(6) World Health Organization. 1964. Report of Seminar on Cholera Control, Manila, World Health Organization.
(7) Griesinger, W. 1857. Infektionskrankheiten, Malariakrankheiten, Gelbes Fieber, Typhus, Pest, Cholera. In: Virchow, R., Handbuch der specillen Patholgie and Therapie, Erlangen.
(8) World Health Organization. 1948. Report of The OIHP/WHO Joint Study Group on Cholera. Geneva, World Health Organization.
(9) Dönitz, W. 1886. Bemerkungen zur Cholerafrage. Z. Hyg. 1: 405.

Person-to-Person Transmission of Cholera[1]

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Cholera is a disease of man caused by *Vibrio cholerae*. It is accepted that the organism gains entry through the mouth and leaves the body in the feces. Urine is not generally considered an important source of organisms, although the vibrios have been demonstrated in the urine of cholera patients (Greig, 1913). The object of this report is to review selected factors involved in transfer of organisms from one person to another. The data presented are drawn from several studies in progress at our laboratory of populations among whom cholera is endemic.

Transmission by contact has been tested in the hospital ward of the Cholera Research Laboratory in several ways: (1) Standard bedside nursing procedures are used, with precautions to prevent the spread of vibrios from the ward, but individual isolation technics are not practiced. Among 1,722 admissions during a 25-month period, 804 cases of cholera were confirmed bacteriologically. Thus, 918 noncholera patients were managed side-by-side with the infected group discharging large numbers of vibrios. No noncholera patient acquired infection while in hospital. (2) It is customary in Dacca for family members to stay in the hospital to serve as attendants. Usually extra beds are not available for such relatives and they often sleep in the same bed as the patient. There was no evidence that any family attendant acquired infection on the ward. (3) Approximately 20 persons serve daily in the hospital as orderlies, ward attendants, and laundrymen. They are in direct contact with large volumes of soiled materials containing many vibrios. None of these ancillary personnel acquired cholera.

It has become generally accepted that cholera is chiefly a waterborne disease and is acquired by drinking contaminated water. The continued endemicity of cholera in Bengal suggested that its countless rivers and ponds (or tanks) are particularly conducive to proliferation and/or survival of cholera vibrios. Studies in this laboratory have confirmed earlier reports showing that classical cholera vibrios survive in local waters for a short period, usually disappearing within 1 week or less if exposed to sunlight. Survival is prolonged as much as 2 weeks and, in some instances, an increase in number of organisms was observed, if the water was boiled or filtered before the vibrios were added. In these studies there was no evidence that *V. cholerae* underwent a change in serotype to the so called nonagglutinating form, as has been reported (Bhattacharja, 1964); as long as vibrios persisted they remained agglutinable in homologous typing serum. Longitudinal surveillance of tanks and wells in Dacca and examination of water sources and storage facilities used by families of cholera patients showed that vibrios were present only at such times as known cases of cholera existed in the area. It would appear that vibrios vanish from water shortly after recovery or removal of known cases, and reappear only when clinical cholera recurs.

An apparent exception to this rule occurred following a sharp epidemic of cholera among a large number of people housed in cotton mills on the bank of a large river. Cases were seen during the period January 23–February 5, 1964; thereafter no further instances of cholera were reported to the health authorities. However, on periodic bacteriological surveillance of the river, *V. cholerae* Inaba was recovered each week through March 30, 7 weeks later (fig. 1). During this period the river was used as customary for washing, bathing, and filling water jars for household purposes.

The relationship between water use and intrafamilial infection has been under investigation during the last

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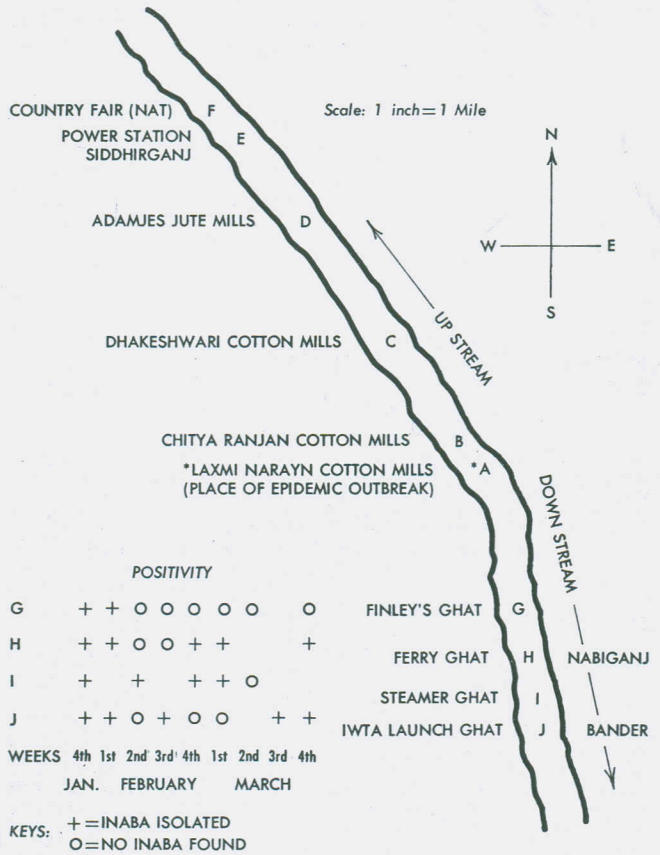

FIGURE 1.—Map of Lakhya River, Narayanganj, showing sampling points under surveillance both upstream and downstream from the point of cholera outbreak in January 1964.

year. Families of CRL-hospitalized patients living in Dacca were selected for study within 2 days of onset of illness in bacteriologically positive index cases. Families were defined as units consisting of two or more persons who lived and ate together. They were observed daily for 10 days by teams comprised of one physician and one health visitor. At the first visit a census was taken and data were collected on housing, water use, and sanitary facilities. Family members were queried daily about the occurrence of diarrhea or any symptom which interfered with usual duties. Rectal swabs were collected daily from all family members. The swabs were placed in tellurite taurocholate peptone enrichment medium and incubated overnight at 37° C. Subcultures were made on gelatin agar and on Monsur's tellurite taurocholate gelatin agar and incubated overnight at 37° C. Suspect colonies were tested for agglutination by specific antisera. Cholera cases were defined as persons with acute diarrheal disease associated with recovery of *V. cholerae* from one or more fecal samples; the term inapparent infection was applied to healthy family members from whom *V. cholerae* was isolated. Water samples too were examined daily: 50 ml. were added to 25 mls. of triple-strength alkaline taurocholate tellurite peptone water; after 6–8 hours' and after 24 hours' incubation at 37° C., respective samples were streaked on gelatin agar and Monsur's medium. These were examined after overnight incubation. In addition, a 0.1 ml aliquot was streaked directly on TTGA in the field to measure the degree of contamination of water from dug wells, tanks, and rivers or canals. Suspect colonies were identified by usual methods.

Forty-two families and their water supplies were studied. The available water sources included: (1) intermittent municipal supply at roadside taps, with flow during two intervals each day; (2) shallow dug wells in courtyards close to the house; (3) deep driven tube wells which serve a number of households; and (4) tanks (or ponds) or rivers or canals. *V. cholerae* was frequently present in water samples collected from dug wells, tanks, and rivers. Vibrios were never found in 136 specimens obtained from the municipal supply or from 179 tube wells (table 1).

TABLE 1.—Bacteriologic findings in water sources used by families of cholera patients during 1964, Dacca, East Pakistan

Water source	Number examined	Number positive for *V. cholerae*	Percent positive for *V. cholerae*
			Percent
Municipal supply	136	0	0
Dug well	150	18	12.0
Tube well	179	0	0
Tanks, rivers, canals	147	32	21.8

Vibrio cholerae infections other than the index case were observed in 17 of the 42 families. The water utilization of these families did not significantly differ from that of the families without multiple infections (table 2).

TABLE 2.—Water sources used by 42 families with cholera, Dacca, East Pakistan, 1964

Water source	Families with multiple infections	Families with index case only	Total
Municipal supply	10	13	23
Dug well	11	13	24
Tube well	6	17	23
Tanks, rivers, canals	2	9	11

The frequency distribution of infection in family members by first appearance of positive culture and the presence of *V. cholerae* in water samples was similar. There was no secondary wave of infections which might have

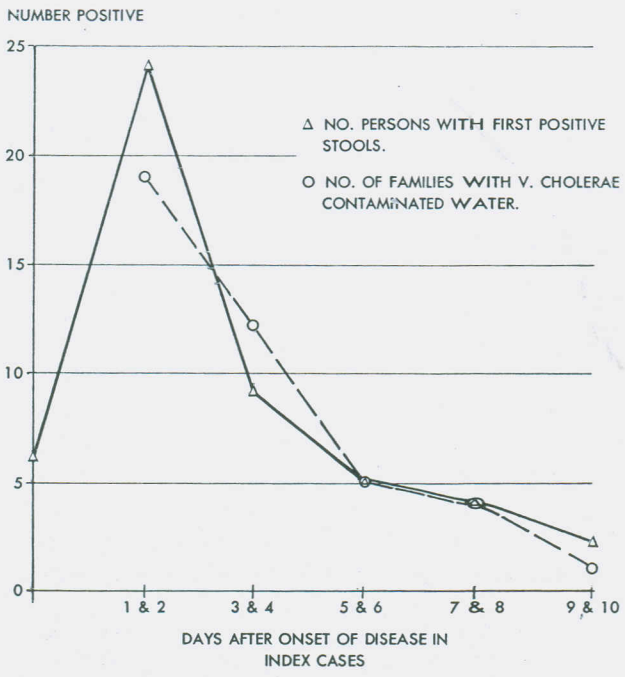

FIGURE 2.—First positive *V. cholerae* culture in secondary cases and *V. cholerae* contamination of family waters.

been related to contamination of water early in the study interval (fig. 2). Vibrios were found more frequently and over a longer period among families with several infected individuals than among those without subsequent intrafamilial infection (table 3). In 14 of 17 households in which other members became infected, household water supplies generally used for washing and bathing were positive; and, conversely, when no further infections were observed samples tended to be negative. Some of these infections may have resulted from exposure to contaminated water. However, the presence of vibrios in

TABLE 3.—Presence of *V. Cholerae* in water used by 42 families with cholera

Days after onset of disease in index case	Number of families with positive water		
	Multiple infections	Families with index case only	Total
1–2	12	7	19
3–4	9	3	12
5–6	5	0	5
7–8	3	1	4
9–10	1	0	1
Total positive at any time	14	9	23
Total families	17	25	42

TABLE 4.—Reported water used in 17 families with multiple cholera infections

	Number of families using water for—			
	Washing and bathing	Drinking	Cooking	Not used
Municipal supply	10	10	9	7
Dug well	11	2	3	6
Tube well	6	5	6	11
Tanks, rivers, canals	2	.....	.....	15

dug well water may have been secondary to pollution by a large number of infected individuals by bathing, clothes washing, and surface runoff from latrines. The riddle of which came first cannot be answered unequivocally with the information now available. Analysis of the specific water habits of the 17 families with multiple infection (table 4) revealed that only 2 of the 17 used dug well water for drinking, and only 3 stated that they prepared food with dug well water. Thus, multiple intrafamilial infection was not associated with drinking and cooking water shown to contain vibrios.

Since endemic infection in these East Pakistan families does not seem to be due to drinking water containing *V. cholerae,* one must turn to food as a possible infecting vehicle. Contaminated water could serve to inoculate foodstuffs in which vibrios can multiply to achieve an infective dose or to elaborate some accessory factor necessary for establishing disease. Accordingly, our interest has been directed toward rice, the main staple of the local diet, as a possible infecting medium. The cholera-prone population is poor; fuel is short; and facilities for refrigeration do not exist. Most foods are prepared daily and consumed completely, but a single batch of rice often serves for the evening meal and breakfast the following day. Leftover rice is covered with water after the evening meal and remains at ambient temperature until completely consumed. This form of rice, known as "panta bhat" (water rice), is characteristic of Bengal. When these conditions were simulated in the laboratory and the added water was inoculated with cholera vibrios, the organisms frequently multipled 1,000-fold or more (table 5). Such multiplication was consistently observed when 0.1–0.5 percent sodium chloride was added. Growth occurred in the absence of salt, but not invariably. Multiplication of vibrios in these experiments appeared to depend in part on the type of rice employed. In 7 of 16 instances, organisms were not recovered 4 hours after they had been added to cooked "boro" rice. "Boro" is a type of rice which is harvested in the spring and is higher in

TABLE 5.—Effect of salt and rice variety on vibrio multiplication in panta bhat

Quality of rice	Change in titer after 8–12 hours	No added salt		0.1 or 0.5 percent salt added	
		Rice boiled with excess water	Rice boiled with minimal water	Rice boiled with excess water	Rice boiled with minimal water
Boro	Fall	8(5)	6(2)	.....	.....
	Rise $\times 10^1$	.....	1	.....	.....
	Rise $\times 10^3$	.....	1	.....	.....
Aus	Fall	3	4	.....	.....
	Rise $\times 2$	1	1	.....	.....
	Rise $\times 10^1$	.....	1	.....	.....
	Rise $\times 10^2$	.....	.....	1	1
	Rise $\times 10^3$	.....	.....	3	1
	Rise $\times 10^4$	.....	.....	1	7

( ) No vibrios recovered after 4 hours.

price than other varieties. The highest bacterial counts were obtained with "aus" rice, which is harvested in the early fall and is the cheapest variety available. However, the use of "panta bhat" does not necessarily imply exposure to cholera; contamination of rice by other common bacteria which reduce the pH may make vibrio growth in rice impossible.

If cholera infection is spread by contaminated food, the pattern of appearance of cases in families should be informative. Fifty Dacca families were observed as described above for a 10-day period after the index case was hospitalized at CRL. Subsequent infection with *V. cholerae* was observed in 22 of these 50 families. Fifty infections occurred; two-thirds were symptomatic; and infections

TABLE 6.—Secondary cases in 50 cholera families, by age and symptom status, Dacca, East Pakistan

Age group	Number with positive cultures		Total	Number at risk	Percent infected
	Symptoms				
	Present	Absent			
Under 15	23	11	34	139	24.5
15 and over	10	6	16	155	10.3
Total	33	17	50	294	17.0

TABLE 7.—Day of first appearance of cholera vibrios among secondary cases

Day after onset of disease in index case	Number becoming positive	Cumulative percentage
Same day	6	12
1–2	24	60
3–4	9	78
5–6	5	88
7–8	4	96
9–10	2	100
Total	50	100

were concentrated among those under 15 years of age (table 6). Of the 50 infected individuals 39 became bacteriologically positive within 4 days after the onset of symptoms in the index case (table 7). This pattern is consistent with common-source infection. The following family outbreak is illustrative (fig. 3).

These results are similar to those obtained in the preceding year, when 57 subsequent cases of cholera occurred in 33 of 85 families from which a cholera patient has been hospitalized (Pakistan-SEATO Cholera Research Laboratory, in press). They contrast with the experience in other countries in southeast Asia.

In the 1958 Thailand epidemic of classical cholera, multiple cases occurred in only 30 of 1,024 studied households (Pradith Siddhichai and Grayston, 1960). In the 1961 Hong Kong outbreak, caused by El Tor strains, all 76 primary cases were sporadic and none could be traced to a previous primary case (MacKenzie, 1961). Dizon (1962) reported second cases in the families of only 6 of 96 consecutive El Tor cases in the Philippines in 1962, but 58 percent of one group of studied households had at least one other infected person in the household, based on rectal swab studies; Wallace and others (1964) found other cases in only 1.9 percent of 264 cholera families in Manila in 1961. In the Taiwan outbreak of 1962, Yen (1964) observed secondary cases in only 1 percent of households, but there was at least one symptomless carrier in approximately 24 percent of households in which there had been an active case.

We remain ignorant of any predisposing conditions or physiological states which may be necessary for the appearance of the symptoms of cholera, but physiological aberrations confined to a relatively restricted geographic area within one part of the world become increasingly unlikely. We believe that infection by cholera vibrios requires exposure to a relatively large number of organisms.

Family 64–82

Individual number	Relation to head of household	Age (years)	Sex	First appearance of *V. cholerae* infection				
				Nov. 25	Nov. 26	Nov. 27	Nov. 28, 29, 30	Dec. 1, 2, 3, 4
1	Head	55	M		+Sick (0500)			
2	First wife	40	F					
3	Daughter	19	F					
4	.....do	17	F					+Carrier.
5	.....do	15	F					
6	.....do	13	F		0	+Carrier		
7	.....do	6	F	+Sick (1400)				
8	Son	8	M		0	+Carrier		
9	Second wife	25	F					
10	Daughter	6 mos.	F					
11	Niece	26	F					
12	Grand nephew	6	M		+Carrier			
13	Grand niece	5	F	Index+sick (0400)				
14	Grand nephew	4	M		0	+Carrier		
15	Grand niece	2 mos.	F					
16	Employee	14	M					
17	.....do	12	M		+Carrier			
18	.....do	14	M	+Sick (1100)				
19	Daughter	10	F		0	+	+Sick (0800)	
20	Aunt	50	F					

FIGURE 3.—Pattern of cholera in one family.

An adequate infectious dose is most easily obtained by eating a food which has served as a good culture medium for the vibrios. Epidemiological differences might not be due to differences in immunological susceptibility, physiological alterations, or temperature and humidity of the area, but may be based on culture differences in preparing and handling foods. In areas where the more common foods can effectively support vibrio growth, so that there develops a high vibrio count evenly distributed, all diners are offered a relatively high and uniform challenge dose resulting in multiple cases within a hearth group. Where organisms multiply on surfaces, such as on the Hong Kong meat blocks, or on the food itself, the organisms may be presented only at subinfectious levels, or the infectious level is achieved erratically, so that only single members of a family might acquire a dose adequate to produce disease; others, with a lower exposure, may become infected but do not develop overt symptoms. Thus, the different epidemiological patterns can be related to infecting doses, without involving any difference in the virulence or pathogenicity of the infecting organism or in susceptibility of the population. This hypothesis does not discount the importance of varying population susceptibility on an immunological, nutritional, or other basis, or of organism variation, should these be demonstrated.

SUMMARY

The precise manner in which the fecal-oral spread of cholera occurs probably varies from place to place and from time to time. Person-to-person spread has not occurred in our hospital enviroment. Drinking contaminated water has not appeared to be a significant factor in our study population. Preliminary findings are consistent with the hypothesis that endemic cholera in East Pakistan is a variety of food infection in which water, fingers, or other factors may serve as vehicles to inoculate a widely consumed staple, such as rice. These findings point to the need for more intense studies of the relevant culture and habit patterns of the populations afflicted by cholera.

REFERENCES

Bhattacharji, L. M. and Benoy Bose. Indian J. Med. Res. 52: 777, 1964.

Dizon, J. J. Epidemiological Studies on Cholera El Tor. Disease Intelligence Center, Manila, 1962.

Greig, E. D. W. Indian J. Med. Res. 1: 90, 1913.

MacKenzie, D. J. M. Report on the Outbreak of Cholera in Hong Kong, Government Press. 1961, Hong Kong.

Siddhichai, P. and Grayston, J. T. Amer. J. Hyg. 72: 149–161, 1960.

Wallace, C. K., Fabie, A. E., Mangubar, O., Velasco, E., Iuinio, C., and Phillips, R. A. Bull. Wld. Hlth. Org. 30: 995, 1964.

Yen, C. H. Bull. Wld. Hlth. Org. 30: 811, 1964.

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