Natural Cholera Infection–Derived Immunity in an Endemic Setting

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Background. Live oral cholera vaccines may protect against cholera in a manner similar to natural cholera infections. However, information on which to base these vaccines is limited.

Methods. The study was conducted in a cholera-endemic population in Bangladesh. Patients with cholera (index patients) detected between 1991 and 2000 were age-matched to 4 cholera-free controls and then followed up during the subsequent 3 years.

Results. El Tor cholera was associated with a 65% (95% confidence interval [CI], 37%–81%; $P < .001$) lower risk of a subsequent El Tor episode. Reduction of the risk of subsequent El Tor cholera was similar for children <5 years and for older persons and was sustained during all 3 years of follow-up. Having El Tor Inaba cholera was associated with lower risks of both El Tor Inaba and El Tor Ogawa cholera, but having El Tor Ogawa cholera was associated only with a reduced risk of El Tor Ogawa cholera. O139 cholera was associated with a 63% (95% CI, –61% to 92%; $P = .18$) lower risk of subsequent O139 cholera, but there was no evidence of cross-protection between the O1 and O139 serogroups.

Conclusions. Live oral cholera vaccines designed to protect against the O1 and O139 serogroups should contain at least the Inaba serotype and strains of both serogroups.

Vibrio cholerae serogroup O1 and O139 organisms cause acute, watery diarrhea, with an estimated 100 000–150 000 deaths annually, and both endemic and epidemic disease [1]. There are 2 biotypes of *V. cholerae* O1 organisms, classical and El Tor. Based on differences in antigenic determinants of the lipopolysaccharide O antigen, the biotypes are further subclassified into Inaba and Ogawa serotypes. The current (seventh) pandemic of cholera is caused by *V. cholerae* O1, El Tor biotype. *V. cholerae* O139, newly emerged in 1992 [2, 3]. After its emergence, *V. cholerae* O139 predominated for a time as the cause of clinical cholera in areas of Asia but was eventually largely replaced by El Tor cholera.

Despite global efforts to improve drinking water quality and sanitation in developing countries, there has been little evidence of a decline in the global burden of cholera in recent years. Interest has therefore increased in the use of cholera vaccines as adjuncts to other preventive and therapeutic measures. Modern approaches to creating cholera vaccines have focused primarily on orally delivered vaccines, either with inactivated or live antigens, the latter typically consisting of genetically attenuated cholera strains.

Live oral cholera vaccines have the theoretical advantage of simulating infection by natural cholera. Experimental infection of North American volunteers has been shown to protect against cholera upon rechallenge, raising the possibility that live oral vaccines may be protective after single-dose regimens. This expectation has been borne out for several live oral vaccines tested in challenge studies in North American volunteers [4–7]. However, to date no live oral vaccine has conferred protection to cholera-endemic populations when tested in a randomized trial, suggesting that the predictions from studies of volunteers lacking preexisting immunity to cholera may not be readily generalizable to cholera-endemic populations.

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One way to estimate the level of protection potentially achievable by live oral cholera vaccines in cholera-endemic populations is to study the protection conferred by natural cholera infections in these populations. Several cohort studies of this type have been conducted in the past [8–10]. None was designed to evaluate the comparative protection conferred by O1 and O139 infections studied concurrently, nor cross-protection conferred by the 2 El Tor serotypes—Inaba and Ogawa—against one another, and none evaluated the protection conferred by natural cholera in young children vs older persons, nor the duration of natural protection. In this paper, we report on a cohort study done in Bangladesh to address these gaps in knowledge.

METHODS

Study Area

The study was conducted in Matlab, a research site of the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) with endemic cholera documented over several decades. The site is approximately 50 km southeast of Dhaka, the capital of Bangladesh. The study area is 184 km², which is bisected by the Dhonagoda River into almost 2 equal portions. About 85% of the population is Muslim. It is a densely populated area with about 1000 people per square kilometer, and a total population of about 200 000 [9]. Like other rural areas of Bangladesh, educational attainment is low.

Initiated in 1966, the Matlab health and demographic surveillance system keeps reliable records of all vital demographic events of the study area population [11, 12]. Each individual is identified by a registration number given by the surveillance system. In Matlab people live in groups of patrilineally linked households sharing a courtyard, called *baris*. In 1996 a bari-level geographic information system (GIS) was created that maintains the temporal sequence of locations of all *baris* ever registered in the surveillance system. The GIS also contains other important features such as rivers, roads, and hospitals in the study area. For the period for this study, 1 January 1988–31 December 2003, demographic and geographic data were arranged into a linked dynamic population database.

Surveillance for cholera was conducted during the study period in the ICDDR,B hospital in Matlab. During the period 1988–1990, surveillance was also conducted in 2 community diarrheal treatment centers, that had been established for a field trial of killed oral cholera vaccines [13]. However, the surveillance in these treatment centers was discontinued after the trial was over in 1990. In all 3 of these treatment settings, all patients presenting with diarrhea were clinically evaluated and treated. For those residing in the ICDDR,B study area, fecal samples were collected and evaluated for *V. cholerae*, by serogroup, biotype, and serotype, using conventional microbiological methods [14, 15]. All patient services in these facilities were free of charge,

and there was a free boat service for patients who needed to be transported for care on an emergency basis. Therefore, it can reasonably be assumed that the surveillance captured virtually all clinically significant cholera cases among residents of the ICDDR,B study area.

Selection of Cholera Index Patients and Controls

In this study, we compared 2 cohorts, 1 consisting of cholera index patients and the other consisting of noncholera controls, for the occurrence of cholera during the 3 years following selection. Cholera was defined as an illness in which the patient complained of diarrhea and in which a fecal specimen yielded *V. cholerae* O1 or O139. The date of presentation for care was taken as the date of selection for the index patient and 4 controls matched to the index patient.

To select index patients, we first assembled all treatment visits for cholera during the period 1 January 1988–31 December 2003. Beginning with a date of selection of 1 January 1991 and proceeding forward in time, we included all cases meeting the following criteria: (1) the patient had resided in Matlab during the 3 years before selection and there was no history of cholera during that interval or, (2) for patients <3 years of age on the date of selection, the patient had resided in Matlab since birth and had no history of cholera from birth until the date of selection. We excluded as an index patient anyone whose treatment visit for cholera occurred in an earlier selected control for which the date of presentation for care was within 3 years following the date of selection of the control (*vide infra*). This selection process yielded 4657 index patients.

For each selected index patient, we selected 4 age-matched controls without cholera. To select controls, we first arranged the selected index patients in temporal order according to the date of selection. Then, beginning with the earliest selected index patient, we sequentially selected 4 randomly sampled persons who were residents of Matlab on the date of selection for the matched index patient and who (1) were the same age (in years) as the index patient on the date of selection; (2) did not reside in the same bari as the matched index patient on the date of selection; and (3) had resided in Matlab during the 3 years before selection and had no history of cholera during that interval, or, if <3 years of age on the date of selection, had resided in Matlab since birth and had no history of cholera from birth until the date of selection. This process yielded 18 628 matched controls.

Analytic Methods

We used survival analysis to compare cohorts of index cholera patients and controls for the occurrence of subsequent cholera [16–18]. We fitted a proportional hazards model to evaluate the association between a clinical episode of cholera and the subsequent occurrence of cholera. These models assessed the time to presentation for care of the first episode of cholera in which the date of presentation was between the day of selection for the index patients and matched controls and 3 years (1095 days)

thereafter, treating deaths and outmigrations as censoring events. We fitted both unadjusted and covariate-adjusted models. In unadjusted models, the only independent variable was a cohort identity variable (index patient vs control). Adjusted models included as independent variables age, the matching variable (age at selection), as well as demographic variables known to be associated with the risk of treated cholera in Matlab, including gender, religion, distance from household to hospital, and distance from residence to the Dhonagoda River [8, 11]. To avoid overfitting these models, we selected a maximum of 1 independent variable per 10 outcome events, selecting those variables that had the lowest *P* values for the association with the time to the occurrence of cholera in bivariate analyses. Hazard ratios (HRs) for the occurrence of cholera in index patients vs controls were estimated by exponentiating the coefficient for the cohort identity variable (index vs control) in these models, and the protection (PE) against recurrent cholera was estimated as $[(1 - \text{HR}) \times 100\%]$. Standard errors for the coefficients were used to estimate 2-tailed *P* values and confidence intervals (CIs) for the HRs. When models failed to converge, we calculated protection as a crude incidence rate ratio, and used Kaplan–Meier survival curves with the log-rank test to estimate *P* values for the compared curves.

RESULTS

We assembled 4657 index cholera patients and 18,628 controls during the period 1991–2000. Twenty-nine percent of the episodes were in children <5 years of age, 27% were in persons 5–14 years of age, and 44% were in persons aged ≥ 15 years. El Tor was the dominant strain (73%), followed by *V. cholerae* O139 (27%). Classical cholera was detected in 26 index patients. In the index patient and control cohorts, only 9% (2013 of 23 285) of subjects died or outmigrated before completion of the third year of follow-up. In total, 211 subsequent cases of cholera were detected among these index patients and their controls.

The baseline characteristics of the cohorts on the date of selection are shown in Table 1. Except for the matching variable (age), other variables showed a significant imbalance ($P < .01$) between index patients and their controls. In all 26 index patients with classical cholera, detection occurred at the beginning of the study period, and no subsequent cases of cholera were observed among these index patients or their matched controls. As shown in Table 2, 12 cases of El Tor were observed among El Tor index patients (1.2 cases per 1000 person-years [py]), and 111 were detected among their matched controls (2.9 cases per 1000 py; adjusted PE, 65%; 95% CI, 37%–81%; $P < .001$). Two cases of O139 cholera were observed among subjects with index episodes of O139 cholera (0.6 cases per 1000 py) as opposed to 18 cases of O139 cholera among controls (1.3 cases per 1000 py; adjusted PE, 63%; 95% CI, –61% to 92%; $P = .18$). There was

no evidence of serogroup-heterologous protection conferred by index episodes of El Tor or O139 cholera (adjusted PE, –40%; 95% CI, –138% to 17%; $P = .21$).

Age-specific analyses of biotype-homologous protection associated with El Tor cholera (Table 3) found an adjusted PE of 66% (95% CI, 29%–84%; $P < .01$) for children infected at <5 years of age and 65% (95% CI 0%–88%; $P = .05$) for older persons. As shown in Table 4, among index patients with El Tor Inaba cholera and their controls, there were 0 and 4 cases, respectively, of subsequent El Tor Inaba cholera (PE, 100%; $P = .32$ in unadjusted analysis). Correspondingly there were 7 and 70 cases of El Tor Ogawa detected among El Tor Ogawa index patients and their controls, respectively, yielding an adjusted PE of 69% (95% CI, 32%–86%; $P < .01$). Among index patients with El Tor Inaba cholera and their controls, there were 0 and 17 cases, respectively, of El Tor Ogawa cholera (PE, 100%; $P = .04$ in unadjusted analysis). In contrast, the incidence of El Tor Inaba cholera in the El Tor Ogawa index patients and their controls was identical (0.6 cases per 1000 py), yielding an adjusted PE of –1% (95% CI, –170% to 62%; $P = .98$).

As shown in Table 5, when examined by the time after selection, biotype-homologous protection associated with El Tor cholera was 62% (95% CI, 11%–84%; $P = .02$) during the first year after selection, 68% (95% CI, 9%–89%; $P = .03$) during the second year, and 72% (95% CI, –22% to 94%, $P = .09$) during the third year.

DISCUSSION

Our findings indicate that index episodes of El Tor cholera were associated with a moderate (65%) reduction of the risk of subsequent El Tor cholera, that this protection was evident both in young children and in older persons, and that the protection showed no evidence of decline over a 3-year period of follow-up. Protection associated with El Tor infection appeared to differ by the serotype of the infection. Inaba infections showed clear evidence of protection against Ogawa infections and apparent protection against Inaba infections, though the latter was limited by a small number of cholera outcomes. In contrast, Ogawa infections were associated with protection only against the homologous serotype. Although not statistically significant, protection associated with serogroup O139 cholera against cholera due to the same serogroup was similar in magnitude (63%) to that associated with serogroup O1, El Tor biotype cholera. There was no evident cross-protection between the O1 and O139 serogroups.

Strengths and Limitations of the Study

Before discussing the implications of these findings, it is important to point out several strengths and limitations of the study. We excluded index patients and controls with episodes of cholera during the 3 years prior to selection to help ensure that measured levels of protection reflected the effect of the index

Table 1. Features of Index Patients and Controls

Variable	Index patients (n = 4657)	Controls (n = 18 628)	P value ^a
Age category			
<5 years	1354 (29%)	5416 (29%)	1.00
5–14 years	1269 (27%)	5076 (27%)	
≥15 years	2034 (44%)	8136 (44%)	
Age (years) ^b	20.18 (21.04)	20.18 (20.04)	1.00
Gender			
Male	2318 (50%)	9931 (53%)	<.001
Female	2339 (50%)	8697 (47%)	
Religion			
Muslim	4299 (92%)	16 241 (87%)	<.001
Hindu	358 (8%)	2387 (13%)	
Distance from residence to hospital (km) ^b	4.62 (2.40)	6.52 (3.84)	<.001
Distance from residence to Dhonagoda River (km) ^b	1.25 (0.99)	1.32 (1.17)	.001
Duration of postselection follow-up (days) ^b	1041 (200)	1043 (192)	.50

NOTE. ^a The *P* values were obtained from the χ^2 test for categorical variables and from the Student *t* test for continuous variables.

^b Mean and standard deviations (in parentheses) are shown for these variables.

episode per se. In addition, selection of index cholera patients and detection of subsequent cholera among index patients and their controls were based on comprehensive, population-based surveillance for cholera, and there was very little loss to follow-up among the cohort. However, it is possible that our estimates of protection were made conservative by the possibility that index patients with cholera may have been more susceptible to cholera than their matched controls or may have been more likely than their matched controls to have sought care for cholera. We attempted to control for these biases by adjusting for potential confounding variables as well as the distance of the subject's residence to the hospital where cholera was detected. On the other hand, all index cholera infections under study were clinically severe enough to prompt solicitation of treatment. Because these clinically severe infections were likely to have

elicited more robust immune responses than clinically mild infections, which might more closely correspond to the immunogenic stimulus of a nonreactogenic oral cholera vaccine, the analysis could have overestimated the level of protection to be expected of live oral vaccines in endemic populations. Finally, the study was not of adequate size to evaluate with suitable precision protection in all subgroups of interest, including infection and reinfection by 0139 cholera, which did not occur with a high frequency throughout the study interval.

Relation to Other Studies

Past cohort studies of the protection conferred by natural cholera infection have yielded widely disparate estimates, ranging from nil to >90% [8–10]. The 1 study finding no protection included mild or asymptomatic cholera infections, which, as

Table 2. Cholera Episodes Among EI Tor and 0139 Index Patients and Controls During 3 Years of Follow-up, Matlab, Bangladesh

	Serogroup- and biotype-homologous index and outcome strains				Serogroup- heterologous index and outcome strains	
	EI Tor outcomes		O139 outcomes		EI Tor O1 or O139 outcomes	
	EI Tor index patients	Matched controls	O139 index patients	Matched controls	EI Tor or O139 index patients	Matched controls
Number of index patients and matched controls	3394	13 576	1237	4948	4631	18 524
Number of cholera episodes detected during follow-up	12	111	2	18	20	48
Person-days of follow-up	3 531 226	14 199 959	1 297 577	5 153 838	4 828 070	19 402 067
Incidence (per 1000 person-years)	1.2	2.9	0.6	1.3	1.5	0.9
Protection (95% CI; <i>P</i> value)						
Unadjusted analysis	56% (20%–70%; <.01)		56% (–90% to 90%; .27)		–68% (–183% to 1%; .052)	
Adjusted analysis	65% (37%–81%; <.001) ^a		63% (–61% to 92%; .18) ^b		–40% (–138% to 17%; .21) ^a	

NOTE. ^a Adjusted for age, gender, religion, distance from residence to hospital, and distance from residence to Dhonagoda River.

^b Adjusted for age and distance from residence to hospital.

Table 3. El Tor Cholera Episodes Among El Tor Index Patients and Matched Controls During 3 Years of Follow-up by Age Group, Matlab, Bangladesh

	Age <5 years at selection		Age ≥5 years at selection	
	Index patients	Matched controls	Index patients	Matched controls
Number of index patients and matched controls	1165	4660	2229	8916
Number of cholera episodes detected during follow-up	8	75	4	36
Person-days of follow-up	1 188 820	4 849 670	2 342 406	9 350 289
Incidence (per 1000 person-years)	2.5	5.6	0.6	1.4
Protection (95% CI; <i>P</i> value)				
Unadjusted analysis	56% (10%–79%; .02)		56% (–25% to 84%; .12)	
Adjusted analysis	66% (29%–84%; <.01) ^a		65% (0%–88%; .05) ^b	

NOTE. ^a Adjusted for age, gender, religion, distance from residence to hospital, and distance from residence to Dhonagoda River.

^b Adjusted for age, gender, and distance from residence to hospital.

noted earlier, may be associated with less robust induction of protective immunity than cholera severe enough to require treatment; all other studies have focused on more severe cholera. Our study found no subsequent episodes of cholera among index patients with classical cholera. Although interpretation of this finding is limited by the absence of cholera in the controls matched to these classical cholera index patients, it is nonetheless interesting that an earlier study done in Bangladesh found that episodes of classical cholera were associated with complete protection against recurrent cholera [9]. However, neither study had sufficient power to be able to state that the protection by classical cholera is serotype-independent.

Biological Implications

Demonstration of protection by episodes of O1 and O139 cholera against the infection by the same serogroup, as well as the absence of cross-protection between the O1 and O139 serogroups, was

expected based on earlier studies [19–21] and the known structural differences of the O antigen of these 2 serogroups. Interestingly, protection by Inaba antigen against both Inaba and Ogawa cholera, but protection by Ogawa antigen only against Ogawa cholera, was observed for parenteral cholera vaccines tested decades ago, and an analysis of trends of cholera in Bangladesh over a 33-year period suggested that Inaba cholera confers greater protection than Ogawa cholera [22–24]. However, to our knowledge, the results of this study are the first to confirm these relationships for natural cholera infections. The Ogawa and Inaba serotypes differ only by a 2-O-methyl group that is present in the nonreducing terminal sugar of the Ogawa O-specific polysaccharide (O-SP) of *V. cholerae* lipopolysaccharide but is absent in the Inaba O-SP [25–27]. It is likely that the effect of these structural determinants on protective epitopes provides the basis for our observations on homologous vs heterologous protection elicited by infections by these 2 serotypes. Secular changes

Table 4. El Tor Cholera Episodes Among El Tor Index Patients and Controls by Serotype of the Index and Outcome Infections During 3 Years of Follow-up, Matlab, Bangladesh

	Serotype of outcome infection homologous to that for index patient				Serotype of outcome infection heterologous to that for index patient			
	Inaba outcomes		Ogawa outcomes		Ogawa outcomes		Inaba outcomes	
	Inaba index patients	Matched controls	Ogawa index patients	Matched controls	Inaba index patients	Matched controls	Ogawa index patients	Matched controls
Number of index patients and controls	546	2184	2848	11 392	546	2184	2848	11 392
Number of cholera episodes detected during follow-up	0	4	7	70	0	17	5	20
Person-days of follow-up	565 754	2 297 598	2 968 005	11 923 054	565 754	2 289 554	2 970 926	11 958 055
Incidence (per 1000 person-years)	0.00	0.6	0.9	2.1	0.00	2.7	0.6	0.6
Protection (95% CI; <i>P</i> value)								
Unadjusted analysis	100% (not applicable; .32) ^a		60% (13%–82%; .02)		100% (not applicable; .04) ^a		0% (–168% to 62%; 0.99)	
Adjusted analysis	100% (not applicable; .32) ^a		69% (32%–86%; <.01) ^a		100% (not applicable; .04) ^a		–1% (–170% to 62%; .98) ^b	

NOTE. Models did not converge, and 95% confidence interval (CI) was not estimable. *P* value was estimated using the log-rank test for Kaplan–Meier curves.

^a Adjusted for age, gender, religion, distance from residence to hospital, and distance from residence to Dhonagoda River.

^b Adjusted for age.

Table 5. El Tor Cholera Episodes Among El Tor Index Patients and Matched Controls by Year of Follow-up After Selection, Matlab, Bangladesh

	1st year		2nd year		3rd year	
	Index patients	Controls	Index patients	Controls	Index patients	Controls
Number of index patients and controls	3394	13 576	3266	13 140	3182	12 773
Cholera episodes	6	51	4	37	2	23
Person-days of follow-up	1 209 989	4 869 405	1 176 519	4 727 761	1 144 718	4 602 793
Incidence (per 1000 person-years)	1.8	3.8	1.2	2.9	0.06	0.2
Protection (95% CI; <i>P</i> value)						
Unadjusted analysis	53% (–10% to 80%; .08)		57% (–21% to 85%; .11)		65% (–48% to 92%; .15)	
Adjusted analysis	62% (11%–84%; .02) ^a		68% (9%–89%; .03) ^b		72% (–22%, 94%; .09) ^c	

NOTE. ^a Adjusted for 4 covariates: gender, religion, distance from residence to hospital, and distance from residence to Dhonagoda River.

^b Adjusted for 3 covariates: gender, religion, and distance from residence to hospital.

^c Adjusted for 1 covariate: distance from residence to hospital.

in prevailing serotypes are frequently observed in cholera-endemic populations, more frequently from Ogawa to Inaba than the reverse. The role of serotype-specific immunity, homologous and heterologous induced by Inaba vs homologous only for Ogawa, in influencing these switches remains to be determined.

Practical Implications

Our findings have several implications for the design, development, and evaluation of live oral vaccines against cholera. They also inform the development of future inactivated oral vaccines against cholera. First, although our data support the immunizing potential of both El Tor and O139 vaccine strains, they are cautionary in the sense that they suggest that the upper limit of protective efficacy that can be expected of these strains may be moderate in magnitude. Second, these moderate levels of protection contrast with the high levels of protection seen in nonimmune North American volunteers participating in studies of the protection conferred against cholera challenges by wild-type cholera infections and by live oral vaccine candidates, underscoring the limitations of the North American cholera challenge model in predicting vaccine performance in populations with endemic cholera. Third, if protection is desired against both El Tor and O139 cholera, vaccines will have to be at least bivalent with respect to the serogroups of their constituent strains. Finally, for vaccine protection against El Tor Inaba cholera, an El Tor Inaba vaccine strain will be needed; such a strain may also be sufficient to protect against El Tor Ogawa cholera if a vaccine parsimonious with respect to the number of strains is desired.

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