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IN RURAL BANGLADESH**

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PREFACE

The Cholera Research Laboratory (CRL) operates under a bilateral project agreement between the government of Bangladesh and the United States of America. Research activities of CRL center on the inter-relationships between diarrheal disease, nutrition, fertility and their environmental determinants. CRL issues two types of papers: scientific reports and working papers which demonstrate the type of research activity currently in progress at CRL. The views expressed in these papers are those of authors and do not necessarily represent views of Cholera Research Laboratory. They should not be quoted without the permission of the authors.

ABSTRACT

During the 1973-74 cholera season an investigation was conducted in the Matlab field trial area to determine the appropriate cholera case finding techniques for the 1974 cholera toxoid field trial, and to elucidate the role of water in cholera transmission. Visits to the house and neighbourhood of cholera index cases identified an average of 5.6 persons infected with V. cholerae and 3.3 symptomatic cholera cases for each index case residing in a small neighbourhood. No cases were detected in control neighbourhoods. Children age 1-14 years and adult females accounted for 4.8 total cases and 2.7 symptomatic cases per index case and were available at home in contrast to adult males. For these reasons this group was chosen for the toxoid vaccine trial in 1974.

Extensive contamination of water was found in neighbourhoods where individuals infected with V. cholerae resided. Forty-three percent of all water sources in infected neighbourhoods were positive for V. cholerae versus 2% in control neighbourhoods. Families using a culture positive water source for drinking, cooking, bathing, or washing were significantly more likely to be infected with cholera than other families. In addition, families using the same water source as the index family for either drinking or bathing were more likely to be infected. These observations suggest that water may be important in transmission of cholera in the Matlab field surveillance area.

INTRODUCTION

The first field trial of a cholera toxoid vaccine was conducted by the Cholera Research Laboratory in the Matlab Vaccine Trial Study (VTS) Area in Bangladesh during the 1974-75 cholera season. During previous field trials in the area, case surveillance was conducted at the Cholera Hospital in Matlab Bazaar; patients with diarrhea presented themselves either directly to the hospital or to ambulance boat stations from which they were transported to the hospital. Cholera cases were then identified bacteriologically. This case detection technique provided an adequate number of cases in the control groups to permit assessment of vaccine efficacy.

Since the completion of the cholera vaccine field trial conducted by CRL during the 1968-69 cholera season¹, Vibrio cholerae, biotype El Tor, has replaced the classical biotype in the area. It is well known that the ratio of asymptomatic or mild cases to severe cases requiring hospitalization is much greater when the disease is caused by the El Tor biotype.² Therefore, it was anticipated that hospital-based case detection might not provide an adequate number of cases in the control group to permit assessment of vaccine efficacy during the 1974-75 season.

Therefore, a study designed to determine the most efficient techniques for detection of mild and asymptomatic cases in the field during the vaccine trial was undertaken. At the same time, an attempt was made to evaluate the role of water in the transmission of cholera in rural Bangladesh.

MATERIALS AND METHODS

I. Selection of Index Cases

Cholera index cases were selected at random from all in-patients and outpatients at the Cholera Hospital in Matlab Bazaar who were residents of the VTS area and had a positive rectal swab culture for V. cholerae on the day of initiation of each study. In all cases, the patient's place of residence was visited on the morning following his arrival at the hospital and frequently before his return to his home. Cholera studies were begun on December 30, 1973.

Control index cases were selected at random from all in-patients and outpatients who were residents of the VTS area and had negative rectal swab cultures for V. cholerae and for non-cholera vibrios on the day of initiation of each study. Control studies were begun on January 18, 1974.

II. Field Study Design

Study neighbourhoods included all families occupying houses on the same elevated piece of land as the index family. Neighbourhoods were arbitrarily classified as small if they contained 17 or fewer families and large if they contained 18 or more families. A family was defined as all individuals eating from the same cooking pot. A bari was defined as all families sharing a courtyard.

In both small and large neighbourhoods, a family questionnaire was completed on the first day of the study during an interview with a responsible adult member of each family. The age and sex of all family members and guests and patterns of water usage for drinking, cooking, bathing and washing (clothes and utensils) by each family were recorded. Information about stool frequency and consistency over the previous week was obtained on the first day of each study for each individual; on subsequent days, this information was obtained for the previous 24-hour period, either through an interview with the individual or a responsible family member. If a person admitted to a change in stool frequency or consistency, more detailed questions on the stool changes were asked. If so, a separate questionnaire was completed on the day the abnormality was identified; associated symptoms were elicited and an effort was made to quantitate the severity of the illness by determining if the person's ability to perform his normal daily activities was compromised.

Studies in small neighbourhoods were designed to evaluate case-finding techniques and to determine the clinical spectrum of cholera in the VTS area. Rectal swabs were obtained daily from all residents who could be located. When a positive culture was obtained from an individual, rectal swabbing was discontinued. If a separate episode of loose motion occurred in the individual (a separate episode was defined as loose motion that was separated from a previous episode by at least two days of normal bowel habits), daily rectal swabbing was resumed.

Studies in large neighbourhoods were designed only to evaluate case-finding techniques. Rectal swabs were obtained on three consecutive days from all individuals who admitted to loose motion. In addition, daily rectal swabs were obtained from all family contacts of culture positive individuals for the duration of the study.

Individuals were included in the questionnaire survey if they were questioned on at least 5 days or gave a history of loose motion. They were included in the culture survey if they had a positive culture for V. cholerae or at least 3 negative cultures.

A cholera case was defined as an individual with a positive culture for V. cholerae. A symptomatic cholera case was defined as an individual who had loose motion within one day of a positive culture for V. cholerae.

Studies in both small and large neighbourhoods were discontinued when no new positive individuals were detected for 9 consecutive days.

III. Bacteriology

Rectal swabs were obtained from hospitalized patients or outpatients using tellurite-impregnated cotton-tipped swabs moistened in bile peptone water; these were used to inoculate a direct plate of TTGA and a tube of bile peptone water for enrichment, both cultures were incubated for 18 to 24 hours at 37°C.

Suspicious colonies on TTGA were then suspended in normal saline and slide agglutination tests with O group 1 polyvalent and Ogawa and Inaba monovalent antisera were performed. The broth culture was then streaked on TTGA (Monsur agar) and incubated at 37°C for 24 hours after which slide agglutination tests were performed.

V. cholerae colonies were tested for their ability to agglutinate a 2.5% suspension of chicken red blood cells for Mukherjee phage IV sensitivity, and for polymyxin B sensitivity.

Rectal swabs obtained in the field were placed directly in bile peptone water and processed as described above.

IV. Collection of Water Samples

Approximately 60 cc of water was collected from each tube-well, ditch, tank, canal and river used by members of each neighbourhood for drinking, cooking, bathing, or washing. Water samples were placed in approximately 30 cc 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 6 hours and then subcultured onto TTGA. Subsequent identification of *V. cholerae* was performed as described in the previous section. Culture results were recorded as either positive or negative; no quantitative results were obtained. Only sources cultured on at least three occasions were included in subsequent analyses.

RESULTS

I. Case Detection

Of the 163 cases seen at the Matlab Hospital from August 18, 1973 through March 23, 1974, 77 (47%) were residents of the VTS area.

Studies were conducted in 14 cholera infected and 14 control neighbourhoods. Ten cholera and 9 control neighbourhoods were defined as small (i.e. less than 18 families).

The age and sex distribution of the cholera index cases is shown in Table 1. Sixty-seven percent of the cases were less than 15 years old.

TABLE 1
AGE AND SEX OF *VIBRIO CHOLERA*
INDEX CASES

Age Group	Male	Female	Total	% of Total
<1	1	0	1	7%
1-14	7	2	9	60%
≥15	2	3	5	33%
Total	10	5	15	100%

one large neighbourhood had 2 index cases.

In the small cholera neighbourhoods, 82% of individuals participated in the questionnaire survey and 77% participated in the culture survey. Females participated in both portions of the study significantly more frequently than males ($P < .001$); this difference can be explained by the relatively infrequent participation of males 15 and older who were frequently working outside the village.

Eighty-three percent of cases in small cholera neighbourhoods were either asymptomatic or had only mild diarrhea (Table 2). When index cases were eliminated, only 1 of 56 cases (2%) discovered during active surveillance had diarrhea severe enough to require medical attention.

TABLE 2

SPECTRUM OF CLINICAL DISEASE - ALL AGES
SMALL NEIGHBOURHOODS ONLY

	Total	% of Total
Asymptomatic ¹	23	35%
Field Diarrhea ²	32	48%
Severe ³	11 ⁴	17%
Total	66	100%

1. Normal bowel habits during period 24 hours before and 24 hours after the positive culture.
2. Stools softer or more watery than normal or increased in frequency within 24 hours of positive culture.
3. Diarrhea severe enough to result in a hospital visit.
4. Ten persons were index cases identified at the hospital.

Age - and sex-specific attack rates in the small neighbourhoods are shown in Table 3. Although cholera case rates were higher in individuals 1 through 14 years of age than in those 15 and older, the difference was not significant when only individuals included in the culture survey were considered.

TABLE 3

CASE¹ RATES IN SMALL NEIGHBOURHOODS, BY AGE AND SEX,
INCLUDING INDEX CASES.

Matlab Cholera Neighbourhood Study, 1974

Age Group	Total Population			Number With Positive Culture			Cases 100 Population			Cases 100 Cultured		
	Male	Female	Total	Male	Female	Total	Male	Female	Total	Male	Female	Total
<1	20	11	31	3	2	5	15.0	18.2	16.1	15.0	18.2	16.1
1-14	140	116	256	26	14	40	18.6	12.1	15.6	20.3	12.7	16.8
≥15	150	156	306	5	16	21	3.3	10.3	6.9	8.8	12.6	11.4
UNK. Age	6	4	10	0	0	0	0	0	0	0	0	0
UNK. Age/Sex			14			0			0			0
Total	316	287	617	34	32	66	10.8	11.1	10.7	16.3	12.7	13.9

1. Individual with a positive culture for Vibrio cholerae.

However, individuals between 1 and 14 were significantly more likely to be symptomatic cases than those over 14 (Table 4). Neither the total nor the symptomatic case rate was significantly different in males than in females in any age group. Case rates were significantly lower in the last 5 small neighbourhood studies which were conducted near the end of the winter cholera season than in the first 5 studies.

Total case rates in the 4 large neighbourhoods, shown in Table 5, are significantly lower than in small neighbourhoods ($X^2 = 11.12$, $p < .001$). Symptomatic case rates, however, were not significantly different between large and small neighbourhoods. These findings are not unexpected given the differences in study design in the two types of neighbourhoods.

In small neighbourhoods there was an average of 5.6 infected persons and 3.3 symptomatic infected persons for each index case. In large neighbourhoods 2.3 infected and 2.0 symptomatic cases were found per index case. In small neighbourhoods children age 1-14 and females older than 14 had a mean of 4.8 cases and 2.7 symptomatic cases per index case. The yield of total and symptomatic cases was not significantly influenced by the age or sex of index cases.

Ninety-four percent of all field cases and 98% of all symptomatic cases were identified by day 12. Seventy-two percent of all cases and 70% of symptomatic cases occurred by day 7.

In the field case rates were significantly higher in index families (12 of 44 positive) than other families (40 of 364 positive) in small neighbourhoods ($X^2 = 7.95$, $p < .01$). The index bari, excluding the index family, had a similar rate to that of other bars in the small neighbourhoods. In large neighbourhoods the proportion of persons ill in the index household (2 of 16) in the index bari, excluding the index household (1 of 22), and in other bars (7 of 211) were similar.

The value of questions regarding stool frequency and consistency and associated symptoms was assessed in cholera neighbourhoods. This information was available for 96% of symptomatic cholera cases and 77% of symptomatic non-cholera cases. Quantitative questions did not clearly differentiate between culture-positive and culture-negative individuals of all ages or of children aged 1-14 and all females over 1 year of age.

TABLE 4

SYMPTOMATIC CASE¹ RATES IN SMALL NEIGHBOURHOODS, BY AGE
AND SEX, INCLUDING INDEX CASES

Matlab Cholera Neighbourhood Study, 1974

Age Group	Total Population			Number of Symptomatic Cases			Symptomatic Cases 100 Population			Symptomatic Cases 100 Cultured		
	Male	Female	Total	Male	Female	Total	Male	Female	Total	Male	Female	Total
<1	20	11	31	2	1	3	10.0	9.1	9.7	10.0	9.1	9.7
1-14	140	116	256	19	10	29	13.6	8.6	11.3	14.8	9.1	12.2 ²
≥15	150	156	306	5	6	11	3.3	3.8	3.6	8.8	4.7	6.0 ²
UNK. Age	6	4	10	0	0	0	0	0	0	0	0	0
UNK. Age/Sex			14			0			0			0
Total	316	287	617	26	17	43	8.2	5.9	7.0	12.4	6.7	7.0

¹ Symptomatic Case = Individual complaining of loose motion or diarrhea within one day of a positive rectal swab culture.

² $\chi^2 = 3.96$, $p < 0.05$.

TABLE 5

CASE YIELD IN LARGE NEIGHBOURHOODS,¹
INCLUDING INDEX CASES

Matlab Cholera Neighbourhood Study, 1974

Study No.	Date Started	Total Pop.	Number Cultured	Number Positive	<u>Cases</u> 100 Pop.	<u>Cases</u> 100 Cult.	Number Symptomatic	<u>Number</u> <u>Symptomatic</u> 100 Pop.	<u>Number</u> <u>Symptomatic</u> 100 Cult.
3	1/3/74	250	47	2	0.8	4.3	2	0.8	4.3
4	1/4/74	913	160	7	0.8	4.4	6	0.7	3.8
6	1/9/74	135	22	2	1.5	9.1	2	1.5	9.1
25	2/4/74	230	25	3	1.3	12.0	3	1.3	12.0
Total		1528	254	14	0.9	5.5	13	0.9	5.1

¹ Individuals complaining of loose motion or diarrhea were cultured.
Household contacts of cholera cases were also cultured.

Results of questions regarding stool characteristics and associated symptoms are shown in Table 6. Individuals with loose motion in association with a positive culture for V. cholerae were significantly more likely to complain of having watery stools and less likely to complain of having soft stools. Symptomatic cases were also more likely to complain of vomiting and more likely to be unable to perform their daily activities. However, when the index cases who were seen at the hospital were eliminated, the association of vomiting and inability to perform normal activities with a positive culture for V. cholerae was no longer significant.

Similar studies conducted in 14 control neighbourhoods did not detect a single case of cholera.

II. Water Data

Available water sources in the VTS area include tubewells, ditches, tanks, canals, and rivers. Eleven of 14 (79%) cholera infected neighbourhoods had at least one water source contaminated with V. cholerae compared with 1 of 14 (7%) non-infected neighbourhoods ($\chi^2 = 11.81$, $p < .001$). In cholera-infected neighbourhoods, 43% of all cultured water sources were positive for V. cholerae, compared with 2% of sources in control neighbourhoods (Table 7). V. cholerae was isolated from all types of water sources with rivers and canals positive most frequently. Positive cultures for V. cholerae were obtained from one tubewell on the second, third and fourth days of one neighbourhood study. Cultures obtained on day 1 and days 5-10 were negative.

Families in cholera and control neighbourhoods were equally likely to use single water sources for drinking and bathing. However, families in cholera neighbourhoods were significantly less likely to use single sources for cooking and washing clothes and utensils (Table 8).

Tubewells and tanks were found less frequently and ditches more frequently in cholera than in control neighbourhoods. The numbers of canals and rivers were comparable (Table 9). Thus case families would be expected to use canal, river and ditch water more frequently, and tank and tubewell water less frequently, than control families. Table 10 shows this to be true. Cholera families used canal and river water more frequently for all purposes, and ditch water more frequently for all purposes other than drinking. Cholera families used water from tanks less frequently

TABLE 6

SYMPTOM COMPLEX AND REPORTED STOOL CHARACTERISTICS FOR SYMPTOMATIC CHOLERA CASES AND SYMPTOMATIC CULTURE-NEGATIVE INDIVIDUALS RESPONDING TO LOOSE MOTION QUESTIONNAIRE

Matlab Cholera Neighbourhood Study, 1974

	Cholera Cases			Non-cholera Cases		
	No. with Symptom	Total	% with Symptom	No. with Symptom	Total	% with Symptom
Watery St Stool	32	54	59.3 ¹	166	469	35.4 ¹
Soft Stool	24	54	44.4 ²	338	473	71.5 ²
Mucus in Stool	17	54	31.5 ³	227	470	48.3 ³
Blood in Stool	2	53	3.8	52	472	11.0
Foul Smell	35	54	64.8	291	471	61.8
Fever	9	31	29.0	105	317	33.1
Chills	8	30	26.7	44	313	14.1
Abdominal Pain	27	52	51.9	270	457	59.1
Abdominal Gurgling	39	52	75.0	372	470	79.1
Nausea	13	54	24.1	104	470	22.1
Vomiting	14 ¹	54	25.9 ⁴	45	473	9.5 ⁴
Tenesmus	5	29	17.2	77	291	26.5
Performing Usual Daily Activity	41	54	75.9 ⁵	454	463	98.1 ⁵
If No, Is It Due to Illness	9	54	16.7	1	463	0.2

1 $\chi^2=10.73$, $p<.01$

2 $\chi^2=15.21$, $p<.001$

3 $\chi^2= 4.85$, $p<.05$

4 $\chi^2=11.53$, $p<.001$

5 Fisher's exact test
 $p<.01$

TABLE 7

WATER BACTERIOLOGY RESULTS

	Study Neighbourhoods					Control Neighbourhoods				
	Number With Source	Total Number Sources	Number Cultured	Number Positive	Percent Positive	Number With Source	Total Number Sources	Number Cultured	Number Positive	Percent Positive
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	11	44	41	1	2
Ditch	7	21	14	3	21	6	9	6	0	0
Tubewell	4	4	3	1	33	6	8	7	0	0
Total	14	61	51	22	43 ¹	14	75	63	1	2 ¹

¹ $\chi^2 = 27.67$, $p < .001$.

TABLE 8

UTILIZATION OF SINGLE
VERSUS MULTIPLE WATER SOURCES

	<u>Cholera Infected Neighborhoods</u>			<u>Control Neighborhoods</u>		
	<u>Families¹</u>	<u>Using Single Source</u> <u>Number</u>	<u>Percent</u>	<u>Families¹</u>	<u>Using Single Source</u> <u>Number</u>	<u>Percent</u>
Drinking	368	325	88	373	328	88
Cooking	368	236	64 ²	373	309	83 ²
Bathing	367	261	71	373	252	68
Washing	366	227	62 ³	371	281	76 ³

1 Includes only families for whom water utilization information is available.

2 $\chi^2 = 32.38$, $p < .001$

3 $\chi^2 = 15.56$, $p < .001$

TABLE 9

NUMBER OF WATER SOURCES AVAILABLE

	<u>Cholera Neighborhoods</u> <u>(370 families)</u>	<u>Control Neighborhoods</u> <u>(374 families)</u>
River	5	7
Canal	8	7
Tank	23	44
Ditch	21	9
Tubewell	4	8

TABLE 10

WATER SOURCE UTILIZATION PATTERNS

Source	Cholera Neighborhood								Control Neighborhoods							
	Drinking		Cooking		Bathing		Washing		Drinking		Cooking		Bathing		Washing	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Using	Using	Using	Using	Using	Using	Using	Using	Using	Using	Using	Using	Using	Using	Using	Using	Using
Tubewell	45	12.2 ¹	0	-	0	-	0	-	127	34.0 ¹	0	-	0	-	0	-
Ditch	19	5.2 ²	76	20.7 ⁶	27	7.4 ¹⁰	113	30.9 ¹⁴	31	8.3 ²	51	13.7 ⁶	50	13.4 ¹⁰	53	14.6 ¹⁴
Pank	57	15.5 ³	152	41.3 ⁷	126	34.3 ¹¹	158	43.2 ¹⁵	80	21.4 ³	288	77.2 ⁷	229	61.4 ¹¹	302	83.0 ¹⁵
Canal	97	26.4 ⁴	150	50.8 ⁸	101	27.5 ¹²	118	32.2 ¹⁶	46	12.3 ⁴	41	11.0 ⁸	47	12.6 ¹²	40	11.0 ¹⁶
River	196	53.3 ⁵	103	28.0 ⁹	187	51.0 ¹³	83	22.7 ¹⁷	135	36.2 ⁵	38	10.2 ⁹	124	33.2 ¹³	21	5.8 ¹⁷

1 $\chi^2 = 48.26$, $p < .001$

2 $\chi^2 = 2.44$ NS

3 $\chi^2 = 3.98$, $p < .05$

4 $\chi^2 = 22.51$, $p < .001$

5 $\chi^2 = 21.51$, $p < .001$

6 $\chi^2 = 5.87$, $p < .02$

7 $\chi^2 = 97.54$, $p < .001$

8 $\chi^2 = 84.25$, $p < .001$

9 $\chi^2 = 36.95$, $p < .001$

10 $\chi^2 = 6.62$, $p < .02$

11 $\chi^2 = 53.20$, $p < .001$

12 $\chi^2 = 24.81$, $p < .001$

13 $\chi^2 = 23.09$, $p < .001$

14 $\chi^2 = 28.11$, $p < .001$

15 $\chi^2 = 113.18$, $p < .001$

16 $\chi^2 = 49.11$, $p < .001$

17 $\chi^2 = 42.63$, $p < .001$

for all purposes, and were less likely to use tubewell water for drinking (which is the only purpose for which tubewell water was used).

Analysis of water source preference for drinking, cooking, bathing, and washing patterns in small cholera-infected neighbourhoods revealed only one significant association; families with cholera were actually more likely to drink from tubewells than families without cholera ($\chi^2 = 9.01$, $p < .01$). This unexpected association may be explained in part by the fact that 6 of the 8 families drinking tubewell water were infected in the one neighbourhood with a contaminated tubewell.

In small cholera-infected neighbourhoods, families using at least one culture-positive water source for drinking, cooking, bathing, and washing were significantly more likely to be infected than those using only culture-negative sources (Table 11). Considering the families with positive cooking, bathing, and washing water, the attack rate in families with positive drinking water (49%) was not significantly different from that in families with negative drinking water (60%).

Families using the same water source for drinking, cooking, bathing or washing as the index family had higher attack rates than families using a different source (Table 12). These differences were not statistically significant for cooking and washing.

Analysis of data from the small cholera-infected neighbourhoods by individual attack rates rather than family attack rates yielded interesting results. Individuals drinking from at least one culture-positive source were not significantly more likely to be infected than those drinking only from negative sources. However, those cooking with, bathing in, or washing in water from at least one positive source were significantly more likely to be infected than those using only negative water sources for these purposes (Table 13). Comparison of individual attack rates for those individuals using water from culture-positive sources for drinking and other uses with those using water from culture-negative sources for drinking but culture-positive sources for cooking, bathing, and washing revealed no significant difference (16% vs 20%, $\chi^2 = 0.44$).

Individuals using the same water source as individuals in the index family for drinking, cooking, bathing or washing were more likely to be infected than those using different sources; however, the difference was significant only for bathing (Table 14).

TABLE 11

FAMILY ATTACK RATES BY USE OF CULTURE POSITIVE
OR CULTURE NEGATIVE WATER SOURCES

	Total Number Families	Number Infected	Percent Infected
Drank from ≥ 1 positive source	45	22	48.9 ¹
Drank from negative sources	47	12	25.5 ¹
Cooked from ≥ 1 positive source	56	29	51.8 ²
Cooked from negative sources	36	5	13.9 ²
Bathed in ≥ 1 positive source	57	29	50.9 ³
Bathed in negative sources	35	5	14.3 ³
Washed in ≥ 1 positive source	57	29	50.9 ⁴
Washed in negative source	35	5	14.3 ⁴

1 $\chi^2 = 4.43, p < .05$

2 $\chi^2 = 11.93, p < .001$

3 $\chi^2 = 10.94, p < .001$

4 $\chi^2 = 10.94, p < .001$

TABLE 12

FAMILY ATTACK RATES BY USE OF SAME OR DIFFERENT
WATER SOURCES THAN INDEX FAMILY

	Total Families	Number Infected	Percent Infected
Drank from same sources	57	22	38.6 ¹
Drank from different sources	25	2	8.0 ¹
Cooked from same sources	43	17	39.5 ²
Cooked from different sources	39	7	17.9 ²
Bathed in same sources	52	19	36.5 ³
Bathed in different sources	30	4	13.3 ³
Washed in same sources	43	15	34.9 ⁴
Washed in different sources	39	9	23.1 ⁴

1 $\chi^2 = 6.45$, $p < .02$

2 $\chi^2 = 3.62$, NS

3 $\chi^2 = 3.99$, $p < .05$

4 $\chi^2 = 0.87$, NS

TABLE 13

INDIVIDUAL ATTACK RATES BY USE OF CULTURE-
POSITIVE OR CULTURE-NEGATIVE WATER SOURCES

	Total No. Individuals	No. Infected	% Infected
Drank from ≥ 1 positive source	258	42	16.3 ¹
Drank from negative sources	210	22	10.5 ¹
Cooked from ≥ 1 positive source	320	55	17.2 ²
Cooked from negative sources	148	9	6.1 ²
Bathed in ≥ 1 positive source	324	55	17.0 ³
Bathed in negative sources	144	9	6.3 ³
Washed in ≥ 1 positive source	324	55	17.0 ⁴
Washed in negative sources	144	9	6.3 ⁴

1 $\chi^2 = 2.83$, NS

2 $\chi^2 = 9.65$, $p < .01$

3 $\chi^2 = 8.83$, $p < .01$

4 $\chi^2 = 8.83$, $p < .01$

TABLE 14

INDIVIDUAL ATTACK RATES BY USE OF
SAME OR DIFFERENT WATER SOURCES
THAN INDEX FAMILY

	Total Individuals	No. Infected	% Infected
Drank from same sources	290	35	12.1 ¹
Drank from different sources	126	7	5.6 ¹
Cooked from same sources	223	28	12.6 ²
Cooked from different sources	193	14	7.8 ²
Bathed in same sources	255	34	13.3 ³
Bathed in different sources	161	8	5.0 ³
Washed in same sources	207	25	12.1 ⁴
Washed in different sources	209	17	8.1 ⁴

1 $\chi^2 = 3.42$, NS

2 $\chi^2 = 2.65$, NS

3 $\chi^2 = 6.71$, $p < .01$

4 $\chi^2 = 1.37$, NS

The data on individual attack rates should be interpreted with caution since water source preferences were obtained for the family as a unit. Some family members, especially young children, may have used water from sources other than those indicated by an adult family member.

DISCUSSION

I. Vaccine Trial Design

Case detection techniques utilized in this study identified a mean of 5.6 total cases and 3.3 symptomatic cases of cholera for each cholera index case residing in small neighbourhoods. No cases were detected in control neighbourhoods, indicating that initiating surveillance in randomly selected neighbourhoods is an inefficient way to detect cases. Children between 1 and 14 years of age (the age group traditionally used in cholera vaccine field trials conducted by the CRL) and adult females yielded a mean of 4.8 total cases and 2.7 symptomatic cases. Children less than one year of age did not contribute a significant number of cases. Although adult males were not significantly less likely to be infected than adult females, they were less likely to be available in their village for culturing. Therefore, the population predicted to contribute the greatest number of cholera cases during the toxoid field trial included children between 1 and 14 years of age and adult females.

Case detection techniques identified the greatest number of cases in the index bari; index bari members were significantly more likely to be cases than members of other baris in the same neighbourhood. Over 90% of all cases were identified during the first 12 days of the study in each bari.

Responses to questions concerning stool quality and quantity and associated symptoms did not differentiate clearly between culture-positive and culture-negative individuals. These questions were also difficult for the population to comprehend; use of such questions would not improve case detection techniques.

In summary, the study suggests that the appropriate population to vaccinate is children between 1 and 14 and adult females, and that field case detection techniques consisting of obtaining daily rectal swab cultures from all members of these groups in the index bari for 12 consecutive days is the most efficient way to detect cholera cases in the field.

II. Water Source Data

The El Tor organism is more resistant to environmental stress than the classical organism.³ Nevertheless, the isolation of the organism from over 40% of water sources in infected neighbourhoods is remarkable in view of the small quantity of water sampled. The fact that over 60% of canals and rivers used by cholera infected neighbourhoods were positive is also noteworthy in view of the potential for dilution of vibrios gaining access to these bodies of water. One of 3 cultured tubewells was positive for V. cholerae on 3 consecutive days. This neighbourhood had the highest cholera attack rate. This tubewell was in good repair and was not primed by the addition of water. However, neighbourhood residents admitted to hanging their laundry on the well to facilitate drying. Contaminated water may have leaked into the well around the vertical pump rod while clothes were drying.

Families in the infected neighbourhoods were significantly more likely to use canal and river water for drinking, cooking, bathing, and washing. On the other hand, families in control neighbourhoods were more likely to use water from tanks for these purposes and more likely to obtain their drinking water from tubewells. While V. cholerae would be expected to be found frequently in the environment of cholera cases when systematic excreta disposal is absent, these data suggest that water may be important in the transmission of cholera in the VTS area. In small neighbourhoods where case-finding techniques were most intensive, families using a culture-positive water source for drinking, cooking, bathing, or washing were significantly more likely to be infected with cholera than other families. In addition, those families using the same water source as the index family for either drinking or bathing were more likely to be infected.

Several findings imply that drinking contaminated water may not be the only important factor in the transmission of El Tor cholera, as suggested by other CRL studies (4-7). Families drinking from culture-positive sources were no more likely to be infected than those drinking from culture-negative sources who used positive sources for cooking, bathing, or washing. In addition, when individuals rather than families were considered, those drinking from culture-positive sources were no more likely to be infected than those drinking from culture-negative; however, those using culture-positive sources for cooking, bathing, or washing were more likely to be infected than those using negative sources for these purposes. Finally, individuals using the same water

source as members of the index family for bathing (but not drinking, cooking, or washing) were significantly more likely to be infected than those using different sources.

In summary, extensive environmental contamination occurred in neighbourhoods where individuals infected with V. cholerae resided. The data support the hypothesis that contaminated water is important in the transmission of cholera, and suggest that providing facilities for adequate sewage disposal to decrease the contamination of surface water may be important in areas where V. cholerae, biotype El Tor, is endemic.

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