MICROBIOLOGICAL SURVEILLANCE OF INTRA-NEIGHBOURHOOD EL TOR CHOLERA TRANSMISSION IN RURAL BANGLADESH

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Dacca, Bangladesh
March, 1980

Scientific Report No. 35

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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

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. In this study, cholera-infected neighbourhoods were placed under intensive microbiological surveillance to pinpoint contaminated vectors and subsequent infections during the course of actual outbreaks. Results of this study show clearly that almost all cholera transmission is via contaminated surface water and that exposure occurs at these sources or when water is taken into households for cooking or drinking. It was observed that infections result from a daily dose not exceeding 10^5 organisms and the importance of exposure frequency rather than total dose as a determinant of infection rate was pointed out. The importance of these data to environmental interventions and particularly the provision of tubewells is discussed.

INTRODUCTION

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 such as drinking; bathing; washing clothes, bedding, food, utensils and household items; cooking and playing; thus assuring frequent exposure of persons using the water. The intuitive viewpoint, then, is that improvements in water supply should reduce the incidence of cholera. In Bangladesh, this viewpoint has led to massive efforts to provide handpump tubewells to the rural population as a source of safe water.

Four studies on 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; G.T. Curlin, *et al.**). 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. As explanation, the authors point out that tubewells are used only for drinking and that surface water is used for all other purposes. Elsewhere in Bangladesh where tubewell water is used for other purposes as well, tubewells may be protective. However, for this flooded region, the consensus of opinion is 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" (G.T. Curlin, *et al.**).

This explanation, if correct, has important implications for our understanding of the process of acquiring cholera infection and for the development of future intervention strategies. Conscious drinking accounts for a large fraction of the total water taken in each day and other uses of water, though more frequent, represent a much smaller volume. If the other exposures overwhelm the protective effects of safe drinking water, the frequency with which contaminated water is ingested may be an important factor in acquiring cholera even though a low number of organisms are taken at each exposure.

Others, however, have interpreted these results as evidence that cholera may not be primarily waterborne in this region (3)

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* 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.

or that the transmission cycle is more complex and that water actually serves as an inoculum for another vector 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. While epidemiological evidence currently available suggests that these hypotheses are not correct, the path and kinetics of transmission of the organism have not been definitely established during actual outbreaks (6). A recent investigation of El Tor cholera in rural Bangladesh by Hughes, *et al.**, 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. While these findings suggested the importance of water in the transmission of El Tor cholera, the surveillance was insufficient for a precise determination of the pathway.

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 which actually lead to infection. We hope that this will, in turn, lead to a better understanding of the requirements and constraints for a successful environmental intervention to prevent cholera.

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 CRL Hospital for severe diarrhoea and who had *V.cholerae* isolated from rectal swab. The home of the index case was visited on the morning after admission. A detailed map of the neighbourhood was made showing all bodies of water and their ghats (water access points); the location of houses, tubewells and latrines; and any existing connections between bodies of water. 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 as called a "point". For open water systems, such

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* 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.

as rivers or canals, all points within 50 meters of a point used by the index family were considered to be part of the same source.

Surveillance was maintained for up to 12 days. Each day, all persons were asked if they had diarrhoea, which was defined as three sequential loose motions or a large liquid stool possibly containing blood, pus or both within 24 hours. Any person with diarrhoea who appeared to be dehydrated was urged to go to the CRL Hospital for possible admission. 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, washing or bathing. Parents supplied this information for small children. A rectal swab was taken from each interviewee and cultured for *V.cholerae* after a six hour enrichment in alkaline bile peptone (ABP). Suspicious colonies on taurocholate-tellurite-gelatine aggar (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 finger stick blood sample (50 lamda) 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).

Water samples were collected from the following sites on each day of surveillance: all points used by persons in the neighbourhood for activities through which ingestion of water might occur; one to two points in sources not used by persons in the study; and all water jars in 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 0900 and 1300 hours. Samples taken from water sources were collected at a depth of 20 cm at a distance of about one meter from 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 onto TTGA. A 100 ml aliquot was enriched in ABP for six hours, then streaked onto TTGA.

Foods held at ambient temperature for several hours 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 foods held overnight and those prepared during the day and held until the evening meal. Morning samples were returned to the

laboratory within five hours of collection; evening samples were kept in the village and brought back the next day. All samples were collected in sterile plastic bags and maintained at 10 C or less at all times. Specimens were blended in ABP (10g/90 ml) and plated directly (0.2 ml) and after enrichment (remaining blended material) onto 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. This rinse was enriched and plated as above. In similar fashion, cooking pots, utensils, and eating dishes were also examined for *V.cholerae*. The surface of cutting and food preparation boards were checked using RODAC plates containing TTGA (5).

The laboratory results of the previous day's samples were available early each morning. On this basis, new households were included if they were exposed to demonstrably contaminated water and new sources were added if they were used by an infected person. No sampling point or household, however, was dropped from the schedule once it was included. A neighbourhood was dropped if all specimens collected on three consecutive days were negative for *V.cholerae*.

RESULTS

Characterization of Study Neighbourhood:

Nineteen cholera-infected neighbourhoods were placed under surveillance in the course of this study. 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. In all, 792 people in 149 families (5.3 persons/family + 2.9, 1 S.D.) 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 2-17 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 due to its tendency to form a brown sediment upon standing overnight.

This study was carried out during the post-monsoon season. Consequently most of the land around neighbourhoods was flooded and virtually all active latrine sites emptied onto 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 four days we have classified 15/19 neighbourhoods as "cholera-positive" and the remaining 4 as "cholera-negative". In the former, surveillance was maintained for 9-12 days; in the latter it was stopped after day 4. The criteria for this classification are given in Table 1. In the cholera-positive neighbourhoods, 25 new infections were found (4.1% of study population) on days 2-4 and approximately one-quarter of all environmental samples collected on the first three days yielded *V.cholerae*. In contrast, no new infections were detected after the first day in cholera-negative neighbourhoods and *V.cholerae* was detected in less than 1% of the environmental samples. The two positive samples were collected on the first day.

The number of infections detected by the end of the first day, including index cases, was of the same frequency in both sets of neighbourhoods. The frequency of persons reporting diarrhoea in the 5 days before surveillance began was somewhat higher in cholera-positive neighbourhoods, but the difference is not significant. All 8 infections in cholera-negative neighbourhoods were detected within index families. In positive neighbourhoods, 11 out of 35 infections detected by day 1, occurred in other than the index household (P = .073, Fishers Exact Test).

The two sets of neighbourhoods are similar in number of families and family size (Table 2). Tubewells were used significantly more frequently by families in cholera-negative neighbourhoods (P = .020) but the use of other water sources were similar. The water use habits of index families also appeared to be the same. Comparing just those persons in index families from whom *V.cholerae* was isolated by day 1, there is no difference between groups as to the type of water source used for various activities. In cholera-negative neighbourhoods, 7/8 infected persons used either a tank or ditch for bathing, latrine and taking cooking water. In cholera-positive

TABLE 1

CLASSIFICATION OF NEIGHBOURHOODS ON THE BASIS OF CONTINUING PRESENCE OF CHOLERA

	Cholera-Positive		Cholera-Negative	
	Number	%	Number	%
Number of individuals	652	-	140	-
Number of infections detected by day 1 (including index)	35	5.4	8	5.7
Number of persons reporting diarrhoea in 5 days prior to study	47	7.2	8	5.7
Number of new infections detected on days 2-4	25	4.1	0	0
Frequency of detectable contamination on days 1-3 of:				
surface water - at source	69/262	26.0	2/96	2.1
surface water - stored in house	95/419	23.0	0/118	0.0

TABLE 2

CHARACTERISTICS OF NEIGHBOURHOODS PLACED UNDER SURVEILLANCE

Characteristics	Neighbourhoods Classified as Cholera*	
	Positive	Negative
Number	15	4
Number of families	120	29
Families per neighbourhood - median (range)	8 (2-17)	7 (6-9)
Number of individuals	652	140
Individuals per family (mean ± 1 S.D.)	5.4 ± 2.8	4.8 ± 2.3
Number of families using:		
river/canal	31 (26.0)**	6 (21.0)
tank	79 (66.0)	22 (76.0)
ditch	12 (10.0)	2 (6.9)
tubewell	46 (38.0)	18 (62.0) (X^2 = 5.37, P=.020)

* In cholera negative neighbourhoods, no new infections were detected after the first day nor were environmental samples positive for *V.cholerae*.

** Numbers in parentheses are percent.

neighbourhoods, 19/24 followed the same practice. We have no information, however, about whether the intensity with which these sources were used varies between groups, though this does not seem very likely.

The sex and age of index cases is given in Table 3. Though more females than males were index cases and more children than adults, the differences are clearly not significant. The age/sex distribution of cases in the two sets of neighbourhoods is also virtually the same.

There appear to be few differences between cholera-positive and negative neighbourhoods that would explain why *V.cholerae* was present in some and not others. It is possible that the fact that there was no environmental contamination of significance in some instances may just be a chance occurrence. Though surveillance was stopped in cholera-negative areas after the fourth day, subsequent examination of paired sera for increased vibriocidal titre indicated that no further cholera had occurred in these families. In contrast, almost 60% of all infections occurred on or after the third day in the cholera-positive neighbourhoods. Environmental contamination is strongly associated with the acquisition of *V.cholerae* in these outbreaks. This appears to be true even for index households, since those in cholera-negative neighbourhoods were free of new infections after the first day, even though the incidence of detectable infection to that point was the same as in cholera-positive neighbourhoods.

The remainder of this paper will deal only with the cholera-positive neighbourhoods. In these, 37% of families regularly separated their defecation and bathing sites either by using different sources or by using opposite sides of the same source. The rest used nearby points of the same source. Families using tubewells were more likely to use separate sites than non-users (21 of 46 versus 22 of 74), but this diference 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% families took some or all of their household water from the point used for bathing.

Pattern of Infection:

Considering only the cholera-positive neighbourhoods, 65 infections with *V.cholerae* biotype El Tor serotype Inaba were detected by bacteriological methods during the 9-12 day surveillance. This number does not include index cases. The

TABLE 3

SEX AND AGE OF INDEX CASES IN CHOLERA- POSITIVE OR NEGATIVE NEIGHBOURHOODS

Classification	Number of Neighbourhoods	No. of neighbourhoods in which index case was: Male		Female		Total
		Adult	Child	Adult	Child	
Cholera positive	15	3	3	4	5	15
Cholera negative	4	1	0	1*	3*	5

* One neighbourhood had two index cases, a female child and female adult.

median duration of infection from first detection was three days. Six additional infections were detected only on the basis of seroconversion. A more detailed description of the time distribution of infections is given in Table 4. In all, 11% of the persons examined showed evidence of infection. Of these, 28% were detected by day one and 41% by day two. 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, but we hope to show how many of the later infections probably came about through exposure to contaminated environmental sources. Thus, the early infections will be excluded from the analyses which follow this section. For a similar reason the six infections detected by seroconversion will also be deleted. None of these six reported having diarrhoea. The rate of infection was higher in index families than in other families (24% versus 8.9%, P= .00003). By day two, 65% of all infections occurred in the former group in contrast to only 29% in the latter (X^2 = 6.95, P = .008). For infections occurring after day two, however, the differences in overall rate (9.8% versus 6.5%, respectively) and daily pattern are not significant. With respect to tubewell use, the rate of later cases was 7.3% for users and 6.5% for non-users. Infections among non-users occur earlier and are more tightly clustered than those among users; 16 of 19 occurred on days 3-5 for the former, and 11 of 17 on days 6-10 for the latter (P = 0.005, FET). The relationship between a person not using tubewell water and also being in an index family was not an important determinant of the above patterns. Of the index family members, 41 were tubewell users and 55 non-users, about the same ratio as was found in non-index families.

The age and sex specific infection rates are shown in Table 5. Overall, children (less than 10 years old) experienced a rate 4.5 times higher than adults in early infections, but approximately the same rate as adult females during later infections. There was no difference due to sex among children, but adult males experienced a significantly lower rate than the other groups in later infections (X^2 = 3.817, P = 0.051).

The spectrum of clinical illness among the cases detected in the field is given in Table 6. There were an average of 4.7 infected persons and 2.6 symptomatic infected persons for each index case. Approximately one person for each two index cases had symptoms considered severe enough to warrant a hospital visit. Only three of these nine, however, required hospitalization. There was a dramatic difference between the spectrum of illness in early cases and that in later cases. The ratio of asympto-

TABLE 4

RATE OF NEWLY DETECTED INFECTIONS IN CHOLERA-POSITIVE NEIGHBOURHOODS (INDEX CASES EXCLUDED) DURING 12-DAY SURVEILLANCE

	No.infected/ Total	(%)	% Detected On Day												
			1	2	3	4	5	6	7	8	9	10	11	12	VTR*
Total	71/637	(11.0)	28	13	18	4	9	4	7	6	1	2	0	0	8
Index family	23/96	(24.0)	**39	26	9	0	4	5	4	0	4	0	0	0	9
Non-index	48/541	(8.9)	**23	6	23	6	11	4	8	9	0	2	0	0	8
TW users	33/286	(12.0)	25	14	9	0	10	9	9	9	3	3	0	0	9
Non-TW users	38/351	(11.0)	29	13	26	8	8	0	5	3	0	0	0	0	8

* Detected by vibriocidal titre rise.

** $\chi^2 = 17.2$, P = .00003.

TABLE 5

AGE AND SEX-SPECIFIC INFECTION RATES

Characteristic	Day *V.cholerae* first detected		
	Early (days 1-2)	Later (days 3-12)	Total
Male adult	4/202 (10)*	6/198 (3.8)	10/202(5.0)
Male child	12/133 (45)	9/121 (9.3)	21/133(16)
Female adult	4/196 (10)	13/192 (8.5)	17/196(8.7)
Female child	9/100 (45)	8/91 (8.8)	17/100(17)
Total	29/631 (23)	36/602 (7.5)	65/631(10)

* No. infected/no. examined (rate/1000 per day).

TABLE 6

SPECTRUM OF CLINICAL DISEASE AMONG ALL CASES DETECTED IN THE FIELD

	Early		Later		Total	
Severity	Number	%	Number	%	Number	%
Asymptomatic	7	24.1	19	52.8	26	40.0
Mild diarrhoea	15	51.8	15	41.7	30	46.2
Moderate-severe	7	24.1	2	5.5	9	13.8
Total	29	100.0	36	100.0	65	100.0

matic:mild:moderate/severe illness among early cases was 1:2.1:1, while among later cases it was 9.5:7.5:1. The infected-to-hospitalized case ratio among all contacts was 22:1. Since early infections were more likely to be symptomatic and also disproportionately comprised of children, we have examined the relationship between age and severity of illness more closely (Table 7). There is no difference between children and adults in the frequency of overt illness in early infections. For both groups 75% of persons infected showed signs of illness. In later infections, however, the frequency among adults drops to 32% (P = .061, FET). Among children the frequency decreases only slightly. Male and female children experienced the same frequency of symptomatic infection at both times. Adult females were somewhat more likely to have symptoms than were males though this difference is not statistically significant. The incidence of moderate/severe illness in early infection was approximately 1 in 4 for both age groups.

Contamination of Vectors and Patterns of Transmission:

The results of our microbiological surveillance for *V.cholerae* in water sources are given in Table 8. In all, 13.9% of the surface water point samples were contaminated. The frequency of contamination differed with the type of water sampled - 16.2% for tanks, 11.7% for canals and rivers, and 4.6% for ditches - but only the difference between tanks and ditches is significant (X^2 = 9.11, P = .003). The contamination rate for sources (usually containing three or more sampling points) is higher, 3-4 times that of individual points. Tubewell water consistently proved to be free of detectable *V.cholerae*.

Table 9 shows the contamination of potential vehicles examined in households. The rate of *V.cholerae* isolation from drinking water taken from a surface water source and stored in the house (9.3%) did not differ significantly from that of cooking water stored in the house (12.9%). The distinction made between them by members of the household was in this regard meaningless. The contamination rate of tubewell water stored in houses (1.2%) was significantly lower than that of other drinking water (P = 0.004, FET). In strong contrast to water, other vectors examined were virtually never found to be contaminated with *V.cholerae*, even when intensive enrichment techniques were used. The rate of contamination of food samples was only 0.13%, while that of the left hands of family members was 0.30%. Utensils and food preparation surfaces were never shown to be contaminated. In the two cases in which foods were positive, there were no subsequent infections among family members who consumed them.

TABLE 7

AGE AND SEX DISTRIBUTION IN EARLY VS. LATER INFECTIONS AND INCIDENCE OF DIARRHOEA

		Early	Later	Total
Child	Male	9/12 (.75)*	6/9 (.67)	15/21 (.71)
	Female	7/9 (.78)	5/8 (.63)	12/17 (.71)
Total Child		16/21 (.76)	11/17 (.65)	27/38 (.71)
Adult	Male	2/4 (.50)	1/6 (.17)	3/10 (.30)
	Female	4/4 (1.00)	5/13 (.38)	9/17 (.53)
Total adult		6/8 (.75)	6/19 (.32)	12/27 (.44)
TOTAL		22/29 (.76)	17/36 (.47)	39/65 (.60)

* Number with diarrhoea/total infections (ratio).

TABLE 8

CONTAMINATION OF SURFACE WATERS IN CHOLERA-POSITIVE NEIGHBOURHOODS DURING 12-DAY SURVEILLANCE

	Sources		Samples	
	Positive/Total	%	Positive/Total	%
Tanks	15/22	68.2	89/549	16.2
Canals/rivers	5/11	45.5	14/120	11.7
Ditches	4/9	44.4	5/109	4.6
Total surface water	24/42	57.1	108/778	13.9
Tubewell	0/12	0.0	0/38	0.0

TABLE 9

CONTAMINATION OF VEHICLES IN HOUSEHOLDS IN CHOLERA-POSITIVE NEIGHBOURHOODS DURING 12-DAY SURVEILLANCE

	Samples Positive/Total	%
Water in jars in household:		
Drinking (from TW)	1/85	1.2
Drinking (from surface)	27/275	9.8
Cooking (from surface)	106/823	12.9
Food	2/1593	0.13
Left hand rinse	2/677	0.30
Utensils and food preparation surfaces	0/437	0.0

A closer examination of contamination rates in the water of tubewell users and non-users (Table 10) shows that their surface water at the source is contaminated with equal frequency for both groups. For non-users, surface water taken into the household for either drinking or cooking is equally often contaminated. The frequency is only half of that of the source. However, we presume that much of this difference may be due to *V.cholerae* dying off in household jars which are not recontaminated to the extent that the source is.

A more intriguing comparison can be made between tubewell users and non-users' household water, i.e. that the advantage enjoyed by users in having much less frequently contaminated drinking water in their household is offset by a significant increase, compared to non-users, in the frequency with which their cooking water contained *V.cholerae* ($X^2 = 7.23$, $P = .007$). As has been noted, index families form a similar proportion of each group, so this is not an issue. The explanation for this difference which we tend to favour is that contaminated household water is a major vehicle for transmission of cholera in the families studied. By the study criteria used, we have deliberately selected families with similar rates of infection among users and non-users (see Table 4). Therefore, we might expect to see an increased risk of exposure in one vehicle of transmission (cooking water) if another (drinking water) is protected. In the situation seen here, tubewell users have a jar of safe water and a jar of very often contaminated water in the house. Non-users simply bring two jars of not-quite-as-often contaminated water into the house. The force of exposure and, hence, the rate of infection is the same. The data from tubewell users also shows, quite clearly, that there is a low frequency of cross-contamination between water or contamination of water from other sources within the house. Water that is safe when brought into the house remains safe.

We have plotted (Fig. 1) 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 four. The plot of surface water contamination, presumably reflecting the presence of active cholera shedders among users, seems to have plateaus which coincide with the apparent peaks in new infections. A distinct trough occurs on day nine, after which a small peak in isolations occurs, ending by day twelve.

The range of concentrations of *V.cholerae* in contaminated water is shown in Table 11. The spectrum of contamination is skewed greatly toward very low concentrations of cholera vibrios

TABLE 10

CONTAMINATION OF COOKING AND DRINKING WATER USED BY TUBEWELL-USERS AND NON-USERS

	Tubewell Users		Non-users	
Water at source:				
Tubewell	0/38	(0.0)*	---	
Surface	39/179	(21.8)	34/183	(18.6)
Drinking jars	1/85	(1.2)	27/275	(9.8)
Cooking jars	67/416	(16.1)	39/407	(9.6)

* Times positive/times sampled (%).

FIGURE 1

RATE OF *V. CHOLERAE* ISOLATION FROM PERSONS AND WATER IN CHOLERA-POSITIVE NEIGHBOURHOODS OVER 12-DAY SURVEILLANCE (SYMBOLS: O-NEWLY DETECTED INFECTIONS; O-HOUSEHOLD WATER SAMPLES; A-SURFACE WATER SAMPLES TAKEN FROM SOURCE)

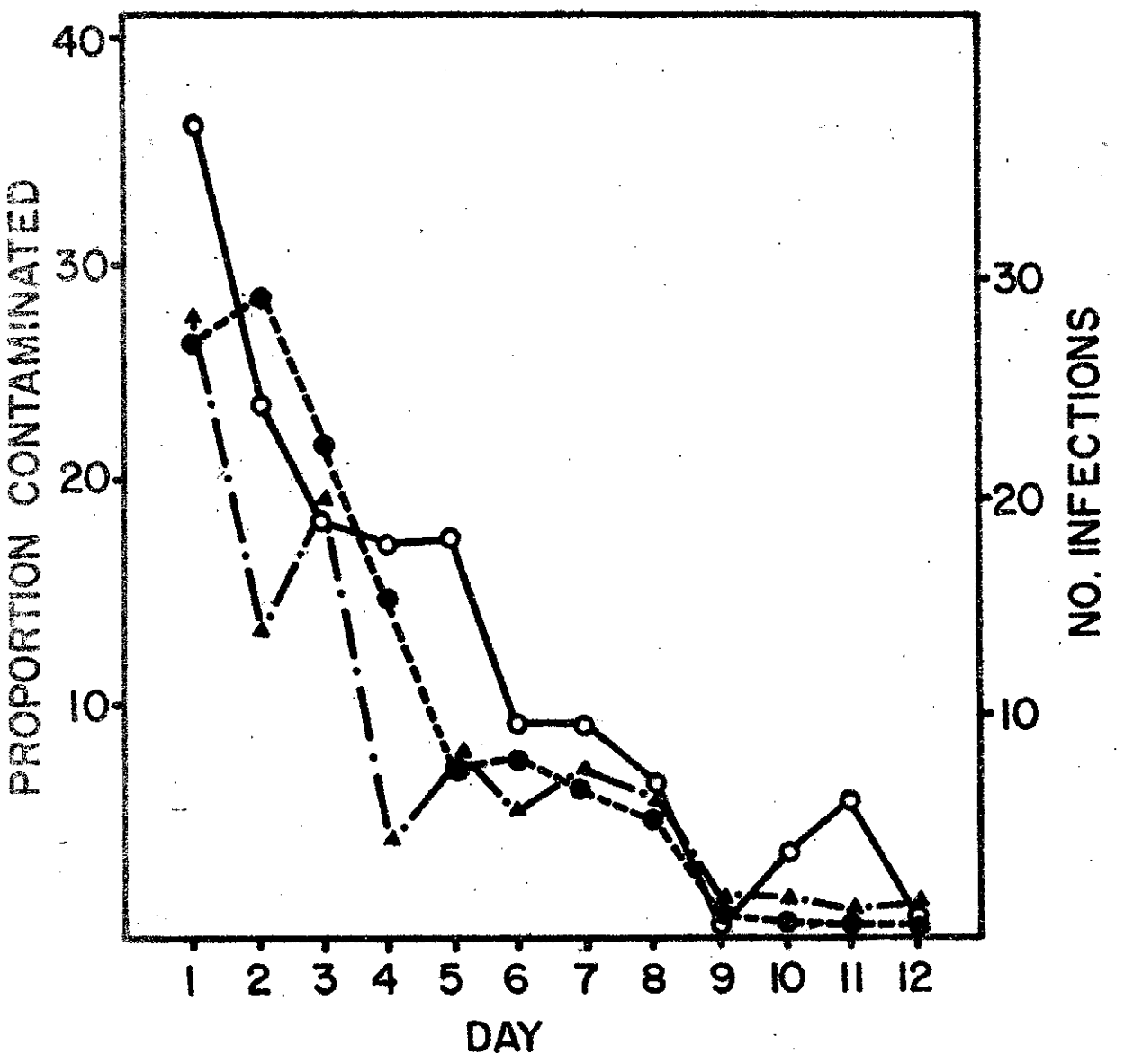

TABLE 11

CONCENTRATION OF *V. CHOLERAE* IN CONTAMINATED WATER

Type of water	Total No. detectably contaminated	<5*	5-10	11-99	100-499	>499
Household:						
Cooking	106	93.4	3.8	1.9	0.9	-
Drinking (surface)	27	85.2	14.8	-	-	-
Surface Water Points:						
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	-	-	-	-

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

in all water types. All isolations included in the lowest category (less than 5 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 household water jars (P = 0.001, FET). The highest concentrations were all found in water samples taken in the first three days.

One caution that must be applied to this data is that the procedure used for enumerating *V.cholerae* involved direct plating on a selective medium. Reported counts of $\geq$ 5 CFU/ml all resulted from this type of plating. The actual counts may underestimate the true concentration because injured but still viable cells may not grow out on selective media, such as TTGA, though this medium has proven in our laboratory to be much less inhibitory for *V.cholerae* than the more commonly used TCBS (thiosulfate citrate bile salts) agar. Such injury is known to result from a variety of environmental stresses but its extent in *V.cholerae* biotype El Tor existing in natural waters is unknown. However, counts made on water samples with gelatine agar (GA), a permissive medium, indicate that TTGA may miss up to half the *V.cholerae* detected on GA (unpublished data). A further complication is that we do not know the extent to which sub-lethally 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. $\geq 10^4$/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 12 shows how the frequency of water contamination affects the rate of bacteriologically detectable infection among users. Most people used household and source water which was contaminated at least once each. This was associated with the highest infection rate, 113 per thousand. Those people using ostensibly uncontaminated water only had an infection rate of twelve per thousand. Both of the infections in this group were detected on the third day of surveillance and it is possible that these persons were exposed before our surveillance started. If only source water was demonstrably contaminated, the rate was 7.5 per thousand, but if household water only was contaminated, the rate was 63 per thousand. 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. Even on the basis of this imprecise measure of exposure, there are significant differences between groups, even though all persons lived

TABLE 12

INFECTION RATE AMONG PERSONS USING SURFACE WATER FOUND CONTAMINATED AT ITS SOURCE OR IN THE HOUSEHOLD*

		Source		
		Positive	Negative	Total
Household	Positive	31/274 (113)**	2/32 (63)	33/306(108)
	Negative	1/134 (7)	2/162 (12)	3/296(10)
Total		32/408 (78)	4/194 (20)	36/602(40)

* 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.

** Number of person infected/number exposed (rate/1000).

in cholera-positive neighbourhoods and all shared at least one water source with the index family. The difference in rates between the group exposed only to contaminated household water and that with only source water contaminated is particularly striking. If the water in the house is negative even though the surface water is positive, the infection rate is just as low as if both are negative. On the other hand, if the water in the home is positive, there is an infection rate which is eight times higher than if the water in the house is negative. This difference (P = .095, F.E.T.) merits further documentation as it has important practical implications for control of cholera transmission.

If contaminated household water is 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 13, we have calculated the frequency with which samples of household water were contaminated for each of the 602 persons included in Table 12. We are using this as a surrogate for the frequency with which persons are exposed to contaminated household water. Of course, this latter parameter will also be influenced by how often people used or ingested this water. 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 (X^2 = 17.23, P = .0006).

Though water stored in the household contained relatively low concentrations of recoverable *V.cholerae*, it was, if found to be contaminated 25% or more of the time, associated with a very high rate of infection (193 per thousand). It appears that how often a person is exposed to cholera vibrios, and under what circumstances, is more important than the ingestion of high numbers of vibrios in determining infection under natural conditions.

DISCUSSION

The post-monsoon cholera season in the riverine delta region of Bangladesh has been characterized by scattered, apparently random out-breaks occurring throughout the area for both classical (10) and El Tor cholera* with cases frequently

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* 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.

TABLE 13

INFECTION RATE AS A FUNCTION OF EXPOSURE TO CONTAMINATED HOUSEHOLD WATER

Contamination of Water	No. infected/No. exposed	Rate/1000
If all water samples taken from household were found to be contaminated with an overall frequency of:*		
Not contaminated	3/296	10
0.1 - 4.9%	2/47	43
5.0 - 24.9%	15/177	85
≥ 25.0%	16/82	193

* See Table 12 for explanation of classification.

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, the data of Khan, *et al.* (7) showing that villages whose people use isolated water sources, such as tanks, have a lower attack rate than those whose people use open water sources (rivers and canals) suggest strongly that the passage of *V.cholerae* between villages is primarily waterborne.

We have demonstrated 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* enters the shared surface water sources presumably when shed directly during defecation or bathing, or when contaminated clothing is washed. In neighbourhoods in which contamination of surface waters did not occur, outbreaks ended after the initial wave of infection, suggesting the importance of this pathway in transmitting the organism.

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

Contamination of household water is primarily the result of its being drawn from a contaminated source rather than in-house exposure to an infected individual. Water taken from a clean source, such as tubewells, remained clean in the house, while that taken from a surface water source was contaminated about as often as the source. The difference in *V.cholerae* isolation rate that was shown between tubewell and surface water-filled jars used only for drinking (Table 9) would not be expected if contamination occurred after jars were brought into the house. It is also noteworthy that the most likely vehicles for in-house transmission (fingers and utensils) were rarely found to be contaminated.

Infections appear to have resulted from frequent exposure to low concentrations of *V.cholerae* rather than from a single high dose exposure. The distribution of counts we obtained in contaminated water samples indicates that the daily dose probably falls between 10^3 - 10^4, and about 10^5 at most. The daily dose for tubewell users should be lower since they consciously drink a much smaller volume of contaminated water. However, a more precise

definition of the infectious dose under natural conditions will required careful measurement of all water ingested by persons at risk and the quantitation of *V.cholerae* in these waters. This effort was beyond the scope of the current investigation.

We found at least 36 bacteriologically detectable infections which occurred two or more days after we began surveillance of potential vectors. This is a rate of 60 per thousand in the 10-day observation period. We have not included serologically detected infections because of our uncertainty about when they occurred, so the actual rate may be somewhat higher. Exposure to contaminated water in the household was a greater risk factor than exposure at water sources. Persons whose household water yielded *V.cholerae*, but whose sources did not, had an infection rate of 63 per thousand. Those persons with the reverse situation experienced a rate of only 7.5 per thousand. Presumably, this reflects a greater frequency of exposure to water stored in the household and indicates that such water was the predominant vehicle of transmission. Infection rates rise significantly as people use household water which is increasingly often contaminated. Contamination frequency and we presume, therefore, frequency of exposure has important explanatory value in developing a model of how infection is acquired in natural cholera outbreaks.

Rather than looking for "the exposure"-one in which a person comes in contact with a large number of cholera vibrios - we should emphasize instead the many low level exposures a person experiences and seek those conditions that finally allow a few organismes to establish an infection.

The apparent infectious dose in this study is much lower than the number required to elicit a similar pattern of clinical illness in volunteers in the U.S. whose gastric acid was not neutralized by $NaHCO_3$(2). It is similar, however, to the experience of those volunteers who ingested cholera vibrios with $NaHCO_3$. Two major differences between the two studies are that the current work is dealing with El Tor vibrios while the volunteer study used classical strains and that infection is a function of multiple exposures in this study while volunteers experienced only one dose of the challenge organism. However, these comparisons emphasize the importance of questions about host resistance under natural conditions. The frequency of gastric hypoacidity, for example, may be a major factor in determining the incidence of cholera in this population. Pierce, *et al.*, (12) found an increased frequency of achlorhydria in convalescent cholera patients compared to controls and postulated that this state may have preceded the infection. It is also possible that

the effectiveness of the gastric acid barrier may vary according to the conditions under which the organisms are ingested. Acid-sensitive non-pathogenic bacteria have been demonstrated in the duodenum immediately after volunteers drank contaminated water (9). The organisms were apparently washed through the stomach so quickly that the stomach acid was incapable of killing all of them. If a similar process occurs with *V.cholerae*, the infectious dose could be very low.

The reasons why tubewell use has failed to decrease appreciably the cholera attack rates of users in the Matlab area discernable in our results. For tubewell users, all water interactions besides drinking lead to frequent exposure to *V.cholerae*. Since the frequency of exposure appears to be as, if not more, important a determinant of infection as the total dose, the value of drinking safe water under these circumstances is limited. On the other hand, our results indicate that some protection from drinking tubewell water does exist. Users and non-users had almost identical rates of infection (a result of our selection process), yet the surface water in the households of users was contaminated twice as often as that of non-users. Presumably, this should have resulted in a higher infection rate for users. That it did not may have been the result of drinking safe water.

While the four previous studies to assess the effect of tubewell use on cholera rates may have been insufficiently sensitive to detect this protection, it is also possible that tubewell users have practices which minimize the value of tubewell use. Levine *et al.*, (8) pointed out that the 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 in general, the resulting increased frequency of exposure could explain the observed failure of tubewell use to protect. Another possibility that has been raised by Briscoe* is that the cholera-prone population, i.e. children, may not use tubewell water even if their families do, and would not therefore, show any protection from cholera infection. He reported, however, that children in ten representative tubewell-using households who were observed in their water use habits always drank tubewell water. He concluded that the explanation he raised was not likely to be correct. A larger survey may show, however, that non-use by children is a factor in some communities. It is evident that

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* Briscoe, J. The role of water supply in improving health in poor countries (with special reference to Bangladesh). Scientific Report No. 6, Cholera Research Laboratory, Dacca, Bangladesh, 1977.

any further assessment of the impact of tubewells must take a comprehensive view of the water use habits of the population into account.

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 in addition to using tubewell water for drinking, will establish a safe source of water just for household use, as well. This should be a much more approachable goal than also providing safe sites for bathing. 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. If surface water must be used, the sources would need to be isolated at all times of the year from open water and protected from all other uses by person in the community. It may also be feasible to disinfect the water in jars prior to using it in the household. Simply leaving water untouched in the sun for one day before use would reduce the pathogen load significantly, though the requirement for additional jars would, for many families, be an economic burden. The moderately protected water supply so developed should, in theory, limit the introduction of *V.cholerae* into a community and should also minimize the organism's transmission in cases where it does gain entrance. If it works, this approach would also have the advantage of relying on community-generated efforts and awareness, and of requiring no equipment or supplies which were not readily available among the people directly involved. This traditional approach to cholera control deserves further consideration and testing.

SUMMARY

The people and environment actually involved in localized cholera outbreaks common to rural neighbourhoods in the flooded region of Bangladesh were subjected to intensive microbiological surveillance. The object of this activity was to define the pathway of *Vibrio cholerae* transmission unambiguously, pinpointing when and how potential vectors became contaminated, the concentration of organisms present, and what subsequent infections ensued following exposure. The results indicate clearly that intra-neighbourhood and intra-family transmission is via contaminated surface water. Exposure occurs either at the source or when water is taken into households for cooking or drinking. The latter situation was the most important risk factor in the current study. Contamination of other potential vectors - food, utensils, fingers - is so infrequent as to be a negligible factor in transmission. Water brought into households is not contaminated after entry; safe water remains safe even if a cholera shedder is present in the house. Contamination of surface water sources is necessary for the continuation of the cholera infection cycle after the introduction of *V.cholerae* into a neighbourhood. Infections result from frequent exposures at a daily dose not exceeding 10^5 organisms. Frequency of exposure more than total dose appears to have value as an explanation for differences in rates of infection. These findings emphasize the importance of questions about host susceptibility and point out possible limitations to proposed environmental interventions to reduce cholera in this region. In particular, the demonstrated failure of drinking safe water from handpump tubewells to protect against cholera infection is discussed in light of our results.

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.

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