Epidemic and Endemic Cholera Trends over a 33-Year Period in Bangladesh

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Despite nearly 200 years of study, the mechanisms contributing to the maintenance of endemic cholera and the causes of periodic epidemics remain poorly understood. To investigate these patterns, cholera data collected over 33 years (1966–1998) in Matlab, Bangladesh, were analyzed. Time-lagged autocorrelations were stratified by *Vibrio cholerae* serogroup, serotype, and biotype. Both classical and El Tor biotypes alternated and persisted between 1966 and 1988; the classical biotype disappeared by 1988, and the O139 serogroup first appeared in 1993. Both the Ogawa and Inaba serotypes circulated the entire time. The autocorrelations revealed that both Inaba and Ogawa epidemics were followed 12 months later by epidemics of the same serotype. Ogawa epidemics, however, were also followed by further Ogawa epidemics only 6 months later. Thus, epidemics of Inaba may selectively confer short-term population-level immunity for a longer period than those of Ogawa. These observations suggest that the Inaba antigen should be maximized in cholera vaccine designs.

The epidemic and pandemic characteristics of cholera, although well appreciated and described for ~200 years, remain poorly understood. Although the virulence factors that cause diarrhea have been elucidated, those factors that account for its transmissibility and ability to move around the world are still largely unknown [1]. Epidemic *Vibrio cholerae* has 2 major serogroups (O1 and O139); the O1 serogroup has 2 biotypes (classical and El Tor), and each biotype has 2 major serotypes (Ogawa and Inaba). Usually, 1 of the 2 serotypes is responsible for the majority of cases in any 1 geographic area, but 1 serotype usually replaces the other with time in an endemic area [2, 3] or during a prolonged outbreak [4]. This change in serotype has been thought to be correlated with the immune status of the population and has been documented in animal models [5]. Furthermore, the molecular mechanism for this serotype change has been elucidated elsewhere [6]. What role any of these antigens has in the transmissibility or virulence of the organism, however, is not known.

In Matlab, Bangladesh, we have had a unique opportunity, since 1966, to study the epidemiological patterns of cholera, including serogroup, biotype, and serotype of the infecting organisms. Matlab is a rural area, located 45 km south of Dhaka, that is highly endemic for cholera. We present 33 years of descriptive data, with particular attention to the protection afforded by infection by one or the other biotype or serotype.

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Methods

Study methods. Since 1966, cholera surveillance has been carried out in a well-defined, demographic surveillance population in Matlab, Bangladesh. The mean (±SD) midyear population size for the period of 1966–1998 was 207,854 ± 36,325. There was a mean of ~100,000 people under surveillance for the first 2 years of the study. After that, the mean tended to be ~200,000 people. All patients with severe diarrheal disease are treated at a single diarrhea hospital and have their stools cultured for *V. cholerae*. Standard bacteriologic methods have been employed [7]. Stools are cultured on selective media (thiosulfate citrate bile salts sucrose agar and taurocholate tellurite gelatin agar), and suspect colonies are agglutinated with specific antisera for *V. cholerae* O1, Inaba, and Ogawa serotypes. Positive colonies are confirmed as *V. cholerae* with biochemical testing, and the 2 biotypes (either classical or El Tor) are differentiated. Since 1993, when the new *V. cholerae* serogroup O139 was first identified [8], all *Vibrio* species isolates have also been agglutinated in specific antiserum to identify this serogroup.

Statistical methods. Let Y_t be the number of reported cholera cases at time t. We use the lagged autocorrelation coefficient (ACC) to measure the degree of association between the number of reported cases at times t and $t - \tau$, where τ is the time lag. We let r_τ be the sample ACC with lag τ for the correlation between $Y_{t-\tau}$ and Y_t, where $t \geq \tau$. Thus, r_τ measures the autocorrelation (AC) of the series $\{Y_t\}$ with lag τ. We use the standard product moment estimator for r_τ [9]. Simple 95% confidence intervals are constructed for the ACC, using Bartlett's approximation [9]. For the analyses

in this paper, the time units are months, so that, for example, r_6 is the AC in cholera cases 6 months apart. For example, if $r_6 = 0.5$, then the correlation between current cholera cases and those 6 months in the future is 0.5, or the current case level can explain 25% of the variation in the number of cases 6 months into the future. We give correlograms of the ACs for lags 1–15 months of cholera cases, stratified by biotype and serotype. A correlogram is a plot of r_τ over a range of values for τ. We also estimate lagged cross-correlations. These measure the correlation between a series $\{Y_t\}$ and a different series $\{X_t\}$ at various lags. Correlations are dimensionless quantities, and, therefore, they are not affected by variations in the mean number of people under surveillance. $P \leq .05$ was considered to be statistically significant.

Results

Figure 1 gives the yearly reported number of cholera cases, by biotype and serotype, for the 33-year period 1966–1998. With regard to the biotypes, during the period 1966–1972, nearly all the reported cases were the classical biotype *V. cholerae* O1. Then, starting in 1973, the El Tor biotype *V. cholerae* O1 began to circulate in the absence of classical cholera. For the period 1982–1988, classical cholera returned and cocirculated with El Tor cholera. After that period, classical cholera has not been reported. In 1993, cases of the non-O1 serogroup, *V. cholerae* O139, were reported in Matlab. Since that time, El Tor and O139 cholera have cocirculated. With regard to the serotypes, both the Inaba and Ogawa serotypes were present over the entire 33-year period. For the period 1966–1981, Inaba predominated, and, in the period 1982–1997, Ogawa predominated. The cycles ran for 2–8-year periods: Inaba predominated for 17 years, Ogawa predominated for 15 years, and there was 1 year in which they were essentially equal.

Figure 2 gives the mean monthly number of cholera cases over the 33-year period. There is a clear large annual fall outbreak in the months of September through December (at the end of the monsoons) and a smaller spring outbreak around April (just before the monsoons) of each year. Figure 2 also gives the correlogram and the 95% confidence intervals for the

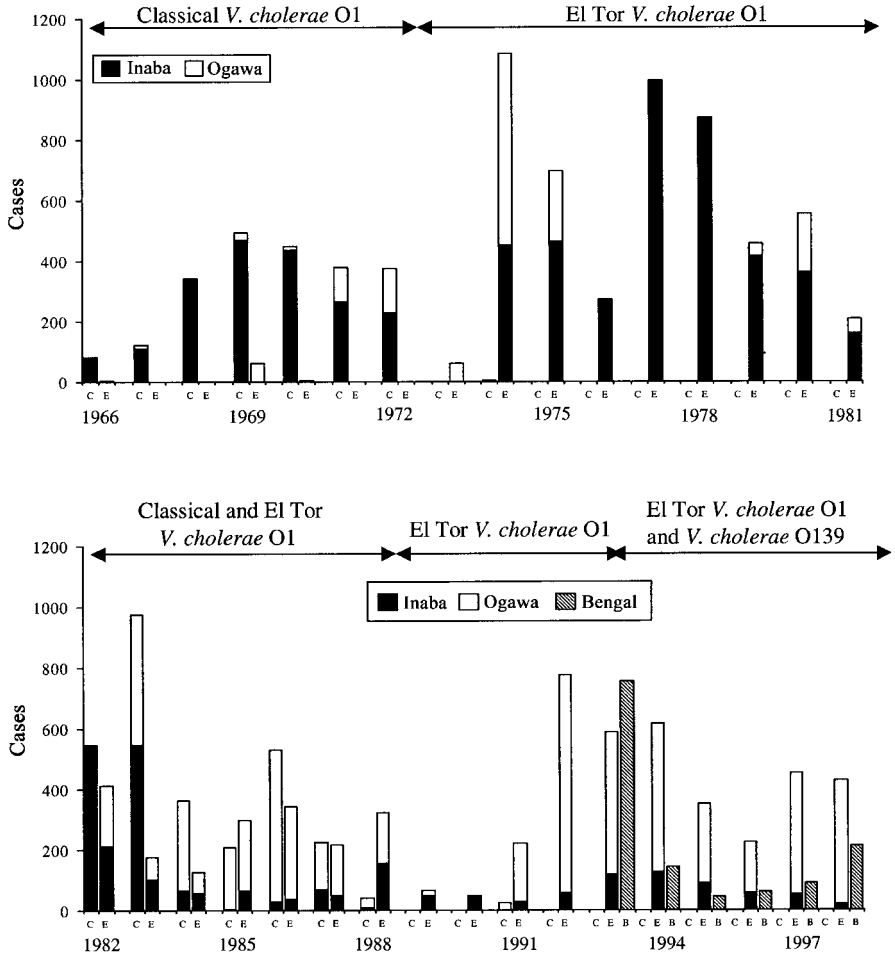

Figure 1. Yearly reported number of cholera cases, by *Vibrio cholerae* biotype and serotype, for the 33-year period 1966–1998, in Matlab, Bangladesh. Arrows indicate the periods of various dominant serogroups and biotypes. B, Bengal; C, classical; E, El Tor.

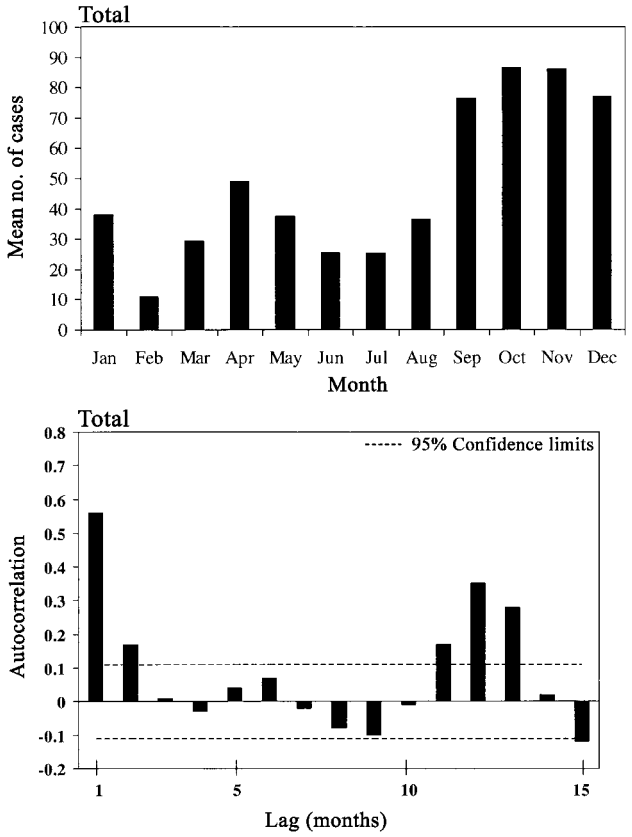

Figure 2. Mean monthly number and correlogram for total cholera cases, for the 33-year period 1966–1998, in Matlab, Bangladesh.

total cholera cases over the 33-year period. Statistically significant ACs (i.e., values that exceed the upper positive 95% confidence limits) occur at lags of 1, 2, and 11–13 months. The estimated 1-month-lagged ACC is 0.56. Thus, if we have Y_t current cases of cholera, we would expect the number of cases in the next month to be moderately correlated with the current number of cases. In addition, the estimated 2-month-lagged ACC is 0.17. Thus, if we have Y_t current cases of cholera, then we would expect the number of cases of cholera 2 months in the future to be weakly correlated with the current number of cases. The estimated 3-month-lagged ACC is not significantly different from 0. Thus, current cholera cases tell us little about what to expect 3 months into the future. This short-term AC indicates that cholera comes in sharp outbreaks that last for ~2 months. The statistically significant estimated ACC of 0.35 at a 12-month lag indicates that current cholera cases predict the number of cases 12 months later. This provides evidence of a strong yearly seasonality in total cholera cases. These cholera seasonality results confirm previous observations [10, 11].

Figure 3 gives the correlograms for the Inaba and Ogawa serotypes, respectively, over the 33-year period. The correlogram for Inaba indicates strong yearly seasonality for this agent. For Ogawa, we see the strong yearly seasonality and 1 and 2 months AC, plus significant ACCs at lags of 3–6 months. This indicates that current Ogawa cases positively predict more Ogawa cases ~6 months later. There is no evidence of this for Inaba. The implication is that Inaba gives good short-term immunity, so that a current outbreak tends not to be followed by another outbreak 6 months later. One possible confounding factor for this difference in the 2 serotypes is the biotype. Figures 4 and 5 give the correlograms for Inaba and Ogawa stratified by the classical and El Tor biotypes. The plots for El Tor cholera were tabulated over the 16-year period of 1973–1998, during which El Tor cholera was reported in Matlab. The plots for classical cholera were tabulated over the 14-year period of 1966–1972 and 1982–1998, during which classical cholera was reported in Matlab. For the most part, Ogawa shows significant AC at ~6 months lag, whereas Inaba shows little or no AC at around a 6-month lag. In figure 4, the lack of a significant 12-month-lagged AC for classical/Ogawa cholera is due to the timing of the relatively small number of classical/Ogawa cholera outbreaks. There does not appear to be a difference in short-term immunity provided by classical or El Tor cholera when we control for serotype.

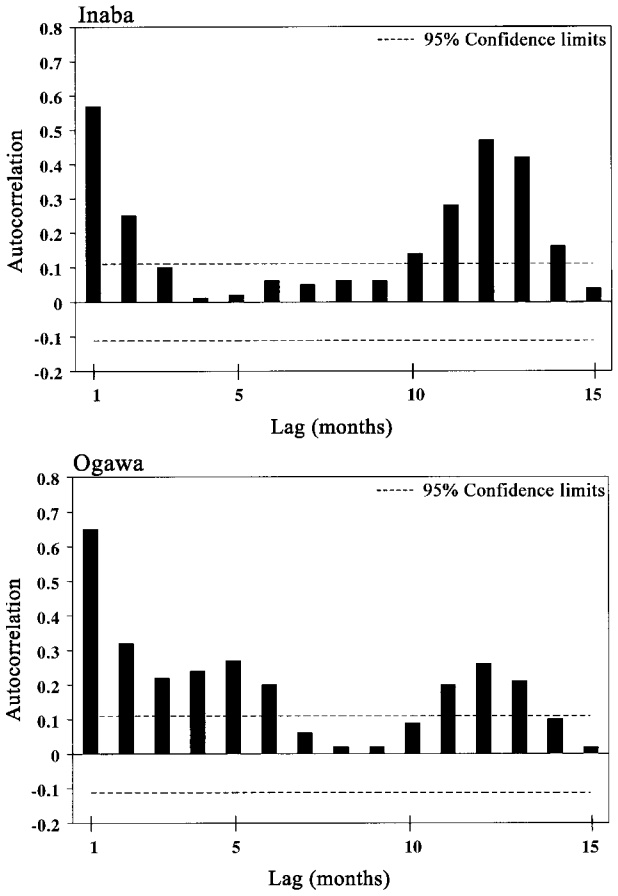

Figure 3. Correlograms for the autocorrelation of Inaba and Ogawa cholera cases, for the 33-year period 1966–1998, in Matlab, Bangladesh.

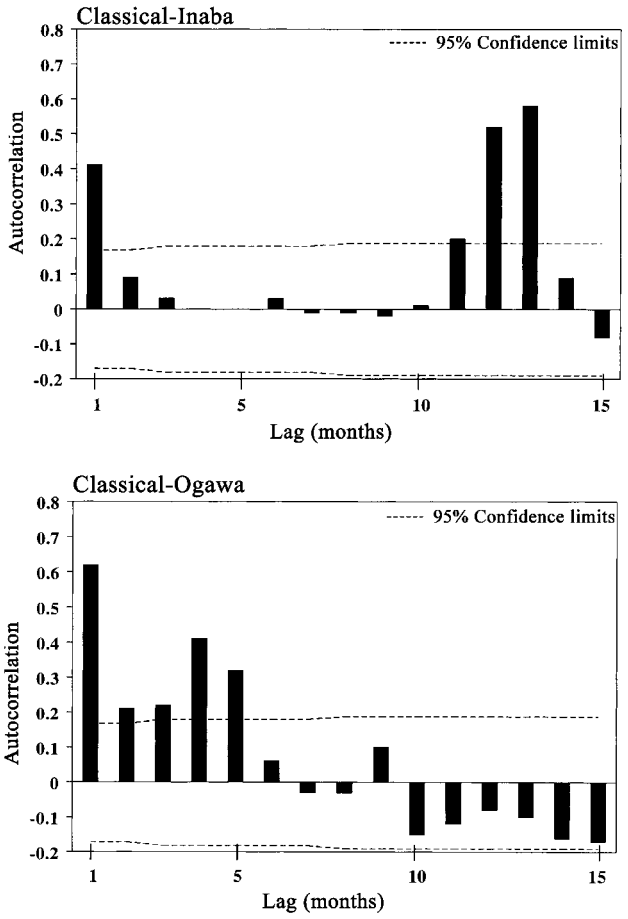

Figure 4. Correlograms for the autocorrelation of classical-Inaba and classical-Ogawa cholera cases, for the 14-year period 1966–1972 and 1982–1998, in Matlab, Bangladesh.

Figure 6 gives the correlogram for the lagged cross-correlation between both serotypes of serogroup O1 cholera, lagged against Inaba, over the 33-year period. The lack of a significant correlation at lags of 3–6 months indicates that Inaba provides significant short-term immunity against all serogroup O1 cholera.

We investigated the lagged AC for *V. cholerae* O139 over the 6-year period of 1993–1998. Although this is too short a time series for rigorous statistical analysis, the 6-month-lagged AC is estimated to be 0.35 ($P < .01$). This indicated that *V. cholerae* O139 did not provide short-term protection against subsequent *V. cholerae* O139 on a population level.

Discussion

This 33-year period of observation has allowed us to define and confirm several aspects of the epidemiology of cholera in a highly endemic area. Although the incidence of cholera fluctuates from year to year, the seasonality remains much the same with spring and fall outbreaks of disease, regardless of the serogroup or biotype of the organism. In Matlab, these seasonal outbreaks are predicted by increases in water temperature (lagged 4–8 weeks before the spring epidemics) and in copepod (simultaneously) and cyanobacteria (lagged 2 weeks before) concentrations in local water bodies, among other environmental factors [12]. However, the frequency of the 2 serotypes fluctuates with time, in what seems to be a long-term ongoing competition.

These data allow us to predict the subsequent occurrence of cholera following cholera outbreaks due to either serotype, thus indirectly measuring the degree of protection an outbreak provides to a population. Since infection with *V. cholerae* produces 10–50 more asymptomatic infections than symptomatic ones [13], during an outbreak, the same serotype circulating in the population would also add to the immune protection provided by that serotype. An outbreak thus is a marker for an even wider circulation of that serotype in the population.

Our data corroborate previous studies indicating that the Inaba serotype may be a more protective antigen than the Ogawa serotype [14, 15]. This may be explained by the differences in antigenic formulas of the O antigens on the lipopolysaccharide

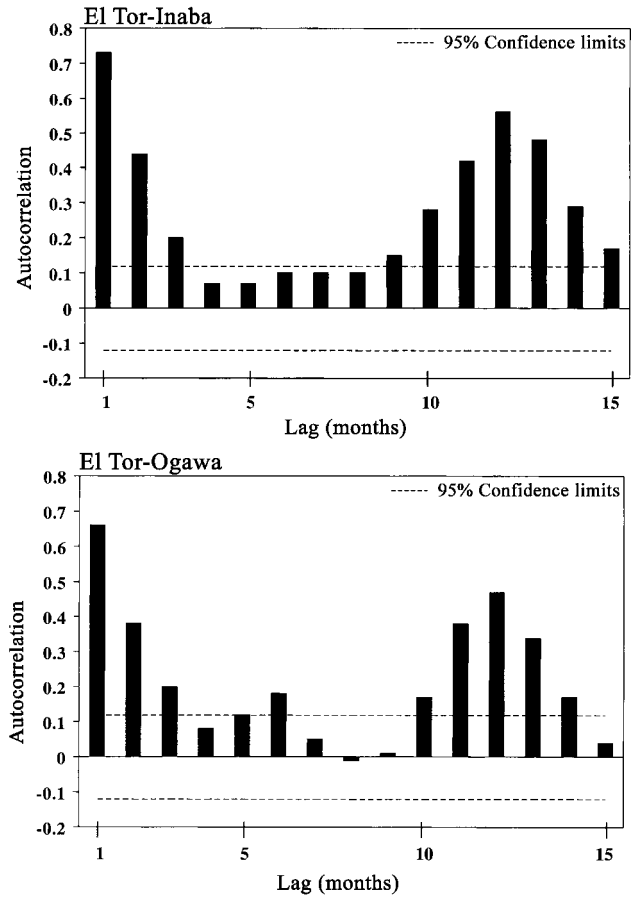

Figure 5. Correlograms for the autocorrelation of El Tor–Inaba and El Tor–Ogawa cholera cases, for the 16-year period 1973–1998, in Matlab, Bangladesh.

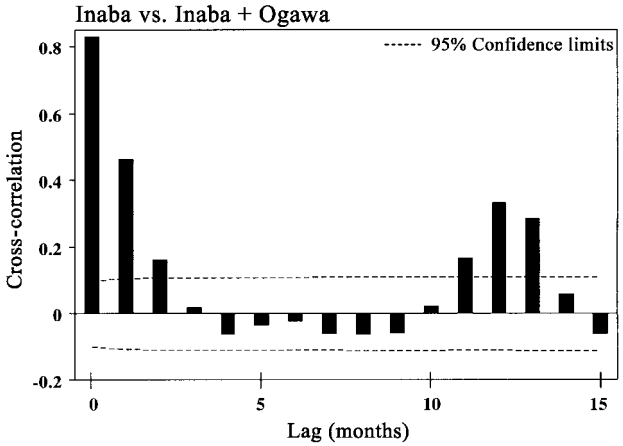

Figure 6. Correlogram for cross-correlation between both serotypes of serogroup O1 cholera, lagged against Inaba, for the 33-year period 1966–1998, in Matlab, Bangladesh.

of the 2 serotypes [6]. The O antigen has 3 components, designated A, B, and C. Inaba strains possess a large amount of C antigen, compared with a small amount in Ogawa, suggesting that this may be the primary protective antigen. The A and B antigens seem to be less important, since A is found in both serotypes in equal amounts, and B is absent in Inaba strains.

Our findings also contradict the previous suggestions that infections with classical strains provide more protection than infections with El Tor strains against subsequent cholera [16, 17]. These data provide the most direct information on protection afforded by cholera outbreaks in populations and resolve some of the uncertainties from previous field studies, which looked for second cholera infections in people who had been infected previously. These studies, all based on small number of patients, reported varying degrees of protection, from near zero [16] to near complete protection [10] to intermediate protection, estimated at 61% [17]. The first study [16] found that, although the overall protection was low, Inaba provided more protection than Ogawa. In the third study [17], it was found that infection with classical strains appeared to provide complete protection against reinfection with either classical or El Tor strains, whereas infection with El Tor provided no protection against subsequent cholera. Of the 7 subjects monitored, 5 were reinfected with Ogawa, 1 was reinfected >2 years later with Inaba, and 1 was infected with Ogawa and then with Inaba >1 year later. Three of the Ogawa reinfections occurred <6 months later. This observation also supports our hypothesis that infection with Ogawa provides little or no short-term immunity.

Several large vaccine field trials have examined the protection afforded by Inaba and Ogawa vaccines. The first, reported in 1964, using parenteral vaccines [14], showed that a whole-cell vaccine consisting of both serotypes was more protective than one containing only a purified Ogawa antigen. In the second trial of parenteral vaccines [15], both a monovalent Inaba vaccine and a purified Inaba antigen vaccine provided significant protection against cholera, whereas a monovalent Ogawa vaccine did not. The authors categorically stated that "the [Ogawa] monovalent vaccine was ineffective against Inaba disease" [15, p. S8].

A large field trial in Bangladesh of oral killed vaccines [18–20] used a vaccine preparation consisting of 3 parts classical organisms to one part El Tor organisms and an equal number of Inaba and Ogawa serotypes. This 3-year efficacy study showed protection against both classical and El Tor cholera, but protection was greater against classical infection. The vaccine significantly protected against both serotypes of classical cholera, but only against the Ogawa serotype of El Tor cholera [19]. Protection waned over the 4.5-year follow-up period, with no significant protection provided against any biotype or serotype after 3 years [20].

Since there have been several cholera vaccine field trials in this area, we tried to evaluate whether these might have affected our results. Seven major cholera vaccine field trials were conducted in Matlab between 1963 and 1989. The first trial was in 1963, and, from that time until 1968, there was a total of 5 trials of injectable vaccines: whole-cell, serotype-specific, and purified antigens. In 1974, there was a trial of a toxoid vaccine, and, in 1985, there was the first and only oral cholera vaccine trial, mentioned above. We do not believe, however, that any of these trials could have altered the relationships among the biotypes and serotypes that we observed in this analysis.

In several volunteer challenge studies, cholera infection has been found to provide solid protection against reinfection with a homologous strain for up to 3 years [21, 22]. In rechallenge experiments using shorter intervals, it was shown that challenge with either an Inaba or Ogawa strain gave solid protection against rechallenge with either serotype [23]. In vaccine studies in volunteers, CVD103HgR, a live vaccine derived from a classical strain, Inaba 569B, gave short-term protection against challenge with either Inaba or Ogawa serotypes of either classical or El Tor strains [24]. These vaccine and volunteer studies have shown that both classical and El Tor biotypes and Inaba and Ogawa serotypes are protective to some extent, although the Inaba serotype clearly provides better protection. Our data strongly support these previous observations.

The data on the protective efficacy of classical and El Tor infections and vaccines have been much less clear. Some studies indicated that classical strains and vaccines may be more protective than El Tor strains [17, 19, 21]. Our data, however, do not support this suggestion. We found no difference in the protection afforded by either classical or El Tor cholera against subsequent disease.

On a population level, the build-up of durable immunity results in a lengthening of the interepidemic interval for many infectious diseases [25]. For example, the onset of pertussis vaccination coincided with a significant increase in the interepidemic interval, from 2.0–2.5 to ~4 years in England and

Wales [26]. No such pattern has been observed for cholera in general, indicating a lack of general durable immunity. However, our observation of fewer serogroup O1 infections 6 months after the initial outbreak with Inaba implies the presence of short-term immunity induced by that serotype.

It is possible that some factor other than immunity in humans is contributing to our observation that Inaba infections may provide short-term protection against subsequent infections. If Ogawa were more viable in the environment for longer periods of time than Inaba, then we could have a similar observation. Little work has been done comparing the environmental viability of various cholera serotypes in the environment. In copepod attachment studies, no difference has been found in the ability of either O1 versus non-O1 or El Tor versus classical cholera to attach to copepods [27, 28].

Our observations suggest some basic guidelines for possible cholera vaccine development. First, since classical cholera has not been identified in Matlab in the last decade and since El Tor strains protect equally well in the field, it would seem that El Tor strains for vaccines should be preferred. Finally, since Inaba antigens may be more protective against Inaba and possibly against Ogawa, vaccine candidates should preferentially contain large amounts of Inaba (which has the greatest amount of C antigen) as the preferred or major antigen.

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