A 4-Year Study of the Epidemiology of *Vibrio cholerae* in Four Rural Areas of Bangladesh

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How *Vibrio cholerae* spreads around the world and what determines its seasonal peaks in endemic areas are not known. These features of cholera have been hypothesized to be primarily the result of environmental factors associated with aquatic habitats that can now be identified. Since 1997, fortnightly surveillance in 4 widely separated geographic locations in Bangladesh has been performed to identify patients with cholera and to collect environmental data. A total of 5670 patients (53% <5 years of age) have been studied; 14.3% had cholera (10.4% due to *V. cholerae* O1 El Tor, 3.8% due to O139). Both serogroups were found in all locations; outbreaks were seasonal and often occurred simultaneously. Water-use patterns showed that bathing and washing clothes in tube-well water was significantly protective in two of the sites. These data will be correlated with environmental factors, to develop a model for prediction of cholera outbreaks.

Cholera, whose “traditional home” has been the Ganges Delta and Southeast Asia, is one of man’s oldest scourges. Over the past 40 years, as part of the 7th pandemic, cholera has spread worldwide and is now a major public health problem in >75 countries in Asia, Africa, and South America [1]. *Vibrio cholerae* is one of a few pathogenic bacteria that have the potential for pandemic spread. More recently, in the latter part of 1992, a new serogroup, O139 (the first known serogroup other than O1 to cause epidemic cholera) appeared in areas surrounding the Bay of Bengal [2–4], produced major epidemics in India and Bangladesh, spread to neighboring countries, and continues to cause epidemic cholera in many of these areas. Unfortunately, in spite of many years of study, it is still not known what determines the seasonal pattern of epidemic peaks of *V. cholerae*, how it has spread around the world, and what determined the evolution of the new, O139 strain [5].

Cholera is well known as the classical “water-borne” disease, and the importance of aquatic ecology in its epidemiology is suggested by the close association between *V. cholerae* and surface water [5–17]. Changes in aquatic ecology that result in increased numbers of these pathogenic bacteria result in their being more easily transmitted to humans.

Cholera remains a major public health problem with significant mortality wherever it occurs, despite the fact
that it is a simple and inexpensive disease to treat when ade-
quately medical supplies and knowledgeable, committed health
workers are available. Unfortunately, mortality rates remain
unacceptably high in many areas of the developing world
[18–20].

Specifically, cholera continues to be a major public health
problem in Bangladesh. In Dhaka, the capital city, and in the
well-studied, rural population of Matlab [21–23], cholera oc-
curs year-round, but with well-described peaks of disease
during the spring and fall (before and after the monsoons) [21,
23, 24]. Little is known of the patterns of cholera in the rest
of the country, however, because of lack of surveillance [23,
24].

Here we report 4.5 years of year-round bimonthly clinical
cholera surveillance involving 4 widely separated areas of rural
Bangladesh. These data will provide the basis for the investi-
gation of possible associations between cholera outbreaks and
changes in environmental factors relating to water ecology; the
latter data are presented in a separate publication.

MATERIALS AND METHODS

Surveillance

Establishing the surveillance sites. The 4 cholera surveillance
areas—Bakerganj, Matlab, Chhatak, and Chaugachha (figure
1)—were selected to represent different geographical areas of
Bangladesh. Bakerganj is on the upper edge of the estuarine
area of the southern coastal region of Bangladesh. Chhatak is
situated in the flood plains of the river Brahmaputra. Chau-
gachha is on the western edge of the tidal plains of the river
Ganges. Criteria for choosing these areas were as follows: (1)
each had a known history of past cholera outbreaks (on the
basis of information from the Epidemic Control Preparedness
Programme of the International Center for Diarrheal Disease
Research, Bangladesh (ICDDR,B), based in Dhaka, which col-
labrates with the Health Service of the Government of Bang-
ladesh in the investigations and intervention of major cholera
outbreaks); (2) travel to each site was possible throughout the
year, in a timely fashion; (3) except for the Matlab diarrhea
hospital, which is an ICDDR,B facility, each had a Thana Health
facility (of the Government of Bangladesh) that was recognized
as the treatment hospital for diarrheal diseases in that area
and had a medical staff that was willing to facilitate the clinical
surveillance; and (4) each had, for the hospital, a catchment
area of ~140,000–200,000 persons.

Matlab, a riverine area that has a well-defined population of
~200,000, has been under cholera surveillance by the ICDDR,B
for the past 36 years and is known to be a highly cholera-
endemic area [21]. Chhatak and Bakerganj had had a history
of regular cholera outbreaks; Chaugachha, on the other hand,
had had no history of cholera outbreaks during the past 10
years and was designated as the control area (figure 1).

Once the clinical areas of study had been selected, 4 stations

Table 1. Patients with bacteriological diagnosis of cholera.

<table>
<thead>
<tr>
<th></th>
<th>Matlab</th>
<th>Bakerganj</th>
<th>Chhatak</th>
<th>Chaugachha</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total patients</td>
<td>2316</td>
<td>897</td>
<td>1295</td>
<td>1162</td>
<td>5670</td>
</tr>
<tr>
<td>Patients with <em>V. cholerae</em> O1/O139</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>V. cholerae</em> El Tor Ogawa</td>
<td>275 (12)</td>
<td>73 (8)</td>
<td>72 (6)</td>
<td>28 (2)</td>
<td>448 (8)</td>
</tr>
<tr>
<td><em>V. cholerae</em> El Tor Inaba</td>
<td>63 (3)</td>
<td>25 (2)</td>
<td>51 (4)</td>
<td>3 (0)</td>
<td>142 (3)</td>
</tr>
<tr>
<td><em>V. cholerae</em> O139</td>
<td>122 (6)</td>
<td>70 (8)</td>
<td>16 (1)</td>
<td>10 (1)</td>
<td>218 (4)</td>
</tr>
<tr>
<td>Total</td>
<td>460 (20)</td>
<td>168 (19)</td>
<td>139 (11)</td>
<td>41 (4)</td>
<td>808 (14)</td>
</tr>
<tr>
<td>Male:female ratio</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.3:1</td>
</tr>
<tr>
<td>Patients &lt;2 years of age</td>
<td>1047 (45)</td>
<td>259 (29)</td>
<td>393 (30)</td>
<td>467 (40)</td>
<td>2166 (38)</td>
</tr>
</tbody>
</table>

NOTE. Data are no. (%), unless indicated otherwise.
Figure 2. Number of patients with acute watery diarrhea, including cholera, during the period of surveillance. Surveillance in Matlab and Chhatak began in March 1997; surveillance in Bakerganj and Chaugachha began in June 1997.

(in rivers, ponds, and lakes) from each surveillance area were chosen to determine physical, microbiological and plankton data for surface-water samples.

Surveillance methods. Clinical and environmental surveillance every 15 days was begun at 2 sites, Matlab and Chhatak, in March 1997 and at the remaining 2 sites, Bakerganj and Chaugachha, in June 1997. No sampling periods were missed. Surveillance continued through December 2001, except in the case of Chaugachha, where, for financial reasons, it was discontinued in June 2001.

Clinical surveillance. Each of the surveillance hospitals was visited by a physician who stayed for a 3-day period, during which time he personally saw all patients presenting with acute watery diarrhea. After each patient had given informed consent, a standardized history (including a questionnaire regarding water use) was taken, a physical exam was given, standard treatment (consisting mainly of fluid replacement) for watery diarrhea was administered, and a rectal swab, which was placed in Cary Blair media for culture of \( V.\) cholerae, was done. Rectal swabs from the patients in Matlab were cultured on the same day, in the Matlab laboratory; rectal swabs from the other 3 sites were transported, within 72 h, to the Environmental Microbiology Laboratory at ICDDR,B. Standard bacteriological methods were employed [25]. Rectal swabs were cultured both directly and after a 6-h enrichment in alkaline peptone water, on selective media (thiosulfate citrate bile-salts sucrose agar and taurocholate tellurite gelatin agar) at 37°C. Suspect colonies were agglutinated with antisera specific for \( V.\) cholerae O1 (Inaba and Ogawa serotypes) and for \( V.\) cholerae O139. Positive colonies were confirmed as \( V.\) cholerae by biochemical testing, and the two biotypes of O1 serogroup (either classical or El Tor) were differentiated by standard methods [25].

Statistical Methods
Clinical data collected through December 2001 were used for the analyses in this report.

Descriptive statistics. The observed number of cases of cholera (O1 and O139) and diarrhea were plotted over time, for each study area.
Figure 3. Age of patients and occurrence of diarrhea associated with cholera (due to either *V. cholerae* O1 or *V. cholerae* O139) and of diarrhea not associated with cholera (*n* = 5670). The age distributions of the 2 groups are significantly different (*P* < .01).

**Association between age and occurrence of cholera.** The age distributions of patients with and without cholera were compared, as were those of patients with cholera serogroups O1 and O139, by *χ*² tests of association. [26].

**Association between water source used and occurrence of cholera.** The clinical surveillance data included information on water sources (tube wells, rivers, ponds, and canals) and uses (drinking, bathing, and washing of clothes and utensils.) Age-adjusted associations between the occurrence of cholera and the water-source and water-use variables were investigated by use of multivariable logistic regressions. [26] Odds-ratio estimates and 95% confidence intervals were computed for significant risk factors in the logistic regressions.

**RESULTS**

**Clinical Surveillance**

Through December 2001, a total of 5670 patients had been seen at clinical surveillance sites. The numbers of patients with the bacteriological diagnosis of cholera are given in table 1. *V. cholerae* O1 El Tor, Ogawa, was the predominant organism, although both serotypes and serogroups of *V. cholerae* were identified. The cholera attack rate in Matlab, the only surveillance area for which we had detailed demographic information, was 3.28/1000/year.

Epidemiological information from the 4 surveillance sites—that is, the total number of patients with acute diarrhea who were seen every 15 days and the number with *V. cholerae* O1 or O139 isolated from their rectal swabs—is shown in figure 2. The findings are as follows: Most cases of acute diarrhea were caused by agents other than *V. cholerae*. Matlab had by far the greatest number of cases of cholera, whereas the designated control area (Chaugachha) had the least. Although O1 vibrios were predominant in all 4 areas, O139 vibrios also were isolated at all sites at some time during the surveillance. In 2 periods, fall of 1998 and fall of 2000, all 4 sites had simultaneous outbreaks of cholera. The seasonal patterns of cholera were similar, although not identical, in all areas.

The age distribution of patients, divided according to whether they had cholera diarrhea or noncholera diarrhea, is shown in figure 3. Most of the patients were children; 53% were <5 years of age. The age distribution of patients with cholera was significantly different (*P* < .01) from that of patients without cholera. Noncholera diarrhea occurred primarily in very young patients, whereas cholera diarrhea occurred more frequently in older children and adults.

The age distribution of patients with cholera serogroup O1 was significantly different (*P* < .01) from that of patients with cholera serogroup O139, as shown in figure 4. *V. cholerae* O1 occurred in a significantly greater number of younger patients, whereas *V. cholerae* O139 occurred primarily in older patients, who are presumed to have little immunity to this relatively new organism. Similar age differences were seen in all of the 4 surveillance areas. These differences in age distribution are similar to those previously reported for hospitalized patients in Bangladesh [2].

**Associations between Cases of Cholera and Water Use**

Logistic regressions with cholera as the outcome and with water-source and water-use variables as the risk factors were performed for each study area, with adjustment for age. Bathing with tube-well water had a statistically significant protective effect in Bakerganj: the age-adjusted odds of cholera for patients who exclusively used tube-well water for bathing were 0.4 times as large (95% confidence interval 0.2–0.8) as those for patients who used pond and/or river and/or canal water (table 2). In Matlab, washing of clothes and utensils with tube-well water was protective. The age-adjusted odds of cholera for people
who used tube-well water exclusively for washing of clothes and utensils were 0.5 times as large (95% confidence interval 0.3–0.9) as those for people who used a different water source.

The potential protective effect of drinking tube-well water could not be assessed, because very few people reported using any other source of drinking water.

**DISCUSSION**

This study is the first to investigate the epidemiology of cholera in rural Bangladesh, outside Matlab. It shows cholera to be widely distributed and present in all 4 areas studied, including the designated control area. Each area had a different pattern of cholera outbreaks, although there were clear similarities between the 4 areas.

In all of the areas, most of the cholera outbreaks occurred during either the pre- or the post-monsoon season. (The monsoon season is approximately June–September, throughout the country). The fall peak, however, appeared to be more predictable in 2 areas, Chhatak and Matlab, whereas the spring peak was more common in Bakerganj.

Matlab clearly had the largest number of cases of cholera, and these occurred throughout the year, except for the year 2000, when cases of cholera were very few. Cases of cholera also decreased during this period in Bakerganj and Chaugachha; only Chhatak had a large outbreak during the fall of 2000.

*V. cholerae* O1 and *V. cholerae* O139 were found in all surveillance sites, although *V. cholerae* O1 was more frequently seen. On several occasions, however, *V. cholerae* O139 clearly predominated, and, on one occasion, it was the sole organism causing the epidemic (June 1999 in Chhatak). It should be noted that *V. cholerae* O139 has been isolated with *V. cholerae* O1 in India [3] and that, for short periods of time, each serogroup has been temporarily displaced by the other.

It was of considerable interest that, on two occasions (fall of 1998 and fall of 2000), all 4 sites had simultaneous outbreaks. This suggests that the synchrony in these widely scattered areas was determined by common factors, most likely environmental.

It was noted that there were many periods of “epidemic” diarrhea, when the number of patients with cholera was extremely high, suggesting that this organism caused most of the illness; but there also were outbreaks of diarrhea in which few or no cases of cholera were seen (April 1998 and June 2000 in Chattak; December 1999 in Chaugachha), suggesting that other etiologic agents were responsible. In this study, we identified only *V. cholerae* and, therefore, can only speculate on what caused these outbreaks of noncholera diarrhea. Because, in Matlab and Dhaka, as noted in a previous study [27], rotavirus and enterotoxigenic *Escherichia coli* are, respectively, the most frequent viral and bacterial causes of acute diarrhea in children, they are the most likely responsible agents.

The patients seen in this study were primarily children <5 years of age. When the ages of patients with cholera were compared with those of patients without cholera, there were marked differences, as would be expected. In children <2 years of age, noncholera diarrhea occurred much more often than did cholera diarrhea. In the older children and young adults, however, cholera diarrhea was seen more frequently than noncholera diarrhea. This age-related phenomenon may suggest a severity of the illness for which this age group sought treatment. In previous studies in Matlab, in adults, enterotoxigenic *E. coli* was found to be more than twice as frequent as cholera [28].

There were also clear age differences when *V. cholerae* O1 and *V. cholerae* O139 were compared: *V. cholerae* O1 occurred more frequently in the young children, whereas *V. cholerae* O139 was seen in all age groups, but more frequently in older adults. These findings are compatible with earlier studies that showed that, in a nonimmune population, *V. cholerae* O139 occurred with similar attack rates at all ages [2]. These data suggest that *V. cholerae* O139 has not yet become endemic in those areas where *V. cholerae* O1 has been heavily endemic for many years.

There were significant associations of cholera and water sources used for bathing and washing. Since nearly all patients used tube wells for drinking water, we could not determine the protective effect that this practice may have had. Other studies have had difficulty in showing a protective effect of tube wells [29, 30], because other sources of drinking water also were used. In the present study, the use of tube-well water for bathing and washing, however, was shown to be protective against cholera in Bakerganj and Matlab, respectively. It should be pointed out that these comparisons were made between patients with cholera diarrhea and those with noncholera diarrhea; there was no control group without diarrhea. Since our control group consisted of patients with diarrhea who also may have had some

<table>
<thead>
<tr>
<th>Area</th>
<th>Water source and use</th>
<th>No. of individuals</th>
<th>Odds ratio (95% CI)a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bakerganj</td>
<td>Bathing exclusively in tube well water</td>
<td>897</td>
<td>0.4 (0.2–0.8)</td>
</tr>
<tr>
<td>Matlab</td>
<td>Washing exclusively with tube well water</td>
<td>2311</td>
<td>0.5 (0.3–0.9)</td>
</tr>
</tbody>
</table>

* Odds ratio compares odds of cholera for people who used water as described versus odds for people who used other sources of water for the described purpose. CI, confidence interval.
similar risk factors, the differences seen here are even more significant.

The clinical surveillance of cholera in 4 widely separated areas of Bangladesh provides a basis for determining which environmental factors may play a role in defining the outbreaks of cholera. The clinical observations in this study indicate that cholera is both seasonal in all areas, sometimes being synchronous in all areas, and is related to water use in some areas, and all suggest that environmental factors play a significant role. Clearly, *V. cholerae* O139 is widespread in Bangladesh and, although not yet endemic, appears to be competing with the O1 serogroup for an environmental niche. In a separate publication, these clinical surveillance data will be correlated with the environmental data collected from the same geographic areas to determine which factors are associated with cholera outbreaks and to develop a model that can be used to predict outbreaks, thus facilitating the early introduction of preventative and treatment measures.

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### References