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September 1981

Scientific Report No. 52

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PREFACE

The International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) is an autonomous, international, philanthropic and non-profit centre for research, education and training as well as clinical service. The Centre is derived from the Cholera Research Laboratory (CRL). The activities of the institution are to undertake and promote study, research and dissemination of knowledge in diarrhoeal diseases and directly related subjects of nutrition and fertility with a view to develop improved methods of health care and for the prevention and control of diarrhoeal diseases and improvement of public health programmes with special relevance to developing countries. ICDDR,B issues two types of papers: scientific reports and working papers which demonstrate the type of research activity currently in progress at ICDDR,B. The views expressed in these papers are those of authors and do not necessarily represent views of International Centre for Diarrhoeal Disease Research, Bangladesh. They should not be quoted without the permission of the authors.

ABSTRACT

This study was designed to define the role of water used for drinking, cooking, bathing, and washing in the transmission of *Vibrio cholerae* biotype El Tor infections in an area with endemic cholera. Surveillance was initiated in neighbourhoods of culture-confirmed cholera index cases and controls with non-cholera diarrhoea. In neighbourhoods with cholera infection, 44 percent of surface water sources were positive for *V.cholerae* as were only 2 percent of surface sources in control neighbourhoods. Canals, rivers, and tanks were most frequently positive. Increased risk of infection in families was associated with use of water from culture-positive sources for drinking, cooking, bathing, or washing and with water sources used by index families for drinking, cooking, or bathing. Increased risk of infection for individuals was associated with using water from culture-positive sources for cooking, bathing, or washing, but not with using water from culture-positive sources for drinking. Individuals who used the same water source as an index family for bathing were more likely to be infected than those using different sources. For families drinking from a culture-negative source, there was an association between infection and bathing in a positive source. For families with a bathing source different from the index family, there was an association between infection and drinking from the same source as the index family, and for families with a drinking source different from the index family, there was an association between infection and bathing in the same source as the index family. These data suggest that use of surface water is important in the transmission of *V.cholerae* and that, in addition to providing safe drinking water, educating persons regarding the risk of transmission of infection by water from potentially contaminated sources used for other purposes, especially bathing, may also be necessary to control transmission in areas where El Tor cholera is endemic.

INTRODUCTION

Transmission of *Vibrio cholerae* by contaminated drinking water was first documented 130 years ago (1). However, the importance of drinking water in transmission of cholera in an area with endemic cholera in rural Bangladesh is still debated (2,3) because the anticipated reduction in cholera case rates has not been observed for persons with easy access to tube-well water of presumed good microbiologic quality (4) or for those who usually drink tube well water [a,b] (5). A number of hypotheses have been proposed to explain the failure of tube well water use to protect individuals from cholera infection (6), but none have satisfactorily explained the relationship of water use to cholera transmission.

To evaluate the relationship between water used for drinking and for other purposes and the transmission of cholera in Matlab Bazar Thana, a rural area in Bangladesh (7), longitudinal studies were conducted in neighbourhoods with a resident who had symptomatic *V.cholerae* infection and in control neighbourhoods with a resident with non-cholera diarrhoea. Methodology varied with community size. Studies in small neighbourhoods were designed to assess the role of water in cholera transmission and to define the clinical spectrum of cholera. Studies in large neighbourhoods were designed to assess transmission of *V.cholerae* in families of persons with cholera. The results confirm the observation that surface water in proximity to persons with *V.cholerae* infection is frequently contaminated with *V.cholerae* (8,9) and suggest that water used for drinking and other purposes, especially bathing, is important in cholera transmission in this environment.

MATERIALS AND METHODS

Index patients were selected from persons who had diarrhoea, lived in villages in the Matlab vaccine trial surveillance (VTS) area, and were seen as either inpatients or outpatients at the Cholera Hospital. Each candidate for the study had a positive rectal swab culture for *V.cholerae* the day the neighbourhood study was begun; if more than one patient has a positive culture, we used a table of random numbers to select the index case. Patients' residences were visited the morning after presentation at the hospital, frequently before they returned home. Using a table of random numbers, we selected control

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a Khan M *et al.* Water sources and the incidence of cholera in rural Bangladesh. Scientific Report No. 16. Dacca, International Centre for Diarrhoeal Disease Research, Bangladesh, 1978.

b Curlin G *et al.* The influence of drinking tubewell water on diarrhoea rates in Matlab Thana, Bangladesh. Working Paper No. 1. Dacca, International Centre for Diarrhoeal Disease Research, Bangladesh, 1977.

patients from all inpatients and outpatients with diarrhoea who lived in the VTS area and had a negative rectal swab culture for *V.cholerae* the day each study was begun.

Study areas included all families living in the bari (a cluster of houses located on the same elevated courtyard or compound and inhabited by patrilineally related families) in which the index patient lived. A neighbourhood was defined as all households on the same elevated piece of land and was arbitrarily classified as "small" if it contained 17 or fewer families and "large" if it contained 18 or more families. Studies were conducted in 14 cholera and 14 control neighbourhoods between December 1973 and February 1974 during the cool dry post-monsoon period. Ten cholera and 9 control neighbourhoods were defined as small.

A family was defined as all individuals sharing the same dwelling or dwellings and eating food prepared in one kitchen. After obtaining free and informed consent from all families in each neighbourhood, a questionnaire was completed the first day of the study by interviewing an adult family member, usually a female. Information was obtained on the age and sex of all family members and guests and the source(s) of water currently used by all persons in the family for drinking, cooking, bathing, and washing clothes and utensils.

Tanks were defined as rectangular ponds formed when monsoon rains fill depressions resulting from removal of earth to build mounds on which houses were built. Tanks retain water all year. Ditches were defined as smaller depressions which contain water during part of the year.

Information about stool patterns of each individual during the previous week was obtained on the first day; on subsequent days, information was obtained about stool patterns during the previous 24 hours, either by interviewing each person or an adult family member. Diarrhoea was defined as a history of stools that were more loose or watery or occurred more frequently than normal for that individual. Cholera infection was diagnosed on the basis of a positive culture for *V.cholerae*. A clinical case of cholera was defined as the presence of diarrhoea within one day of the person's having a positive culture for *V.cholerae*.

In small neighbourhoods, rectal swabs were obtained each day of the study from all available residents; when a positive culture was obtained, no more swabs were obtained from that person. If an individual had a second episode (defined as being separated by at least two days of normal stool patterns) of diarrhoea, daily culturing was resumed. In large neighbourhoods rectal swabs were obtained on three consecutive days from all individuals with diarrhoea. Rectal swabs were also obtained each day from all family contacts of culture-positive individuals for the rest of the study. Studies were discontinued when no new culture-positive individuals were identified for nine consecutive days.

Data for individuals were included in the questionnaire survey if they were questioned on at least five days or had a history of diarrhoea. They were included in the culture survey if they had a positive culture or three negative cultures for *V.cholerae*.

Rectal swabs were cultured for *V.cholerae*, and isolates were biotyped as described (4). Approximately 60 ml. of water was collected from each tube-well, ditch, tank, canal, and river used by residents of both small and large neighbourhoods for drinking, cooking, bathing, or washing. Individual samples were obtained on several days beginning on the first day of each study near the periphery of each source at site where villagers obtained water. Each water sample was placed in approximately 30 ml of triple-strength bile peptone water; tellurite was added to a dilution of 1:200,000, and the sample was incubated at 37°C for six hours and then subcultured onto tellurite-taurocholate-gelatin (TTGA) agar. Colonies thought to be *V.cholerae* were identified (4). Cultures were recorded as either positive or negative; no quantitative results were obtained. Sources were considered adequately cultured if a positive culture or at least three negative cultures were obtained.

Statistical analyses were performed using the two-tailed Fisher's exact test, the chi-square test with Yates' correction, the t test on proportions with variance adjusted for cluster effect, and the Mantel-Haenszel test.

RESULTS

In the small cholera neighbourhoods, 504 of 617 individuals (82%) participated in the questionnaire survey and 476 of 617 (77%) participated in the culture survey. When the ten index cases are eliminated, only one of 56 infections (2%) identified in small neighbourhoods involved diarrhoea severe enough to require medical attention whereas 32 (57%) involved mild diarrhoea, and 23 (41%) were asymptomatic.

All infections were caused by *V.cholerae* biotype El Tor. Cholera infection rates were similar for all age groups. Case rates were highest in individuals 5-9 years of age and were significantly higher than the rate in adults (Table I). Sex-specific rates for symptomatic or asymptomatic infection were comparable. Ninety-four percent of infections and 98% of cases were identified by day 12 of the studies (Figure 1). By day five, only 59% of infections and 59% of cases had been identified.

Rates of infection identified in both small and large neighbourhoods were significantly higher for index families (23%) than other families (8%) in the neighbourhood ($p<.001$); rates were higher in the index bari (11%), when data for

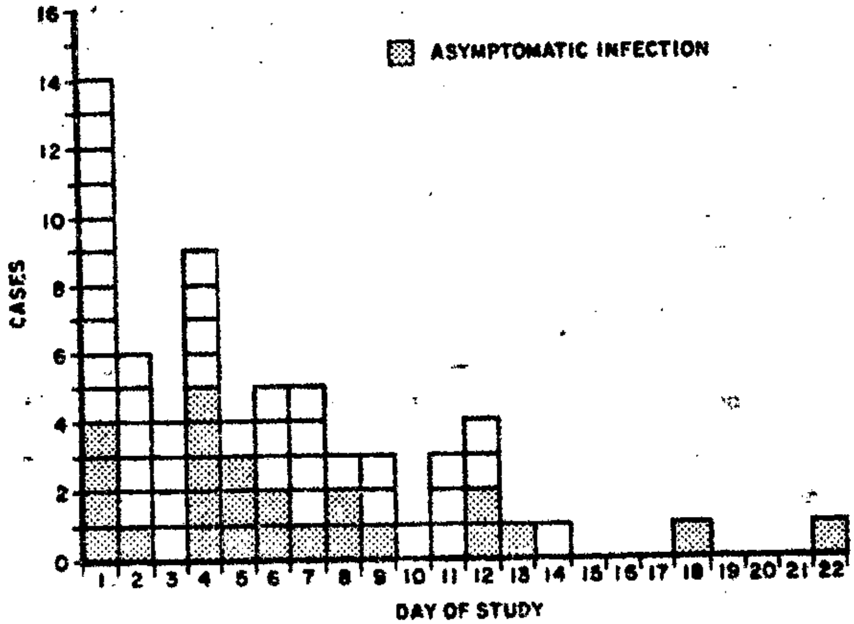
TABLE I--INFECTION AND CASE RATES, BY AGE GROUP, SMALL NEIGHBOURHOODS

Age Group	No. Cultured	No. (%) Infected	No. (%) Ill
<1	30	4 (13.3)	2 (6.7)
1-4	76	11 (14.5)	6 (7.9)
5-9	79	12 (15.2)	10 (12.7)[b]
10-14	76	10 (13.2)	6 (7.9)
$\geq$15	182	19 (10.4)	9 (4.9)[b]
Total	443[a]	56 (12.6)	33 (7.4)

a Index cases excluded.

b $p = .037$.

Fig. I V. *CHOLERAE* CASES AND ASYMPTOMATIC INFECTIONS, BY DAY OF DETECTION, LARGE AND SMALL NEIGHBORHOODS, BANGLADESH



the index family were excluded, than in other baris in the neighbourhood (4%) ($p<.01$). Similar studies conducted in 14 control neighbourhoods did not document a single *V.cholerae* infection.

Eleven of 14 (79%) neighbourhoods with an index *V.cholerae* infection had at least one water source contaminated with *V.cholerae*, as did only one of 14 (7%) control neighbourhoods ($p<.001$). In cholera-infected neighbourhoods, 44% of all cultured surface water sources were positive for *V.cholerae*, as were only 2 percent of surface sources in control neighbourhoods (Table II). Rivers were most frequently positive, and then canals, tanks, and ditches in that order. Positive cultures for *V.cholerae* were obtained from one tube-well on days 2, 3, and 4 of one study. Cultures of water obtained from this well on day 1 and on days 5-10 were negative. Cultures of water obtained from two tube wells in two other cholera neighbourhoods were negative.

Similar types of water sources were available in cholera and control neighbourhoods. When the percentages of families in cholera and control neighbourhoods that used water from each of the five types of sources were compared (Table III), more families in neighbourhoods with a cholera index case used canal and river water for drinking, cooking, bathing, and washing, and water from ditches for washing. Conversely, significantly fewer families in neighbourhoods with a cholera index case than families in control neighbourhoods used water from tanks for cooking, bathing, and washing, and water from tube-wells for drinking.

When choices of water source for drinking, cooking, bathing, and washing in small neighbourhoods with a cholera index case were evaluated, only one significant association was found. Families with an infected individual were more likely to drink from tube-wells than were families without infection ($p<.01$); however, only eight infected families used tube-well water for drinking. Six of these eight infected families lived in the same neighbourhood, which had a contaminated tube-well, and the families drank water from that well.

In small neighbourhoods with a cholera index case, families using at least one culture-positive water source for drinking, cooking, bathing, or washing were significantly more likely to have a person infected with *V.cholerae* than those using only culture-negative sources (Table IV). Infection rates for families using culture-positive sources for drinking and other purposes (51%) and those for families using culture-negative sources for drinking but culture-positive sources for cooking, bathing, or washing (58%) were similar; however, infection rates for both groups were higher than for families using culture-negative sources for all purposes (14%, $p<.001$ and $p<.01$, respectively). Families using the same water source(s) as the index family for drinking, cooking, or bathing were significantly more likely to have a person infected with *V.cholerae* than those using different sources (Table V).

TABLE II--RESULTS OF WATER CULTURES FOR *V. CHOLERAE* IN SMALL AND LARGE NEIGHBOURHOODS WITH AND WITHOUT CHOLERA INFECTION

Water Source	Cholera Neighbourhoods (n=14)					Control Neighbourhoods (n=14)				
	Number With Source	Total Number Sources	Number Cultured	Number Positive	Percent Positive	Number With Source	Total Number Sources	Number Cultured	Number Positive	Percent Positive
Surface Water										
River	5	5	4	3	75	7	7	4	0	0
Canal	8	8	8	5	63	7	7	5	0	0
Tank	9	23	22	10	44[a]	11	44	41	1	2[a]
Ditch	7	21	14	3	21	6	9	6	0	0
Subtotal	-	57	48	21	44	-	67	56	1	2
Tube-well	4	4	3	1	33	6	8	7	0	0

a p<.001.

TABLE III—PERCENTAGE OF FAMILIES USING WATER SOURCES, BY TYPE AND PURPOSE IN SMALL AND LARGE NEIGHBOURHOODS WITH AND WITHOUT CHOLERA INFECTION

	Tube-well	Ditch	Tank	Canal	River
Drinking					
Cholera	12	5	16	26	53
Control	34[a]	8	21	12[a]	36[a]
Cooking					
Cholera	0	21	41	51	28
Control	0	14	77[a]	11[a]	10[a]
Bathing					
Cholera	0	7	34	28	51
Control	0	13	61[a]	13[a]	33[a]
Washing					
Cholera	0	31	43	32	23
Control	0	15[a]	83[a]	11[a]	6[a]

a $p<.001$.

TABLE IV—FAMILY INFECTION RATES BY CULTURE STATUS OF WATER SOURCES, SMALL NEIGHBOURHOODS

	Total Families	Families Infected	Percent Infected	P Value
Drank water from ≥1 positive source	43	22	51.2	.017
Drank water from negative sources	47	12	25.5	
Cooked with water from ≥1 positive source	54	29	53.7	.0001
Cooked with water from negative sources	36	5	13.9	
Bathed in ≥1 positive source	55	29	52.7	.0003
Bathed in negative sources	35	5	14.3	
Washed with water from ≥1 positive source	55	29	52.7	.0003
Washed with water from negative source	35	5	14.3	

TABLE V—FAMILY INFECTION RATES BY USE OF INDEX FAMILY WATER SOURCES, SMALL NEIGHBOURHOODS

	Total Families	Number Infected	Percent Infected	P Value
Drank from same sources	55	22	40.0	.004
Drank from different sources	25	2	4.0	
Cooked from same sources	41	17	41.5	.03
Cooked from different sources	39	7	17.9	
Bathed in same sources	50	19	38.0	.05
Bathed in different sources	30	5	16.7	
Washed in same sources	41	15	36.6	.09
Washed in different sources	39	9	23.1	

Analysis of data for individuals in small neighbourhoods with a cholera index case revealed that persons who drank from at least one culture-positive source were not significantly more likely to be infected than those who drank only from negative sources. However, those who cooked with, bathed in, or washed with water from at least one positive source were significantly more likely to be infected than those who used only negative water sources for these purposes (Table VI). The proportion of individuals using water from culture-positive sources for drinking and other uses who were infected was similar (.167) to that for those using water from culture-negative sources for drinking, but culture-positive sources for cooking, bathing, and/or washing (.194).

Individuals who used the same water sources as those in the index family for bathing were significantly more likely to be infected than those who used different sources (Table VII). Individuals who used the same sources as members of the index family for drinking, cooking, or washing were no more likely to be infected than those who used different sources.

TABLE VI--PROPORTION OF INDIVIDUALS INFECTED, BY CULTURE STATUS OF WATER SOURCES, SMALL NEIGHBOURHOODS

	Total No. Individuals	No. Infected	Proportion Infected (S.E.)
Drank water from ≥ 1 positive source	265	44	.166(.027)
Drank water from negative sources	211	22	.104(.032)
Cooked with water from ≥ 1 positive source	328	57	.174(.026)
Cooked with water from negative sources	148	9	.061(.029)[a]
Bathed in ≥ 1 positive source	332	57	.172(.026)
Bathed in negative sources	144	9	.063(.030)[a]
Washed with water from ≥ 1 positive source	332	57	.172(.026)
Washed with water from negative so sources	144	9	.063(.030)[a]

a $p<.01$

TABLE VII--PROPORTION OF INDIVIDUALS INFECTED, BY USE OF WATER SOURCE USED BY INDEX FAMILY, SMALL NEIGHBOURHOODS

	Total Individuals	No. Infected	Proportion Infected (S.E.)
Drank water from same sources	295	37	.125(.022)
Drank water from different sources	127	7	.055(.034)
Cooked with water from same sources	223	28	.126(.024)
Cooked with water from different sources	199	16	.080(.028)
Bathed in same sources	255	34	.133(.026)
Bathed in different sources	167	10	.060(.024)[a]
Washed with water from same sources	207	25	.121(.025)
Washed with water from different sources	215	19	.088(.027)

a $p<.05$.

In an attempt to define the relative importance of drinking and bathing in the transmission of *V.cholerae*, families were stratified by drinking and bathing source positivity (Table VIII) and use of the same or different sources than the index family for drinking or bathing (Table IX). For families bathing in positive sources (Table VIII), there was no association between infection and use of positive sources for drinking. In contrast, for families drinking from negative sources, there was an association between infection and use of positive sources for bathing. For families using the same source as the index family for bathing (Table IX), there was no association between infection and drinking from the same source used by the index family; however, for those bathing in different sources, there was an association between infection and using the same source used by the index family for drinking. For families drinking from the same source as the index family, there was no association between infection and bathing in the same source used by the index family; however, for those drinking from sources different from the index family, there was an association between infection and bathing in the same source used by the index family. For all families, Mantel-Haenszel analysis indicated that there was a borderline association between drinking and infection when controlling for bathing but not between bathing and infection when controlling for drinking.

TABLE VIII--FAMILY INFECTION RATES BY CULTURE POSITIVITY OF DRINKING AND BATHING WATER SOURCES, SMALL NEIGHBOURHOODS

Drinking Source	Bathing Source	Total Families	Number Infected	Percent Infected
+	+	43	22	51.2[a]
+	-	0	0	-
-	+	12	7	58.3[a,b]
-	-	35	5	14.3[b]

a For families with culture-positive bathing source, not significant.

b For families with culture-negative drinking source, p=.005.

TABLE IX--FAMILY INFECTION RATES BY USE OF SAME OR DIFFERENT WATER SOURCES USED BY INDEX FAMILY FOR DRINKING AND BATHING, SMALL NEIGHBOURHOODS

Drinking Source	Bathing Source	Total Families	Number Infected	Percent Infected
Same	Same	46	17	37.0[a,c]
Same	Different	9	5	55.6[b,c]
Different	Same	4	2	50.0[a,d]
Different	Different	21	0	0[b,d]

Mantel-Haenszel test: p=.06 drinking (controlling for bathing)
p=.88 bathing (controlling for drinking)

a For families with same bathing source as index family, not significant.

b For families with bathing source different from index family, p=.001.

c For families with same drinking source as index family, not significant.

d For families with drinking source different from index family, p=.02.

DISCUSSION

Previously reported studies of the epidemiology of *V.cholerae* biotype El Tor infections in Matlab Bazar have failed to demonstrate convincingly a significant role for contaminated drinking water in transmission[c,d] (4,5). Explanations include the possibility that cholera is not primarily waterborne in this environment, that people report using tube-well water when they do not, that only some family members use tube-well water while others use alternative sources, or that the protection provided by use of tube-well water is negated by the use of contaminated surface water for cooking, bathing, or washing (5,6). Results of this study, although qualified by the possibility that not all water sources used by each family member were specified and by the lack of information on water storage practices and possible contamination in the home, suggest that contaminated surface water used for drinking and for other purposes, especially bathing, is important in transmission of *V.cholerae* in this environment.

Rates of infection with *V.cholerae* in the neighbourhood studies were comparable for all age groups. From 1968-1977, the age-specific cholera case rate for patients seen at the Matlab Hospital was highest for children 2-4 years old (4.5/1000) followed by that for children 5-9 years old (3.8/1000) (10). Results of our neighbourhood surveillance indicate that case rates were highest for children 5-9 years old and were significantly higher for this group than for persons $\geq$15 years but not significantly higher than for other age groups. These differences probably reflect the different case detection techniques in the two studies. In our study, no *V.cholerae* infections were detected in control neighbourhoods, an observation consistent with earlier reports that classical *V.cholerae* cases occurred in localized outbreaks in both Dacca (11) and Matlab (7).

The highest infection rate was in cholera index families in which 23% of individuals were infected. This infection rate is comparable to infection rates of 11%, 13%, 21%, and 25% reported for families with classical *V.cholerae* infection (7,12-14) and to infection rates of 21% and 31% reported for families with biotype El Tor *V.cholerae* infection in Bangladesh (14,15). However, in contrast to observations in the study of El Tor infection in Dacca, which indicated that none of 136 neighbourhood residents were infected, index bari residents were significantly more likely (11%) than residents of other baris in the same neighbourhood (4%) to be infected. Only 59% of all infections were identified by day five of the study in each bari, in contrast to 86% of all infections during the first five days of a ten-day study of classical cholera infections in Dacca (14). These observations suggest that *V.cholerae* biotype El Tor frequently infects other members of index households and baris in this environment and that the risk of acquiring infection exists for at least one to two weeks.

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c Khan M *et al.* Water sources and the incidence of cholera in rural Bangladesh. Scientific Report No. 16. Dacca, International Centre for Diarrhoeal Disease Research, Bangladesh, 1978.

d Curlin G *et al.* The influence of drinking tubewell water on diarrhea rates in Matlab Thana, Bangladesh. Working Paper No. 1. Dacca, International Centre for Diarrhoeal Disease Research, Bangladesh, 1977.

It is well documented that El Tor strains survive longer than classical strains in both water and food (16). In one study, El Tor *V.cholerae* survived 19.3 $\pm$ 5.1 days in shallow well water whereas classical strains survived 7.5 $\pm$ 1.9 days (16). The isolation of El Tor strains from 44% of surface water sources in infected neighbourhoods is comparable to the 45% rate reported from an urban environment where classical *V.cholerae* cases were occurring (11) and to the 57% rate in Matlab in neighbourhoods with infections caused by El Tor organisms (9). The observation that over 60% of canals and rivers used by cholera-infected neighbourhoods were positive is noteworthy because organisms entering these bodies of water would be diluted and is consistent with earlier observations that use of water from these sources is associated with increased risk of classical (8) and El Tor *V.cholerae* infections.[e] Although the availability of canals and rivers in neighbourhoods with and without cholera index cases was comparable, families in neighbourhoods with cholera were significantly more likely to use canal and river water for drinking as well as for cooking, bathing, and washing. Whether water usage patterns might reflect differences in socioeconomic status which might in turn influence susceptibility to *V.cholerae* infection was not addressed.

Only one water source in a neighbourhood not infected with *V.cholerae* was positive; in a study conducted in Matlab in 1965-66, only 10 of 5,670 surface water cultures were positive for classical *V.cholerae*, and all positive samples were from neighbourhoods with documented *V.cholerae* infection (7).

One of three cultured tube-wells was positive for *V.cholerae* on three consecutive days. The use of water from this well by six families that had at least one person infected with *V.cholerae* accounts for the observed association of tube well use with infection. This tube-well was in good repair and was not primed by adding water. However, neighbourhood residents admitted to hanging their laundry on the well to dry. Contaminated water may have leaked into the well around the vertical pump rod. The observation of Spira *et al.* that none of 12 wells they cultured was contaminated (9) suggests that tube well water contamination with *V.cholerae* is uncommon.

The importance of water in the transmission of cholera is illustrated by the fact that, in small neighbourhoods where case-finding techniques were most intensive, families who used a culture-positive water source for drinking, cooking, bathing, or washing were significantly more likely to have a cholera infection than families who used negative sources. These findings are compatible with observations by Levine *et al.* that high cholera case rates were associated with use of contaminated canal water (17) and by Spira *et al.* that the infection rate for families increased as the percentage of positive water cultures in the home increased (9). Spira's observations strongly suggested that water was

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e Khan M *et al.* Water sources and the incidence of cholera in rural Bangladesh. Scientific Report No. 16. Dacca, International Centre for Diarrhoeal Disease Research, Bangladesh, 1978.

contaminated almost exclusively at the source rather than in the home. We found that families who used the same water source(s) as the index family for either drinking, cooking or bathing were more likely to have an infected individual.

Several findings imply that drinking contaminated water may not be the only important factor in the transmission of El Tor cholera, a hypothesis suggested previously by others (4,5,8,9). Families who drank from culture-positive sources were no more likely to be infected than those who drank from culture-negative sources but used positive sources for cooking, bathing, or washing; both groups were more likely to be infected than families using only culture-negative sources. In addition, when data from individuals rather than families were considered, those who drank from culture-positive sources were no more likely to be infected than those who drank from culture-negative ones but used culture-positive sources for other purposes. Individuals who used the same water source as members of the index family for bathing were significantly more likely than those using different sources to be infected.

For families with culture-negative drinking sources, the association between infection and bathing in a positive source suggests that bathing in contaminated water is a risk factor for infection independent of drinking, but could also be interpreted as indicating that infected persons defecate where they bathe. However, the association between infection and drinking from the same source as the index family in families bathing in different sources from the index family and between infection and bathing in the same source as the index family in families drinking from different sources than the index family suggest that both drinking and bathing in contaminated water are risk factors for *V.cholerae* infection in this environment. The association of bathing and infection is compatible with a previous suggestion that ingestion of water while bathing may be important in the transmission of classical cholera (8) and raises a question concerning the relative concentrations of *V.cholerae* in contaminated water stored in the home and that at bathing sites. This hypothesis is compatible with the custom in West Bengal of taking handfuls of water into the mouth during bathing (18).

In summary, extensive environmental contamination occurred in neighbourhoods with residents who were infected with *V.cholerae*. Although not providing information regarding the mode of introduction of *V.cholerae* into a neighbourhood, the data support a role for contaminated water in transmission once the organism is introduced. These observations suggest that, in addition to providing safe drinking water, educating persons about the risk of transmission of infection by water from potentially contaminated sources used other purposes, especially bathing, may be necessary to control transmission in areas where *V.cholerae* biotype El Tor is endemic.

FULL SUMMARY

Transmission of *Vibrio cholerae* by contaminated drinking water was first documented 130 years ago. However, the importance of drinking water in the transmission of cholera in an endemic area in rural Bangladesh is still debated. This study was designed to define the role of water used for drinking, cooking, bathing, and washing in the transmission of *V.cholerae* biotype El Tor infections in Matlab Bazar Thana, a rural area in Bangladesh. Longitudinal studies were conducted in neighbourhoods of culture-confirmed cholera index cases and controls with non-cholera diarrhoea. During daily visits to the households in the neighbourhoods, histories of diarrhoea and rectal swab cultures were obtained from residents, and specimens were obtained from water sources used by neighbourhood residents for culture for *V.cholerae*.

In neighbourhoods with cholera infection, 44 percent of surface water sources were positive for *V.cholerae* as were only 2 percent of surface sources in control neighbourhoods. Canals, rivers, and tanks were most frequently positive. Although similar types of water sources were available in cholera and control neighbourhoods, families in cholera infected neighbourhoods were more likely to use canal and river water for drinking, cooking, bathing, and washing than families in control neighbourhoods.

Increased risk of infection in families was associated with use of water from culture-positive sources for drinking, cooking, bathing, or washing; infection rates were similar for families using culture-positive sources for drinking and other purposes and for families using culture-negative sources for drinking but culture-positive sources for cooking, bathing, or washing. Infection rates for both groups were higher than for families using culture-negative sources for all purposes. Increased risk of infection in families was also associated with use of water sources used by index families for drinking, cooking, or bathing. Increased risk of infection for individuals was associated with using water from culture-positive sources for cooking, bathing, or washing, but not with using water from culture-positive sources for drinking. Individuals who used the same water source as an index family for bathing were more likely to be infected than those using different sources. For families drinking from a culture-negative source, there was an association between infection and bathing in a positive source. For families with a bathing source different from the index family, there was an association between infection and drinking from the same source as the index family, and for families with a drinking source different from the index family, there was an association between infection and bathing in the same source as the index family.

These data suggest that use of surface water is important in the transmission of *V.cholerae* and that, in addition to providing safe drinking water, educating persons regarding the risk of transmission of infection by water from potentially contaminated sources used for other purposes, especially bathing, may also be necessary to control transmission in areas where El Tor cholera is endemic.

ACKNOWLEDGEMENTS

We are grateful to Mr. J. Chakraborty and Mr. M.R. Khan who supervised the field staff during this study, to Mr. A.R.M.A. Alim who assisted in identification of the isolates, to Roger A. Feldman, M.D. and Michael H. Merson, M.D. for their helpful suggestions during preparation of the manuscript, to Robert A. Pollard, M.A., for statistical assistance, and to the National Institutes of Health and the International Centre for Diarrhoeal Disease Research, Bangladesh, for support during the study. Finally, we are grateful to Ms. Peggy Hutton and Ms. Ruth Greenberg for assistance in preparing the manuscript.

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