REPORT ON REACTIGENICITY AND IMMUNOGENICITY OF WELLCOME CHOLERA TOXOIDS IN BANGLADESHI VOLUNTEERS

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PREFACE

The International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) is an autonomous, international, philanthropic and non-profit centre for research, education and training as well as clinical service. The Centre is derived from the Cholera Research Laboratory (CRL). The activities of the institution are to undertake and promote study, research and dissemination of knowledge in diarrhoeal diseases and directly related subjects of nutrition and fertility with a view to develop improved methods of health care and for the prevention and control of diarrhoeal diseases and improvement of public health programmes with special relevance to developing countries. ICDDR,B issues two types of papers: scientific reports and working papers which demonstrate the type of research activity currently in progress at ICDDR,B. The views expressed in these papers are those of authors and do not necessarily represent views of International Centre for Diarrhoeal Disease Research, Bangladesh. They should not be quoted without the permission of the authors.

ABSTRACT

This report summarizes the results of two pre-trial assessments of three aluminum adjuvanted cholera vaccines prepared by the Wellcome Research Laboratories of the United Kingdom. These were a whole cell preparation, a toxoid, and a mixture of these two vaccines. They were compared to a standard tetanus toxoid. In the first pre-trial done in January, 1978 skin rashes were observed in recipients of all groups including the tetanus toxoid control vaccine. A second assessment was therefore done in April and May of 1979 to assess this problem. The results of these pre-trials indicate that there is no unusual reaction associated with the use of any of the vaccines tested. Both the whole cell and toxoid vaccines produced excellent immune responses in a range that would be expected to be protective. There were significant rises in vibriocidal titres in recipients of the toxoid vaccine indicating the residual presence of cell wall antigens in the toxoid preparation. The conclusion of the assessment is that these aluminum adjuvanted whole cell and toxoid cholera vaccines are safe and produce good antibody responses.

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I. INTRODUCTION

In April and May 1979, we conducted reactigenicity and immunogenicity assessments on three cholera vaccines[1/] - a cholera toxoid (300 µg/dose), a whole cell vaccine, and a whole cell vaccine-toxoid (300 µg/dose) mixture. Each vaccine was adjuvanted with aluminum hydroxide. These assessments were primarily to determine the suitability of the whole cell vaccine-toxoid mixture for a field evaluation of protection from cholera currently scheduled for January 1980.

A previous pre-trial study was conducted in January 1978; however, due to the observation of possibly allergic skin rashes in 1 percent of recipients of all types of vaccines, only one of the planned two injections were given (see "Interim Report on Reactions in the Pre-Test of Cholera Vaccines for 1978 Field Trial," February 14, 1978). It was therefore necessary to repeat the reactigenicity studies before a full field evaluation could be considered. The most important question of this second pre-test study was whether there were allergic reactions associated with the vaccines and the design of the pre-test was altered accordingly. The number of volunteers receiving the whole cell vaccine-toxoid mixture, the experimental vaccine proposed for the field trial in 1980, was increased to 1,000 to permit better evaluation of reactions having an incidence of 1 percent or less. In addition, two control

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1/ Produced by Wellcome Research Laboratories.

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vaccines, tetanus toxoid and "normal" saline, were each given to 500 volunteers to allow comparison between these groups and that receiving the whole cell vaccine-toxoid mixture. Approximately 100 persons each were given cholera toxoid or whole cell vaccine to permit serologic studies of the immunogenicity of the experimental vaccines.

As the 1980 field study plans to utilize two injections of the vaccines separated by six weeks, it was also necessary to assess local and systemic reactigenicity in volunteers during this pre-trial study. Thus, all recipients of a vaccine in April were sought for a second injection of the same vaccine, six weeks later.

After informed consent and a thumbprint were obtained from each volunteer or guardian (for children) an intramuscular injection (0.5 ml) was given by Ped-O-Jet injector. The numbers of volunteers receiving first and second injections of the various vaccines are shown in Tables 1 and 2.

II. REACTIGENICITY

A. METHODS

After each injection Bangladeshi physicians conducted house-to-house surveillance for local and systemic reactions. For four days after the innoculation, each vaccinee was questioned and examined to determine

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the presence and severity of reactions, which were scored by defined criteria (Table 3). Temperatures were measured for all vaccinees on days 1 and 2. Temperatures were taken on days 3 and 4 only if a temperature of 100°F or greater had been noted on either day 1 or 2. Temperatures were taken rectally in children 1 to 4 and orally in older children and adults. Visits to each neighborhood were made on days 7 and 14 following vaccination to permit the detection of delayed reactions.

In addition to the reactions listed in Table 3, each vaccinee was asked about the presence of skin rash and the skin was examined for the occurrence of urticaria or other skin rashes. Special attention was also directed to looking for periorbital edema and other possible manifestations of allergic reactions.

At the time of the examination, neither the physicians nor the vaccinees knew the type of vaccine that had been administered.

B. RESULTS

1. First Injection

Of the 2,279 persons receiving the first injection, 2,035 (89 percent) were available for reaction assessment on the first day after innoculation. The percentage of persons available for examination on day 1 varied slightly by age: 94 percent were available in the 1 to 4 year

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age group, 84 percent in the 5 to 14 year age group, and 92 percent in the 15 year or more age group. Of the total vaccinees, 2,165 (95 percent) were examined on at least one of the four days following innoculation.

(a) Pain

As illustrated in Tables 4-6, on day 1, pain was most frequently reported by recipients of whole cell vaccine and of cholera toxoid, followed by the whole cell vaccine-toxoid mixture, tetanus toxoid, and saline. The severity of pain appeared to be slightly less in the 5 to 14 age group but the subjective nature of this reaction makes comparison among age groups difficult. In all age and vaccine groups the pain severity had decreased substantially by day 2 and was rarely above grade 1 by day 4.

(b) Tenderness

Tenderness was noted in 80 percent or more of recipients of each of the vaccines on day 1 (Tables 7-9). The severity of tenderness was highest for recipients of cholera toxoid, whole cell vaccine or the mixture but even in these cases was usually only grade 2 (tenderness on light pressure). The severity of tenderness did not differ substantially by age.

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By day 2 the severity had diminished in all vaccine groups. Only three persons had tenderness greater than grade 2 by day 4; one had received the whole cell vaccine and two the mixture.

(c) Redness

After the first injection, redness appeared to be more common and more extensive with the whole cell vaccine-toxoid mixture than with any other vaccine (Table 10-22). This was seen in all age groups and the difference persisted to day 2. However, even in this group the redness rarely exceeded 6 cm and there were only two adults who had redness extending more than 10 cm. In one case, this degree of redness persisted to day 4, but in all other vaccinees the severity was reduced to grade 2 or less by that time.

(d) Induration

The degree of induration noted after the first injection is shown in Tables 13-15. On day 1 the whole cell vaccine-toxoid mixture resulted in more grade 2 induration (2-5.9 cm) than the other vaccine in all three age groups. Induration was rarely more than 10 cm in any group; however, more cases of induration extending 6-10 cm were seen in the groups

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receiving the mixture. The higher degree of induration with the mixture was also clearly seen on day 2 and even by day 4, when 6 percent of recipients still had grade 2 induration.

(e) Fever

The rate of fever (>100°F) following innoculation did not differ substantially in the different age groups, so Table 16 includes vaccinees of all ages. Fever following any of the vaccines was unusual; being seen in approximately 7 percent of recipients of cholera toxoid, whole cell vaccine, or the mixture. Fevers were usually low grade, with temperatures of 101°F or greater occurring in less than 1 percent of recipients. The temperature usually returned to normal by day 2 although 2 percent of fevers associated with the mixture persisted until the day 2 (Table 17).

(f) Skin rash and other clinical findings

Although carefully sought in all vaccinees, skin rashes, including urticaria, were not observed, nor were other allergic manifestations such as periorbital edema. Glandular enlargement was unusual, occurring in 0.4-0.9 percent of each vaccine group.

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(g) Limitation of activity

Limitation of activity because of arm discomfort was more commonly reported in the cholera toxoid, whole cell vaccine, and mixture groups (Table 18). On day 1 the degree of limitation did not differ among these three groups; however, limitation persisted to day 2 more often in the whole cell vaccine and mixture groups. Limitation was usually mild, with only 1 to 2 percent of vaccinees unable to work and of short duration resolving by day 2 or 3.

2. Second Injection

Of the 1,680 persons receiving the second injection, 1,468 (87 percent) were available during reaction surveillance on day 1. The percentage of persons available in the three age groups was similar to after the first injection: 93 percent in the 1 to 4 year age group, 83 percent in the 5 to 14 year group, and 89 percent in the 15 year or more age group. Of the total vaccinees, 1,649 (98 percent) were seen by the examining physicians on at least one of the four days following innoculation.

Since the reactions on day 1 following the second injection were the most frequent and the most severe, as had been the case after the first injection, only reactions on day 1 will be shown in tables. Unless noted in the text, recovery from the reactions followed a similar time course to

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that reported after the first injection.

(a) Pain

The frequency and severity of pain reported after the second injection was comparable to that after the first injection (Table 19). In fact, the reactions to the cholera toxoid, the whole cell vaccine, and the mixture tended to be a little less severe, with a lower frequency of reported grade 3 or 4 pain.

(b) Tenderness

As with pain, tenderness reactions had a slightly lower reported severity than after the first injection (Table 20). Reactions of grade 3 were unusual and grade 4 rare.

(c) Redness

The generally more extensive redness with the whole cell vaccine-toxoid mixture after the first injection was not seen after the second injection. Redness on day 1 was similar for the mixture and the whole cell vaccine alone, with the exception of slightly more frequent grades 3 and 4 reactions in adults receiving the mixture (Table 21). Likewise, on day 2 these two vaccines had a similar percentage

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of grades 2 and 3 reactions, but adults receiving the mixture had more grades 3 and 4 reactions observed.

(d) Induration

As after the first injection, induration was slightly more prominant on day 1 in recipients of the mixture than in other vaccinees (Table 22). However, there was very little difference in the persistence of induration to days 2-4 between the groups getting the mixture or the whole cell vaccine and induration was rarely greater than 6 cm in any group.

(e) Fever

Fever again occurred in a low proportion of vaccinees (Table 23). Low grade fever (<101°F) was most common in the whole cell vaccine group but the difference did not persist to day 2. Fever rarely persisted beyond day 2 (Table 24).

(f) Skin rash and other clinical findings

An urticaria-like generalized rash was noted in one child on day 4 following vaccination with the mixture. As the child had a history of previous identical rashes unassociated with vaccination, this was not considered an allergic reaction

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due to the vaccine.

Glandular enlargement was rare and not associated with a particular vaccine.

(g) Limitation of activity

Limitation of activity on days 1 and 2 following innoculation was very similar to that noted after the first injection (Table 25). Again, the limitation was usually mild and of short duration.

C. DISCUSSION

The cholera vaccines evaluated here, resulted in local and systemic reactions which were similar to those produced by previously tested cholera vaccines. The whole cell vaccine and the vaccine-toxoid mixture resulted in slightly higher reaction rates than the tetanus and cholera toxoids. As expected, the recipients of saline injections had the lowest reaction rates.

Slightly less severe reactions were reported after the second injection than after the first, although differences among the vaccines were similar following each injection. Since the same physicians conducted the reaction surveillance the difference is not likely to be due to differing criteria for severity. However, since only 74 percent of

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persons getting the first injection were available for the second injection, it is possible that a higher proportion of persons with more severe reactions to the first injection refused the second dose.

Because of the observation of allergic-like rashes during the 1978 pre-test of these vaccines, the evaluation of allergic reactions following innoculation was a major concern of this pre-test. Thus, the number of vaccinees was increased to permit better confidence of eliminating a reaction of low incidence. In this pre-test, no allergic manifestations were seen following 1,089 innoculations containing cholera toxoid which were given as the first injection of the pre-test. Of the 798 persons receiving a second innoculation containing toxoid, no allergic reactions were thought to be associated with the vaccines.

From this pre-test, we find no evidence to suggest that these vaccines would result in unacceptable or dangerous side effects. The reactions seen in recipients of the whole cell-toxoid mixture were comparable to those observed with the aluminum-adjuvanted whole cell vaccine and with other currently used vaccines. Thus, from all available evidence, it appears that the whole cell vaccine-toxoid mixture can be used safely in a field evaluation of protection from cholera.

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III. IMMUNOGENICITY

A. METHODS

During the 1979 pre-test, an age-stratified sample of volunteers was preselected to have blood obtained for immunologic studies. A blood sample was taken by fingerstick prior to and 14 days following each of the two injections received by the volunteers. As the injections were separated by 42 days the bloods will be referred to as day 0, 14, 42, and 56. Table 26 illustrates the number of blood specimens obtained by age and vaccine type for each of the four collections.

In each case, 100 µl of capillary blood was diluted in 0.9 ml of sterile saline in 1 dram vials. These diluted bloods were refrigerated until they were delivered to Dacca within 24 hours. In Dacca the vials containing diluted blood were centrifuged at 1,500 RPM for 5 minutes. Using disposable Pasteur pipettes the sera were separated to sterile 1-dram vials which were stored at -20°C until testing.

A sub-sample of lactating women was selected for studies of breast milk and saliva, which may provide information on the local intestinal immune response to vaccination. Milk and saliva specimens were obtained from vaccinated women on days 0, 5, and 14 for each injection and blood was taken according to the regular schedule. At least three milk specimens were

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obtained from 52 women and complete collections of six specimens were obtained from 32 women. Studies will begin shortly with these specimens.

The serologic response to immunization was assessed by the vibriocidal antibody (Inaba) assay and by the passive hemagglutination technique for cholera antitoxins (1, 2).

Paired sera (days 0 and 56) from 145 adult (10 or more years of age) recipients of cholera toxoid, whole cell vaccine-cholera toxoid, mixture or saline have been submitted for determination of IgE titers to equine protein by the RAST assay. Specimens from these vaccinees will also be sent to Wellcome Laboratories for determination by radioimmunassay of IgG titers to equine protein.

B. RESULTS

1. Vibriocidal Antibody Titers

The geometric mean vibriocidal antibody titers on day 0 in all four vaccine groups show the expected increase with age (Table 27). In recipients of tetanus toxoid mean titers within each age group remain unchanged after innoculation.

Mean vibriocidal titers in cholera toxoid vaccinees increased following the first injection in all age groups, as noted in the 1978 pre-test. After the second injection a further rise in titer was noted only in

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the 1-4 year age group.

In recipients of the whole cell vaccine mean titers increased substantially after the first injection. This was true in all age groups although titers in the 1-4 year group were lower than those in the other two age groups. Mean titers in all age groups decreased by day 42 and increased again after the second injection. However, only the 1-4 year group had higher titers on day 56 than on day 14. The addition of cholera toxoid to the whole cell vaccine did not seem to have an adverse effect on vibriocidal titers in vaccinees.

2. Passive Hemagglutination Antitoxin Titers

The geometric mean serum passive hemagglutination titers in antitoxin units/ml are shown in Table 28. Mean titers remained unchanged in recipients of tetanus toxoid or whole cell vaccine, but increased substantially in persons receiving either of the two vaccines containing cholera toxoid. Titers in all age groups decreased by day 42 and increased again after the second injection of the same vaccine. However, in all groups the mean titer on day 14 was higher than on day 56 (day 14 following the second injection). Antitoxin titers in recipients of the whole cell vaccine-toxoid mixture did not differ significantly from those in recipients of the toxoid alone.

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As previously noted in the 1978 pre-test, day 0 titers (prior to innoculation) did not decrease with age as has been observed with titers determined by neutralizing assays, such as the adrenal cell or rabbit skin assays. The frequency distribution of day 0 antitoxin titers shown in Table 29 corroborates this finding. The proportion of persons with low and high titers does not differ significantly by age group.

C. DISCUSSION

The aluminum-adjuvanted whole cell vaccine used in this pre-test resulted in substantial vibriocidal antibody titers in volunteers of all age groups. This observation and the favorable results of field testing of aluminum-adjuvanted cholera vaccines in Indonesia and India, suggest that this vaccine may provide excellent protection from cholera for a longer period than currently available vaccines.

Increases in vibriocidal antibodies were also found in recipients of purified cholera toxoid, suggesting that this vaccine also contains somatic antigen. This further confirms similar observations made in immunized volunteers in Texas, in immunized dogs and in Matlab vaccinees in the 1978 pre-test. Although these rises in titer in toxoid recipients are of much lower magnitude than those in whole cell vaccine recipients, they preclude the possibility of determining the protective value of purified toxoid. Without knowing the protective effect of toxoid, it will

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also not be possible to determine if whole cell vaccine and toxoid are synergistic in providing protection from cholera. However, it is still possible to determine if parenteral cholera toxoid adds to the protective value of aluminum-adjuvanted whole cell vaccine.

The aluminum-adjuvanted cholera toxoid caused sizable rises in antitoxin titer when given to Matlab volunteers of all ages. Titers were higher on day 14 after the first injection than on day 14 after the second injection. This higher hemagglutinating titer may be the result of a relatively higher proportion of IgM rather than IgG after the first injection, or alternately may be the result of an inhibition of the immune response with the second injection. The immuglobin class-specific antitoxic antibodies and neutralization antibody titers will be determined in a sample of specimens to help define this observation.

The geometric mean titers of hemagglutinating antitoxic antibody from sera collected before immunization (day 0) did not differ with age. This is different from neutralizing antibody titers which show a decrease with age. This difference in titers by age may be the result of a lack in sensitivity or accuracy at the lower range in titers, or it may be the result of the two assays measuring different classes of antibody.

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IV. CONCLUSION

The aluminum-adjuvanted Wellcome whole cell vaccine, cholera toxoid, and combination vaccine which were evaluated in this pre-test result in local and systemic reactions comparable to those produced by previously tested and currently available cholera vaccines. The lack of allergic reactions or other severe complications indicates that these vaccines are suitable for further evaluation to determine their value in protection from cholera.

Available evidence from studies done in Indonesia and India, suggest that an aluminum-adjuvanted whole cell vaccine may be significantly better than currently available vaccines. The vibriocidal antibody titers in Matlab volunteers receiving this vaccine in the 1978 and 1979 pre-tests indicate substantial immunogenicity. Thus, it seems appropriate to field test this vaccine in Matlab where previous studies of non-adjuvanted cholera vaccines have taken place and where the evaluation can be continued for several years if necessary.

Although it is not possible to evaluate the protection from cholera offered by a purified cholera toxoid, it is important to determine if such a toxoid adds to the protective value of a whole cell vaccine. The Wellcome toxoid is highly immunogenic, resulting in high antitoxin

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titers, as assessed by the passive hemagglutination, adrenal cell, and rabbit skin assays and thus seems a good candidate for field testing in combination with an aluminum-adjuvanted whole cell vaccine.

TABLE 1

NUMBER OF PERSONS RECEIVING FIRST INJECTION DURING THE WELLCOME CHOLERA TOXOID PRE-TEST, 1979

Vaccine	Age Group (years)			Total
	1-4	5-14	15+*	
Tetanus toxoid	98	220	229	547
Cholera toxoid	33	40	44	117
Whole cell vaccine-cholera toxoid	158	414	400	972
Whole cell vaccine	35	42	50	127
Saline	61	205	250	516
Total	385	921	973	2,279

* Females only

TABLE 2

NUMBER OF PERSONS RECEIVING SECOND INJECTION DURING THE WELLCOME CHOLERA TOXOID PRE-TEST, 1979

Vaccine	Age Group (years)			Total
	1-4	5-14	15+*	
Tetanus toxoid	62	166	165	393
Cholera toxoid	26	37	34	97
Whole cell vaccine-cholera toxoid	128	280	293	701
Whole cell vaccine	25	34	42	101
Saline	37	154	197	388
Total	278	671	731	1,680

* Females only

TABLE 3

SCORING CRITERIA FOR VACCINE REACTIONS

LOCAL REACTIONS

Pain:	0	- No pain
	1+	- Dull ache with or without movement of limb
	2+	- Pain only on movement of limb
	3+	- Pain at rest with partial limitation of movement
	4+	- Pain at rest with total limitation of movement
Tenderness:	0	- No tenderness
	1+	- Tenderness on firm pressure
	2+	- Tenderness on light pressure
	3+	- Tenderness on firm touch
	4+	- Tenderness on light touch
Redness:	0	- No redness
	1+	- Redness only at injection site
	2+	- Redness extending 2-5.9 cm
	3+	- Redness extending 6-10 cm
	4+	- Redness extending >10 cm
Induration:	0	- No induration
	1+	- Induration extending up to 2 cm
	2+	- Induration extending 2-5.9 cm
	3+	- Induration extending 6-10 cm
	4+	- Induration extending >10 cm

GENERAL REACTIONS

Glandular Enlargement:	0	- Absent
	+	- Present
Activity:	0	- Normal activity
	1+	- Interferred with work but not totally bedridden
	2+	- Totally bedridden - no work possible

Temperature:

- Record oral temperature after 1 minute in all vaccines aged 5 and over.
- Record rectal temperature after 1 minute in all children aged 1-4.

TABLE 4

PERCENTAGE OF VACCINEES, AGE 1-4 YEARS, REPORTING PAIN ON DAYS 1 AND 2 FOLLOWING VACCINATION I

Vaccine	Pain Severity on Day 1					
	0	1	2	3	4	Unk
Tetanus toxoid	31	42	14	1	0	12
Cholera toxoid	15	48	33	3	0	0
Whole cell vaccine-cholera toxoid	13	57	22	4	<1	3
Whole cell vaccine	9	31	49	9	0	3
Saline	46	43	5	0	0	7

Vaccine	Pain Severity on Day 2					
	0	1	2	3	4	Unk
Tetanus toxoid	65	18	1	0	0	15
Cholera toxoid	49	27	12	0	0	12
Whole cell vaccine-cholera toxoid	48	33	11	1	<1	6
Whole cell vaccine	29	57	6	3	0	6
Saline	77	8	0	0	0	15

TABLE 5

PERCENTAGE OF VACCINEES, AGE 5-14 YEARS, REPORTING PAIN ON DAYS 1 AND 2 FOLLOWING VACCINATION I

Vaccine	Pain Severity on Day 1					
	0	1	2	3	4	Unk
Tetanus toxoid	27	48	8	1	0	16
Cholera toxoid	20	38	20	0	0	22
Whole cell vaccine-cholera toxoid	10	56	18	3	0	13
Whole cell vaccine	17	33	26	2	0	21
Saline	44	36	2	0	0	18

Vaccine	Pain Severity on Day 2					
	0	1	2	3	4	Unk
Tetanus toxoid	61	17	4	0	0	18
Cholera toxoid	32	35	2	0	0	30
Whole cell vaccine-cholera toxoid	48	30	4	<1	0	17
Whole cell vaccine	43	36	5	0	0	17
Saline	71	4	0	0	0	25

TABLE 6

PERCENTAGE OF VACCINEES*, AGE 15 OR MORE YEARS, REPORTING PAIN ON DAYS 1 AND 2 FOLLOWING VACCINATION I

Vaccine	Pain Severity on Day 1					
	0	1	2	3	4	Unk
Tetanus toxoid	18	56	15	2	1	9
Cholera toxoid	4	39	43	7	0	7
Whole cell vaccine-cholera toxoid	8	46	31	8	0	8
Whole cell vaccine	6	38	42	14	0	0
Saline	42	40	7	1	0	10

Vaccine	Pain Severity on Day 2					
	0	1	2	3	4	Unk
Tetanus toxoid	57	24	6	1	0	12
Cholera toxoid	50	32	7	0	0	11
Whole cell vaccine-cholera toxoid	35	39	14	1	0	11
Whole cell vaccine	46	24	24	2	0	4
Saline	71	9	2	<1	0	18

* Females only

TABLE 7

PERCENTAGE OF VACCINEES, AGE 1-4 YEARS, REPORTING TENDERNESS ON DAYS 1 AND 2 FOLLOWING VACCINATION I

Vaccine	Tenderness Severity on Day 1					
	0	1	2	3	4	Unk
Tetanus toxoid	4	54	28	2	1	11
Cholera toxoid	6	46	39	9	0	0
Whole cell vaccine-cholera toxoid	1	40	42	11	2	3
Whole cell vaccine	3	40	37	14	3	3
Saline	20	59	15	0	0	7

Vaccine	Tenderness Severity on Day 2					
	0	1	2	3	4	Unk
Tetanus toxoid	12	68	3	0	1	15
Cholera toxoid	9	64	9	6	0	12
Whole cell vaccine-cholera toxoid	4	60	25	3	2	6
Whole cell vaccine	9	60	20	6	0	6
Saline	36	46	3	0	0	15

TABLE 8

PERCENTAGE OF VACCINEES, AGE 5-14 YEARS, REPORTING TENDERNESS ON DAYS 1 AND 2 FOLLOWING VACCINATION I

Vaccine	Tenderness Severity on Day 1					
	0	1	2	3	4	Unk
Tetanus toxoid	8	54	20	1	1	16
Cholera toxoid	8	42	22	2	2	22
Whole cell vaccine-cholera toxoid	2	36	40	7	2	13
Whole cell vaccine	5	33	26	12	2	21
Saline	16	58	7	<1	0	18

Vaccine	Tenderness Severity on Day 2					
	0	1	2	3	4	Unk
Tetanus toxoid	20	56	5	<1	<1	18
Cholera toxoid	18	40	10	2	0	30
Whole cell vaccine-cholera toxoid	5	61	16	1	0	17
Whole cell vaccine	10	64	10	0	0	17
Saline	40	36	0	0	0	25

TABLE 9

PERCENTAGE OF VACCINEES, AGE 15 OR MORE YEARS, REPORTING TENDERNESS ON DAYS 1 AND 2 FOLLOWING VACCINATION I

Vaccine	Tenderness Severity on Day 1					
	0	1	2	3	4	Unk
Tetanus toxoid	2	46	39	4	<1	9
Cholera toxoid	0	30	52	9	2	7
Whole cell vaccine-cholera toxoid	<1	28	47	15	2	8
Whole cell vaccine	0	24	58	14	4	0
Saline	15	56	18	<1	<1	10

Vaccine	Tenderness Severity on Day 2					
	0	1	2	3	4	Unk
Tetanus toxoid	8	64	14	2	<1	12
Cholera toxoid	11	59	18	0	0	11
Whole cell vaccine-cholera toxoid	2	50	33	4	<1	11
Whole cell vaccine	12	50	26	8	0	4
Saline	35	44	2	0	0	18

TABLE 10

PERCENTAGE OF VACCINEES, AGE 1-4 YEARS, WITH REDNESS ON DAYS 1 AND 2 FOLLOWING VACCINATION I

Vaccine	Redness Severity on Day 1					
	0	1	2	3	4	Unk
Tetanus toxoid	35	51	4	0	0	10
Cholera toxoid	46	46	9	0	0	0
Whole cell vaccine-cholera toxoid	12	63	22	<1	0	3
Whole cell vaccine	26	63	9	0	0	3
Saline	39	52	2	0	0	7

Vaccine	Redness Severity on Day 2					
	0	1	2	3	4	Unk
Tetanus toxoid	58	26	1	0	0	15
Cholera toxoid	52	33	3	0	0	12
Whole cell vaccine-cholera toxoid	34	49	10	<1	0	6
Whole cell vaccine	54	40	0	0	0	6
Saline	74	12	0	0	0	15

TABLE 11

PERCENTAGE OF VACCINEES, AGE 5-14 YEARS, WITH REDNESS ON DAYS 1 AND 2 FOLLOWING VACCINATION I

Vaccine	Redness Severity on Day 1					
	0	1	2	3	4	Unk
Tetanus toxoid	38	42	4	< 1	0	16
Cholera toxoid	50	28	0	0	0	22
Whole cell vaccine-cholera toxoid	16	50	19	2	0	13
Whole cell vaccine	43	29	7	0	0	21
Saline	56	25	2	0	0	18

Vaccine	Redness Severity on Day 2					
	0	1	2	3	4	Unk
Tetanus toxoid	60	20	2	0	0	18
Cholera toxoid	50	20	0	0	0	30
Whole cell vaccine-cholera toxoid	40	37	6	<1	0	17
Whole cell vaccine	57	26	0	0	0	17
Saline	71	4	<1	0	0	25

TABLE 12

PERCENTAGE OF VACCINEES, AGE 15 OR MORE YEARS, WITH REDNESS ON DAYS 1 AND 2 FOLLOWING VACCINATION I

Vaccine	Redness Severity on Day 1					
	0	1	2	3	4	Unk
Tetanus toxoid	23	56	11	1	0	9
Cholera toxoid	43	39	11	0	0	7
Whole cell vaccine-cholera toxoid	9	47	27	9	<1	8
Whole cell vaccine	30	54	14	2	0	0
Saline	56	30	3	0	0	10

Vaccine	Redness Severity on Day 2					
	0	1	2	3	4	Unk
Tetanus toxoid	50	31	7	0	0	12
Cholera toxoid	61	23	4	0	0	11
Whole cell vaccine-cholera toxoid	24	44	17	4	<1	11
Whole cell vaccine	52	36	6	2	0	4
Saline	72	9	<1	0	0	18

TABLE 13

PERCENTAGE OF VACCINEES, AGE 1-4 YEARS, WITH INDURATION ON DAYS 1 AND 2 FOLLOWING VACCINATION I

Vaccine	Induration Severity on Day 1					
	0	1	2	3	4	Unk
Tetanus toxoid	11	69	9	0	0	10
Cholera toxoid	21	70	9	0	0	0
Whole cell vaccine-cholera toxoid	8	56	29	3	0	3
Whole cell vaccine	6	69	23	0	0	3
Saline	36	56	2	0	0	7

Vaccine	Induration Severity on Day 2					
	0	1	2	3	4	Unk
Tetanus toxoid	18	66	0	0	0	15
Cholera toxoid	12	73	3	0	0	12
Whole cell vaccine-cholera toxoid	6	61	25	<1	<1	6
Whole cell vaccine	9	77	9	0	0	6
Saline	51	34	0	0	0	15

TABLE 14

PERCENTAGE OF VACCINEES, AGE 5-14 YEARS, WITH INDURATION ON DAYS 1 AND 2 FOLLOWING VACCINATION I

Vaccine	Induration Severity on Day 1					
	0	1	2	3	4	Unk
Tetanus toxoid	22	54	8	0	0	16
Cholera toxoid	18	60	0	0	0	22
Whole cell vaccine-cholera toxoid	13	50	24	1	0	13
Whole cell vaccine	31	43	5	0	0	21
Saline	34	45	2	0	0	18

Vaccine	Induration Severity on Day 2					
	0	1	2	3	4	Unk
Tetanus toxoid	20	56	6	0	0	18
Cholera toxoid	28	42	0	0	0	30
Whole cell vaccine-cholera toxoid	8	63	12	<1	0	17
Whole cell vaccine	24	57	2	0	0	17
Saline	42	34	0	0	0	25

TABLE 15

PERCENTAGE OF VACCINEES, AGE 15 OR MORE YEARS, WITH INDURATION ON DAYS 1 AND 2 FOLLOWING VACCINATION I

Vaccine	Induration Severity on Day 1					
	0	1	2	3	4	Unk
Tetanus toxoid	15	54	20	2	0	9
Cholera toxoid	7	77	9	0	0	7
Whole cell vaccine-cholera toxoid	7	44	32	8	<1	8
Whole cell vaccine	16	70	12	2	0	0
Saline	38	48	4	0	0	10

Vaccine	Induration Severity on Day 2					
	0	1	2	3	4	Unk
Tetanus toxoid	17	58	13	1	0	12
Cholera toxoid	18	64	7	0	0	11
Whole cell vaccine-cholera toxoid	6	52	28	2	<1	11
Whole cell vaccine	22	64	8	2	0	4
Saline	47	34	1	0	0	18

TABLE 16

PERCENTAGE OF VACCINEES WITH FEVER ON DAYS 1 AND 2 FOLLOWING VACCINATION I

Vaccine	Temperature (°F) on Day 1					
	<100.0	100.0-100.9	101.0-101.9	102.0-102.9	103.0 or higher	Unk
Tetanus toxoid	84	3	0	<1	<1	13
Cholera toxoid	82	7	0	0	0	11
Whole cell vaccine-cholera toxoid	83	6	<1	<1	<1	10
Whole cell vaccine	83	7	<1	<1	0	10
Saline	85	1	0	0	<1	14

Vaccine	Temperature (°F) on Day 2					
	<100.0	100.0-100.9	101.0-101.9	102.0-102.9	103.0 or higher	Unk
Tetanus toxoid	80	1	0	0	0	19
Cholera toxoid	82	0	0	0	0	18
Whole cell vaccine-cholera toxoid	80	2	<1	<1	<1	17
Whole cell vaccine	89	<1	0	0	0	10
Saline	76	<1	0	0	<1	23

TABLE 17

VACCINATION I

PERCENTAGE OF VACCINEES BY DURATION OF FEVER AND VACCINE TYPE

Vaccine	Duration of Fever (days)					
	0	1	2	3	4	Unk
Tetanus toxoid	91	4	<1	0	0	5
Cholera toxoid	88	8	0	0	0	4
Whole cell vaccine-cholera toxoid	88	6	2	<1	0	4
Whole cell vaccine	87	9	0	0	0	3
Saline	90	3	0	0	0	7

TABLE 18

PERCENTAGE OF VACCINEES REPORTING LIMITATION OF ACTIVITY ON DAYS 1 AND 2 FOLLOWING VACCINATION I

Vaccine	Limitation of Activity on Day 1			
	0	1	2	Unk
Tetanus toxoid	62	26	< 1	12
Cholera toxoid	47	41	2	10
Whole cell vaccine-cholera toxoid	44	45	1	9
Whole cell vaccine	45	46	2	8
Saline	75	12	0	13

Vaccine	Limitation of Activity on Day 2			
	0	1	2	Unk
Tetanus toxoid	77	8	<1	15
Cholera toxoid	72	9	1	18
Whole cell vaccine-cholera toxoid	67	20	<1	13
Whole cell vaccine	74	17	0	9
Saline	78	1	0	21

TABLE 19

PERCENTAGE OF VACCINEES, BY AGE AGROUP, REPORTING PAIN ON DAY 1 FOLLOWING VACCINATION II

Vaccine	Pain Severity on Day 1					
	0	1	2	3	4	Unk
Age 1-4 Years						
Tetanus toxoid	36	48	13	0	0	3
Cholera toxoid	27	62	8	0	0	4
Whole cell vaccine-cholera toxoid	18	51	24	1	0	6
Whole cell vaccine	36	24	32	0	0	8
Saline	54	30	0	0	0	16
Age 5-14 Years						
Tetanus toxoid	31	35	9	1	0	24
Cholera toxoid	62	30	0	0	0	8
Whole cell vaccine-cholera toxoid	15	50	18	1	0	16
Whole cell vaccine	29	38	24	0	0	9
Saline	54	28	3	0	0	16
Age 15 or More Years						
Tetanus toxoid	13	57	17	2	0	11
Cholera toxoid	6	59	24	0	0	12
Whole cell vaccine-cholera toxoid	7	50	24	9	0	11
Whole cell vaccine	7	50	38	0	0	5
Saline	45	38	4	2	0	12

TABLE 20

PERCENTAGE OF VACCINEES, BY AGE GROUP, REPORTING TENDERNESS ON DAY 1 FOLLOWING VACCINATION II

Vaccine	Tenderness Severity on Day 1					
	0	1	2	3	4	Unk
Age 1-4 Years						
Tetanus toxoid	2	76	19	0	0	3
Cholera toxoid	4	88	4	0	0	4
Whole cell vaccine-cholera toxoid	1	56	34	3	0	6
Whole cell vaccine	0	48	44	0	0	8
Saline	19	62	3	0	0	16
Age 5-14 Years						
Tetanus toxoid	2	60	11	2	<1	24
Cholera toxoid	8	73	11	0	0	8
Whole cell vaccine-cholera toxoid	1	46	34	4	0	16
Whole cell vaccine	0	62	29	0	0	9
Saline	20	62	3	0	0	16
Age 15 or More Years						
Tetanus toxoid	4	50	32	4	0	11
Cholera toxoid	0	44	41	3	0	12
Whole cell vaccine-cholera toxoid	<1	32	45	11	<1	11
Whole cell vaccine	2	40	45	7	0	5
Saline	26	52	9	2	0	12

TABLE 21

PERCENTAGE OF VACCINEES BY AGE GROUP WITH REDNESS ON DAY 1 FOLLOWING VACCINATION II

Vaccine	Redness Severity on Day 1					
	0	1	2	3	4	Unk
Age 1-4 Years						
Tetanus toxoid	29	61	6	0	0	3
Cholera toxoid	19	73	4	0	0	4
Whole cell vaccine-cholera toxoid	13	66	12	1	1	6
Whole cell vaccine	24	52	16	0	0	8
Saline	62	22	0	0	0	16
Age 5-14 Years						
Tetanus toxoid	26	40	9	<1	0	24
Cholera toxoid	46	43	3	0	0	8
Whole cell vaccine-cholera toxoid	12	54	18	<1	0	16
Whole cell vaccine	21	50	21	0	0	9
Saline	61	23	0	0	0	16
Age 15 or More Years						
Tetanus toxoid	14	55	16	3	0	11
Cholera toxoid	18	47	21	3	0	12
Whole cell vaccine-cholera toxoid	4	50	25	8	2	11
Whole cell vaccine	10	45	40	0	0	5
Saline	52	32	4	1	0	12

TABLE 22

PERCENTAGE OF VACCINEES BY AGE GROUP WITH INDURATION ON DAY 1 FOLLOWING VACCINATION II

Vaccine	Induration Severity on Day 1					
	0	1	2	3	4	Unk
Age 1-4 Years						
Tetanus toxoid	23	69	5	0	0	3
Cholera toxoid	38	58	0	0	0	4
Whole cell vaccine-cholera toxoid	12	60	20	1	0	6
Whole cell vaccine	8	56	28	0	0	8
Saline	30	54	0	0	0	16
Age 5-14 Years						
Tetanus toxoid	18	51	7	<1	0	24
Cholera toxoid	27	60	5	0	0	8
Whole cell vaccine-cholera toxoid	10	48	26	<1	0	16
Whole cell vaccine	38	38	15	0	0	9
Saline	36	48	<1	0	0	16
Age 15 or More Years						
Tetanus toxoid	16	47	22	4	0	11
Cholera toxoid	29	29	29	0	0	12
Whole cell vaccine-cholera toxoid	15	36	31	7	<1	11
Whole cell vaccine	17	40	36	2	0	5
Saline	44	38	6	<1	0	12

TABLE 23

PERCENTAGE OF VACCINEES WITH FEVER ON DAYS 1 AND 2

FOLLOWING VACCINATION II

Vaccine	Temperature (°F) on Day 1					
	<100.0	100.0-100.9	101.0-101.9	102.0-102.9	103.0 or Higher	Unk
Tetanus toxoid	81	3	<1	0	<1	15
Cholera toxoid	85	7	0	0	0	8
Whole cell vaccine-cholera toxoid	80	7	1	<1	0	12
Whole cell vaccine	77	13	3	0	0	8
Saline	85	1	0	0	0	14

Vaccine	Temperature (°F) on Day 2					
	<100.0	100.0-100.9	101.0-101.9	102.0-102.9	103.0 or Higher	Unk
Tetanus toxoid	79	2	<1	0	0	18
Cholera toxoid	94	1	0	0	0	5
Whole cell vaccine-cholera toxoid	77	4	<1	<1	0	19
Whole cell vaccine	89	2	0	0	1	8
Saline	80	<1	0	0	0	20

TABLE 24

VACCINATION II

PERCENTAGE OF VACCINEES BY DURATION OF FEVER AND VACCINE TYPE

Vaccine	Duration of Fever (days)					
	0	1	2	3	4	Unk
Tetanus toxoid	94	2	1	<1	0	2
Cholera toxoid	90	9	0	0	0	1
Whole cell vaccine-cholera toxoid	88	6	3	<1	<1	3
Whole cell vaccine	81	15	2	0	1	1
Saline	93	2	0	0	0	5

TABLE 25

PERCENTAGE OF VACCINEES REPORTING LIMITATION OF ACTIVITY ON DAYS 1 AND 2 FOLLOWING VACCINATION II

Vaccine	Limitation of Activity on Day 1			
	0	1	2	Unk
Tetanus toxoid	61	23	2	15
Cholera toxoid	58	33	1	8
Whole cell vaccine-cholera toxoid	49	38	2	12
Whole cell vaccine	41	44	9	7
Saline	77	9	0	14

Vaccine	Limitation of Activity on Day 2			
	0	1	2	Unk
Tetanus toxoid	76	9	0	15
Cholera toxoid	86	8	0	5
Whole cell vaccine-cholera toxoid	66	19	<1	15
Whole cell vaccine	69	24	0	8
Saline	83	1	<1	16

TABLE 26

BLOOD SPECIMENS COLLECTED DURING 1979

WELLCOME CHOLERA TOXOID PRE-TEST

Age Group	Vaccine Type					Total
	Tex Tox	Chol Tox	Chol Tox/WCV	WCV	Saline	
Blood No. 1 - Day 0						
1-4 yrs.	30	36	36	39	-	141
5-14 yrs.	42	38	40	38	35	193
15+ yrs.	48	43	41	49	71	252
Blood No. 2 - Day 14						
1-4 yrs.	24	29	29	30	-	112
5-14 yrs.	36	27	32	31	29	155
15+ yrs.	39	33	32	41	61	206
Blood No. 3 - Day 42						
1-4 yrs.	25	28	30	31	-	114
5-14 yrs.	35	32	30	31	30	158
15+ yrs.	40	37	31	41	62	211
Blood No. 4 - Day 56						
1-4 yrs.	23	26	31	31	-	111
5-14 yrs.	33	32	26	28	26	145
15+ yrs.	35	35	32	39	65	206

TABLE 27

GEOMETRIC MEAN VIBRIOCIDAL (INABA) ANTIBODY LEVELS IN MATLAB VACCINEES IN THE 1979 PRE-TEST

Vaccine Type* and Age Group	Time of Blood Collection			
	Day 0	Day 14	Day 42	Day 56
Tetanus Toxoid				
1-4 years	8.1**	8.2	6.8	8.1
5-14 years	20.9	20.1	17.1	14.6
15+ years	30.8	26.5	28.8	38.3
Cholera Toxoid (300 µg)				
1-4 years	8.0	16.8	13.6	50.0
5-14 years	21.8	52.1	41.4	76.5
15+ years	34.1	66.1	42.0	62.8
Whole Cell Vaccine-Cholera Toxoid (300 µg)				
1-4 years	8.2	129.9	48.4	316.2
5-14 years	11.2	452.0	101.9	250.1
15+ years	45.4	353.0	183.2	215.7
Whole Cell Vaccine				
1-4 years	13.6	148.5	85.1	256.6
5-14 years	15.6	410.0	78.5	212.9
15+ years	49.3	616.2	321.0	370.0

* Vaccines (0.5 ml dose) given on days 0 and 42.

** Geometric mean vibriocidal antibody titers expressed in vibriocidal units/ml, based on comparison with the NIH vibriocidal reference serum.

TABLE 28

GEOMETRIC MEAN SERUM PASSIVE HEMAGGLUTINATION ANTITOXIN LEVELS IN MATLAB VACCINEES IN THE 1979 PRE-TEST

Vaccine Type* and Age Group	Time of Blood Collection			
	Day 0	Day 14	Day 42	Day 56
Tetanus Toxoid				
1-4 years	11.1**	8.3	7.6	9.7
5-14 years	8.0	7.8	6.2	5.7
15+ years	9.4	8.7	7.4	9.4
Cholera Toxoid (300 µg)				
1-4 years	8.6	252.3	61.2	180.0
5-14 years	12.3	458.4	250.0	344.0
15+ years	9.5	346.3	286.4	295.8
Whole Cell Vaccine-Cholera Toxoid (300 µg)				
1-4 years	8.4	247.0	73.1	166.2
5-14 years	8.1	259.1	69.7	131.8
15+ years	7.8	292.6	132.9	284.3
Whole Cell Vaccine				
1-4 years	10.5	10.2	10.2	10.8
5-14 years	8.6	22.0	12.9	12.2
15+ years	12.0	18.2	20.2	25.5

* Vaccines (0.5 ml dose) given on days 0 and 42.

** Geometric mean passive hemagglutination titer expressed in antitoxin units/ml, based on comparison to the Swiss Reference Serum No. EC3 (A-2/67) - B (SEM in each case 1.1-1.4).

TABLE 29

DISTRIBUTION OF DAY 0* SERUM PASSIVE HEMAGGLUTINATION TITERS BY AGE IN THE 1979 MATLAB PRE-TEST

Age Group	Antitoxin Units/ml**			
	<10 (%)	10-39 (%)	40+ (%)	Total
1-4	70 (55)	47 (37)	10 (8)	127
5-14	87 (58)	55 (37)	8 (5)	150
15+	97 (55)	57 (33)	21 (12)	175

* Prior to innoculation.
** Based on comparison to the Swiss Reference Serum No. EC3 (A-2/67) - B.

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