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A Simple Filtration Method To Remove Plankton-Associated *Vibrio cholerae* in Raw Water Supplies in Developing Countries

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Plankton to which cells of *Vibrio cholerae* O1 and/or O139 were attached was introduced into 0.5‰ Instant Ocean microcosms maintained at 25°C. The bulk of the plankton and associated particulates was removed with a filter constructed from either nylon net and one of several different types of sari material, the latter being very inexpensive and readily available in villages in Bangladesh, where *V. cholerae* is endemic. *V. cholerae* was enumerated before and after filtration to evaluate the efficiency of the filtration procedure. The results obtained indicate that 99% of *V. cholerae*, i.e., those cells attached to plankton, were removed from the water samples. Epidemic strains of *V. cholerae* O1 and O139 from various geographical sources, including Bangladesh, Brazil, India, and Mexico, were included in the experiments. Removal of vibrios from water by this simple filtration method was found to yield consistent results with all strains examined in this study. Thus, it is concluded that a simple filtration procedure involving the use of domestic sari material can reduce the number of cholera vibrios attached to plankton in raw water from ponds and rivers commonly used for drinking. Since untreated water from such sources serves as drinking water for millions of people living in developing countries (e.g., Bangladesh), filtration should prove effective at reducing the incidence and severity of outbreaks, especially in places that lack fuel wood for boiling water and/or municipal water treatment plants. The results of this study provide the basis for determining such reductions, which are to be carried out in the near future.

Cholera is endemic in countries of the world where socioeconomic conditions are poor, sanitary systems and public hygiene are rudimentary, and safe drinking water is not available, especially during floods. Such situations are even more hazardous to human health in countries in which fuel for boiling water is in scarce supply, such as in Bangladesh and other developing countries of that region of the world. Cholera is an epidemic disease in many of these countries, including Bangladesh. In 1991, an epidemic of cholera was responsible for an estimated 8,000 deaths in a 12-week period (23). Cholera occurs worldwide, and in 1992 to 1993, 800,000 cholera cases were reported by 21 countries in the Western Hemisphere, mostly in coastal areas, with more than 8,000 of the cases resulting in death (26). In July 1994, 14,000 deaths from cholera were reported in refugee camps in Rwanda (22). It has long been known that cholera is a waterborne disease; i.e., the infectious agent, *Vibrio cholerae*, is transmitted via water (8). Furthermore, it is now established that *V. cholerae* O1 and O139 strains, which are both capable of producing epidemic cholera, are autochthonous to the aquatic environment (2, 5, 6, 11), with surface water being implicated in the transmission of epidemic cholera in Bangladesh (9).

Filtering water at the time of collection and just before drinking is a successful means of removing cyclops, a planktonic crustacean copepod, vector of the guinea worm, which causes dracunculiasis. The crustacean cyclops-associated worm is removed with polyester cloth, and this is now recommended as an effective method of preventing dracunculiasis, a life-threatening disease common in Africa (27). Although the boiling of water prior to drinking it will kill cyclops and guinea worm larvae, as well as pathogenic microorganisms, it is a time-consuming procedure and expensive as well, especially in a country like Bangladesh, where fuel wood is in very short supply. Furthermore, the boiling of water is not socially acceptable to the inhabitants of most rural villages in Africa (27), much like the situation in Bangladesh. Moreover, during severe flooding, which occurs every year, some areas of Bangladesh experience deterioration of conditions to those of mere survival; i.e., even the barest necessities become difficult to obtain, and building a fire to boil water is simply not possible.

The association of vibrios, specifically, *V. cholerae*, with phytoplankton (16) and zooplankton (12, 14, 24) has been studied extensively in our laboratory for the past 25 years. The accumulated data show that planktonic copepods play a major role in the multiplication, survival, and potential transmission of cholera (3, 4, 7, 10). It has been determined on the basis of human volunteer trials that, depending on the health of a given individual, the ingestion of ca. 10^4 to 10^6 *V. cholerae* O1 organisms is likely to produce clinical cholera (1). It is evident from our previous work that 1 to 10 copepods can harbor enough *V. cholerae* cells to produce clinical symptoms of cholera in a susceptible host (3). Copepods are a dominant group of the zooplankton community of aquatic organisms in many aquatic systems, both fresh- and saltwater. During the summer, phytoplankton blooms occur in Bangladesh, followed by zooplankton blooms in both September and October. Cholera

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TABLE 1. Strains employed in this study

V. cholerae strain	Biotype	Serogroup	Year of isolation	Source
ATCC 14035	El Tor	O1	Unknown	Clinical specimen, ATCC[a]
569B (ATCC 25870)	El Tor	O1	Unknown	Clinical specimen, ATCC
332	Unassigned	O1	1992	Clinical specimen, Mexico (J. A. K. Hasan)
455	Unassigned	O1	1992	Clinical specimen, Mexico (J. A. K. Hasan)
CT7021	El Tor	O1	1994	Sewage, Brazil (M. Martins)
CT7649	El Tor	O1	1993	Sewage, Brazil (M. Martins)
CT7606	El Tor	O1	1993	Sewage, Brazil (M. Martins)
CTMARMI2	El Tor	O1	1992	Sewage, Brazil (M. Martins)
HH330	Unassigned	O139	1993	Clinical specimen, India (G. B. Nair)
34003	Unassigned	O139	1993	Clinical specimen, Bangladesh

[a] ATCC, American Type Culture Collection.

outbreaks almost always follow a plankton bloom (11). The majority of the members of the zooplankton population are crustaceans, which are known to support the attachment of vibrios. Furthermore, they demonstrate a seasonal distribution, both in size of individuals and in species composition (17, 18). Recently, several investigators have reported the presence of *V. cholerae* in cargo ship ballast water, suggesting the occurrence of international dissemination of *V. cholerae* via aquatic organisms carried in ballast water that is discharged in harbors remote from the original source of the water (19). Our work on ballast water shows that *V. cholerae* cells are attached to copepods in the ballast water of ships arriving in Baltimore, Md., from ports of origin elsewhere in the world. The major component of ballast water plankton is copepods (21a). Thus, zooplankton offer a means of global transport of *V. cholerae* (unpublished data).

Taking into consideration all of the findings described above, we have hypothesized that a simple and inexpensive filtration method to sieve out plankton to which *V. cholerae* is attached in raw water supplies, such as ponds, rivers, and other natural water supplies, could be an effective way to curb or, at the least, reduce the number of cholera epidemics if the number of *V. cholerae* organisms per volume unit of drinking water could be reduced below the potential infectious dose. The results of experiments reported here suggest that simple filtration, in fact, may be a useful practice to institute, since it is affordable to even the poorest of the poor, to reduce the incidence of cholera, especially during extreme weather conditions, e.g., cyclones, floods, and monsoons.

MATERIALS AND METHODS

Laboratory microcosm experiments. Planktonic copepods, phytoplankton, and water samples were collected at different sites, including the Little Patuxent River, Sunderland, Md.; Chesapeake Bay, Solomon's Island, Md.; a duck pond in Hyattsville, Md.; and a freshwater pond in College Park, Md. Also, samples of water and plankton were collected in Matlab, Bangladesh. Plankton tow nets with mesh sizes of 20 to 100 μm were used to collect plankton. Laboratory microcosms, with and without plankton, to which *V. cholerae* O1 or O139 was added were used in different sets of experiments (described below). All experiments were repeated at least twice and were always done in duplicate. Plankton was prepared for testing by washing with filter-sterilized 0.5‰ Instant Ocean (IO) (Aquarium Systems, Mentor, Ohio) and was acclimatized prior to addition of *V. cholerae.*

V. cholerae isolates of different geographic origin and from a variety of sources (Table 1) were grown in 250 ml of Luria-Bertani (LB) broth in 1-liter flasks incubated at 35°C on a shaker (150 rpm). The cells were concentrated by centrifugation at 3,000 × *g* for 15 min at 20°C, washed twice in IO, resuspended in 250 ml of sterile IO, and incubated at 25°C for 18 h to ensure starvation (21). This step (i.e., starvation) ensured that *V. cholerae* cells attached to plankton would be retained on the filter, and it also allowed the determination of whether starved cells (which are smaller in size) would remain attached and/or whether they would pass through the filter more readily if they were not attached or if they had detached because of starvation. Plankton collected from the different sources cited above was washed with 4 liters of sterile IO, collected by filtration through a nylon net with a mesh size of 20 or 64 μm, and resuspended in 250 ml of sterile IO.

Starved cells of *V. cholerae* were added to washed plankton to a final concentration of 10^8/ml. The mixtures of plankton and *V. cholerae* were incubated at 25°C for 18 h to allow attachment of *V. cholerae* to the plankton. After incuba-

TABLE 2. Counts of *V. cholerae* organisms attached to plankton in raw water samples after filtration through layers of sari material and nylon net

Filter material[a]	*V. cholerae* viable count[b] (CFU/ml ± SD) and % count reduction determined on:			
	LB agar		TCBS agar	
	Count	% Reduction	Count	% Reduction
None	$(9.10 \pm 0.22) \times 10^4$	NA[c]	$(6.40 \pm 0.24) \times 10^3$	NA
N-1	$(7.18 \pm 0.23) \times 10^2$	99.21	$(1.00 \pm 1.00) \times 10^1$	99.84
S-1	$(6.93 \pm 0.41) \times 10^2$	99.23	$(1.00 \pm 1.00) \times 10^1$	99.84
S-2	$(4.98 \pm 0.64) \times 10^2$	99.45	$(1.00 \pm 1.00) \times 10^1$	99.84
S-4	$(4.55 \pm 0.54) \times 10^2$	99.50	<10	99.99
S-6	$(4.75 \pm 0.34) \times 10^2$	99.48	$(1.25 \pm 0.50) \times 10^1$	99.80
S-8	$(2.38 \pm 0.42) \times 10^2$	99.74	$(1.50 \pm 1.00) \times 10^1$	99.77

[a] None, prefiltration; N, nylon net; S, sari material; numbers refer to the number of layers of material (i.e., folds).
[b] Viable count in filtrate (not retained by filter).
[c] NA, not applicable (control).

TABLE 3. Efficiencies of nylon net and different kinds of sari material employed as filters for raw water (containing plankton to which *V. cholerae* had attached)

Filter material[a]	*V. cholerae* viable count[b] (CFU/ml ± SD) and % count reduction determined on:			
	LB agar		TCBS agar	
	Count	% Reduction	Count	% Reduction
None	$(1.35 \pm 0.10) \times 10^5$	NA[c]	$(8.00 \pm 1.10) \times 10^4$	NA
Green sari	$(5.70 \pm 0.30) \times 10^3$	95.78	$(2.90 \pm 0.60) \times 10^3$	96.38
Blue sari	$(2.98 \pm 0.25) \times 10^3$	97.79	$(6.80 \pm 0.06) \times 10^2$	99.15
Red sari	$(4.08 \pm 0.48) \times 10^3$	96.98	$(1.76 \pm 0.28) \times 10^3$	97.80
White sari	$(3.70 \pm 0.35) \times 10^3$	97.26	$(1.08 \pm 0.35) \times 10^3$	98.65
Nylon net	$(4.75 \pm 0.30) \times 10^3$	96.48	$(2.50 \pm 1.35) \times 10^3$	96.88

[a] None, prefiltration. Four folds of cloth were used for all materials except nylon net. Different colors represent different levels of coarseness of weave; the green sari in this experiment was the coarsest, followed by blue and red, and the white sari material was the finest.
[b] Viable count in filtrate.
[c] NA, not applicable (control).

TABLE 4. Efficiencies of removal of plankton-associated *V. cholerae* by filtration through sari and nylon material relative to source (clinical versus environmental) and serogroup (O1 versus O139)

V. cholerae strain (serogroup) and filter material	*V. cholerae* viable count[a] (CFU/ml ± SD) and % count reduction determined on:			
	LB agar		TCBS agar	
	Count	% Reduction	Count	% Reduction
ATCC 14035 (O1)				
Control	$(9.10 \pm 1.22) \times 10^4$	NA[b]	$(6.40 \pm 0.41) \times 10^3$	NA
Nylon net	$(7.18 \pm 0.72) \times 10^2$	99.21	$(1.00 \pm 1.00) \times 10^1$	99.84
Sari	$(4.98 \pm 0.26) \times 10^2$	99.45	$(1.00 \pm 1.00) \times 10^1$	99.84
569B (O1)				
Control	$(3.35 \pm 0.93) \times 10^3$	NA	$(2.05 \pm 0.57) \times 10^2$	NA
Nylon net	$(5.50 \pm 0.15) \times 10^1$	98.36	$(5.00 \pm 0.50) \times 10^0$	97.56
Sari	$(3.00 \pm 0.55) \times 10^1$	99.10	$(1.00 \pm 1.00) \times 10^1$	95.12
CT7021 (O1)				
Control	$(2.57 \pm 1.38) \times 10^4$	NA	$(4.37 \pm 3.47) \times 10^3$	NA
Nylon net	$(3.80 \pm 0.30) \times 10^2$	98.52	$(2.10 \pm 0.15) \times 10^2$	95.19
Sari	$(2.62 \pm 0.67) \times 10^2$	98.98	$(1.50 \pm 1.50) \times 10^1$	99.66
CT7649 (O1)				
Control	$(1.15 \pm 0.23) \times 10^4$	NA	$(2.75 \pm 0.65) \times 10^3$	NA
Nylon net	$(3.23 \pm 0.13) \times 10^2$	97.19	$(9.25 \pm 2.25) \times 10^1$	96.64
Sari	$(3.58 \pm 1.97) \times 10^2$	96.89	$(3.25 \pm 1.25) \times 10^1$	98.82
CTMARMI2 (O1)				
Control	$(3.87 \pm 0.98) \times 10^5$	NA	$(3.22 \pm 0.43) \times 10^4$	NA
Nylon net	$(2.39 \pm 0.22) \times 10^3$	99.38	$(1.45 \pm 0.02) \times 10^3$	95.50
Sari	$(1.33 \pm 0.03) \times 10^3$	99.99	$(4.97 \pm 0.57) \times 10^2$	98.46
CT7606 (O1)				
Control	$(9.30 \pm 2.30) \times 10^4$	NA	$(4.03 \pm 1.70) \times 10^4$	NA
Nylon net	$(2.18 \pm 0.04) \times 10^3$	97.66	$(1.11 \pm 0.32) \times 10^3$	97.25
Sari	$(1.61 \pm 0.21) \times 10^3$	98.27	$(1.04 \pm 0.30) \times 10^3$	97.42
332 (O1)				
Control	$(1.25 \pm 0.07) \times 10^5$	NA	$(3.78 \pm 0.78) \times 10^4$	NA
Nylon net	$(2.50 \pm 0.27) \times 10^3$	98.00	$(9.63 \pm 1.32) \times 10^2$	97.45
Sari	$(2.31 \pm 0.51) \times 10^3$	98.15	$(8.25 \pm 1.95) \times 10^2$	97.82
455 (O1)				
Control	$(2.10 \pm 0.65) \times 10^4$	NA	$(4.37 \pm 1.37) \times 10^3$	NA
Nylon net	$(2.70 \pm 0.75) \times 10^2$	98.71	$(6.75 \pm 0.25) \times 10^1$	98.46
Sari	$(1.60 \pm 0.55) \times 10^2$	99.24	$(3.75 \pm 3.75) \times 10^1$	99.14
HH330 (O139)				
Control	$(7.25 \pm 1.15) \times 10^4$	NA	$(1.81 \pm 0.76) \times 10^4$	NA
Nylon net	$(1.09 \pm 0.29) \times 10^3$	98.50	$(4.65 \pm 0.95) \times 10^2$	97.43
Sari	$(1.09 \pm 0.32) \times 10^3$	98.50	$(3.92 \pm 1.47) \times 10^2$	97.83
34003 (O139)				
Control	$(1.33 \pm 0.19) \times 10^4$	NA	$(6.55 \pm 0.52) \times 10^3$	NA
Nylon net	$(1.35 \pm 0.74) \times 10^2$	98.98	$(5.50 \pm 0.38) \times 10^1$	99.16
Sari	$(4.50 \pm 0.26) \times 10^2$	96.62	$(2.40 \pm 0.18) \times 10^2$	96.34

[a] Counts for controls are prefiltration counts; counts given for nylon net and sari material are the counts of the filtrate (postfiltration, with plankton removed).
[b] NA, not applicable (control).

tion, the suspensions of plankton (90% copepods, as determined by direct microscopic count) were collected by filtration through nylon nets of appropriate mesh size and washed with 4.0 liters of sterile IO at 25°C (room temperature). Equal amounts (by volume) of the bacterium-carrying plankton were inoculated into 250-ml flasks containing 200 ml of sterile IO.

V. cholerae cells which had attached to plankton in the microcosms were filtered through simple filters consisting of folds of autoclaved sari material or nylon net. The sari is worn by village women in Bangladesh and is usually made of 100% cotton, which is approximately 5 to 6 yards of unstitched, flat cloth. The particular variety worn by villagers is usually made of a coarse material, the majority of which comes in a blue or green color. The filtration experiments were done with filters consisting of different layers, i.e., multiple folds of each type of filtering material. Plate counts of *V. cholerae* cells in the filtrates and, similarly, in each microcosm were determined. Plate counts of filtrate and of homogenized plankton separated from the 250-ml IO suspension were obtained by previously described methods (14) by using a Teflon-tipped tissue grinder, and then 0.1-ml aliquots of appropriate dilutions were spread onto both thiosulfate-citrate-bile salt-sucrose (TCBS) agar and LB agar.

Attachment of *V. cholerae* to plankton. A variety of sari materials differing in quality was obtained from village women in Bangladesh, and nylon net material with a maximum pore size of 200 μm was obtained from a commercial source (a gift from Precision Fabrics Group, Inc., Greensboro, N.C.).

Samples of plankton were exposed to 10^8 cells of starved *V. cholerae* per ml for 18 to 20 h, as described above. Starved cells were employed to simulate the state of the cells in the natural environment as closely as possible. Plankton-associated cells that remained culturable were counted on agar media, as described above.

TABLE 5. Comparison of attachment of *V. cholerae* to plankton with diameters of ≥64 μm and those with diameters of ≥20 μm but <64 μm[a]

Microcosm	Diameter of plankton collected	*V. cholerae* viable counts (CFU/g [wet wt] of plankton ± SD) determined on:	
		LB agar	TCBS agar
A	≥64 μm	$(1.34 \pm 0.25) \times 10^9$	$(9.19 \pm 0.70) \times 10^7$
B	≤64 μm but ≥20 μm	$(8.97 \pm 0.09) \times 10^6$	$(5.99 \pm 0.85) \times 10^5$
C	Total (A + B)	$(1.86 \pm 0.10) \times 10^9$	$(1.58 \pm 0.95) \times 10^8$

[a] Plankton samples were collected by using a net with a mesh size of 20 or 64 μm. A 64-μm-mesh plankton net was used to separate ≥64-μm-diameter plankton. The plankton obtained by using the 64-μm-mesh net yielded predominantly copepods, as observed directly under the microscope. All plankton samples were washed with 4 liters of 0.5‰ sterile IO. Equal amounts, by wet weight, of plankton collected by filtration of raw water samples were resuspended in two separate microcosms, each containing 400 ml of 0.5‰ sterile IO. A third microcosm contained plankton with diameters between the two mesh sizes. All plankton suspensions were inoculated with *V. cholerae* (see Materials and Methods) to a final concentration of 10^8 cells per ml. All microcosms were incubated at room temperature for 18 to 20 h. Plankton to which *V. cholerae* cells were attached were collected with a nylon net filter, washed with 0.5‰ sterile IO, resuspended in 10 ml of 0.5‰ sterile IO, and homogenized, and the viable number of plankton-associated *V. cholerae* cells was then determined.

Decontamination of filtering device between experiments. Laboratory experiments were done to determine the optimal conditions for decontamination of the filter material after each filtration to remove plankton, with the objective of reducing and preventing bacterial carryover if possible. Several types of treatment were employed, with cost-effectiveness and simplicity of the procedure always being kept in mind, e.g., air drying in the shade, air drying by exposure to sunlight, or washing with a disinfectant, e.g., chlorine bleach or UltraKleen (Sterilex Corporation, Narberth, Pa.). UltraKleen has been approved by the U.S. Department of Agriculture for food handlers, hard surfaces, and mechanical cleaning involved in preparing food and drink for human consumption.

The sari material and nylon net were decontaminated by several procedures described as follows. Contaminated sari materials and nylon net were spread out to dry in the shade for 24 h at 25°C, followed by exposure to direct sunlight for 2 h at 25°C or soaking in a detergent for 30 min. All treated filters were placed in 100 ml of alkaline peptone water and incubated at 35°C for 48 h to allow the growth of any *V. cholerae* present, with subsequent plating onto TCBS agar.

RESULTS AND DISCUSSION

The results obtained from the experiments carried out in this study showed decreased counts of *V. cholerae* cells in water, i.e., removal of *V. cholerae* attached to plankton. Previous studies have shown that an individual copepod will have 10^4 to 10^5 cells of *V. cholerae* attached; this is thus a measure of the carrying capacity of the plankton (3). The bacterial cells attached themselves to the plankton, yielding a 2-log reduction of *V. cholerae* when two or four layers of sari material or a single layer of nylon material was used (Table 2). The nylon material used in this study is identical to that used to eradicate dracunculiasis from Africa and from many countries elsewhere in the world. The counts on LB agar were a log higher than those obtained with TCBS, since TCBS is highly selective for *V. cholerae*. The plankton, although washed several times (see Materials and Methods), was not sterile; hence, growth of bacteria other than *V. cholerae* occurred on LB agar. In addition, by recording the number of yellow colonies of typical morphology appearing on TCBS plates, an assessment was made of the extent of the presence of non-*V. cholerae* cells, as well. The reduction in counts confirmed that attachment to plankton is an important phenomenon in aquatic systems, especially for vibrios. The results obtained by using different kinds of sari material showed no variation related to the type of material in removal of *V. cholerae* attached to plankton (Table 3). Isolates of *V. cholerae* from both clinical and environmental sources showed the same extent of attachment to plankton and the same degree of removal by filtration (Table 4); i.e., there were no significant differences associated with the source (i.e., clinical versus environmental) or the serogroup (i.e., O1 versus O139).

Attachment of *V. cholerae* to zooplankton (i.e., copepods) has been established, with many data now published in several reports (11, 13–15, 24). In fact, it has been suggested that phytoplankton populations will also support growth of *V. cholerae* and serve as attachment sites (16, 25). Therefore, we were interested in assessing whether sari material would be effective in removing *V. cholerae* since the mesh size of sari material not only lacks uniformity but, more importantly, in most cases is too large to retain the noncolonial forms of phytoplankton. Data from the attachment experiments showed that 99% of the *V. cholerae* cells became attached to plankton and particulates (Table 5). Counts of *V. cholerae* in the filtrate were subtracted from prefiltration counts of the water containing plankton and particulates. This provided counts of cells that were not retained by the sari filter, which were considered to be unattached cells of *V. cholerae*. The data clearly indicate that sari material can be highly effective for removal of *V. cholerae* from plankton. However, any *V. cholerae* cells that are unattached or are attached to small planktonic species may pass through the sari filters; they were present but much fewer in number and, thus, less likely to present an infectious dose, as determined by studies with human volunteers (a minimum of 10^4 to 10^6 culturable cells per ml represents an infectious dose) (1). A thickness of four folds of sari cloth is recommended for good efficiency of filtration and of retaining smaller-sized particulates. More than four folds increased the probability of clogging and slow passage of the water without improving the efficiency of removal of plankton and particulates.

It is important to provide a simple method of decontamination of sari material since *V. cholerae* could grow and accumulate in biofilms over a period of time after many filtrations. Chemical or heat treatment is an effective decontamination procedure. However, a practical and feasible method acceptable to rural villagers and, therefore, likely to be practiced is exposure to sunlight. Results obtained in experiments carried out in this study showed that exposure of unfolded sari cloth to direct sunlight for 2 h resulted in complete drying of the cloth (Table 6). This proved to be an effective method for decontamination of the filter material. The approach taken in this study was to find a method that would add no cost to villagers and would be acceptable culturally. Use of Clorox or UltraKleen after filtration to decontaminate sari material would add some cost but would be recommended during monsoon season,

TABLE 6. Decontamination of materials used to filter *V. cholerae*-associated plankton from raw water used as drinking water by rural villagers in areas in which cholera is endemic

Treatment	No. of *V. cholerae*-positive samples per total no. of tested filters composed of:	
	Sari material	Nylon net
None (control)	2/2	2/2
Drying in shade (24 h)	2/2	1/2
Drying in direct sunlight (2 h)	0/3	0/3
Clorox[a] (5.0%)	0/2	0/2
Clorox[a] (2.5%)	2/2	2/2
UltraKleen (0.8%)[a]	0/2	0/2
UltraKleen (0.5%)[a]	0/2	0/2

[a] Filters were soaked for 30 min.

when full sunlight does not occur. However, thorough rinsing with raw water suffices to remove the accumulated plankton, and that, coupled with the second rinse with filtered water, will reduce the risk of ingestion of *V. cholerae* in numbers constituting an infectious dose, especially during plankton blooms, when the concentration of copepods increases significantly. Since each copepod can carry up to 10^5 cells (3), a reduction in the number of *V. cholerae* cells can be critical.

It is important to note that cholera epidemics in Bangladesh occur in April and May and in September and October, in bimodal peaks, characteristically. Plankton blooms generally occur during March and April as well as August and September (17, 20). Thus, the filtration procedure, although valuable year round in a country like Bangladesh, where cholera is endemic, should prove vital at times when plankton blooms occur, with the potential of significantly reducing the scale of a cholera epidemic. Since some of the experiments were done in Bangladesh, using plankton and water collected from rivers in Matlab, Bangladesh, the approach recommended will work. Furthermore, experiments to determine the effectiveness of adding alum to the raw water to enhance the effectiveness of filtration are in progress. It should be possible to institute a very simple, cost-effective, and efficient method of sari cloth filtration that can be as effective at reducing or eliminating cholera epidemics as the method that has been successfully implemented in Africa to eradicate dracunculiasis.

It is concluded from this study that, when attached to plankton, both *V. cholerae* O1 and O139, which are responsible for causing cholera epidemics, can be removed from water by simple filtration through domestic sari cloth, a material which is readily available in every household in Bangladesh, during flooding and monsoons, which are characteristically followed by the development of plankton-enriched waters favoring the growth of *V. cholerae*. Simple methods for public health improvement, when rooted in scientific information and scientifically validated procedures, can prove highly effective, at least until sophisticated methods for water purification at the community level can be instituted. For countries like Bangladesh, such a prospect is, sadly, at least a decade away, whereas simple filtration can be implemented immediately.

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