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The detection of cholera vibrios in Calcutta waters: the River Hooghly and canals

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INTRODUCTION

Bengal has long been regarded as the principal endemic centre of cholera in India; indeed Koch (1884) considered it to be the true home of the disease. Year after year, with the coming of the hot weather, outbreaks of varying intensity have occurred among the dense populations in the Calcutta area and these have continued up to the present time. Once an outbreak has occurred the spread of the infection through direct or indirect contact is well understood and contaminated water supplies play the most important role in this. Although the Calcutta Corporation provides a piped supply of purified water, either directly to dwellings or to public stand-pipes, there is a very considerable consumption of raw river, canal or tank water by sections of the city's population for domestic purposes.

There is overwhelming epidemiological evidence to incriminate raw water supplies as the most important vehicle in the spread of infection during cholera epidemic periods, and many bacteriological investigations in recent years (Venkatraman, 1940, 1941; Panja & Ghosh, 1947; Krishnan, 1953; Pillay, Dutta & Rajgopal, 1954; Lahiri, 1954; Roy, 1959) have shown that *Vibrio cholerae* can be isolated from natural waters of the Hooghly River and the canals of Calcutta during the period of high incidence of cholera in the early spring—the beginning of the hot weather. During the inter-epidemic periods few workers have, however, been successful in isolating cholera vibrios from natural waters; indeed there has been a suggestion that true cholera vibrios tend to disappear during these periods but that the vibrios that replace them (vibrios with a different antigenic constitution) are related variants. Pillay *et al.* (1954) examined nearly 1000 samples of the Hooghly waters and isolated *V. cholerae* from ten of the samples collected during the period of maximum incidence of the disease but failed to demonstrate cholera vibrios in any of the samples collected during inter-epidemic periods or at times when the incidence was low, although non-agglutinating (NAG) vibrios were found on many occasions. Taylor & Ahuja (1938), working in Northern India in a non-endemic area and away from any contact with cholera, isolated NAG vibrios belonging to Heiberg's group I (the biochemical group which includes *V. cholerae*) during the hot weather from natural open waters, but during the colder weather the same waters yielded vibrios belonging to Heiberg's groups V and VI only;

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group I vibrios were not found. Venkatraman, Krishnaswami & Ramkrishnan (1941) reported that in a cholera-free area in Southern India they had isolated from open natural waters agglutinating non-haemolytic vibrios 'indistinguishable from true *Vibrio cholerae*'. El Tor vibrios were also isolated from similar sources in the same area.

The suggestions that the NAG vibrios belonging to Heiberg's group I that have been isolated from waters in endemic areas of cholera are examples of specific antigen loss variation and have epidemiological importance, and also the possibility of transmutation of such cholera-like vibrios to true *V. cholerae*, were fully considered by Pollitzer & Burrows (1955). After an exhaustive study of all the evidence available these authorities were unable to accept any as convincing. More recently Korobov & Kolesnikova (1956) have claimed to have isolated, in the laboratory under certain special conditions, true cholera vibrios from cultures of NAG vibrios. These findings have so far not been confirmed by other workers.

In spite of the fact that some of the investigations of the Hooghly waters were spread over several months of the year and that the results had nearly always indicated that *V. cholerae* disappeared from the waters on the termination of the epidemic period, it seemed desirable to re-examine the problem from a somewhat different angle. The present communication is concerned with the bacteriological results of water examinations continued over a period of some 8 months in an attempt to bridge the gap between the epidemic periods. In this investigation it was decided to fix the sampling points for the periodic collection of samples of water only after an intensive study of the detailed statistics related to the distribution of notified and fatal cases of cholera in the environs of Calcutta over the last 20 years. Such sampling points, selected as a result of the information obtained from this study, would then be the most likely points where cholera-infected material could be expected to pollute the water in sufficient concentration to facilitate the isolation of *V. cholerae* from suitably collected specimens of water.

EPIDEMIOLOGICAL AND STATISTICAL STUDY

The city of Calcutta like most other big cities is divided up geographically into a number of administrative units. For many years this system comprised thirty-two wards, but in 1953 a new system of administrative divisions was set up. This was unrelated to the old ward system; the new units, called constituencies, are smaller in size and number eighty. Both systems are used side by side for various administrative purposes.

In a detailed study of the distribution of notified cases and deaths of cholera in the various divisions of the city over a number of years it would have been an obvious advantage to have been able to use the records relating to the constituency system with its smaller units. Owing, however, to the short time it had been in operation it was felt that the information obtained by this method would not give a sufficiently comprehensive picture of the cholera situation, and it was therefore decided to use the old ward system and to study the appropriate records over a period extending from 1938 to 1957. The published figures (*Annual Reports of Calcutta Medical Officer*, 1939–51) of the cholera specific mortality rates did not provide sufficient information, but it was possible through the kindness of the Statistical Department of the Calcutta Corporation to obtain the data required.

The mid-year population of each ward was estimated by the geometric method of estimation for the period of study on the basis of the three census figures of 1931, 1941 and 1951. Every year was considered to start on 1 April and end on 31 March of the following year; the mid-year population was then taken as that of 1 October. The same method of estimating the monthly population of the city was used throughout the study. The annual specific death-rates from cholera per 100,000 of the population in each ward, the monthly death-rates from cholera in the whole city, the quinquennial, decennial and the 20 years' averages (arithmetic means) were calculated and the results were plotted on epidemiological maps in accordance with the ward boundaries marked on the maps. Histograms were drawn on each map to show the average (arithmetic mean) monthly death-rates from cholera per 100,000 of the population of the whole city over the various periods. Maps 1–3 illustrate, by different degrees of shading, the annual death-rates per 100,000 of the population by wards over a 5-, 10- and 20-year period. Map 4 shows the position of the various points selected for the collection of the water samples and their relation to the configuration of the river and canal systems (to be considered later).

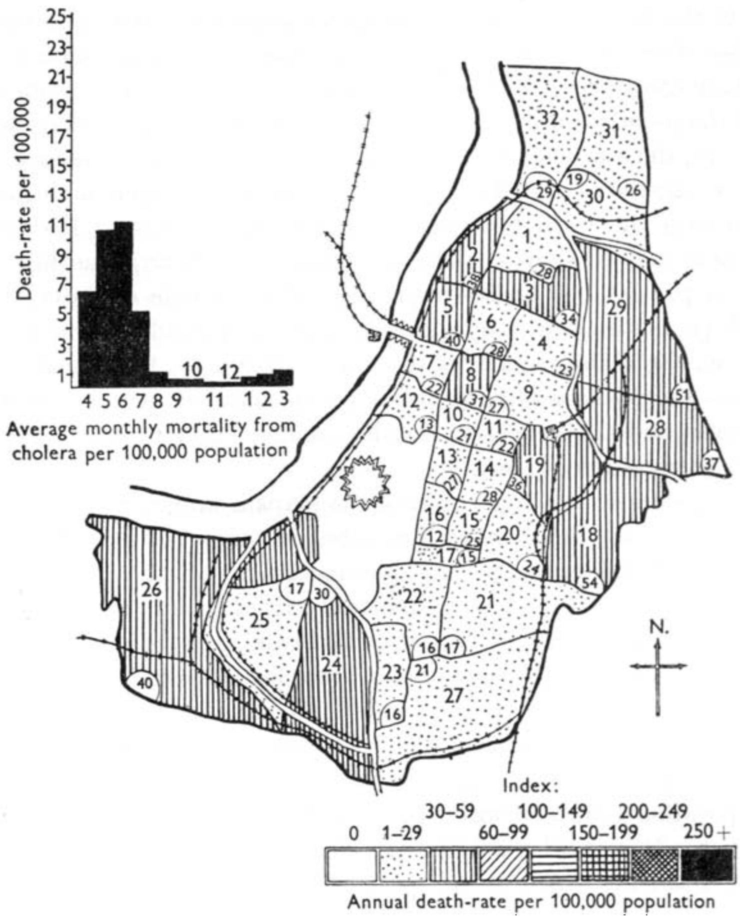
The canal system in the upper part of the maps is known as the Circular Canal and in comparing the death-rates illustrated in maps 1–3 it will be noted that ward 18, which is traversed by the horizontal limb of the Circular Canal, has consistently experienced the highest mortality from cholera among all the wards. Investigation of this locality revealed that this canal, especially in its lower reaches, is much used by the local people to obtain water for domestic purposes as a supplement to the piped supply of purified water. At the same time the banks of this stretch of the canal are used by canal boatmen, moving labourers and homeless people for promiscuous defaecation. To avoid the long waiting at the stand-pipes of the Corporation's purified water or those from the tube-wells the people here readily take the raw canal water for drinking purposes.

Similarly, ward 24, which is traversed by the Chetla Canal (see lower part of map 4) had experienced a high mortality rate from cholera throughout the period and inquiries yielded information of the same order as that obtained in relation to ward 18. Other wards do not call for special mention at this stage.

The histograms accompanying the maps show the peak mortality figures to occur in the month of April, followed by May and then June. In the quinquennial period 1953–57, however, the peak was in June (see map 1). Occasionally a second smaller peak occurs in October and this is shown in map 3 which covers the period 1938–58. It is important to note that the official records showed that over the 20 years studied there was no month in the year in which some deaths from cholera were not reported; this is illustrated in maps 1–3.

SELECTION OF WATER-SAMPLING POINTS

The selection of these sampling points on the Hooghly River and the canals was largely influenced by the results of the epidemiological inquiries outlined above, but in addition by the information about places likely to be heavily contaminated



Maps 1–3. Maps of Calcutta showing the average death-rates per 100,000 from cholera by wards. The approximate rates are indicated by the degree of shading in accordance with the index key included in the map. Each ward can be identified by the centrally placed number and the exact death-rate in each ward is shown within a partial circle at one edge of each ward boundary. The accompanying histogram represents the average monthly mortality for the whole of the city over each particular period. Map 1 covers the 5-year period 1953–58. Map 2 covers the 10-year period 1948–58. Map 3 covers the 20-year period 1938–58.

by man excreting infecting vibrios, bathing places on the river and shore contamination as a result of promiscuous defaecation, by sewage or other means. Sampling points nos. I, II, IV, VI and X (see map 4) are adjacent to bathing ghats and point no. III is the place on the river where the ashes and remains from the burning ghat are discharged. (All dead, including those dying from cholera, are burnt here.) Point no. V, originally chosen as specially important and later found

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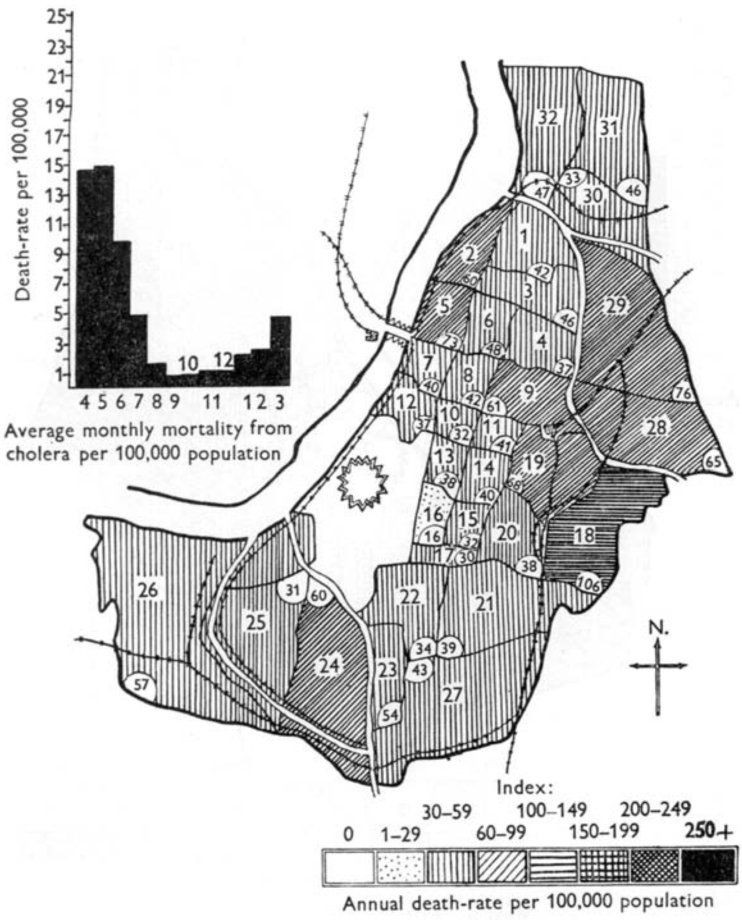
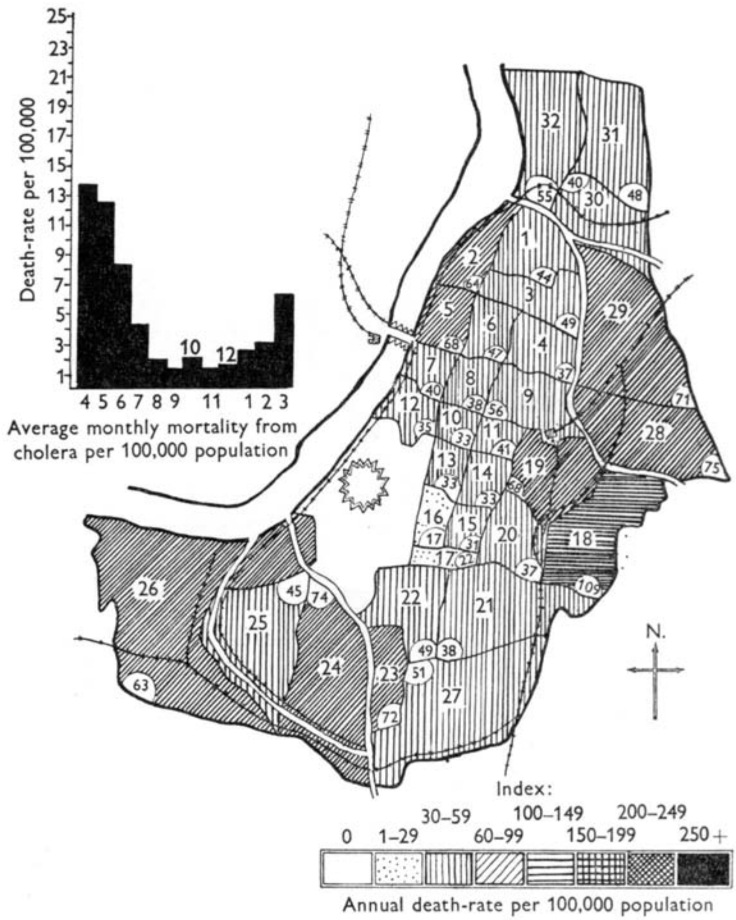
not to be so, served as an additional river point. Nos. VIII and IX on the Circular and Chetla Canals respectively were chosen as suitable points for collection of samples because the shores here were much used by the local population from wards with very high average mortality rates from cholera. Pillay *et al.* (1954) in their cholera studies in the Hooghly estuary observed that the main endemic foci were at the confluence of rivers, and it was decided to check this observation by placing sampling point no. VII at the meeting of the two arms of the Circular Canal.



Map 2. For legend see p. 24.

COLLECTION OF SAMPLES AND BACTERIOLOGICAL METHODS

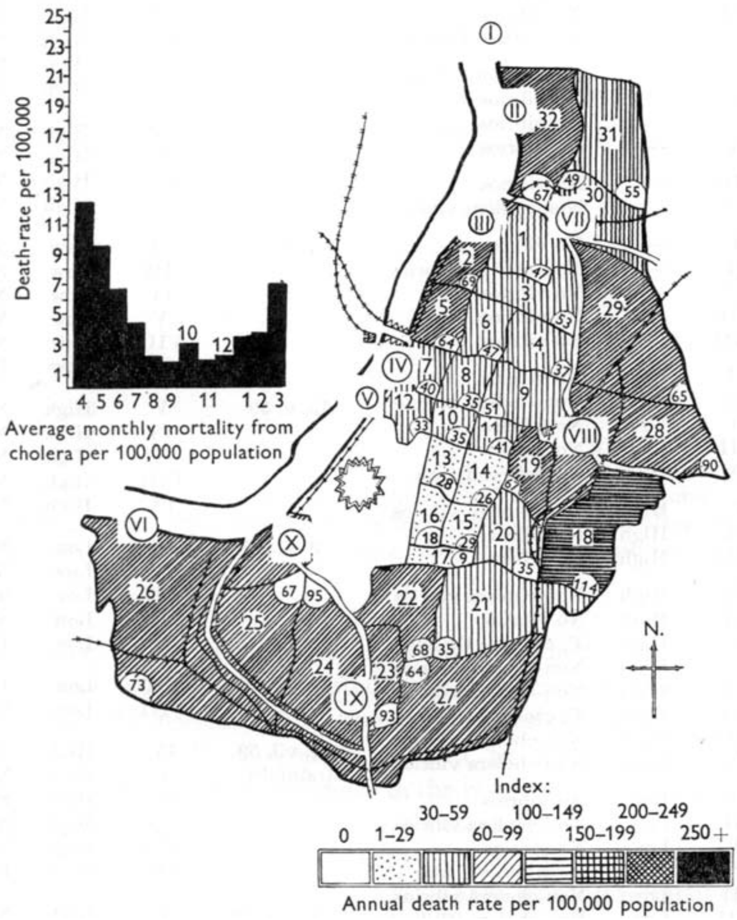
Samples of surface water were collected in $2\frac{1}{2}$ or 5 l. quantities at the designated sampling points between 11 a.m. and 1 p.m. during which times there were large collections of people at the bathing ghats and the canal points were well frequented. The samples were immediately taken to the laboratory and subjected to the Kieselguhr filtration technique recommended by the Joint OIHP/WHO Study Group on Cholera (World Health Organization, 1950). At the beginning of this investigation in December 1958, $2\frac{1}{2}$ l. samples were filtered, but from 9 June 1959 onwards the size of the samples was increased to 5 l. Whenever the water was very turbid with much suspended matter, as during the monsoon period, or contained collections of algae, it was first filtered through sterile absorbant cotton-wool before being passed through the Kieselguhr filter-papers. In both cases the filter pads were transferred with aseptic precautions directly to a modified Wilson and Blair liquid medium with a pH of 9·0, which was subsequently adjusted to



Map 3. For legend see p. 24.

pH 8·8. The inoculated media were incubated for 18–20 hr. at 37° C. after which subculture platings were made on to nutrient agar and to bile-salt agar. Colony selection followed and the various cultures were submitted to biochemical, serological and haemolytic tests in addition to the primary microscopical examination, to ensure that the isolations were in fact pure cultures of vibrios.

The biochemical reactions were classified in accordance with Heiberg's method using lactose, sucrose, mannose and arabinose as the differentiating carbohydrate media. Serological identification depended primarily on the slide agglutination test with pure O agglutinating serum of *V. cholerae*. All positive slide-agglutination tests were confirmed by tube agglutination with the same serum, after which the culture was examined with the absorbed specific Inaba and Ogawa sera for the determination of the subtype. Two haemolytic tests were done in parallel on each culture under test. In the first 1 ml. of a 24 hr. culture in isotonic Douglas broth was mixed with 1 ml. of a 5 % suspension of freshly washed sheep's R.B.C.'s and in the second test 1 ml. of a 24 hr. culture in ordinary isotonic meat broth was mixed



Map 4. Map of Calcutta showing the position of the water sampling points, indicated in roman numerals within circles, in relation to the river and the canal systems. This map also illustrates cholera death rates on lines similar to those in maps 1–3 but for the period 1938–48.

with a 3 % suspension of sheep's R.B.C.'s made up from the same stock of freshly washed packed cells as used in the first test. In both cases the mixtures were incubated at 37° C. for 2 hr. and then transferred to the cold room for storage overnight. The next morning the tubes were removed from the cold room and examined at room temperature for any sign of haemolysis. Tubes failing to show haemolysis were subcultured on to nutrient agar as a further check on the cultures.

Full controls were employed throughout the series of tests to ensure that all test reagents used were satisfactory.

Table 1. *Details of the isolation of vibrios from the Hooghly River and its branching canals, showing the results of the bacteriological examination of samples collected on particular dates from the various sampling points*

Date	Sampling point	Tide	Result
8. xii. 58	II	—	No vibrios
	III	—	No vibrios
	VII	—	*V. cholerae* Ogawa
10. xii. 58	IV	—	Non-cholera vibrios
	V	—	No vibrios
	VI	—	No vibrios
	X	—	No vibrios
22. xii. 58	VIII	—	No vibrios
	IX	—	Non-cholera vibrios
24. xii. 58	I	—	No vibrios
	II	—	Non-cholera vibrios
	III	—	No vibrios
	VII	—	No vibrios
	VIII	—	No vibrios
5. i. 59	II	Low	No vibrios
	III	Low	Non-cholera vibrios
	IV	Low	No vibrios
	VII	Low	No vibrios
	IX	Low	*V. cholerae* Inaba
7. i. 59	V	High	Non-cholera vibrios
	VI	High	No vibrios
	VIII	High	No vibrios
5. ii. 59	II	High	Non-cholera vibrios
	III	High	No vibrios
	IV	High	*V. cholerae* Ogawa
	V	High	Non-cholera vibrios
	VI	High	Non-cholera vibrios
	VII	High	*V. cholerae* Ogawa
	VIII	High	Non-cholera vibrios
	IX	High	Non-cholera vibrios
23. ii. 59	II	Low	No vibrios
	III	Low	Non-cholera vibrios
	IV	Low	No vibrios
	V	Low	*V. cholerae* Inaba
	VII	Low	Non-cholera vibrios
	VIII	Low	Non-cholera (El Tor) vibrios
	IX	Low	Non-cholera vibrios
	X	Low	No vibrios
6. iv. 59	II	Low	No vibrios
	III	Low	No vibrios
	IV	Low	Non-cholera vibrios
	VI	Low	No vibrios
	VII	Low	*V. cholerae* Inaba
	VIII	Low	Non-cholera vibrios
	IX	Low	Non-cholera vibrios
17. iv. 59	II	High	No vibrios
	IV	High	Non-cholera vibrio
	VII	High	No vibrios
	VIII	High	Non-cholera vibrio
	IX	High	Non-cholera vibrio
24. iv. 59	IV	High	No vibrios
	VII	High	No vibrios
	VIII	High	Non-cholera vibrio
	IX	High	No vibrios
1. v. 59	II	Low	No vibrios
	III	Low	No vibrios
	IV	Low	Non-cholera vibrio
	V	Low	No vibrios
	VIII	Low	Non-cholera vibrio
	IX	Low	*V. cholerae* Ogawa
15. v. 59	V	High	No vibrios
	VI	High	No vibrios
	VII	High	Non-cholera vibrio
	VIII	High	Non-cholera vibrio
	IX	High	No vibrios
9. vi. 59	III	Low	No vibrios
	IV	Low	No vibrios
	VII	Low	No vibrios
	VIII	Low	Non-cholera vibrio
	X	Low	*V. cholerae* Ogawa
3. vii. 59	VII	Low	*V. cholerae* Inaba
	VIII	Low	No vibrios
22. vii. 59 (rainfall)	IV	High	No vibrios
	V	High	Non-cholera vibrio
	VI	High	Non-cholera vibrio
	VII	High	Non-cholera vibrio
	VIII	High	Non-cholera vibrio
	IX	High	*V. cholerae* Ogawa
7. viii. 59	V	High	Non-cholera vibrio
	VI	High	Non-cholera vibrio
	VII	High	Non-cholera vibrio
	VIII	High	*V. cholerae* Ogawa
	IX	High	Non-cholera vibrio
	X	High	No vibrios
17. viii. 59 (heavy rainfall)	V	High	Non-cholera vibrio
	VI	High	Non-cholera vibrio
	VII	High	*V. cholerae* Inaba
	VIII	High	Non-cholera vibrio
	IX	High	Non-cholera vibrio

Of the thirty-eight non-cholera vibrios isolated from the water samples examined only one strain was agglu tinated by specific anti-cholera sera. It was shown to produce true haemolysin in the haemolytic tests and wa identified as an El Tor vibrio. Of the remaining thirty-seven NAG vibrios only nine belonged to Heiberg's group I and seven of these were isolated during non-epidemic periods from waters which at other times had yielded tru cholera vibrios. It seems doubtful if any significance can be attributed to this observation.

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RESULTS

The detailed results of the vibrio isolations are set out in Table 1 and these are summarized in Table 2. It will be seen from the dates on the extreme left of Table 1 that the examinations of the waters of the Hooghly River and the canals commenced on 8 December 1958 and continued over a period of approximately 8 months.

Table 2. *Summary of vibrio isolations from the Hooghly River and the canals*

Sampling point	No. of samples taken	True *V. cholerae*	El Tor vibrios	NAG vibrios	NAG vibrios Heiberg I group
I	1	—	—	—	—
II	8	—	—	2	1
III	8	—	—	2	—
IV	10	1	—	4	—
V	9	1	—	5	—
VI	8	—	—	4	3
VII	14	5	—	4	3
VIII	15	1	1	9	2
IX	12	3	—	7	—
X	4	1	—	—	—
Total	89	12	1	37	9

The twelve cholera vibrios comprised seven Ogawa and five Inaba strains. Occasionally NAG vibrios were isolated from the same specimen from which *V. cholerae* were isolated. None of these NAG vibrios belonged to Heiberg group I and they are not included in the above table.

During that time eighty-nine specimens were examined and from twelve of these true cholera vibrios were isolated. The positive isolations were spread fairly evenly over the period. While there was no very significant difference in the overall isolations of *V. cholerae* in relation to the periods recognized as epidemic there is a very marked difference in the results obtained from the various sampling points. Because of the proximity of sampling point no. VIII to ward 18, which consistently had the highest mortality rates, it was expected that this point would yield the most satisfactory results, whereas the greatest number of isolations of cholera vibrios was obtained at some distance from this point, at the junction of the two arms of the Circular Canal (sampling point no. VII). No less than five of the twelve positive specimens were taken at this point and during the period 8 December 1958 to 5 February 1959 Ogawa strains were twice isolated from specimens taken here. It was a common experience to find many more NAG vibrios immediately after a heavy rainfall than after a dry day or during the dry weather; nevertheless, cholera vibrios were isolated from five specimens taken during the Monsoon period. It is of interest to note that out of forty-three non-cholera vibrios isolated only one proved an El Tor vibrio and that was recovered from point no. VIII in February.

DISCUSSION

It will be recalled that Koch, speaking at a conference on cholera in Berlin (1884), drew attention to the very significant reduction in the incidence of the disease in the city following the opening of the Calcutta Water Works in 1870, but pointed out that Calcutta did not become free of the infection because a considerable proportion of the population continued to drink water directly from the river and other natural open sources. In its essential features this state of affairs remains unchanged. At the present time although the mean consumption of purified water from the piped supplies of the Calcutta Corporation's water works and tube-wells amounts to some 90 million gallons each day it is estimated that, at least, 60 million gallons of raw untreated river, canal or tank water and open shallow well water is also consumed every day by the population. It may be argued that like Kathmandu (Abou-Gareeb, 1959*a*) the piped supply of purified water is inadequate for the needs of the entire population of Calcutta or that the public stand-pipes, delivering purified water, are too few in some areas and that the supply is not continuous throughout the 24 hr. all the year round. Even if all this is true, it must not be overlooked that there are other important reasons for the continued consumption of raw unfiltered natural water by considerable sections of the population. The Hooghly River is regarded as holy and its waters have a special religious value to the Hindu. There also may be some misunderstanding among less well-educated groups about what is frequently called the self-purifying properties of natural waters. In addition, there are others who appear to be unaware of the dangers that lie in the consumption of raw waters, when purified supplies are not immediately or readily available, and rely, without further thought, on raw natural waters for their requirements.

In spite of the overwhelming epidemiological evidence relating to the spread of infection through the agency of cholera-contaminated water supplies, the actual demonstration of *V. cholerae* by exact bacteriological methods has always been complicated by technical difficulties. The early human experiments on the transmission of the disease by the ingestion of cultures of the vibrios (Metchnikoff, 1893, and many other workers) were often inconclusive. As Koch (1884) pointed out, in so far as the epidemiology of the disease was concerned, negative results were not necessarily conclusive. All the factors relating to the reproduction of the disease under laboratory conditions were not understood and there are still many gaps in our knowledge of the natural history of cholera.

In recent years, however, improved techniques have greatly facilitated the isolation of pathogenic organisms from polluted water supplies. In connexion with cholera investigations the fundamental principle is the filtration of large volumes (up to 5 l. or even more) of the test water through special Kieselguhr filter pads and the direct culture in an enrichment medium of the pads which contain all the residues of the filtered specimens of water. These procedures were followed in the investigation reported here and met with a reasonable degree of success. They were also employed for the examination of the waters of mosque ritual ablution tanks in Calcutta (Abou-Gareeb, 1959*b*). Before the congregation had arrived for prayer, specimens taken from the tanks failed to show cholera vibrios yet from some of the specimens taken shortly after the ritual ablutions true cholera vibrios were isolated in cultures.

The viability of *V. cholerae* in raw natural water is of short duration, and although the period of viability may be affected by a number of factors, such as temperature, degree of pollution, etc., the isolation of cholera vibrios from open natural waters may, as a general rule, be taken as an indication of recent pollution rather than an example of prolonged viability of the vibrios. It seems probable, in this connexion, that the question of the length of survival of *V. cholerae* in natural waters has less practical importance than is often assumed; the role of contaminated water supplies in the spread of cholera, as Pollitzer (1957) indicates, depends more on their repeated reinfection with *V. cholerae* than a single invasion of the organism.

The examination of the cholera records maintained by the Statistical Department of the Calcutta Corporation revealed that cases and deaths continued to occur throughout the year; in no period of the year was the city free from infection. During some parts of the year conditions might not be favourable to the development of an epidemic, or to a serious increase in the incidence of the disease, nevertheless, the infection did not die out. Moreover, the isolation during inter-epidemic periods of true cholera vibrios from the canal waters (personal communication from Lahiri and published report by Roy (1959)), which has been confirmed in the present investigation, strongly suggests that these results are due more to the continued reinfection of the waters with excreta containing cholera vibrios than to some possible prolongation of the survival time of the vibrios during the cooler weather. Indeed it would seem probable from the evidence available that the overall potential infectivity of the canal waters changes but little over the year.

When this investigation was planned it was considered that the sampling points likely to yield the greatest number of positive isolations would be those proximal to the wards with the highest mortality rates. The fact that sampling point VIII, adjacent to ward 18 where the endemicity was judged to be very high, gave only one specimen from which cholera vibrios were isolated is difficult to explain. It was entirely unexpected. Sampling point VII was chosen on two counts: it was at the junction of the two limbs of the Circular Canal and it was also adjacent to ward 29 where the cholera death-rates were reasonably high. On ten occasions samples were collected simultaneously at points VII and VIII, four samples from point VII were positive but only one from point VIII was shown to contain cholera vibrios and at the time this specimen was collected the sample from point VII was negative. Whether the movement of the canal waters influenced the recovery rates of the vibrios at point VII cannot be stated; several other factors, detailed information concerning which is lacking, obviously played their part.

This investigation has clearly established the continuous potential infectivity of the river and canal waters—particularly the latter—of Calcutta during epidemic and non-epidemic cholera periods. Cases of cholera occur throughout the year in Calcutta and there is a large amount of evidence to show that the open waters in and around the city are continuously being reinfected with cholera vibrios from the faecal material that is allowed to enter these waters. In spite of improved bacteriological methods the isolation of *V. cholerae* from polluted waters is still complicated by technical difficulties. The advantages of a careful study of local conditions before selecting sampling points has been demonstrated in this study and appears to have significantly contributed to the relatively high recovery rates from the best selected sampling points.

SUMMARY

1. Before undertaking a bacteriological survey of the waters of the Hooghly River and the associated canals a detailed epidemiological study over the past 20 years of cholera, as it affected the individual administrative wards of Calcutta, was undertaken. Sampling points were selected in accordance with the results of this study. Samples of water from the various points were collected at intervals extending from December 1958 to August 1959 in $2\frac{1}{2}$–5 l. amounts. The whole sample in each case was filtered through special filter pads. The pads were first cultured in an enrichment medium from which plate cultures were subsequently made for colony isolation, serological and biochemical examination.

2. The sampling points on the canals were all adjacent to areas where the local endemicity was judged to be high; other points were by bathing ghats, etc. A total of eighty-nine samples covering all the sampling points were examined and *Vibrio cholerae* were isolated from twelve of these samples, eight of which came from twenty-six samples collected from two sampling points on the Chetla and Circular Canals, respectively.

3. The positive isolations were spread fairly evenly over the whole period of the study which covered both epidemic and non-epidemic periods including the monsoon. Although the incidence of cholera in Calcutta may fall to a low level during non-epidemic periods cases continue to occur throughout the year and the relationship of the maintenance of the infection in the city to the continuous potential infectivity of the open natural waters of Calcutta is discussed.

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