# Research Recherche

*Bulletin of the World Health Organization*, 58 (5): 731-740 (1980)

# Microbiological surveillance of intra-neighbourhood El Tor cholera transmission in rural Bangladesh*

W. M. SPIRA,[1] M. U. KHAN,[2] Y. A. SAEED,[3] & M. A. SATTAR[4]

*The apparent failure of handpump tubewells to reduce the incidence of cholera among users in the flooded rural area of Bangladesh has stimulated interest in defining precisely the means of* Vibrio cholerae *transmission during localized outbreaks. Cholera-infected neighbourhoods were placed under intensive microbiological surveillance to pinpoint contaminated sources and subsequent infections. The results show that cholera transmission was via contaminated surface water, particularly water taken into households for cooking or drinking. Infections resulted from a daily dose not exceeding $10^5$ organisms and the frequency of exposure appeared to be a major determinant of the infection rate. The importance of these data in environmental interventions and particularly in the provision of tubewells is discussed.*

The transmission of cholera in Bangladesh appears to be associated with surface water contaminated with *Vibrio cholerae* (*6*). This surface water is used for a great variety of purposes, thus assuring frequent exposure of persons using it. Improvements in water supply should therefore reduce the incidence of cholera. In Bangladesh, this has led to massive efforts to provide handpump tubewells to the rural population as a source of safe water.

Four studies of the impact of tubewells on the incidence of cholera have been conducted by the Cholera Research Laboratory (CRL) in Matlab Thana, a rural area in the Meghna River floodplain (*7*, *8*, *13*, and Curlin et al.[a]). All four show that for El Tor cholera, at least, there is no difference in attack rates between those who use tubewells and those who do not. In explanation, the authors point out that tubewells were used only for drinking and that surface water was used for all other purposes, so that the small amount of protection afforded by drinking bacteriologically safe water may be overwhelmed by the exposure to polluted surface water through bathing, food preparation and utensil washing.

Others, however, have interpreted these results as evidence that cholera may not be primarily waterborne in this region (*3*) or that the transmission cycle is more complex and that water actually serves as an inoculum for another source, such as food, in which multiplication of the organism occurs (W. Verwey, personal communication, 1976). In either case, tubewell use would not be expected to decrease the incidence of cholera. A recent investigation of El Tor

* From the Cholera Research Laboratory, Dacca, Bangladesh (now the International Centre for Diarrhoeal Disease Research, Bangladesh).

[1] Investigator, Microbiology Branch, Laboratory Division. Present address: Baltimore City Hospitals, Division of Geographic Medicine, 4940 Eastern Avenue, Baltimore, Maryland 21224, USA.

[2] Investigator and Head, Community Studies Branch, Epidemiology Division.

[3] Senior Research Assistant, Microbiology Branch, Laboratory Division.

[4] Assistant Supervisor, Field Surveillance Activities Branch, Epidemiology Division.

[a] CURLIN, G. T. ET AL. *The influence of drinking tubewell water on diarrhea rates in Matlab Thana, Bangladesh.* Working Paper No. 1, Cholera Research Laboratory, Dacca, Bangladesh, 1977.

cholera in rural Bangladesh by Hughes et al.[b] found extensive contamination of surface water sources in the area around the cholera-infected person. They further showed that families using a culture-positive water source for any purpose were significantly more likely to become infected than other families, as were families sharing the same source as the index family for either drinking or bathing.

We have attempted through microbiological surveillance to identify *V. cholerae* in people and the environment in the neighbourhood of cholera patients in order to define its transmission and to estimate the conditions and level of exposure that actually lead to infection.

## MATERIALS AND METHODS

The study was conducted during the 1976 post-monsoon cholera season (October–January) in the CRL rural study area in Matlab Thana, Bangladesh. Index cases were randomly selected from patients who had been admitted to the CRL Hospital with cholera-like diarrhoea and from whom *V. cholerae* had been isolated from a rectal swab. A check of hospital records was made to ensure that these patients were the first admitted from their neighbourhood during the current cholera season. The home of the index case was visited on the morning after admission. All families who shared any water source for any purpose with the index family were questioned about their water use habits. In this context, 'source' was taken to mean a single definable body, while the actual site at which water was taken or used was called a 'point'. For open water systems, such as rivers or canals, all points within 50 metres of a point used by the index family were considered to be part of the same source. The term 'neighbourhood' includes all persons who shared any water source for any purpose with the index family. In practice, the houses of such people usually formed a geographical cluster around a major water source that was distinguishable from other clusters in the vicinity.

Surveillance was maintained for up to 12 days. On each day, all persons in each household were asked if they had diarrhoea, which was defined as 3 sequential loose motions or a large liquid stool possibly containing blood, pus, or both within 24 hours. A rectal swab was taken from each interviewee and cultured for *V. cholerae* after a 6-hour enrichment in alkaline bile peptone (ABP). Suspicious colonies on taurocholate-tellurite-gelatine agar (TTGA) (*11*) were confirmed by testing for agglutination in polyvalent and group-specific *V. cholerae* antiserum and for direct agglutination of chicken red cells (*4*). A fingertip blood sample (50 μl) was collected at the time of the first interview and a second one 15 days later. These were diluted 1:10 in physiological saline in the field and centrifuged upon return to the laboratory. The vibriocidal antibody response was then determined by the method of Benenson et al. (*1*).

The female head of each household was questioned about which water points were used on each day for taking water kept in the house for drinking and cooking. When possible, each person was asked directly about which points he or she used for latrine toilet, washing, or bathing. Parents supplied this information for small children. Water samples were collected from all water jars in the study households. If households had multiple jars for the same purpose, each jar was sampled. If any were found positive, the water for that purpose was considered positive. All samples were collected between 09h 00 and 13h 00. Samples were taken at all surface water points used persons in the neighbourhood of the index case for bathing, dish washing, playing, or obtaining water for drinking or cooking. Tubewells were also sampled. Samples taken from water sources were collected at a depth of 20 cm at a distance of about 1 metre from the shore using sterile plastic bottles. Samples were cooled and cultured within five hours of collection. We conducted preliminary experiments to confirm that viable counts did not decrease detectably during this holding period (data not shown). From each sample, 0.2 ml was plated directly on TTGA. A 100-ml aliquot was enriched in ABP for 6 hours, then streaked on TTGA.

All left-over foods present in study households were sampled for *V. cholerae*. These consisted mostly of rice, fish, and vegetable curry. Samples were usually taken just before foods were to be reheated for a meal. These included: (1) foods left over from an evening meal and eaten the following day; and (2) foods prepared in the morning to be eaten at midday and evening. Samples of foods in category (1) were usually collected just prior to the midday meal and returned to the laboratory within 5 hours of collection. Foods in category (2) were sampled early in the evening. These samples were placed in sterile plastic bags and maintained at 10 °C or less until they were returned to the laboratory on the following morning. Specimens were blended in ABP (10 g/90 ml) and plated directly (0.2 ml) and after enrichment (remaining blended material) on TTGA.

The left hand of persons engaged in water handling or food preparation in each household was rinsed with 20 ml of ABP for 30 seconds. These rinsings were generally done between 09h 00 and 13h 00. The rinse was enriched and plated as above. In similar fashion, cooking pots, utensils, and eating dishes were also

[b] HUGHES, J. M. ET AL. Water and the transmission of El Tor cholera in rural Bangladesh, Working Paper No. 2, Cholera Research Laboratory, Dacca, Bangladesh, 1977.

examined for *V. cholerae*. The surfaces of cutting and food preparation boards were checked using RODAC plates containing TTGA (*5*).

## RESULTS

### *Characterization of study neighbourhoods*

Nineteen neighbourhoods were placed under surveillance in the course of this study. In all, 792 people in 149 families (5.3 persons/family ± 2.9 SD) were included. Families were defined on the basis of sharing the same cooking facilities and eating together. The number of families per neighbourhood ranged from 2 to 17 with a median of 8.

Overall, 30 tanks, 12 canals or rivers, and 12 ditches were used by persons in the study. Thirty-seven families (25%) used a river or canal for at least one purpose, while tanks were used by 101 families (68%) and ditches by 14 (9.4%). Sixty-four families (43%) took water from tubewells. Tubewell (TW) water was used only for drinking. Because of the relatively high iron content in TW water, all families interviewed used surface water for cooking, rinsing dishes, and washing hands and feet. Water jars for cooking were replenished daily or every other day. Drinking jars containing TW water were refilled daily on account of its tendency to form a brown sediment upon standing overnight.

This study was carried out during the postmonsoon season. Consequently most of the land around neighbourhoods was flooded and virtually all active latrine sites emptied into water that was contiguous with points used for bathing, washing, and drawing water for household uses.

On the basis of bacteriological surveillance during the first 4 days, we have classified 15 out of 19 neighbourhoods as 'cholera-positive' and the remaining 4 as 'cholera-negative'. The criteria for this classification are given in Table 1. Though the frequency of *V. cholerae* infection on the first day of surveillance was similar for both groups, the environment of 'cholera-negative' neighbourhoods was relatively uncontaminated and no subsequent infections were detected. Surveillance in cholera-negative neighbourhoods was stopped after the fourth day.

We found few demographic or physical differences between cholera-positive and cholera-negative neighbourhoods. There was no difference in the age/sex distribution of index cases. The number of families per neighbourhood and family size were similar. Rivers, tanks, and ditches were used with approximately the same frequency for similar purposes. Tubewells, however, were used by 62% of families in negative neighbourhoods as compared with 38% in positive neighbourhoods ($x^2 = 5.37$, $P = 0.02$). The importance of this is difficult to interpret since the surface waters in cholera-negative neighbourhoods were only infrequently contaminated with *V. cholerae*. Environmental contamination, as we will show, appears to be the factor most strongly associated with intra-village cholera transmission. Tubewell use should have little direct bearing on whether water sources become contaminated. It might, however, be associated with other, as yet unidentified, sanitary practices that do affect such contamination.

Table 1. Classification of neighbourhoods on the basis of the continuing presence of cholera

| | Cholera-positive | | Cholera-negative | |
|---|---|---|---|---|
| | No. | % | No. | % |
| Individuals in sample | 652 | – | 140 | – |
| Infections detected by day 1 (including index cases) | 35 | 5.4 | 8 | 5.7 |
| Persons reporting diarrhoea in 5 days prior to study | 47 | 7.2 | 8 | 5.7 |
| New infections detected on days 2–4 | 25 | 4.1 | 0 | 0 |
| Frequency of contamination detectable on days 1–3: | | | | |
| of surface water— at source | 69/262 | 26.0 | 2/96 | 2.1 |
| of surface water— stored in house | 95/419 | 23.0 | 0/118 | 0.0 |

The remainder of this paper will deal only with the cholera-positive neighbourhoods. In these, 37% of families regularly separated their defaecation and bathing sites, either by using different sources or by using points on opposite sides of the same source. The rest used nearby points of the same source. Families using tubewells were somewhat more likely to use separate sites than were non-users (21 of 46 versus 22 of 74), but this difference was not significant. Even when families used separate latrine and bathing sites, neighbouring families did not follow the same pattern. Most bathing sites were bordered by at least one active latrine. In all, 72% of families took some or all of their drinking and/or cooking water from the point they used for bathing.

### *Pattern of infection*

Sixty-five infections with *V. cholerae* biotype eltor serotype Inaba were detected by bacteriological methods, excluding index cases. The median duration of infection from first detection was 3 days. Six additional infections were detected on the basis of

Table 2. Rate of newly detected infections in cholera-positive neighbourhoods (index cases excluded) during 12-day surveillance

| | Infections detected | | % Detected on day | | | | | | | | | | | | VTR[a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No./Total examined | % | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | |
| Total | 71/637 | 11.0 | 28 | 13 | 18 | 4 | 9 | 4 | 7 | 6 | 1 | 2 | 0 | 0 | 8 |
| Index family | 23/96[b] | 24.0 | 39 | 26 | 9 | 0 | 4 | 5 | 4 | 0 | 4 | 0 | 0 | 0 | 9 |
| Non-index | 48/541[b] | 8.9 | 23 | 6 | 23 | 6 | 11 | 4 | 8 | 9 | 0 | 2 | 0 | 0 | 8 |
| TW users[c] | 33/286 | 12.0 | 25 | 14 | 9 | 0 | 10 | 9 | 9 | 9 | 3 | 3 | 0 | 0 | 9 |
| Non-TW users[c] | 38/351 | 11.0 | 29 | 13 | 26 | 8 | 8 | 0 | 5 | 3 | 0 | 0 | 0 | 0 | 8 |

[a] Infections detected by vibriocidal titre rise.
[b] $\chi^2 = 17.2$, $P = 0.00003$.
[c] 41/96 (43%) persons from index families and 245/541 (45%) persons from non-index families used tubewells.

seroconversion only. A more detailed description of the time distribution of infections is given in Table 2. In all, 11% of the persons examined showed evidence of infection. Of these, 28% were detected by day 1 and 41% by day 2. We will make a distinction between those infections first detected on days 1–2 ('early') and those detected later ('later'). Given the incubation time of cholera, we cannot say much about the source of the exposure of the early infections. Even less can be said about the 6 infections detected by seroconversion, so these will not be included in any of the following analyses. None of these 6 reported having diarrhoea.

The overall rate of infection was higher in index families than in other families and 65% of index family infections were 'early' as opposed to only 29% of non-index family infections ($\chi^2 = 6.95$, $P<0.001$). The proportion of persons who used tubewell water was similar in index and non-index households. Among tubewell users, 39% of infections were 'early' compared with 42% among non-users. Sixteen 'later' infections (8 households) occurred subsequent to an index or 'early' infection in the same household; 20 'later' infections (in 11 families) were not preceded by index infections.

The frequency of 'early' infections among children (less than 10 years old) in index families was 23.8% (10/42) compared to 9.6% (5/52) for index family adults, 5.8% (11/191) for non-index family children, and 0.87% (3/346) for non-index family adults. The frequency for index children is significantly greater ($\chi^2 = 10.8$, $P = 0.001$) and that for non-index adults is significantly lower ($P = 0.0001$, Fisher Exact Test) than that of the remaining groups. The frequency of 'later' infections was 6.2% (2/32) among children and 8.5% (4/47) among adults in index families; and 8.3% (15/180) among children and 4.4% (15/343) among adults in non-index families. The difference between index and non-index families is not significant and that between non-index children and adults is marginal ($\chi^2 = 2.73$, $P = 0.10$). There were no significant differences in frequency between males and females; adult males experienced a somewhat lower rate of 'later' infections than females (6/198 compared with 13/192, $\chi^2 = 2.19$, $P = 0.14$).

The incidence of diarrhoea in infected persons was higher in 'early' infections. Among index family children the frequency was 90% (9/10) and among adults, 80% (4/5); in non-index families the frequency for children was 64% (7/11) and for adults 67% (2/3). These differences are not statistically significant. In 'later' infections, 50% of index family children (1/2) and adults (2/4) had diarrhoea; in non-index families, the frequency was 77% (10/13) for children and 27% (4/15) for adults. The difference between non-index family adults and children is significant ($P = 0.01$, Fisher Exact Test). Overall, the ratio of the three categories of illness—asymptomatic, mild, and moderate/severe—among 'early' infections was 1:2.1:1, while among 'later' infections it was 9.5:7.5:1 ($\chi^2 = 7.65$, $P = 0.02$). There were no significant differences in diarrhoea rates between males and females.

### *Contamination of vehicles and patterns of transmission*

The results of the microbiological surveillance for *V. cholerae* in water sources are given in Table 3. Overall, 57% of surface water sources were contaminated, as were 14% of all samples taken from them. Tubewell water was consistently free of detectable *V. cholerae.*

Table 3. Contamination of surface water in cholera-positive neighbourhoods during 12-day surveillance

| | Sources | | Points | | Samples | |
|---|---|---|---|---|---|---|
| | No. positive/ Total No. | % | No. positive/ Total No. | % | No. positive/ Total No. | % |
| Tanks | 15/22 | 68.2 | 36/66 | 54.5 | 89/549 | 16.2 |
| Canals/rivers | 5/11 | 45.5 | 11/26 | 42.3 | 14/120 | 11.7 |
| Ditches | 4/9 | 44.4 | 4/18 | 22.2 | 5/109 | 4.6 |
| Total surface water | 24/42 | 57.1 | 51/110 | 46.4 | 108/778 | 13.9 |
| Tubewell | 0/12 | 0.0 | — | — | 0/38 | 0.0 |

Table 4 shows the contamination of potential vehicles examined in households. Other than water from surface sources, which was frequently contaminated, the household environment, even in index households, was almost totally free of *V. cholerae*. The contamination rate of tubewell water stored in the house for drinking was significantly lower than that of surface water for the same purpose ($P = 0.004$, Fisher Exact Test). The difference in contamination frequency between surface water designated for cooking and that for drinking was marginally significant ($\chi^2 = 2.72$, $P = 0.10$). Other vehicles examined were virtually never found to be contaminated with *V. cholerae*, even when intensive enrichment techniques were used. Only 0.13% of food samples yielded *V. cholerae*. In the two instances in which foods were positive, there were no subsequent infections among family members who consumed them.

Table 4. Contamination of vehicles[a] in households in cholera-positive neighbourhoods during 12-day surveillance

| Item examined | All households | Tubewell users | Non-users |
|---|---|---|---|
| Water in jars in household: | | | |
| drinking (from TW) | 1/85 (1.2) | 1/85 (1.2) | — |
| drinking (surface water) | 27/275 (9.8) | — | 27/275 (9.8)[b] |
| cooking (surface water) | 106/823 (12.9) | 67/416 (16.1)[b] | 39/407 (9.6)[b] |
| Food | 2/1593 (0.13) | — | — |
| Left-hand rinse | 2/677 (0.30) | — | — |
| Utensils and food preparation boards | 0/437 (0.0) | — | — |

[a] Number contaminated/No. examined. Percentage in parentheses.

[b] Contamination frequency of surface water points used by tubewell users was 21.8% (39/179) and for non-users, 18.6% (34/183).

The surface water points used by tubewell users and non-users were contaminated with equal frequency. For non-users, household water for either drinking or cooking was contaminated only half as often as was the source. We presume that much of this difference may be due to *V. cholerae* dying off in household jars which were not recontaminated to the extent that the source was. A more intriguing point, however, is that the advantage enjoyed by tubewell users in having much less frequently contaminated drinking water in their household was offset by a significant increase, compared to non-users, in the frequency with which their cooking water contained *V. cholerae* ($\chi^2 = 7.23$, $P. = 0.007$).

The surface water points used by 'early' infected households were contaminated significantly more often than other points on days 1 and 2 (46/105 as against 7/75 samples positive; $\chi^2 = 24.3$, $P<0.001$), indicating that infected individuals very quickly transmitted their *V. cholerae* to the environment around their household.

Table 5 indicates strongly that if household water is contaminated, it is because it was drawn from a contaminated source rather than because an infected family member contaminated it after it was brought into the household. The data for the first two days of surveillance were chosen for this analysis because of the high frequency with which *V. cholerae* was isolated from environmental samples and because most persons with 'early' infections had diarrhoea, which ought to have enhanced their capacity to contaminate

Table 5. Contamination rate[a] in household water during the first two days of surveillance as a function of contamination in surface water sources and presence of "early" infections among members of household

| "Early" infection present in household? | Surface water source from which household water drawn | | |
|---|---|---|---|
| | Positive | Negative | Total |
| Yes | 32/67 (48) | 0/8 (0.0)[b] | 32/75 (43) |
| No | 41/122 (34)[b] | 4/96 (4.2) | 45/218 (21) |
| Total | 73/189 (39) | 4/104 (3.8) | 77/293 (26) |

[a] Number contaminated/number examined. Percentage in parentheses.

[b] $P = 0.043$ Fisher Exact Test.

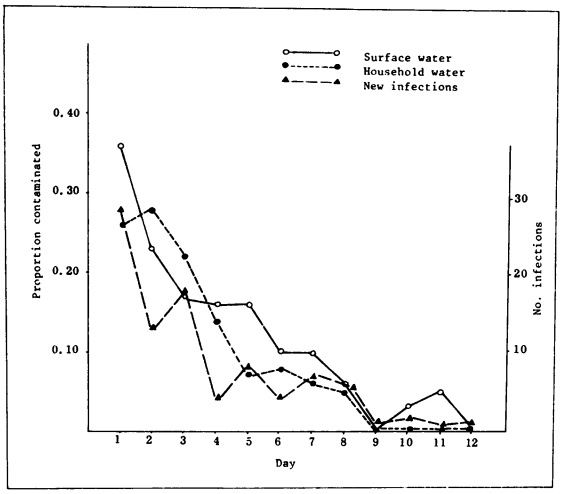


Fig. 1. Rate of *Vibrio cholerae* isolation from persons and water in cholera-positive neighbourhoods over a 12-day period of surveillance.

Table 6. Concentration of *V. cholerae* in contaminated water

| Type of water | No. of samples yielding *V. cholerae* | Percentage of samples in concentration range (CFU/ml): | | | | |
|---|---|---|---|---|---|---|
| | | < 5[a] | 5–10 | 11–99 | 100–499 | ⩾ 500 |
| Household: | | | | | | |
| cooking | 106 | 93.4 | 3.8 | 1.9 | 0.9 | – |
| drinking (surface) | 27 | 85.2 | 14.8 | – | – | – |
| Surface water sources: | | | | | | |
| tank | 89 | 79.8 | 7.8 | 11.3 | 1.1 | – |
| canal/river | 14 | 71.4 | 7.1 | 14.4 | 7.1 | – |
| ditch | 5 | 100.0 | – | – | – | – |

[a] 5 CFU/ml is the minimum concentration detectable by direct plating; all isolations listed in this column were made from enrichment culture.

household water directly. Contamination of surface water at the source has significantly greater association with household water contamination than does the presence of infected persons in the household.

Fig. 1 shows the daily frequency of contamination in household water and in surface water points, and the daily rate of newly detected infections. All three curves tend downward with time and tail after day 4. The plot of surface water contamination, presumably reflecting the presence of active cholera shedders among users, has plateaux that seem to coincide with the peaks in new infections. A distinct trough occurred on day 9, after which a small peak in isolations occurred, ending by day 12.

The range of concentrations of *V. cholerae* in contaminated water is shown in Table 6. The spectrum of contamination is skewed greatly toward very low concentrations of cholera vibrios in all water types. All isolations included in the lowest category (less than 5 colony forming units (CFU)/ml) resulted from enrichment cultures and represent a probable range of concentrations from 1 to 500 per 100 ml of water. Surface water points were contaminated with 10–500 CFU/ml significantly more often than were household water jars ($P = 0.001$, Fisher Exact Test). The highest concentrations were found in water samples taken in the first 3 days.

In interpreting these data it must be borne in mind that the procedure used for enumerating *V. cholerae* involved direct plating on a selective medium, TTGA, which may have underestimated the true concentration owing to the failure of injured cells to grow out. We do not know, however, the extent to which sublethally injured cells retain infective potential. Thus, the true level of exposure to infective organisms cannot be determined precisely. It is clear, however, that high concentrations of *V. cholerae* (i.e., $\geqslant 10^4$/ml) were extremely uncommon and that persons who became infected during the course of this study were unlikely to have ingested more than $10^5$ viable organisms per day.

Table 7 shows how the contamination of water affected the rate of 'later' infections among users. These rates were calculated on the basis of whether the water used was contaminated at any time during the surveillance for persons with no detectable infection, or up to the day before detection for infected persons. There were significant differences between groups, even though all persons lived in cholera-positive neighbourhoods and all shared at least one water source with the index family. In particular, contamination of household water, whether or not water sources were also contaminated, was associated with substantial increases in infection rate. The 32 individuals whose household water was positive, but whose source water was negative, represent 5 non-index households. There were no 'early' infections in any of these families. None were tubewell users. *V. cholerae* was isolated from household water once or twice in each household and from no other vehicles.

If contaminated household water were the predominant vehicle of cholera transmission, one would expect to see a significant increase in infection rate with increasingly frequent exposure to it. In Table 8, we have calculated the frequency with which samples of household water were contaminated for each of the 602 persons included in Table 7. We are using this

Table 7. Infection rate[a] among persons using surface water found contaminated at its source or in the household[b]

| | | Source water | | |
|---|---|---|---|---|
| | | Positive | Negative | Total |
| Household water | Positive | 31/274 (11.3)[c] | 2/32 (6.3)[c, d] | 33/306 (10.8) |
| | Negative | 1/134 (0.75)[c, d] | 2/162 (1.2)[c] | 3/296 (1.0) |
| Total | | 32/408 (7.8) | 4/194 (2.0) | 36/602 (4.0) |

[a] Number infected/number exposed. Percentage in parentheses.

[b] Classification of water is based on the samples collected up to one day prior to the detection of infection in each individual or samples collected throughout surveillance period for uninfected individuals.

[c] Overall differences between groups are highly significant by log-likelihood ratio ($G = 30.845$, $P < 0.001$). An explanation of the application of the log-likelihood ratio will be found in Sokal, R. R. & Rohlf, F. J. *Biometry*, San Francisco, Freeman, 1969.

[d] $P = 0.095$, Fisher Exact Test.

Table 8. Infection rate as a function of exposure to contaminated household water

| Frequency of contamination of household water[a] | No. infected/no. exposed | % |
|---|---|---|
| 0 | 3/296 | 1.0 |
| 0.1 - 4.9% | 2/47 | 4.3 |
| 5.0 - 24.9% | 15/177 | 8.5 |
| ⩾25.0% | 16/82 | 19.3 |

[a] See Table 7 for explanation of classification.

figure in place of the frequency with which persons are exposed to contaminated household water. Of course, the latter parameter will also be influenced by how often people used or ingested this water, but we have little information on this point. In any case, there is an obvious and clearly significant relationship between frequency of household water contamination and the rate of infection among persons using that water ($\chi^2 = 17.23$, $P = 0.0006$).

## DISCUSSION

The postmonsoon cholera season in the riverine delta region of Bangladesh has been characterized by scattered, apparently random outbreaks of both classical (*10*) and El Tor cholera[c] occurring throughout the area, with cases frequently clustered, indicating that spread within a given village may be a fairly common event. The introduction of *V. cholerae* into a village may follow the arrival of an infected person, as suggested by McCormack et al. (*10*). On the other hand, Khan et al. (*7*) have shown that villages where people use isolated water sources, such as tanks, have a lower attack rate than those where people use open water sources (rivers and canals), suggesting strongly that the passage of *V. cholerae* between villages is primarily waterborne.

We have found that intra-neighbourhood and intra-family cholera transmission in the Matlab study area is via contaminated surface water. Once introduced into a neighbourhood, *V. cholerae* from the first infected persons enter the shared surface water sources, presumably when shed directly during defaecation or bathing, or when contaminated clothing is washed. In cholera-negative neighbourhoods, where contamination of surface waters did not occur, outbreaks ended after the initial wave of infection. This provides further evidence of the importance of surface waters in transmitting the organism.

Propinquity to individuals shedding *V. cholerae* and susceptibility to infection appear to have been important determinants of the pattern of infection seen in this study. Thus, children in index families were most likely, and non-index adults least likely, to have 'early' infections. Persons of any age with an 'early' infection were also likely to have diarrhoea, perhaps reflecting increased susceptibility. *V. cholerae* were dispersed throughout the surface water sources coincidentally with 'early' infections and it is reasonable to assume that the risk of exposure at this time may have been similar for all families in the neighbourhood. This might explain why index and non-index households had similar 'later' infection rates.

Vehicles other than water played virtually no role in transmitting *V. cholerae* in these outbreaks. A great

[c] HUGHES, J. M., ET AL. Water and the transmission of El Tor cholera in rural Bangladesh. Working Paper No. 2, Cholera Research Laboratory, Dacca, Bangladesh, 1977.

deal of attention was given to food, in particular, because of its potential for providing a multiplication point for the organism. Foods were sampled at the time they would be most likely to harbour detectable numbers of organisms. Our results clearly show that no multiplication step existed and that water is the critically important mode of transmission of *V. cholerae* in the neighbourhoods studied.

The general pattern for *V. cholerae* transmission within and between households appears to have involved first the contamination of surface water sources, then the bringing of contaminated water into the household. Contamination of household water was significantly associated with its being drawn from a contaminated source rather than with exposure to an infected individual after it was brought into the house. Water taken from a clean source, such as a tubewell, remained uncontaminated even in households with members actively shedding *V. cholerae*. It is also noteworthy that the vehicles most likely to contaminate water in-house (fingers and utensils) were rarely found to be contaminated. In 5 instances, household water was positive while the source of that water was consistently negative, suggesting that the household water was contaminated after it had been drawn. However, none of the 32 family members involved were shedding *V. cholerae* at the time that the water was found to be contaminated, though 2 developed infections later. A more plausible explanation is that our sampling techniques were insufficiently sensitive in these few instances to detect *V. cholerae* that were in the source at the time the household water was drawn.

Exposure to contaminated water in the household was a greater risk factor than exposure at water sources. The 32 individuals whose household water yielded *V. cholerae*, even when their sources did not, had an infection rate of 6.3%. Those persons with the reverse situation experienced a rate of only 0.75%. Infection rates rose significantly with increasing frequency of contamination in household water. Contamination frequency and presumably, therefore, frequency of exposure may have an important explanatory value in developing a model of how cholera infection is acquired. The low concentration of *V. cholerae* in contaminated samples indicates that the infected individuals in this study were probably not exposed to large numbers of cholera vibrios. Instead, they appear to have had more frequent contact with small numbers. The rate of infection we observed suggests that the circumstances under which relatively few organisms can establish an asymptomatic or mildly symptomatic infection must be fairly common.

Conditions that reduce gastric acidity may be a major factor. The number of vibrios needed to elicit clinical symptoms in volunteers is reduced greatly when gastric acidity is neutralized by sodium bicarbonate prior to challenge (*2*). Recent studies in volunteers whose gastric acidity was neutralized showed that $10^3$ El Tor vibrios elicited mild diarrhoea in 4 out of 6 persons challenged (M. Levine, personal communication, 1980). Pierce et al. (*12*) found an increased frequency of achlorhydria in convalescent cholera patients in Calcutta and concluded that persons with this condition may be predisposed to cholera infection.

Other circumstances may also modify the effectiveness of the gastric acid barrier. It has been reported that, in some cases, acid-sensitive organisms can pass through the stomach so quickly that some may escape acid-mediated killing (*9*). Ingesting vibrios with natural buffers, such as proteinaceous foods, may protect them from gastric acid. An interesting finding in this regard is that volunteers who ingested $10^6$ El Tor vibrios during a meal of rice, fish, and milk but with no bicarbonate treatment had a significantly higher rate of clinical illness than volunteers receiving the same challenge dose in water alone (M. Levine, personal communication, 1980). Typical mealtime practices followed by our study population bring small volumes of water in contact with already cooked food through hand washing, rinsing of raw vegetables and dinnerware, and direct addition of water to food to cool or thin it. These activities would tend to contaminate food after it was taken from the cooking pot. The meal is also accompanied by the drinking of water, usually immediately after eating. It is possible that this combination of circumstances in which contaminated water is ingested with potential buffering material is an important determinant of infection.

The criteria used in our study selected families with similar rates of infection among tubewell users and non-users (see Table 2). Tubewell users had a jar of safe water and a jar of very often contaminated water in the house. Non-users simply had two jars of not-quite-as-often contaminated water in the house. Presumably, since the rates of infection were the same, the force of exposure was similar, and the value of drinking safe water was balanced by a greater risk of exposure in another area. Yet this implies that tubewell use does give some protection that ought to be seen as a reduced cholera infection rate in a randomly selected population of tubewell users. However, the effect of protecting water that is used only for drinking might be exceedingly small, and this may have accounted for the failure of the four Matlab studies mentioned previously to find differences in cholera rates between tubewell users and non-users. It is also possible that tubewell users differ from non-users in other practices that negate the value of tubewell use. Levine et al. (*8*) pointed out that tubewell users in the area they studied 'used 35% more water for all purposes than non-tubewell users, including more surface water'. If this proves to be characteristic of tubewell

users, the increased likelihood of exposure could explain the observed failure of tubewell use to provide any protection. It is evident that any further assessment of the impact of tubewells must take into account the water use habits of the population.

The findings of Khan discussed earlier suggest that an alternative to tubewells for controlling cholera in the flooded regions of Bangladesh may be feasible if communities can be persuaded to set aside isolated surface water sources for bathing and for taking drinking and cooking water. Our findings suggest that a significant improvement can be obtained if communities establish a safe source of water just for household use. This should be a much more approachable goal than also providing safe sites for bathing. Alternatively, it may be feasible to disinfect water in jars prior to using it in the household. It may even be possible in some cases to provide tubewell water that is acceptable in quality and availability for household use in this part of Bangladesh.

## ACKNOWLEDGEMENTS

The authors wish to acknowledge the excellent technical assistance of the members of the CRL Matlab microbiology and field staff who assisted in this project. We also wish to thank Dr W. H. Mosley for his review of this manuscript and for his many helpful comments and suggestions.

## RÉSUMÉ

### TRANSMISSION DU CHOLÉRA EL TOR DANS LA FAMILLE ET LE VOISINAGE: SURVEILLANCE MICROBIOLOGIQUE DES ZONES RURALES ENDÉMIQUES DU BANGLADESH

La population rurale de la région du Bangladesh sujette aux inondations et où sévissent des épidémies localisées de choléra a fait l'objet d'une surveillance microbiologique intensive qui a porté également sur l'environnement. Il ressort clairement des constatations faites que la transmission qui se produit au sein d'une même famille ou celle qui intéresse le voisinage est due à l'utilisation d'eaux de surface contaminées, que l'infection ait été acquise à la source d'eau elle-même ou lorsque l'eau rapportée à la maison est employée pour la boisson ou la cuisson des aliments. L'étude a montré que le principal facteur de risque réside dans ce dernier cas. La contamination des aliments eux-mêmes, des ustensiles ou des mains—autres véhicules potentiels—s'est révélée si peu fréquente qu'elle peut être négligée en tant que facteur de transmission. Selon les données recueillies, il est extrêmement probable que l'eau utilisée aux fins domestiques était contaminée parce qu'elle provenait d'une source elle-même contaminée, et non parce que des personnes infectées se trouvaient au foyer. En effet une eau saine demeurait saine même en présence d'excréteurs du vibrion au foyer. Après introduction de *Vibrio cholerae* dans une localité, la transmission ne peut donc persister que par la contamination des sources d'eau de surface. On peut raisonnablement avancer que l'infection se produit à la suite d'une exposition répétée, sans doute à l'occasion des repas, à une dose journalière ne dépassant pas $10^5$ micro-organismes. Rien ne permet de penser qu'une des personnes infectées ait été en contact à un moment quelconque avec des concentrations plus élevées de *V. cholerae*. Ces résultats mettent en relief l'importance de tout ce qui touche à la sensibilité de l'hôte, et indiquent les limites à ce qu'on peut attendre des interventions proposées tendant à réduire la transmission dans cette région en agissant sur l'environnement. Les auteurs commentent en particulier, au vu des résultats de l'étude, l'échec évident de la tentative d'approvisionnement en eau de boisson saine pompée à la main dans des puits instantanés, alors qu'une eau contaminée continuait d'être utilisée pour d'autres usages comme la toilette, la préparation des repas et la vaisselle.

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