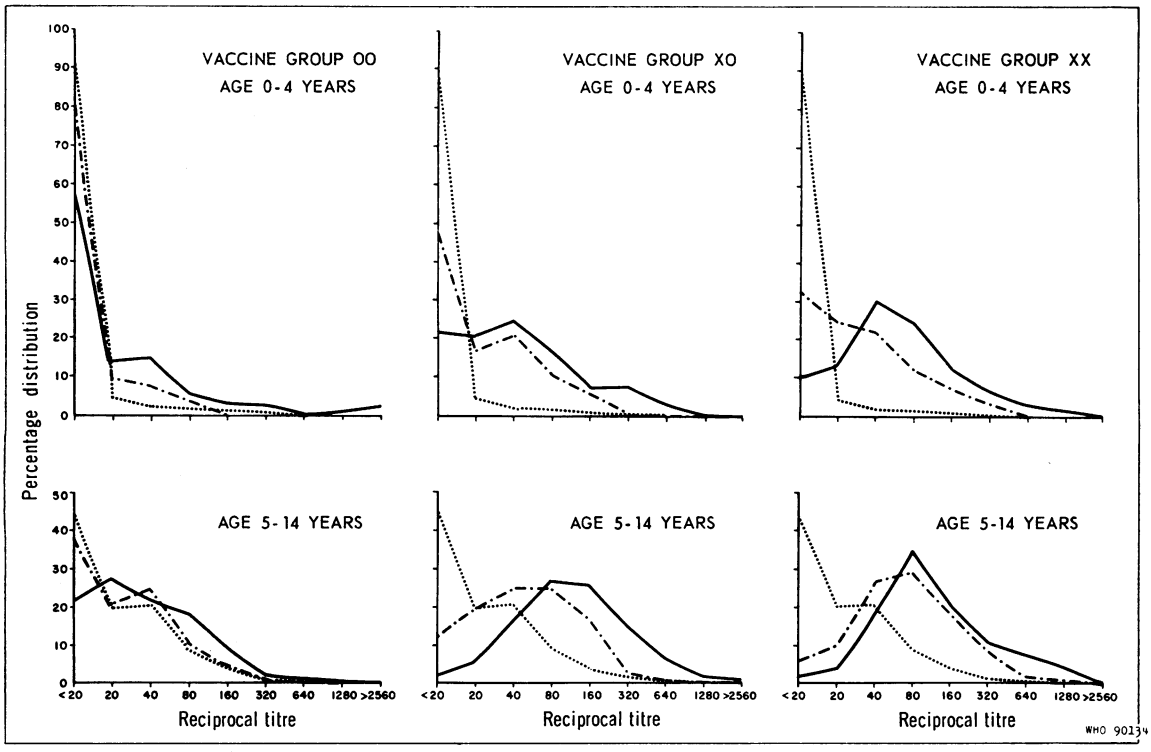
DISTRIBUTION OF VIBRIOCIDAL TITRES AGAINST INABA ORGANISMS BY VACCINE GROUP AND AGE IN MAY 1967

Age-group (years)	Reciprocal titre									Total	GMT [a]	Percentage of titres 1 : 20 or greater
	<20	20	40	80	160	320	640	1280	>2560			
Vaccine group OO												
0–4	77	9	7	3						96	13	19.8
5-14	65	35	42	18	6	3	1			170	24	61.8
Total	142	44	49	21	6	3	1			266	19	46.6
Vaccine group XO												
0-4	46	16	20	10	5					97	21	52.6
5-14	16	26	33	33	22	4	1		1	136	49	88.2
Total	62	42	53	43	27	4	1		1	233	35	73.4
Vaccine group XX												
0-4	64	49	43	23	14	6				199	27	67.8
5-14	18	29	76	84	53	26	4	2		292	69	93.8
Total	82	78	119	107	67	32	4	2		491	48	83.3

[a] Geometric mean titre (reciprocal).

FIG. 1

PERCENTAGE DISTRIBUTION OF VIBRIOCIDAL TITRES AGAINST INABA ORGANISMS BY VACCINE GROUP AND AGE IN THE SEROLOGICAL SURVEYS OF SEPTEMBER 1966, JANUARY 1967 AND MAY 1967



to May 1967, only the cases occurring in a limited period from 2 months prior to, to 2 months following, the January survey are analysed. There were 51 hospitalized cholera patients with onsets between 1 December 1966 and 31 March 1967. Table 5 shows the distribution of antibody titres at the time of admission of these patients, as well as the distribution of antibody titres for the entire population found in the January 1967 serological survey. Over-all, 59% of the hospitalized patients had titres of 1 : 20 or greater on admission. The admission titres in cases from the vaccinated groups were higher than in the control group; titres of 1 : 20 or greater on admission were found 12 of 26 (46%) of the control group, 6 of 11 (55%) of vaccine group XO and 12 of 14 (86%) of vaccine group XX. Low titres were significantly more frequent among the cholera patients than in the population survey. Titres of less than 1 : 20 were found in 41% of the 51 cholera patients but in only 15% of the 834 samples in the population survey.

Based on the distribution of antibody titres found in the January serological survey, it is possible to make an estimate of the actual number of persons in the study population at each level of antibody and thus calculate the cholera case rate, specific for titre. This calculation, shown in Table 6, reveals that the case rate was 36 per 10 000 in individuals with titres of less than 1 : 20. There was a decline in the case rate to 3 per 10 000 in individuals with titres of 1 : 160 or greater. These rates are illustrated in Fig. 2 to demonstrate the progressive decline in case rate with each doubling of the antibody titre. The slope of the line fitted the points shown in Fig. 2

TABLE 5

DISTRIBUTION OF ADMISSION VIBRIOCIDAL ANTIBODY TITRES AGAINST INABA ORGANISMS OF THE 51 HOSPITALIZED PATIENTS FROM THE STUDY POPULATION ADMITTED BETWEEN 1 DECEMBER 1966 AND 30 MARCH 1967, AND DISTRIBUTION OF THE VIBRIOCIDAL TITRES AGAINST INABA ORGANISMS FOUND IN THE STUDY POPULATION IN THE JANUARY 1967 SEROLOGICAL SURVEY

Reciprocal Inaba vibriocidal titre	No. of cases of cholera in hospitalized patients					Study population; January 1967 survey	
	Vaccine group OO	Vaccine group XO	Vaccine group XX	Total cases	%	Number	%
2 560						5	0.60
1 280						16	1.92
640						33	3.96
320			1	1	2.0	65	7.79
160			2	2	3.9	122	14.63
80	3	2	3	8	15.7	198	23.74
40	2	1	5	8	15.7	173	20.74
20	7	3	1	11	21.6	100	11.99
<20	14	5	2	21	41.2	122	14.63
Total	26	11	14	51	100.0	834	100.00

TABLE 6

ESTIMATED DISTRIBUTION OF STUDY POPULATION BY VIBRIOCIDAL TITRE AGAINST INABA ORGANISMS BASED ON THE JANUARY 1967 SEROLOGICAL SURVEY AND THE NUMBER OF CHOLERA CASES FROM THE STUDY POPULATION BY VIBRIOCIDAL TITRE AND CASE RATES

Reciprocal Inaba vibriocidal titre	Estimated number [a] in study population	Cases of cholera in the study population by vibriocidal titre (1.12.66–30.3.67)	Rate of cholera per 10 000 persons
> 2 560	239	0	—
1 280	765	0	—
640	1 578	0	—
320	3 105	1	3.2
160	5 831	2	3.4
80	9 463	8	8.5
40	8 267	8	9.7
20	4 783	11	23.0
< 20	5 831	21	36.0
Total	39 862	51	12.8

[a] Calculated by multiplying the total study population, (39 862) by the proportion in each antibody titre (from Table 3).

suggests that the case rate in the population fell an average of 44% with each doubling of the vibriocidal antibody titre.

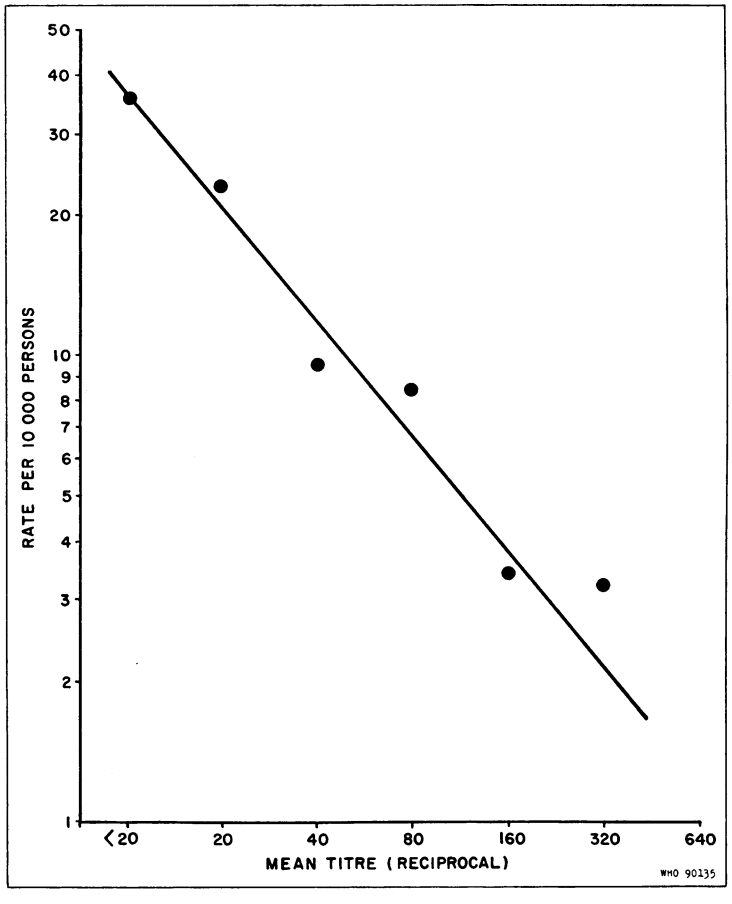
The case rates in hospitalized cholera patients from 1 December 1966 to 30 March 1967, arranged by vaccine group, are shown in the following tabulation:

Vaccine group	*No. of cases*	*Rate per 10 000*
OO	26	26.2
XO	11	11.0
XX	14	7.0

During this 4-month period, the case rate in the control group was 26.2 per 10 000 persons. One dose of cholera vaccine reduced the rate by 58% to 11.0 per 10 000 and 2 doses reduced the rate by 73% to 7.0 per 10 000 persons.

Fig. 3 is a correlation diagram illustrating the relationship of the case rates by vaccine group (see tabulation above) to the geometric mean titres found in the January serological survey (Table 3). This demonstrates that the relative reduction in the case rate produced by cholera vaccine can be closely correlated with the geometric mean titre of the vaccine groups. The slope of the line shown in Fig. 3 indicates that a doubling of the antibody titre

FIG. 2
CHOLERA CASE RATE BY VIBRIOCIDAL ANTIBODY TITRE[a]



[a] Calculated from admission titres of patients hospitalized from December 1966 to March 1967 and the January 1967 serological survey.

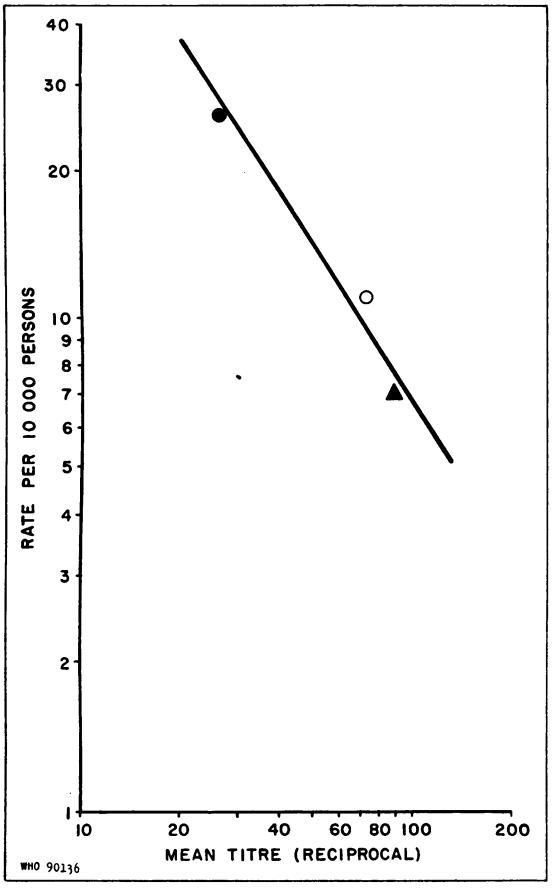
in the population by vaccine was associated with a 52% reduction in the cholera case rate.

DISCUSSION

The serological surveys have proved to be valuable tools in assisting the interpretation of results of the field trials and in elucidating some of the problems in the immunology of cholera. The original serological survey in 1965 demonstrated that there was a progressive increase in the vibriocidal antibody titre in the population with age which could be correlated with a fall in the cholera case rate with age in the endemic cholera area (Mosley et al., 1968b). In addition, the survey demonstrated that there were differences in the antibody titres of the vaccinated and control populations detectable for as long as 2 years after immunization, and that this could be correlated with the persisting effect of the 1963–64 field trial vaccine for more than 18 months (Mosley et al., 1968c). The vaccine used in the 1965 trial was of unusually high potency (Feeley & Pittman, 1965).

In the 1966–67 field trial, in which a commercial cholera vaccine of average potency was used in a 1- and 2-dose schedule, the results, summarized in the first paper of this series, indicated that 2 doses were more effective than 1, but that this enhanced effectiveness was seen only in children under the age

FIG. 3
RELATIONSHIP OF THE CHOLERA CASE RATE IN EACH VACCINE GROUP TO THE MEAN VIBRIOCIDAL ANTIBODY TITRE FOUND IN THE SEROLOGICAL SURVEY [a]



● Group OO
○ Group XO
▲ Group XX

[a] Calculated from patients hospitalized from December 1966 to March 1967 and the January 1967 serological survey.

of 5 years; there was no significant difference in protection by 1- and 2-dose schedules in children aged 5–14 years (Mosley et al., 1969). The results of the serological survey in January 1967, at the peak of the cholera epidemic, were consistent with this observation. There was a significant difference in the antibody titres for the 1- and 2-dose schedules in children aged 0–4 years, but there was no significant difference in titres in children aged 5–14 years. Since the September survey indicated that more than half of the children aged 5–14 years already had detectable antibody titres prior to immunization, this suggests that the first dose of cholera vaccine in this age-group acted as a booster. This response was not enhanced by a second dose of vaccine a month later.

The determination of antibody titres at the time of admission of the cholera patients made it possible to analyse the relationship of the cholera case rate to antibody titre in the study population. The results revealed that there was a progressive reduction in cholera case rate in the population with higher levels of vibriocidal antibody. This fall in case rate approximated to a 44% reduction with each doubling of the vibriocidal antibody titre. This finding is consistent with that in an earlier study in family contacts of cholera cases (Mosley et al., 1968a). When the family contacts were grouped by antibody titre, subsequent infections and cases occurred predominantly in those contacts with low titres (less than 1 : 20) with a progressive fall in the infection rate with higher levels of vibriocidal antibody.

This consistent relationship of vibriocidal antibody titre to case rate makes it possible to estimate the relative effectiveness of a vaccine from serological data only by assuming a 50% reduction in case rate with every doubling of the mean titre of a population group. For example, based on the mean titres found in each vaccine group in the January survey (Table 3), it can be estimated that 1 dose of this vaccine would reduce the case rate by 63% and 2 doses by 68%, compared with the control group. These estimates agree reasonably well with the observed protection of 58% for 1 dose and 73% for 2 doses during the period from 1 December 1966 to 31 March 1967 (see tabulation on p. 193).

While the cholera case rate appears to be related to the vibriocidal antibody titre, this association does not necessarily indicate that vibriocidal antibodies play a direct role in protection from cholera; however, the consistency with which this association has been observed during all field trials in East Pakistan suggests that the determination of vibriocidal response in man may be a valuable tool for the evaluation of cholera vaccines. For example, from the 1966–67 field trial, using a commercial cholera vaccine in 1- and 2-dose schedules, it is evident that this vaccine produces an inadequate serological response, particularly in young children, both in terms of level of antibody titre and in duration of antibody response. Thus, future vaccine develop-

ment should be directed toward a product that will produce a high and sustained antibody response in children. When such a product becomes available, a vaccine field trial should demonstrate sustained protection against the disease. At least it would seem prudent to utilize the vibriocidal response in man as a major criterion for selecting test vaccines for future field trials.

There was a remarkable increase in the titres among the control group between September 1966 and January 1967 with a subsequent fall by May 1967. The proportion of the sample from vaccine group OO with titres of 1 : 20, or higher, was greater by an increment of 27% in the January 1967 survey than in the population sample of September 1966, with the increments by age being 35% in children under 5 years of age and 15% in children aged 5–14 years. This increase is believed to have resulted from a high rate of inapparent infection in the study population, particularly among the young children. Before arriving at this conclusion, other possible explanations should be considered. Among these are chance variations, errors in administration of the assigned vaccines and technical variations in preparing the antibody tests.

Considering the size of the samples, the differences are far beyond the limits of what could be expected from sampling variations only. Incorrect distribution of cholera vaccine to some members of the control group would have resulted in a larger proportion with vibriocidal titres in January than in September. We do not believe such an error occurred. The method of vaccine assignment included a double check on identification. Separate jet-injectors were used for each vaccine and the vaccinations were performed by well-disciplined teams which were constantly supervised. A technical error in performing antibody titrations could have led to some differences between surveys. Again, this is unlikely since the procedure has proved to be quite reproducible in surveys where titrations over a period of days will minimize the effect of day-to-day variations. For example, in this study, the mean titre of the reference sera was 1 : 51 in September (20 determinations), 1 : 50 in January (22 determinations) and 1 : 56 in May (14 determinations).

Thus, the most reasonable hypothesis is that the shift in titres was largely due to inapparent infections with *V. cholerae*. Evidence for a very high rate of inapparent infections with *Vibrio cholerae* has recently been accumulating from many sources. Van de Linde & Forbes (1965) in Hong Kong in 1961–63, were able to demonstrate widespread infection with *V. cholerae* in the city, although there were only sporadic cases of cholera appearing. From their analysis of the data, it was suggested that there had probably been at least 100 infections for every clinical case. Applying a similar method of latrine sampling to Calcutta bustees in 1965–66, Sinha et al. (1967) were able to detect 4 cholera cases and 98 persons with inapparent infection, thus directly demonstrating the presence of 25 inapparent infections for every clinical case.

The 1965 serological survey in the Matlab vaccine trial population revealed that the proportion of the population with vibriocidal titres of 1 : 20 or greater increased at a rate of 5%–10% per year of age from the first year of life (Mosley et al., 1968b). This rate is at least 10 times higher than the annual cholera case rate for the area. Direct evidence for frequent inapparent infection in the field trial area has recently been provided by McCormack et al. (1969b). In an intensive bacteriological and serological surveillance of a village of 1600 persons, they documented 27 infections with *V. cholerae*. The infection rate was over 5% in children under 5 years of age, and yet there was not a single clinically recognizable case of cholera.

In vaccine group OO, the cholera case rate for the period from November 1966 to January 1967 was 26 per 10 000 (Mosley et al., 1969). The serological surveys suggested that the infection rate may have been as high as 2700 per 10 000 (27%) between September 1966 and January 1967. This would indicate that the infection to case ratio was in the range of 100 to 1, a ratio similar to that suggested by van de Linde & Forbes (1965) in Hong Kong.

It may be noted that there is a significant disparity between the results of the 1966–67 study suggesting that the annual cholera infection rate in children may be as high as 27%, and the serological survey taken in September 1965 indicating that the proportion of children with titres of 1 : 20, or greater, only increased at a rate of 5%–10% per year of age. A long-term serological follow-up of hospitalized cholera patients by McCormack et al. (1969a) provides one solution to this apparent disparity in annual infection rates. They found that more than two-thirds of the children under 5 years of age with clinical cholera no longer had a detectable vibriocidal titre of 1 : 20 or greater by 6 months after infection. They have suggested that, in order for children in the endemic cholera area to acquire sustained vibriocidal titres of 1 : 20 or greater at the rate of 5%–10% per

year, not only must the annual infection rate be at least 3 times higher, but reinfection with *V. cholerae* must be common. The marked decline in antibody titres in all 3 vaccine and control groups between the January and May surveys is entirely consistent with this evanescent character of the vibriocidal antibody, whether it arises from natural infections or artificial immunization.

RÉSUMÉ

RAPPORT SUR L'ESSAI PRATIQUE D'UN VACCIN ANTICHOLÉRIQUE DANS UNE RÉGION RURALE DU PAKISTAN ORIENTAL (1966-1967): 2. RÉSULTATS DES ENQUÊTES SÉROLOGIQUES MENÉES DANS LA POPULATION SOUMISE A L'ESSAI — RELATION ENTRE LA MORBIDITÉ CHOLÉRIQUE ET LES TITRES D'ANTICORPS; ÉVALUATION DU TAUX D'INFECTION INAPPARENTE A *VIBRIO CHOLERAE*

Des enquêtes sérologiques ont été entreprises parmi un groupe d'environ 40 000 enfants à l'occasion de l'essai de vaccin anticholérique décrit dans l'article précédent. Des échantillons de sérum ont été prélevés au hasard, dans chaque cas chez 600 à 900 sujets, avant la vaccination, 3 mois après (au moment de l'acmé de la poussée saisonnière de choléra) et 6 mois après (lors de la régression de l'épidémie). On a utilisé une microtechnique pour la recherche des anticorps vibriocides, le sang étant recueilli par piqûre du doigt.

L'examen des sérums prélevés avant la vaccination a montré une similitude du profil immunologique dans les trois groupes vaccinés, avec des titres d'anticorps plus élevés chez les enfants les plus âgés. Au cours des enquêtes postvaccinales, on a constaté chez les enfants ayant reçu le vaccin anticholérique une hausse des titres après 3 mois et une diminution après 6 mois. Chez les enfants de moins de 5 ans, la réponse immunitaire à l'injection de deux doses de vaccin a été plus forte qu'après l'injection d'une dose unique. Chez les enfants âgés de 5 à 14 ans, la réponse immunitaire a été identique après l'administration d'une ou de deux doses. La majorité des sujets de ce dernier groupe possédant des anticorps vibriocides avant la vaccination, on peut admettre que la vaccination à dose unique a fait effet d'injection de rappel et que la seconde dose n'a pas eu d'action appréciable.

L'examen des sérums prélevés au moment de l'admission chez des sujets hospitalisés pour choléra a montré chez la plupart des malades la présence d'anticorps vibriocides à des titres faibles. En comparant la répartition de ces titres et les données sérologiques relatives à l'ensemble du groupe, on a mis en évidence une réduction progressive de la morbidité cholérique en fonction de la teneur du sérum en anticorps. Chez les enfants porteurs d'anticorps à des titres inférieurs à 1:20, le taux de la morbidité a été estimé à 36 pour 10 000, alors qu'il n'était que de 3 pour 10 000 lorsque les titres atteignaient 1: 160 ou plus.

L'étude des sérums prélevés chez les enfants vaccinés par la préparation témoin (anatoxine tétanique ou diphtérique) a montré une hausse des titres d'anticorps vibriocides, succédant à l'acmé de la poussée saisonnière de choléra, suivie d'une diminution 3 mois plus tard. Il semble d'après ces données que dans ce groupe d'environ 10 000 enfants, le taux d'infection par *V. cholerae* a pu être pendant l'épidémie de l'ordre de 27‰, le taux des cas de choléra cliniquement avérés n'étant que de 0,26‰.

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