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Field trial of inactivated oral cholera vaccines in Bangladesh: results from 5 years of follow-up

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To determine the protective efficacy (PE) of three doses of oral B subunit-killed whole cell (BS-WC) or killed whole cell-only (WC) vaccines against cholera, a clinical trial was conducted among 62285 children over 2 years and adult women in rural Bangladesh. During 5 years of follow-up, there were 144 cases of cholera in the BS-WC group (PE=49%; P<0.001), 150 in the WC group (PE=47%; P<0.001), and 283 in the K12 group. Protection by each vaccine was evident only during the first three years of follow-up; long-term protection of young children was observed only against classical but not El Tor cholera; 3-year protection against both cholera biotypes occurred among older persons, but at a higher level against classical cholera.

Keywords: Cholera; oral vaccination; Bangladesh

Recent efforts to develop new vaccines against cholera have focused upon vaccines administered orally, since intestinal mucosal immunity is thought to be most relevant to protection against cholera and since peroral presentation of vaccine antigens is most efficient in eliciting mucosal immune responses[1]. The first oral cholera vaccines to be evaluated for efficacy in preventing cholera in an endemic setting were killed preparations consisting of heat- or formalin-inactivated whole cells, either with (BS-WC) or without (WC) the addition of cholera toxin B subunit[2]. When tested in rural Bangladesh, three doses of each vaccine were well tolerated and conferred about 50% protection over a 3-year period of follow-up[3]. In this paper, we present the results for the final 2 years of follow-up, and examine the manner in which age at vaccination and the biotype of the infecting vibrios modified the magnitude and longitudinal pattern of vaccine efficacy.

MATERIALS AND METHODS

Methods for the trial, which was conducted in the Matlab field studies area of the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), have been presented in detail elsewhere[3]. The trial evaluated two vaccines. The BS-WC vaccine consisted of 1 mg cholera toxin B subunit together with 10^{11} killed whole cells representing the El Tor and classical biotypes and the Inaba and Ogawa serotypes of *Vibrio cholerae* 01. The WC vaccine had the same cellular constituents, but lacked B subunit. In early 1985 persons 2–15 years of age and older females were randomized to BS-WC, WC or an *Escherichia coli* K12 strain placebo (K12). The regimen for each agent consisted of three doses at 6-week intervals. At least one dose was taken by 89 596 persons after giving verbal informed consent. Of these, 62 285 ingested three complete doses.

For the first 3 years after vaccination, surveillance for diarrhoea was conducted at all three diarrhoea treatment centres serving the Matlab population of nearly 200 000 persons. During the final 2 years of the cholera vaccine trial surveillance was maintained as rigorously as before, with two exceptions:

no team was delegated to verify the patient's identity by visiting the patient's stated home address, since during the previous 3 years only 12 of 2904 cases (0.4%) of misidentification had occurred;

one of the original three Matlab diarrhoea treatment centres was closed because during the first 3 years of the trial it had contributed only 189 cases (1.6%) to the trial population.

At each centre clinical data and faecal specimens were systematically obtained from all Matlab residents who presented for care of diarrhoea, and microbiological evaluations of faeces were performed to detect

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V. cholerae 01 and to characterize each isolate by biotype and serotype.

Cholera endpoints were analysed as episodes, as previously described[3]. Vaccine protective efficacy (PE) was calculated as [(1 − Relative risk of cholera in vaccinees vs placebo recipients) × 100%)]. Efficacy was evaluated for recipients of three complete doses of an assigned agent, during the period 14 days to 5 years after the third dose. Only initial cholera episodes occurring during this period were analysed. Denominators for calculation of cholera risk were persons who were still under follow-up at the beginning of each year of follow-up and who had not previously had a detected episode of cholera.

Comparisons of the risk of cholera in vaccinees vs placebo recipients were statistically appraised with χ^2 tests, or with Fisher's exact tests when mandated by sparse strata. Confidence intervals (95%) for protective efficacy were estimated with test-based methods for relative risks. For reasons cited elsewhere[4], all statistical appraisals of protective efficacy employed one-tailed tests to estimate *P* values and 95% confidence intervals (95% CI); for the latter, only lower boundaries are cited. Overall comparisons of vaccinees and placebo recipients employed $P<0.025$ as the threshold of statistical significance, whereas subgroup analyses used $P<0.05$.

RESULTS

During the final years of the cholera vaccine trial, the annual admission rate for cholera patients seen at the Matlab treatment centres had declined considerably, from 1258 in 1986 to 76 in 1989; this trend was noticed nationwide including at our Centre's diarrhoea treatment centre in the capital Dhaka.

As presented elsewhere[4], at baseline the three groups were equivalent in size (each with *ca* 21 000 persons) and in the distributions of factors known to be associated with the risk of cholera in Matlab[5]. *Table 1* shows the occurrence of cholera during the entire follow-up period. A total of 580 episodes of cholera were detected; three (two in the BS-WC group and one in the K12 group) were excluded since they represented recurrent episodes during the follow-up interval. After these exclusions, there were 144 cases of cholera in the BS-WC group (PE: 49%; 95% CI lower boundary (LB): 38%, $P<0.001$), 150 cases in the WC group (PE: 47%; 95% CI LB: 36%, $P<0.001$), and 283 cases in the K12 group.

This pattern reflected relatively sustained and equivalent protection for each vaccine during the initial 2 years (*Table 1*), followed by a substantial drop in efficacy during the third year. During the fourth year, 56 cases were noted in the three groups (15 in BS-WC, 23 in WC and 18 in K12), and no significant protection was noted for either vaccine. During the fifth year, cholera declined to very low levels in Matlab, and no cases were detected among three-dose recipients.

As shown in *Table 1*, there was a rather complex age-related longitudinal pattern of efficacy. Protection conferred by both vaccines was sustained at substantial levels in persons vaccinated at ages of 5 years or greater for the initial 3 years of follow-up, but were not significant in the fourth year. Children vaccinated at ages below 5 years exhibited moderate protection (67%, $P<0.05$, for all vaccinees) during the initial pre-epidemic period of the first year, corresponding to the first 4–6 months of follow-up. More modest protection of this age group was observed for the remainder of the first 2 years, after which protection dropped to nil.

Because age at vaccination and the biotype of the infecting vibrios were earlier noted to be critical determinants of vaccine protection[3], it was of interest to examine the trends of vaccine protection separately for classical and El Tor cholera, by age, during each year of follow-up (*Table 2*). In this analysis the two vaccine groups were combined, in view of the similar constituents of the vaccines and the nearly identical performance of these vaccines in the field, particularly after the pre-epidemic period of the first year.

Among children vaccinated before the age of 5 years, protection against El Tor cholera was evanescent, being observed only in the first 4–6 months of follow-up. In contrast, for classical cholera, significant protection (40–56%) of young children was sustained for the first 2 years of follow-up.

For persons vaccinated at older ages, vaccine protection against El Tor cholera declined gradually from 64% to 48% during the initial 3 years, after which protection disappeared. Vaccine protection against classical cholera was somewhat higher, but also exhibited a year-by-year decline from 76% to 58%. No classical cases were detected in the fourth year.

DISCUSSION

In this trial each vaccine demonstrated moderate (*ca* 60%) protective efficacy for 2 years of follow-up. Thereafter, protection declined but was still evident during the third year. In the fourth year, neither vaccine conferred significant protection. No noteworthy differences in the protection conferred by the two vaccines were detected, apart from the initial pre-epidemic period when the BS-WC provided superior protection[4].

Analyses of all vaccinees in aggregate revealed moderate (PE=40–56%) vaccine protection against classical cholera during each of the first 2 years of follow-up of vaccinees aged 2–5 years, but only evanescent protection against El Tor cholera in this age group. Among older vaccinees, protection against classical cholera was more substantial and was sustained (PE=58–76%) during each of the initial 3 years; somewhat lower protection was maintained during this period against El Tor cholera (PE=48%–64%). It is a matter of speculation whether protection would have been more sustained against classical cholera after 3 years of follow-up of the older group, since no classical cases were detected during the fourth year of follow-up.

These results demonstrate that the killed oral vaccines were able to confer sustained protection against classical cholera for 2 years in both young children and older persons, and for at least 3 years in the older group. In contrast, sustained protection against El Tor cholera was seen only in the older group, in whom protection was evident for only 3 years.

The possible explanations for these biotype- and age-related distinctions in vaccine efficacy are multiple. It has been speculated that the better vaccine performance in older than in younger subjects might reflect the fact that older subjects had a greater likelihood of being immunologically primed at the time of vaccination[6]. This explanation does not, however, account for the

Table 1 Overall occurrence of cholera episodes during the 5 years of surveillance[a], by age at vaccination[b]

	Group BS-WC	WC	Vaccinees[c]	K12[d]
Year 1				
Pre-epidemic				
2–5 years	0 (100%)** (80%)	6 (35%) (−55%)	6 (67%)* (24%)	9
>5 years	4 (76%)** (44%)	5 (71%)** (37%)	9 (73%)*** (50%)	17
Epidemic				
2–5 years	26 (21%) (−20%)	24 (31%) (−7%)	50 (26%) (−6%)	34
>5 years	11 (78%)*** (64%)	17 (66%)*** (47%)	28 (72%)*** (60%)	50
Entire year				
2–5 years	26 (38%)* (7%)	30 (31%) (−1%)	56 (35%)* (9%)	43
>5 years	15 (78%)*** (66%)	22 (67%)*** (51%)	37 (72%)*** (62%)	67
Total	41 (62%)*** (50%)	52 (53%)*** (38%)	93 (57%)*** (47%)	110
Year 2				
2–5 years	16 (47%)* (13%)	24 (24%) (−18%)	40 (35%)* (5%)	31
>5 years	25 (63%)*** (45%)	18 (73%)*** (59%)	43 (67%)*** (56%)	67
Total	41 (58%)*** (44%)	42 (57%)*** (42%)	83 (58%)*** (46%)	98
Year 3				
2–5 years	24 (−36%) (−126%)	18 (2%) (−70%)	42 (−17%) (−85%)	18
>5 years	23 (41%)* (10%)	15 (61%)*** (38%)	38 (51%)*** (30%)	39
Total	47 (18%) (−14%)	33 (42%)** (18%)	80 (30%)* (7%)	57
Year 4				
2–5 years	7 (−44%) (−276%)	6 (−19%) (−220%)	13 (−31%) (−211%)	5
>5 years	8 (38%) (−28%)	17 (−31%) (−140%)	25 (4%) (−69%)	13
Total	15 (16%) (−49%)	23 (−28%) (−114%)	38 (−6%) (−69%)	18
All years				
2–5 years	73 (23%)* (1%)	78 (21%) (−1%)	151 (22%)* (3%)	97
>5 years	71 (62%)*** (52%)	72 (61%)*** (52%)	143 (61%)*** (54%)	186
Total	144 (49%)*** (38%)	150 (47%)*** (36%)	294 (48%)*** (39%)	283

[a]No cases were detected during the fifth year of surveillance. Three recurrent cholera episodes were excluded: two in the BS-WC group (one with episodes in the first and second years; the other with two episodes in the second year); and one in the K12 group (with episodes in the second and third years). [b]The numbers in the table denote: Number of cases (protective efficacy in percentage) (lower boundary of the 95% confidence interval). [c]"Vaccinees" refer to the aggregate of the BS-WC and WC groups. [d]Denominators for (BS-WC, WC, K12) were: Year 1—(3728, 3900, 3830) for 2–5 years, (16 977, 16 843, 17 007) for 5 years; Year 2—(3599, 3745, 3674) for 2–5 years, (16 403, 16 260, 16 338) for 5 years; Year 3—(3532, 3669, 3600) for 2–5 years, (15 838, 15 755, 15 780) for 5 years; Year 4—(3447, 3600, 3557) for 2–5 years, (15 356, 15 305, 15 348) for 5 years. *$P<0.05$; **$P<0.01$; ***$P<0.001$ (one-tailed) for protective efficacy

relatively sustained protection of young children against classical cholera, but the very transient protection of this age group against El Tor cholera.

The better protection against classical cholera might have resulted from the preponderance of classical whole cells in the vaccines: each vaccine contained classical and El Tor cells in a ratio of 3:1. It is also possible that the immunogenicity of the El Tor cells in the vaccines might have been deficient because the inactivation procedures for the vaccines failed to preserve mannose-sensitive hemagglutinin, a potentially important immunogen for El Tor cholera[7]. Finally, it may be relevant that, after being absent for many years in Matlab, classical cholera reappeared during the years immediately prior to the trial and nearly displaced El Tor cholera[8]. As a result, it is conceivable that the field trial population was more effectively "primed" against classical than against El Tor cholera at the time of vaccination.

Whatever the explanation, the credible performance of the vaccines against classical cholera in young children lends optimism to the possibility of protecting young children with killed oral vaccines, if reasons for the selectively poor performance against El Tor disease can be unravelled and overcome by suitable vaccine modifications.

Table 2 Occurrence of cholera episodes among vaccinees during the 5 years of surveillance, by age and biotype[a]

	El Tor		Classical	
	2–5 years	>5 years	2–5 years	>5 years
Year 1				
Pre-epidemic[b]	6 (57%) (−5%)	8 (71%)** (43%)	0 (100%)* (38%)	1 (83%)* (11%)
Epidemic	18 (−13%) (−127%)	7 (50%) (−19%)	32 (36%)* (1%)	21 (75%)**** (63%)
Entire year	24 (20%) (−38%)	15 (64%)*** (39%)	32 (40%)* (9%)	22 (76%)**** (64%)
Year 2	25 (11%) (−55%)	24 (57%)*** (32%)	15 (56%)** (22%)	19 (75%)**** (62%)
Year 3	20 (−11%) (−115%)	22 (48%)* (14%)	22 (−22%) (−134%)	16 (58%)** (28%)
Year 4	13 (−31%) (−211%)	25 (4%) (−69%)	0 (—)	0 (—)
All years	82 (4%) (−31%)	86 (48%)**** (33%)	69 (35%)** (12%)	57 (72%)**** (65%)

[a]The numbers in the table denote: number of cases (protective efficacy in percentage) (lower boundary of the 95% confidence interval). [b]Pre-epidemic period corresponds to the initial 4–6 months of follow-up. Table excludes one episode, detected in a K12 patient during the first year, in which *V. cholerae* 01 vibrios of both biotypes were simultaneously excreted. No cases were detected during the fifth year of surveillance. Three recurrent episodes were excluded, as noted in *Table 1*. Denominators are given in *Table 1*. *$P<0.05$; **$P<0.01$; ***$P<0.001$; ****$P<0.0001$ for protective efficacy

Two earlier trials of parenteral cholera vaccines observed that protection of young children was followed by a "rebound" higher incidence of cholera in vaccinees than of controls[6,9,10]. A similar rebound with long-term follow-up was also reported during long-term follow-up of persons immunized with parenteral vaccines against typhoid[11]. In this trial there was a suggestion of such a rebound in young vaccinated children during the third and fourth years of surveillance (*Table 1*). However, the higher incidence among vaccinated children was not statistically significant. The possibility of such a rebound, and the corresponding need for long-term surveillance, should be considered in the design of future trials of cholera vaccines.

During the 5 years of follow-up, the epidemiological pattern of cholera shifted from balanced coexistence of both biotypes, to El Tor alone. Moreover, the incidence of cholera among placebo recipients began at an unexpectedly high rate of *ca* 5 cases per 1000 placebo recipients, and progressively declined, disappearing completely during the fifth year of surveillance. These data highlight the unpredictability of cholera incidence, even in Matlab where cholera is hyperendemic[5], and underscore the conservatism required for adequate planning of such evaluations[12].

During the years since initiation of this trial, several important developments have occurred in the epidemiology of cholera worldwide. El Tor cholera appeared in a massive outbreak in Peru in 1991, and now has spread to affect much of the remainder of South and Central America[13]. The Latin American epidemic elicited a renewed interest in vaccination against cholera as a public health measure. As a result, two field trials of a newer version of the oral BS-WC containing the same cellular constituents with BS produced by recombinant genetic technology (rBS), currently in progress in Peru, are testing whether the use of booster doses of vaccine can extend the duration of protection against El Tor cholera. Whether this different regimen will extend the rather transient protection observed against El Tor cholera in the Bangladesh trial, particularly in young children, will be of great interest.

The second major development occurred in late 1992, with the appearance of *V. cholerae* 0139 in Asia[14]. This organism is capable of causing life-threatening cholera on epidemic scale, and appears to have pandemic potential. However, because epidemiological and laboratory studies suggest that natural immunity to *V. cholerae* 01 is not protective against *V. cholerae* 0139[12], there is little likelihood that the killed oral vaccines tested in this trial will prove to be useful against this new form of cholera. Thus, in addition to improving long-term vaccine protection against El Tor cholera, especially in young children, future development of killed oral vaccines in a manner that will make them useful public health tools will require inclusion of additional *V. cholerae* 0139 antigens.

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