

Water Studies In Connection With Cholera Epidemics

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SINCE Snow's discovery in 1855, water has been regarded as the main cause of the epidemicity of cholera ; and since the deltaic region of East Pakistan has long been an endemic area, it is believed that water has been the cause of the spread of the disease there. Much work has been done in the past on the isolation of *V. cholerae*, its viability, and its survival in water, but none of this work proves the contention that water serves as a permanent seed-bed for cholera vibrios. The results lead to the finding that water only serves as a vehicle which has to be contaminated from a human source in order to initiate an outbreak.

To gain further information, the water sources of East Pakistan, particularly in the endemic areas, are to be thoroughly studied. The establishment of the Pakistan SEATO Cholera Research Laboratory has provided the opportunity, and work has already started in this direction.

Common water sources in East Pakistan are rivers, tanks, and less frequently open dug wells. These are prone to contamination and are used for all purpose, even in places where safe drinking water is available. In southern coastal regions like Khulna and Barisal where river and ground waters (tube wells) are highly saline, tanks are the only source of drinking water.

Our studies have been based on three aspects :—

(1) Studies on the chemical compo-

sition of water to determine the true nature of different waters and seasonal variations in the constituents.

- (2) Bacteriological analysis to determine the degree of pollution, particularly by both cholera and non-cholera vibrios.
- (3) Inoculation of cholera vibrios into different water samples and studies on their survival under different conditions.

For studies 1 and 2 we have selected a particular endemic area where the water sources are of a representative nature and have checked them periodically for surveillance studies, and we have been investigating the water closely associated with actual cholera cases.

Surveillance studies of the chemical nature of water samples reveal that some tanks, particularly in the dry season, indicate a very high pH, which is favourable for the growth of classical cholera vibrios rather than other gastrointestinal organisms. Since recovery of true cholera vibrios from such tank waters has not been possible, even during an epidemic period, the effect of pH alone on *V. cholerae* is not established.

The bacteriological analysis, i.e. the coliform count done under surveillance studies, has also not established any conclusive results. In fact, the coliform count has not followed any pattern, and some tanks have shown changes from

week to week indicating that the level of pollution has been varying throughout. Only the river water has indicated a set pattern, namely, that during the rainy season when the waters become diluted.

The search for classical cholera vibrios in water has not been very encouraging. Even in connection with cases of cholera in individual outbreaks or during an epidemic, true cholera vibrios have rarely been found in water. Non-cholera vibrios have been found throughout with varying frequency, that is to say, during the epidemic times non-cholera vibrios have been isolated more often than during the inter-epidemic periods. The methods used for the isolation of these vibrios have been the same as those adopted for the isolation of these organisms from stool cultures in cholera patients. The possibility that the viability of the organisms in water is too low to permit their isolation has been investigated, and water samples of larger bulk have been collected and filtered through Millipore filters

which were transferred into enrichment media. Other water samples were put straight into enrichment media. Both the procedures have given the same results. So far, nearly 700 samples of water from all sources have been examined, and about 300 vibrios have been isolated of which only 10 were true cholera vibrios. These amounted to 3.3% of the vibrios isolated and 1.4% of the total numbers of water samples examined. Of these 10 classical cholera vibrios, which were all of the Inaba strain, 4 have been found in dug wells of affected households, 1 from drying ditch mud outside an affected household, and 5 from water stored in affected households, with the exception that 1 cholera vibrio was isolated from an adjacent household pitcher, where cholera had not occurred. Of the 300 vibrios isolated 65% from dug well.

The predominating non-cholera vibrios from water samples have fallen into Heiberg Group II. The grouping of the vibrio isolates is as follows :—

Classical <i>Vibrio cholerae</i> (Inaba)		3.3%
Non-agglutinable Vibrios Heiberg Group	I	21.0%
" " " " "	II	60.0%
" " " " "	III	3.2%
" " " " "	IV	0.4%
" " " " "	V	12.0%
Total:—		99.9%

Survival of true cholerae in water.

Studies on the survival of *V. cholerae* mentioned in the literature vary considerably on account of differences in the character of water samples used and differing conditions of the tests. In order to observe the survival of true cholera vibrios in water from tanks and rivers inhabited

by non-cholera vibrios and other enteric organisms, some studies have been done in this laboratory based on viable counts made before and after inoculation of the water, at fixed time intervals.

The results obtained confirm previous findings that, in general, no natural water serves as an enrichment in which multiplication of cholera vibrios can occur.

The tests carried out under varying conditions, both inside and outside the laboratory, indicate that the survival period was less outside, under the atmospheric conditions than inside (maximum 3 and 12 days, respectively). All the waters inoculated had previously been tested for the presence of vibrios and, when found negative, were then inoculated with classical cholera vibrios. One very interesting observation made during this study was that non-cholera vibrios were recovered from water which had been inoculated with an Ogawa strain of *V. cholerae* and which had no vibrio of any kind before this inoculation.

Besides water, cooked rice was also tested after being inoculated with classical cholera organisms. After being boiled with water the rice was freed of the starch concentrate (as is the common practice in households), and the cooked rice was then mixed with tap water and inoculated

with cholera vibrios. Tests again under varying conditions inside and outside the laboratory showed survival for 3 days and less than 1 day respectively. The starch concentrate served as a very good medium for *V. cholerae*, either when neutralized to pH 7.0 or after the addition of 0.1% NaCl. The inoculum was 10^3 organisms per ml., and after 1 day the viable count had risen to 1 to 3×10^8 organisms per ml. This remained almost constant for up to 7 days without any changes in viability or agglutinating properties. This interesting observation gives rise to suspicions that the habit of adding water to cooked rice left over from supper, and to be eaten next morning, is rather risky. If a contaminated water is added to the cooked rice the organisms will have a breeding ground and may cause disease when ingested subsequently. However, more studies are to be undertaken before drawing any final conclusion.