Infectious Diseases, Massachusetts General Hospital, Departments of Medicine and of Microbiology and Molecular Genetics, Massachusetts General Hospital, Boston, MA 02114 (scalderwood@partners.org).

Gray-Jackson 504, Massachusetts General Hospital, Boston, MA.

Debasish Saha,1,2 Regina C. LaRocque,1,3 Ashraful I. Khan,1 Jason B. Harris,2,3 Yasmin Ara Begum,1 Syed M. Akramuzzaman,1 Abu S. G. Faruque,1 Edward T. Ryan,1,2,4 Firdausi Qadri,1 and Stephen B. Calderwood2,5

1International Centre for Diarrheal Disease Research, Bangladesh: Centre for Health and Population Research, Dhaka, Bangladesh; 2Division of Infectious Diseases, Massachusetts General Hospital, Departments of Medicine and of Microbiology and Molecular Genetics, Harvard Medical School, and 3Department of Immunology and Infectious Diseases, Harvard School of Public Health, Boston, Massachusetts.

The serum vibriocidal antibody is the only recognized predictor of protection from cholera, but no seroepidemiological data have been gathered since the emergence of Vibrio cholerae O139. We assessed the association between the vibriocidal antibody titer and protection from cholera in an endemic setting. Although a higher baseline vibriocidal titer correlated with protection from V. cholerae O1, infection still developed in some contacts with very high titers. No association between baseline vibriocidal titer and protection from V. cholerae O139 infection was found. Our findings suggest that the vibriocidal antibody is an incomplete predictor of protection from V. cholerae infection.

Vibrio cholerae infection causes severe secretory diarrhea, with >100,000 cases/year in endemic areas [1]. It is unique among diarrheal pathogens in its ability to cause global pandemics. Since 1817, there have been 7 pandemics during which disease has spread across Asia, Europe, Africa, and the Western Hemisphere.

Strains of V. cholerae are differentiated serologically by the O side chain of lipopolysaccharide (LPS), and the majority of toxigenic strains belong to serogroup O1 or O139. V. cholerae O1 is divided into 2 biotypes, classic and El Tor; the El Tor biotype is responsible for the current, seventh cholera pandemic. V. cholerae O139 emerged as a cause of epidemic cholera on the Indian subcontinent in 1992–1993 [2, 3] and initially displaced the existing V. cholerae O1 strains. The O139 serogroup appears to have arisen from the El Tor biotype of V. cholerae O1 by substitution of genes encoding the O antigen and acquisition of the ability to produce a capsule [4, 5]. The ongoing spread of V. cholerae O139 may represent the eighth cholera pandemic.

Infection with V. cholerae induces long-lasting immunity [6], but the nature of this adaptive immune response is not completely understood. Both antitoxin and antibacterial immune responses are present after infection [7]. Although serum antitoxin levels increase dramatically after a clinical case of cholera, they have not been shown to protect from subsequent colonization or disease [8]. Furthermore, infection with V. cholerae O1 does not confer protection from infection by V. cholerae O139, even though both strains produce identical toxins [3].

The best-characterized antibacterial immune response induced by V. cholerae infection is the serum vibriocidal antibody, which is bactericidal in the presence of complement. It is directed primarily against V. cholerae LPS [9] but may also include activity against outer-membrane proteins [10, 11].

Prior seroepidemiological studies have demonstrated that vibriocidal antibody titers increase as age increases and correlate with protection from subsequent disease [12, 13]. In an endemic setting, vibriocidal antibodies are detectable in 40%–80% of individuals by 10–15 years of age [13, 14]. A cross-sectional study performed during the sixth cholera pandemic (caused by the V. cholerae O1 classic biotype) suggested a 44% decrease in the attack rate of cholera for every 2-fold increase in the baseline vibriocidal geometric mean titer (GMT) [13]. Furthermore, a prospective study undertaken in Bangladesh in...
1980–1982, during the seventh pandemic, demonstrated that colonization and disease due to *V. cholerae* O1 El Tor were significantly more common among people with vibriocidal antibody titers <20 than among those with higher titers [12]. No population-based studies of vibriocidal antibody titers to *V. cholerae* O139 in an endemic setting have been performed.

*V. cholerae* does not disrupt the intestinal epithelium during infection, and the role of a serum response, such as the vibriocidal antibody, in protection at the mucosal surface is unclear. One possibility is that the vibriocidal antibody response is a surrogate marker for specific intestinal secretory IgA responses or other mucosal immune responses that are the primary mediators of protective immunity. The purpose of our ongoing study is to better understand the nature of protective immunity against *V. cholerae* infection. In this analysis, we characterize vibriocidal antibody responses in the household contacts of patients hospitalized with cholera in Bangladesh and correlate baseline vibriocidal antibody titers with the risk of subsequent infection in these contacts.

**Methods.** This prospective, observational study was undertaken as a collaboration between Massachusetts General Hospital in Boston and the ICDDR,B: Centre for Health and Population Research in Dhaka, Bangladesh. Patients presenting to the ICDDR,B with acute watery diarrhea and a stool culture positive for *V. cholerae* were eligible for this study. Exclusion criteria included age <6 months, severe comorbid conditions, current enrollment in an unrelated interventional study, and having a family member who had received care at the ICDDR,B within the preceding 2 months. Informed consent was obtained from patients or their guardians prior to entry in the study. This study was reviewed and approved by the Institutional Review Board of the Massachusetts General Hospital and the Ethical and Research Review Committees of the ICDDR,B.

Study day 1 was defined as the day of identification of the index case patient. Index patients were enrolled on day 2 if a stool culture was positive for *V. cholerae* O1 or O139. Information regarding clinical features, demographics, and history of cholera were collected from index patients at enrollment. Samples of venous blood were collected, for determining vibriocidal antibody titers, from index patients on day 2 and again at follow-up visits on days 7 and 21.

On the day of enrollment of the index patient, a field team discussed enrollment with contacts who had lived in the same household as the patient for at least the previous 3 days and who shared the same cooking pot. Household contacts from whom consent was obtained were administered a questionnaire, and a rectal swab for *V. cholerae* culture was obtained. Homes were visited by the field team on each of days 2–11 and again on day 21. During these visits, contacts were questioned about diarrheal symptoms, and rectal swabs were obtained for *V. cholerae* culture. Venous blood samples for determining vibriocidal antibody titers were obtained from contacts on days 2, 4, and 21.

Assays for detection of vibriocidal antibody were performed by use of guinea pig complement and the homologous serogroup and serotype of *V. cholerae* as the target organism, as described elsewhere [15]. The concentrations of complement and bacteria were optimized separately for determining the vibriocidal antibody response to *V. cholerae* O1 and to *V. cholerae* O139. Serial 2-fold dilutions of serum were tested. Vibriocidal antibody titers for *V. cholerae* O1 and *V. cholerae* O139 are reported separately, given the biologic differences in the assay.

For groups of patients, vibriocidal antibody titers are presented as the inverse GMT, with the associated range. Means were compared by analysis of variance, for data with a normal distribution, or by the Kruskal-Wallis or Mann-Whitney U test, for data without a normal distribution. The χ² test was used for comparison of categorical variables.

**Results.** One hundred twenty-five patients with clinical cases of cholera and 326 of their household contacts were enrolled in the study between January 2001 and May 2002. The clinical and demographic features of the 125 index patients with cholera are presented in table 1. Forty-three index patients (34%) were infected with *V. cholerae* O139, and 82 patients (66%) were infected with *V. cholerae* O1. Patients with *V. cholerae* O139 infection were older than those with O1 infection (mean age, 32.8 vs. 22.2 years) and had more-severe illness, as measured by liters of intravenous (iv) fluids required, number of bowel movements in the 24 h prior to presentation, and duration of hospitalization.

As expected, index patients infected with *V. cholerae* O1 and O139 almost uniformly mounted strong vibriocidal antibody responses. Only 2 of the 125 patients failed to mount a 4-fold response by day 21. Both of these patients were infected with *V. cholerae* O1; one patient was a 6-month-old infant, and the other was an adult who had a high initial titer.

Higher initial vibriocidal antibody titers were found in patients with *V. cholerae* O1 infection who had symptoms for >24 h, compared with those who had symptoms for a shorter duration of time (GMT, 164.0 vs. 44.9; *P* = .001). This rapid increase in baseline vibriocidal antibody titer suggests an anamnestic response in these patients. This finding did not occur in patients with *V. cholerae* O139 infection.

Even among patients with *V. cholerae* O1 infection who had symptoms for <24 h at presentation, a wide distribution of vibriocidal antibody titers was present on enrollment in the study. Although baseline vibriocidal antibody titers were <40 in the majority of these patients, 7 patients (13%) had titers ≥320, and 4 patients (7.4%) had titers ≥1280 on study day 2.

Of the 326 household contacts evaluated in this study, two-thirds were contacts of patients with *V. cholerae* O1 infection, and one-third were contacts of patients with *V. cholerae* O139...
Table 1. Demographic and clinical features of index case patients with cholera.

<table>
<thead>
<tr>
<th>Feature</th>
<th>Vibrio cholerae serogroup</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O1</td>
</tr>
<tr>
<td>Demographic</td>
<td></td>
</tr>
<tr>
<td>No. of patients</td>
<td>82</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>48 (58.5)</td>
</tr>
<tr>
<td>Male</td>
<td>34 (41.5)</td>
</tr>
<tr>
<td>Age, years</td>
<td></td>
</tr>
<tr>
<td>&lt;15</td>
<td>26 (31.7)</td>
</tr>
<tr>
<td>≥15</td>
<td>56 (68.3)</td>
</tr>
<tr>
<td>Clinical</td>
<td></td>
</tr>
<tr>
<td>Duration of diarrhea at presentation, median (25th, 75th percentile), h</td>
<td>19 (8, 28)</td>
</tr>
<tr>
<td>No. of bowel movements in 24 h prior to presentation</td>
<td></td>
</tr>
<tr>
<td>≤15</td>
<td>22 (26.8)</td>
</tr>
<tr>
<td>&gt;15</td>
<td>60 (73.2)</td>
</tr>
<tr>
<td>Dehydration status at arrival to hospital</td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>67 (81.7)</td>
</tr>
<tr>
<td>Some</td>
<td>15 (18.3)</td>
</tr>
<tr>
<td>Vomiting present</td>
<td>81 (98.8)</td>
</tr>
<tr>
<td>Amount of iv fluid received, median (25th, 75th percentile), L</td>
<td>4 (2, 6)</td>
</tr>
<tr>
<td>Additional pathogens identified in stool</td>
<td>25 (30.5)a</td>
</tr>
</tbody>
</table>

NOTE. Data are no. (%) of patients, except where indicated otherwise. Statistical analyses were performed by use of the \( \chi^2 \) test, for categorical variables, or the Mann-Whitney \( U \) test, for continuous variables. iv, intravenous.

a Ascariis (11 patients), rotavirus (3 patients), Campylobacter (3 patients), Trichuris (2 patients), Blastocystis hominis (2 patients), Hymenolepis nana (2 patients), Salmonella (1 patient), Shigella (1 patient), Entamoeba histolytica (1 patient), Giardia (1 patient), hookworm (1 patient), and Aeromonas (1 patient).

b Ascariis (3 patients), Giardia (2 patients), Campylobacter (1 patient), and hookworm (1 patient).

infection. Sixty-eight contacts were excluded from the analysis: 23 because of symptomatic cholera or asymptomatic passage of \( V. cholerae \) on enrollment, 40 because of watery diarrhea in the week preceding enrollment, and 5 because of infection with a serogroup of \( V. cholerae \) different from that associated with the index patient.

To determine the risk of \( V. cholerae \) disease or colonization after household exposure to an index patient, contacts were categorized by the presence or absence of diarrheal symptoms, whether rectal cultures were positive for \( V. cholerae \), and detection of a 4-fold change in vibriocidal antibody titer (table 2). Overall, transmission of \( V. cholerae \) O1 or O139 occurred in approximately one-third of the household contacts (categories 1–5). There was a trend toward more symptomatic infections among the household contacts of index patients with \( V. cholerae \) O139 infection than among those with \( V. cholerae \) O1 infection (21% vs. 12%, respectively; \( P = .06 \)). No transmission occurred in two-thirds of household contacts (categories 6 and 7); these contacts may represent a heterogeneous group that includes those not exposed to \( V. cholerae \), as well as those who were exposed but who had sufficient protection to prevent colonization or a subsequent immune response.

No significant differences in age, sex, or blood group were noted between the 7 categories of household contacts. However, significant differences in baseline vibriocidal antibody titers were present between the contacts in the various categories (table 2).

To evaluate the association between baseline vibriocidal antibody titer and protection from \( V. cholerae \) infection, household contacts were grouped into those with symptomatic or asymptomatic \( V. cholerae \) infection (categories 1–4) and those in whom no infection occurred (categories 5–7). Among contacts of patients with \( V. cholerae \) O1 infection, a higher baseline vibriocidal antibody titer was present in the group of contacts who did not develop infection, compared with the group of those who did (GMT, 166.9 vs. 39.2, respectively; \( P = .001 \)). Although, as a group, a higher baseline vibriocidal antibody titer correlated with protection among the household contacts of patients with \( V. cholerae \) O1 infection, a number of contacts developed symptomatic infection despite having high baseline vibriocidal antibody titers. In particular, 5 (24%) of the 21 contacts who developed symptomatic infection had baseline vibriocidal antibody titers ≥320, and 4 (29%) of the 14 household contacts who were asymptomatic but who shed \( V. cholerae \)
in their stool had baseline vibriocidal antibody titers $\geq 320$. Thus, a substantial fraction of household contacts with high baseline vibriocidal antibody titers against V. cholerae O1 infection remained susceptible both to symptomatic disease and asymptomatic colonization.

A similar analysis of contacts of patients with V. cholerae O139 infection showed no association between baseline vibriocidal antibody titer and protection from infection (table 2). In fact, the highest baseline vibriocidal antibody titer was observed in contacts who developed symptomatic infection (category 2).

**Discussion.** Since the emergence of V. cholerae serogroup O139 in 1992–1993 [5, 6], V. cholerae O1 El Tor and V. cholerae O139 have coexisted as causes of cholera in Bangladesh. In the present study, we have observed that V. cholerae O139 causes more disease among adults, results in more-severe disease, and has a higher attack rate among household contacts than V. cholerae O1. One possible explanation for these findings is that the Bangladeshi population has not been sufficiently exposed to V. cholerae O139 to develop protective immunity, despite the emergence of this serogroup more than a decade ago. Alternatively, V. cholerae O139 may not lead to durable protective immunity after natural infection due to an immune evasion strategy, such as the presence of the polysaccharide capsule.

One problem with distinguishing these hypotheses is that a validated marker of immunity to V. cholerae O139 has not been established [16]. Although we have demonstrated a robust vibriocidal response among patients infected with V. cholerae O139, the baseline vibriocidal antibody titer among household contacts of these patients did not correlate with protection from subsequent disease or colonization. The vibriocidal antibody is therefore not a useful marker of protective immunity to the O139 serogroup.

The results of the present study illustrate the need to better understand adaptive immunity to V. cholerae O1 and O139 infection and its association with the vibriocidal antibody response. As noted above, the vibriocidal antibody titer did not correlate with protection from infection with V. cholerae O139 among household contacts in our cohort. Furthermore, although the baseline vibriocidal antibody titer correlated with protection from subsequent disease and colonization with V. cholerae O1, this correlation was imperfect, since one-quarter of contacts who developed symptomatic or asymptomatic cholera had high baseline vibriocidal antibody titers. Because V. cholerae causes a noninvasive enteritis and because the correlation between vibriocidal antibody titer and protection from disease is incomplete, it is possible that the vibriocidal antibody response is a surrogate marker for an immune response at the level of the intestinal mucosa. Such a response may be directed against specific in vivo–expressed V. cholerae antigens, such as those recently identified in a novel screen using human serum [17]. In addition, host genetic or nutritional factors may play a significant role in protection from V. cholerae infection and disease [18]. Future studies based on this cohort will address these questions.

**Acknowledgments**

We wish to thank the study participants and the dedicated field and laboratory workers of the Cholera Immune Response...
References


