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A controlled field trial of an aluminium phosphate-adsorbed cholera vaccine in Calcutta*

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A controlled field trial to determine the efficacy of a single dose of an aluminium phosphate-adsorbed cholera vaccine was conducted in Calcutta during 1975–77. An aluminium phosphate-adsorbed tetanus toxoid was used as the placebo. Follow-up of the immunized volunteers for a period of two years showed that the adsorbed cholera vaccine provided 100% protection to children under five years of age for 6 months, 88.9% for 12 months, and 91.7% for 18 months ($P<0.05$). The overall protection for all age groups was 58.5% for 18 months. There were no serious side effects following the anti-cholera inoculations.

Controlled field trials with different types of conventional cholera vaccine were conducted between 1963 and 1973 in several countries. The results of these trials showed that these vaccines protected about 50% of the vaccinated population for a period of 3–6 months (*1*). Increasing the antigenic content did not improve the efficacy of the vaccine (*2*). Moreover, a single dose of conventional vaccine did not offer any significant protection to the children below 5 years of age, who are the main victims of cholera in endemic areas.

An oil-adjuvant cholera vaccine tested in the Philippines and a high-potency vaccine used in Bangladesh offered higher protection for a longer period but could not be recommended for public use because of the severe reactions that they produced (*3*, *4*).

Aluminium compounds have been recommended as the safest adjuvants by a WHO Scientific Group on Immunological Adjuvants (*5*) and the safety, potency, and immunogenicity of cholera vaccine adsorbed by aluminium hydroxide was studied by Joó et al. (*6*, *7*). They demonstrated that this vaccine was more potent and immunogenic than conventional vaccine when used in laboratory animals and young adult volunteers, and that no serious side effects were observed in the human subjects.

A controlled field trial of an aluminium hydroxide-adjuvant cholera vaccine was conducted in Surabaya, Indonesia, during 1973–75 (*8*). In children of 1–4 years of age, the vaccine with adjuvant provided a higher degree of protection for a longer period than the existing conventional vaccine.

This report presents the results of a controlled field trial on the effectiveness of an aluminium phosphate-adsorbed cholera vaccine, conducted in Calcutta between February 1975 and March 1977. The study was performed jointly by the Cholera Research Centre (now the National Institute of Cholera and Enteric Diseases), Calcutta, and the Government of West Bengal.

MATERIALS AND METHODS

Study area

The trial was conducted in 17 municipal wards in eastern Calcutta and two adjoining wards of South Dum Dum municipality having a total population of over 500 000. Most of the population in this part of the city are of low socioeconomic status. The area was selected because it had had a higher cholera incidence than other areas of the city during the previous 10 years. The average annual incidence of hospitalized cases was 5 per 10 000 and it was estimated that a minimum of 100 000 volunteers would have to be inoculated with each vaccine to obtain a statistically significant result. The Infectious Diseases Hospital, which is the only hospital in the city that treats cholera patients, is situated at the centre of the study area.

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The vaccines

A conventional cholera vaccine, containing 4000 million vibrios each of classical Ogawa and Inaba serotypes per 0.5 ml, was prepared following the standard techniques for manufacture of cholera vaccines (*9*). This vaccine was then adsorbed with aluminium phosphate (1.5 mg per 0.5 ml). Tetanus toxoid was used as placebo. Both the vaccines were manufactured by the Central Research Institute, Kasauli, where toxicity and potency tests were performed on the vaccine before it was used in the field trial. The vaccine was found to be non-toxic. The potency tests showed that the Inaba and Ogawa fractions of the adsorbed vaccine were, respectively, 1.62 and 1.02 times more potent than the WHO reference cholera vaccine. A preliminary assessment of reactions to the adsorbed vaccine in a small group of volunteers showed no adverse side effects. The vaccine and the placebo were put in identical bottles and coded with letters A and B. The identity of each sample was known only by the manufacturing laboratory.

Volunteers over 5 years old received 0.5 ml of vaccine and those in the 1–5-year age group received 0.25 ml of vaccine, given as a single dose by deep intramuscular injection. Children below 1 year of age were not included in the trial. Sterile 1-ml syringes and 23-gauge (0.65-mm) needles were used, each needle being used for only one injection. Vaccines were kept refrigerated at 4 °C and any unused vaccine was discarded at the end of the day.

Vaccination

The study area was selected in consultation with the Department of Health of the Government of West Bengal, who agreed that no other agencies would be allowed to carry out anticholera inoculations in the study area during the 2-year period of the trial.

Sixty teams, each consisting of a recorder and a vaccinator, were employed to carry out the vaccinations. During the preparatory phase, they received intensive training in the vaccination and recording techniques. Four such teams were closely supervised by each of 15 health inspectors, who had had previous experience of similar controlled field trials. Two physicians were responsible for supervising and assessing the vaccination programme. The two vaccines were administered strictly at random, as required in a double-blind trial. The volunteers were vaccinated only after their informed consent had been obtained.

The vaccination phase was completed in 43 working days during February and March 1975. The number of cholera cases started to increase in April and reached its peak during May–June that year. A total of 203 170 volunteers were vaccinated, of whom 101 096 received the adjuvant cholera vaccine and 101 030 were given the placebo. The vaccination status of the remaining 1044 subjects was not accurately recorded, and hence they were excluded from the study population. In spite of repeated visits the number vaccinated represents only 38.4% of the total population.

Side effects

Reactions to the vaccination were assessed every 24 hours for 120 hours in a random sample of the vaccinated population. The reactions looked for included erythema, pain, tenderness, swelling, headache, fever, and vomiting.

Follow-up

The follow-up of the vaccinated volunteers started at the hospital level seven days after the start of the vaccination programme and continued for two years. Staff at the Infectious Diseases Hospital took rectal swabs of every case of diarrhoea admitted from the study area before administering antibiotic treatment. The rectal swabs were placed in Cary-Blair transport medium and taken to the laboratory within a few hours. These samples were tested in the laboratory, using standard procedures for detection of *Vibrio cholerae* (*10*). If a sample was found to be bacteriologically positive, the vaccination status of the patient was determined from the records.

Table 1. Age and sex distribution of vaccinated volunteers

Age group (years)	Male			Female			Total
	Cholera vaccine	Tetanus toxoid	Total	Cholera vaccine	Tetanus toxoid	Total	
1 – 4	3678	3662	7340	3481	3470	6951	14 291
⩾ 5	53 546	53 518	107 064	40 391	40 380	80 771	187 835
Total	57 224	57 180	114 404	43 872	43 850	87 722	202 126

Table 2. Percentage distribution of adverse reactions in the vaccinated and control groups

Reactions	Control group (711 subjects)					Vaccinated group (682 subjects)				
	24 h	48 h	72 h	96 h	120 h	24 h	48 h	72 h	96 h	120 h
Erythema	3.4	0.4	–	–	–	5.9	0.3	–	–	–
Pain	21.2	3.2	0.6	–	–	28.9	6.6	0.6	–	–
Local tenderness	20.7	15.3	7.2	1.4	0.1	26.1	22.9	14.4	4.8	0.4
Local swelling	6.2	0.8	–	–	–	13.2	4.0	1.0	–	–
Mild headache	3.2	–	–	–	–	6.2	–	–	–	–
Fever[a]	11.3	0.1	–	–	–	21.7	0.6	–	–	–
Vomiting	0.1	–	–	–	–	1.5	–	–	–	–

[a] Less than 38 °C.

RESULTS

Age and sex distribution

The age and sex distribution of the vaccinated volunteers was very similar in the two groups (Table 1). The male:female ratio of 1000:766 in the vaccinated population is close to the overall ratio in the study area which, according to the 1971 Census, was 1000:726.

Side effects

The reactions of a sample of the volunteers to inoculation are given in Table 2. It can be seen that the cholera vaccine tended to produce slightly more reactions but the difference between the two groups was not significant.

Efficacy of the vaccine

Data on the age-specific effectiveness of the aluminium phosphate-adsorbed cholera vaccine for follow-up periods of 6 months, 1, 1½, and 2 years are presented in Table 3. The overall effectiveness of the adjuvant vaccine during these periods was 60.6%, 62.5%, 58.5%, and 53.4%, respectively. The efficacy rates for children below 5 years of age during the corresponding periods were 100.0%, 88.9%, 91.7%, and 78.7%, respectively, and for volunteers over five years of age were 51.9%, 56.4%, 51.0%, and 47.6% respectively. All these rates were found to be statistically significant ($P<0.05$).

Table 3. Age-specific effectiveness of the adsorbed cholera vaccine

Period of follow-up (months)	1 – 4 years of age					⩾ 5 years of age					All ages				
	Tetanus toxoid		Adsorbed vaccine			Tetanus toxoid		Adsorbed vaccine			Tetanus toxoid		Adsorbed vaccine		
	No. of cases	Attack rate per 10 000	No. of cases	Attack rate per 10 000	Efficacy (%)	No. of cases	Attack rate per 10 000	No. of cases	Attack rate per 10 000	Efficacy (%)	No. of cases	Attack rate per 10 000	No. of cases	Attack rate per 10 000	Efficacy (%)
0 – 6	6	8.4	0	–	100.0	27	2.9	13	1.4	51.9	33	3.3	13	1.3	60.6
7 – 12	3	4.2	1	1.4	66.8	12	1.3	4	0.4	66.7	15	1.5	5	0.5	66.7
13 – 18	3	4.2	0	–	100.0	14	1.5	9	1.0	35.7	17	1.7	9	0.9	47.1
19 – 24	2	2.8	2	2.8	0.4	8	0.9	6	0.6	25.0	10	1.0	8	0.8	20.1
0 – 12	9	12.6	1	1.4	88.9	39	4.2	17	1.8	56.4	48	4.8	18	1.8	62.5
0 – 18	12	16.8	1	1.4	91.7	53	5.6	26	2.8	51.0	65	6.4	27	2.7	58.5
0 – 24	14	19.6	3	4.2	78.7	61	6.5	32	3.4	47.6	75	7.4	35	3.5	53.4

DISCUSSION

During the present trial, the detection of cholera infection in the vaccinated volunteers was carried out at hospital level only because this was the simplest and most economical method. It also reduced the risk of including inapparent cholera infections, on which cholera vaccines apparently have no effect. Any labelling of cholera carriers as cholera patients might have vitiated the results of the study which was carried out in a cholera-endemic area. Moreover, since the number of volunteers was chosen on the basis of attack rates in hospitalized cases only, there was no risk involved in concentrating on hospital level follow-up.

The results of a trial in Surabaya (*8*) showed that aluminium hydroxide-adsorbed vaccine afforded significant protection to children aged 1–4 years, the efficacy rates being 88.2% and 67.6% for periods of 6 and 14 months, respectively. These are comparable with the rates obtained in the present trial in Calcutta. This high degree of protection for young children may be attributed to the adjuvant action of the aluminium salt which results in longer-lasting antigenic stimulation, thus simulating a booster effect. This booster phenomenon was absent in earlier trials conducted with plain cholera vaccines and resulted in poor protection for children. With plain vaccine, the adult volunteers residing in endemic areas, were, however, better protected as a result of prior exposure to subclinical doses of oral infection with *V. cholerae*.

In the present trial, the number of cases in the 1–4-years age group was small although the age-specific attack rate was much higher than in the older subjects (12.6 per 10 000 for children under 5 years of age compared with 4.2 per 10 000 in volunteers over 5 years of age during the first year of observation). The higher level of protection given by the vaccine with adjuvant to children below 5 years of age compared with that given to volunteers aged 5 years and over, cannot be easily explained. One possible explanation is that the vaccinated children below 5 years of age were compared with an "unsensitized" control group whereas the volunteers over 5 years of age were compared with a "sensitized" control population in an endemic area. In the present trial, the protection given to all the groups was insignificant 18 months after inoculation.

Comparison of side effects induced by the adsorbed vaccine with those induced by tetanus toxoid showed that differences in local and general reactions were insignificant. The vaccination campaign was continued for a period of 43 days, and intake of the vaccine did not hamper the volunteers in their routine daily activities.

Practical difficulties prevented the inoculation of a third group of volunteers with a plain cholera vaccine and thus, there was no direct comparison with the aluminium phosphate-adsorbed vaccine. However, several other controlled field trials conducted with plain vaccines under various conditions have shown that it is not possible to protect more than 50% of vaccinated volunteers for more than 3–6 months. None of those vaccines give significant protection to children below 5 years of age. Moreover, a plain vaccine was compared with an aluminium hydroxide-adjuvant vaccine in Indonesia (*8*), and the latter was shown to give better protection in children in the 1–4 year age group.

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RÉSUMÉ

ESSAI CONTRÔLÉ SUR LE TERRAIN D'UN VACCIN ANTICHOLÉRIQUE ADSORBÉ SUR PHOSPHATE D'ALUMINIUM

L'essai contrôlé effectué à Calcutta en 1975–1977 avait pour but de déterminer l'efficacité d'une dose unique de vaccin anticholérique adsorbé sur phosphate d'aluminium, administrée par injection intramusculaire. Le vaccin avec adjuvant a été injecté à 101 096 volontaires, et 101 030 autres sujets ont reçu un placebo sous forme d'anatoxine tétanique, les différentes injections ayant été opérées au hasard.

Les sujets vaccinés ont été suivis dans le cadre hospitalier pendant une période de 24 mois en vue de dépister tout cas de choléra. Cette surveillance a montré que le vaccin adsorbé assurait aux enfants de moins de 5 ans une protection à 100% pendant 6 mois, à 88,9% pendant 12 mois et à 91,7% pendant 18 mois ($P<0,05$). La protection globale pour tous les groupes d'âge s'est établie à 58,5% pendant 18 mois. Aucun effet secondaire grave n'a été provoqué par l'inoculation du vaccin.

On peut donc conclure que le vaccin anticholérique adsorbé sur phosphate d'aluminium confère aux enfants de moins de 5 ans, qui sont les principales victimes du choléra dans les régions endémiques, une meilleure protection que celle obtenue avec le vaccin actuellement en usage.

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