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REVIEW ARTICLE

The Aquatic Environment as a Reservoir of *Vibrio cholerae*: A review

Mohammad Sirajul Islam[1], Bohumil S. Drasar[2] and R. Bradley Sack[1]

[1]Laboratory Sciences Division, ICDDR,B, GPO Box 128, Dhaka 1000, Bangladesh; [2]Department of Clincial Sciences, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, U.K.

1. INTRODUCTION

Cholera is endemic in many parts of the world. Substantial advances in our understanding of treatment, pathophysiology and immunology of cholera have been made but many aspects of cholera epidemiology still remain obscure. In endemic areas of Bangladesh, cholera epidemics occur twice a year and have a regular seasonal pattern (1 - 5). During epidemics, *Vibrio cholerae* 01 is isolated from patients as well as from surface water, but disappears from the environment during inter-epidemic seasons (6). The reservoirs or sites of survival and multiplication of *V. cholerae* 01 between epidemics are not known (7 - 11).

This paper reviews literature which is relevant to the hypothesis that the aquatic environment is a reservoir for *V. cholerae* 01.

2. HISTORICAL ASPECTS OF CHOLERA.

Cholera has been known as a killing disease from time immemorial. The total number of deaths reported to be caused by cholera from 1909 - 1948 in India was 788,000 (12). Cholera still takes a toll of lives in many parts of the world. If one goes back to the history of cholera, a description of cholera was found in India dating from the time of Alexander The Great (12). Subsequently various Portugese, Dutch, French and British observers have described cholera in India (12).

However, from 1817 onwards, the literature and study of cholera are more systematic. The first pandemic started in 1817 in India and spread to other parts of the world. After 1817 cholera began to gain more attention because it was no longer confined to India but rather was becoming a disease of most serious concern to the world.

Seven pandemics have been recorded so far. The seventh pandemic started in 1961 and is still continuing. The origin of all these pandemics and their spread to various countries of the world have been extensively reviewed by Pollitzer (12), Kamal (13) and Barua (14).

It has been observed that most of the countries of the world have been visited by cholera at one time or another. Therefore, it would be simpler to list the countries which have not been invaded. Cholera did not penetrate generally into the northernmost and southernmost parts of the globe. Accordingly in Asia, northern Siberia and Chamchatka were spared. Similarly the most northern parts of the western Europe (Iceland, the Faroe Islands, Shetland and Orkney Islands) as well as North American regions including Newfoundland and Greenland were also spared. In South America, cholera was absent from the southernmost parts of Chile, Argentina and from the Falkland Islands (Figure).

One important aspect of the present pandemic is its place of origin. All the previous pandemics started from the Ganges delta of Bengal (erstwhile East Pakistan, now Bangladesh and West Bengal) but the 7th pandemic started from the island of Sulawesi (Celebes) in Indonesia. However, during this period of the seventh pandemic, there was cholera in Bengal and it is still endemic there. After outbreaks, cholera has sometimes disappeared from many countries but always continued to be recognised in Bengal. Cholera has, therefore, been endemic in Bengal from the very beginning of its history. Bengal has been considered as the homeland of cholera.

However, until now it is not clear why Bengal is the homeland of cholera. Why should it be endemic there? What are the factors responsible for providing a suitable habitat for cholera in Bengal?

3. SEASONALITY OF CHOLERA.

The lower part of Ganges Delta being the

Correspondence and reprint requests should be addressed to: Dr. M Sirajul Islam.

homeland of cholera has maintained a clear seasonal pattern of epidemics. For example, in Bangladesh, the Dhaka (capital city) and Matlab (rural sub-district) areas have been studied as endemic foci of cholera. Epidemiologic studies of cholera were started in Bangladesh (the then East Pakistan) after the establishment of the Pakistan South East Asia Treaty Organisation (SEATO) Cholera Research Laboratory in 1961 (now defunct). Martin *et al.* (1) observed in Dhaka from 1964 to 1966 that the outbreaks of cholera every year reached their peaks during November, December or January. These authors also observed a second peak in April and May in the 1965-1966 epidemic (1).

McCormack *et al.* (2) examined the seasonality of cholera in a study carried out from November 1963 to June 1966 in Matlab, and found that each year the cholera epidemic reached its peak during November to January. In the 1966 epidemic they also observed a small second peak during April and June. Merson *et al.* (3) in a study in Matlab in Bangladesh during 1968-1977 showed that the peak incidence of classical biotype occurred during November, December and January. In the case of El Tor biotype the peak occurred during September, October and November. They also observed a second small peak during March and April. Glass *et al.* (4) in a study in Matlab in Bangladesh compiled 15-years data and examined the seasonality of both El Tor and classical biotypes. They also observed a pattern of seasonality for both El Tor and classical biotypes similar to that observed by Merson *et al.* (3). Samadi *et al.* (5) showed the seasonality of both El Tor and classical biotypes in urban Bangladesh over a 17-year set of data from 1964 to 1980. They observed a significant seasonal pattern for both El Tor and classical cholera. The estimated peak of El Tor outbreak was October and that for classical was December. They found that the peak incidence of El Tor occurred earlier than classical cholera. All these studies are summarised in Table I.

Table I: Studies on seasonality of cholera in Bangladesh.

Source	Date	Place of study	Biotypes	Epidemics: Winter peak	Epidemics: Summer peak
McCormack *et al.* (1969)	1963-66	Matlab	Classical	Nov-Jan	Apr-Jun
Martin *et al.* (1969)	1964-66	Dhaka	Classical	Nov-Jan	Apr-May
Merson *et al.* (1980)	1968-77	Matlab	Classical	Nov-Jan	Mar-Apr
			El Tor	Sep-Nov	Mar-Apr
Glass *et al.* (1982)	1966-80	Matlab	Classical	Nov-Jan	Mar-Apr
			El Tor	Sep-Nov	Mar-Apr
Samadi *et al.* (1983)	1964-80	Dhaka	Classical	Oct-Dec	Mar-May
			El Tor	Sep-Nov	Mar-May

If one evaluates the seasonality of cholera in Bangladesh (the then East Pakistan) from the available sources of information from 1963 to 1980, it is evident that the occurrence of cholera cases in endemic areas is usually seasonal. It is also clear that the cholera epidemic in Bangladesh always reaches its peak during the cooler months of the year with a second small epidemic during the hot season. So far no satisfactory explanation has been put forward to explain this pattern of cholera seasonality.

4. ENDEMICITY OF CHOLERA.

Why is the Ganges delta a reservoir of infection? What is the mechanism by which the endemicity of this area is maintained?

There is at present no satisfactory explanation. From time to time various explanations have been put forward. Sometimes climatic factors have been considered to be responsible for maintaining endemicity. Swaroop (15) investigated the annual rates of cholera mortality in individual districts in India of cholera endemic areas for the period of 1901-1945. In this way he located the endemic foci and then tried to correlate these endemic areas with other topographic conditions. He observed the following factors which were common in all endemic areas:

a) all endemic areas were located around rivers,
b) all these tracts lay in areas of high population density,
c) all of them lay in low lying lands, and
d) all these tracts lay in areas of high absolute humidity.

Among these four factors all except the second seems to indicate that the topography or environment of these places play an important role in maintaining the endemicity of cholera in these areas. He finally concluded that all these features may indirectly affect the causative organism or the human hosts and their interrelationships.

It may be that under these environmental conditions *V. cholerae* 01 may be able to persist in the environment or be able to colonize a plant or animal that lives in these environmental conditions in the aquatic systems in these areas. The answer to maintaining endemicity lies in the mechanisms by which *V. cholerae* 01 survives during inter-epidemic periods in the endemic areas.

As far as the endemicity of these areas is concerned, there are four models available to explain the maintenance of endemic cholera (16,17). These are:

Carrier status in animals; Carrier status in man;
Continuous transmission in man; and
An environmental reservoir.
These will be discussed separately.

4.1. **Carrier status in animals.** No animals have so far been demonstrated as reservoirs of cholera. Sack (18) investigated canines as reservoirs of cholera in

Calcutta, India. Over 500 dogs were examined bacteriologically but no evidence of *V. cholerae* 01 reservoir was found although 14% of dogs harboured *V. cholerae* non-01 in their intestines. These results indicate that non-human reservoirs exist for *V. cholerae* non-01 in contrast to *V. cholerae* 01. However, toxigenic *V. cholerae* 01 have been isolated from domestic animals, e.g. cows, goats and chicken, only in the locality of current cholera cases in man (19). It is likely that these animals are probably picking up the *V. cholerae* 01 from the environment at the time when the disease is occurring locally and can not act as reservoirs.

be isolated from one or more members of 83% (19/23) of households having an index cholera case (27). Among a total of 492 household contacts, 20% (96) became infected. Of the 96 carriers identified, 53 excreted *V. cholerae* 01 on only one day, whereas 2 of the remainder excreted *V. cholerae* 01 for more than 25 days. However, the different properties of *V. cholerae* 01 such as sensitivity to acid conditions (28), short survival time in potable water (29,30) and its high infectious dose for disease (31) make *V. cholerae* 01 unsuitable for continuous transmission in human intestines. Therefore, this mechanism is unlikely to be a successful strategy.

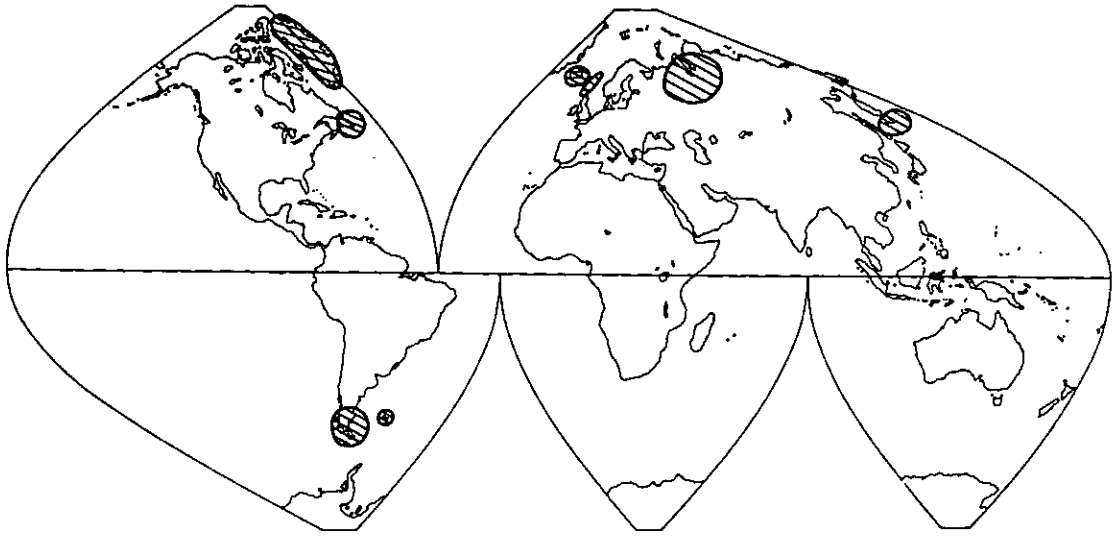

Figure. Global occurrence of cholera.

4.2. **Carrier status in man.** To date only a few chronic gallbladder carriers have been demonstrated and this is in spite of some very extensive studies on populations exposed to cholera (20,21). Chronic carriers of *V. cholerae* 01 in the gallbladder were also found in experimental dogs convalescent from cholera (22). However, it has been observed that rough vibrios can be excreted by convalescent patients for a few months after infection (23,24). These rough vibrios proved non-pathogenic after testing in animal models (24,25). Relatively large numbers of carriers would be required to give the observed epidemiological pattern of the disease. Therefore, chronic carriage of *V. cholerae* 01 by man is unlikely to contribute to the persistence of cholera during inter-epidemic periods.

4.3. **Continuous transmission in man.** The possibility of low level of infection maintaining endemic cholera has some credibility and this is the theory put forward by Gangarosa and Mosley (26). Studies in Calcutta during 1968 showed that *V. cholerae* 01 can

4.4. **Environment as reservoir.** As this is the alternative mechanism to be examined, it may be helpful to list the reasons favouring this hypothesis and why the criticisms against this mechanism are inadequate.

4.4.1 **Historical background.** The first speculation about the environment as a reservoir came from Robert Koch (32) in 1884 when he isolated the comma bacillus from a tank in Calcutta during 1883 epidemic. He wrote:

> "I succeeded in finding the comma bacillus with all its characteristic peculiarities in a tank that supplies water for drinking and household purposes for all the people living around the immediate neighbourhood where a number of fatal cases had taken place"

He then discussed the survival and multiplication of the comma bacillus in the environment.

> "There remains still the important question to be answered, whether the infectious material

> can reproduce or multiply itself outside the human body. I believe that it can. As the comma bacillus can grow on a gelatin plate, as it can grow on a piece of linen, or in meat broth or on potatoes, it must also be in a position to grow in the open air, specially as we have seen that a comparatively low temperature enables it to develop. I would not certainly assume that the multiplication of the comma bacilli outside the human body takes place in well water or river water without any assistance, for these fluids do not possess that concentration of nutritious substances necessary for the growth of the bacilli. But I can easily imagine that, although the whole mass of the water in a tank or reservoir is too poor in nutritious substances for bacilli to flourish in it, yet some spots may contain sufficient concentration of nutritive substances, for example, those spots where a gutter, or an outlet of a cesspool, opens into the stagnant water, where vegetable matter, animal refuse etc., lie and are exposed to putrifaction by bacteria. At such points a very active form of life can develop."

After Dr. Robert Koch's isolation of *Vibrio* and postulation of the environment as the possible reservoir of comma bacilli, several scientists have carried out investigations both in the laboratory and in the field to explore the possibility of the environment as a reservoir. So it would be useful to examine some of the studies which favour the hypothesis or that are against the hypothesis.

4.4.2. **Evidence against environmental hypothesis.** The evidence used against an environmental hypothesis is some of the laboratory studies in which it has been shown that the survival of *V. cholerae* 01 in river and tank water is short. Some of the studies are discussed below:

Lahiri *et al.* (33) studied the viability of *V. cholerae* 01 in natural waters collected from several sources in Calcutta, where the disease was most prevalent. The sources were spring, tap, river and tank. They found that the survival of *V. cholerae* 01 in raw water samples varied from one hour in samples of spring water to a maximum of 72 hours in certain tank waters.

Survival experiments of *V. cholerae* 01 in peptone enriched water, human faeces, food and water were carried out in Taiwan by Cheng (34) in 1963. The water examined had been collected from tap, well, drain, river and sea. He found that *V. cholerae* 01 survived less than 24 hours in well water, 2 days in river water, 1 day in drain water, 5 days in sea water and less than 6 hours in pond water. He concluded that the survival capacity of *V. cholerae* 01 in water varied according to temperature, pH, salt, organic matter, degree of pollution, sunlight and presence of chlorine.

Konchady *et al.* (35) studied the survival of *V. cholerae* 01 in five different sources of water in Calcutta. The sources were tap water, open well water, river water, canal water and pond water. The initial inoculum was 1 x 10^2/ml. They examined the survival of *V. cholerae* 01 in water daily until cultures were negative. They used 2 or 3 loopsful of water and streaked them onto bile salt agar (BSA) plates in duplicate and read the plates after 18 to 24 hours incubation at 37°C. They carried out the experiments at room temperature (about 25°C). They observed that the duration of survival of *V. cholerae* 01 in all water samples was about 5 days.

Mukerjee *et al.* (36) observed the viability of *V. cholerae* 01 in the natural water sources in and around Calcutta, using freshly collected water from the Ganges river, tanks and sewage. Experiments were carried out at room temperature. 0.5 cc of the water sample with *V. cholerae* 01 were plated onto duplicate nutrient agar plates. They carried out these experiments for 16 months. In total, 97 water samples were tested in this time. The survival time in different sources of water varied from 1 to 6 days.

All these studies showed that *V. cholerae* could not survive in water collected from different natural sources. On the basis of these laboratory results, it was considered that the natural bodies of water do not act as reservoirs because of the short survival time of *V. cholerae* 01 in the water. However, this interpretation may be inadequate because any water sample from a river, tank, well, spring, etc. represents only a tiny fraction of the total number of habitats in the aquatic environment. All these natural bodies are composed of hundreds of different kinds of macro and micro flora and fauna. So this kind of negative conclusion based on survival studies of *V. cholerae* 01 in water (excluding its different kinds of plants and animals) in the laboratory is of limited application.

4.4.3. **Evidence consistent with environmental hypothesis.** Some studies which support an environmental hypothesis are discussed below. For convenience of discussion, the data are grouped into different categories.

4.4.3.1. **Taxonomical evidence.** Taxonomical studies of 142 so-called NAG vibrios were carried out by Sakazaki *et al.* (37) in 1967. A total of 80 physiological, morphological and biochemical characters were compared with 5 reference strains of *V. cholerae* El Tor. They also compared the base composition of the deoxyribonucleic acid. They found that the different characters of 5 reference strains coincided with the majority of the strains of the non-agglutinable (NAG) vibrios at a high level of over 90% S value. So on the basis of numerical taxonomy and DNA base composition, the authors suggested that the NAG vibrios are very closely related to *V. cholerae* 01 biotype El Tor.

Colwell (38) studied the polyphasic taxonomy of

the genus *Vibrio*. A total of 86 strains were included in the study of which 30 were strains of *V. cholerae*, 35 strains of *V. parahaemolyticus* and 21 representative strains of *Pseudomonas*, *Spirillum*, *Acromobacter*, *Arthrobacter* and *Vibrio* species. She examined 200 different morphological, physiological and biochemical characteristics of each strain. She also examined overall DNA base composition and ultrastructure under the electron microscope. The taxonomic data were analysed by computer using numerical taxonomy programmes. She found that *V. cholerae* and nonagglutinable vibrios fell into a single relatively homogeneous *V. cholerae* species cluster.

Polynucleotide similarities among bacteria help to assess the genetic and phylogenic relationships amongst bacteria. To find out this relationship, Citarella and Colwell (39) in 1970 studied selected *Vibrio* species in their studies, such as *V. cholerae* biotype El Tor and Classical, *V. parahaemolyticus*, *V. alginolyticus*, and marine vibrios. They examined the polynuclotide sequence relationships among different species by means of DNA reassociation reaction and chromatography on hydroxyapatite. They found that interspecific DNA duplexes between *V. cholerae* DNA and that of non-cholera vibrios exhibited high relative levels of formation at 60°C (>80%) and were slightly reduced at 75°C, whereas in the case of all other interspecific DNA association reactions only a low level of DNA duplex formation was noted at 60°C (<25%) and these were drastically reduced (>50%) at 75°C. On the basis of the degree of reassociation and thermal stability of *V. cholerae* and noncholera vibrio, they concluded that there is little evolutionary divergence between *V. cholerae* and noncholera vibrios.

All these studies have shown that *V. cholerae* 01 and non 01 are very closely related. *V. cholerae* non 01 are found throughout the year in the aquatic environment in the cholera endemic areas like Bangladesh (6). So if non 01 can survive for a long time in the environment it may well be that *V. cholerae* 01 will be able to survive in the same manner as *V. cholerae* non 01. As the number of *V. cholerae* 01 are always less than *V. cholerae* non-01 in environmental waters and as there is no medium to differentiate *V. cholerae* 01 from non-01, there always remains a chance of missing *V. cholerae* 01 from environmental samples.

4.4.3.2. **Epidemiological evidence**. Pollitzer (12) described the simultaneous outbreaks of cholera in different places in Bengal which are quite far from one another and that under favourable climatic conditions, the disease then spread in a wave-like form in areas generally free from cholera.

McCormack *et al.* (2) in an epidemiological study in rural endemic areas in Bangladesh observed the location of cases by village during the early weeks of 1964 and 1966 epidemics. They observed simultaneous outbreaks of the disease in several villages in widely scattered areas so they suggested multiple simultaneous introduction of the cholera cases into different villages.

Glass *et al.* (4) in a study of rural endemic cholera in Bangladesh in 1966-1980 observed that the first recognized cases at the start of the cholera seasons each year occur at distant location in the Matlab area. They also observed that in 1979, the early recognized cases were of different phage types.

Analyses of data on the epidemiological pattern of cholera in an endemic area like Bangladesh, revealed that for a long time simultaneous outbreaks of cholera occurred in distant villages without any interconnection. This epidemiological feature of cholera is consistent with the environmental hypothesis.

4.4.3.3. **Laboratory based survival studies**. Baker *et al.* (40) studied the survival of *V. cholerae* 01 in artificial seawater and natural seawater microcosms. Two strains of *V. cholerae* 01, one of clinical origin and another isolated from the estuarine environment, were used in the experiments. It was observed that the initial numbers of cells inoculated into microcosms increased 2.5 $\log_{10}$ cfu within 3 days. After 75 days the number of viable cells was still 1 to 2 $\log_{10}$ cfu higher than the initial inoculum size.

Miller *et al.* (16) studied the effect of different physicochemical conditions on the survival of toxigenic *V. cholerae* 01 in water in an extensive series of laboratory experiments. They used six Bangladeshi isolates of *V. cholerae* 01 in their experiments. Three of these strains were isolated from cholera patients and three were from polluted water. They found that toxigenic *V. cholerae* 01 can survive for a long period (months) in water having a salinity of 0.25-3% and a pH of around 8.0 at 25°C. These studies have shown that *V. cholerae* 01 can survive for months in the right environment.

Recent laboratory microcosm studies have also demonstrated that toxigenic *V. cholerae* 01 can survive longer in association with aquatic macrophytes (8,11) and algae (9,10) than the surrounding water.

4.4.3.4. **Environmental isolations of *V. cholerae*.** *V. cholerae* 01 and non-01 have been isolated from the environmental sources from different parts of the world, which are discussed below:

India

It has been found that *V. cholerae* 01 have been isolated from surface water of some places in India which were free from cholera for a number of years. Read and Pandit (41) studied the distribution of *V. cholerae* 01 in certain endemic rural areas of Bengal and Bihar in India for one year, 1939-1940. They collected stool and water samples from the endemic areas. As a negative control, they selected Sindh province which was free from cholera for the last 10 years. They collected water samples from tanks, wells, lakes, marshes, streams and rivers from Sindh province. They isolated *V. cholerae* 01 from one of the tanks and jhils in Sindh province for 41

days which suggested that *V. cholerae* 01 can survive in surface water for a long time.

Venkatraman *et al.* (42) studied the occurrence of *V. cholerae* 01 in natural sources of water in the absence of cholera in the Cauvery delta of India during 1940. This region had been free from cholera from May of the preceding year. They examined 1827 stool samples from the inhabitants of the region and all were negative. In total 878 specimens of water were examined from 237 different sources. These sources included rivers, tanks, ponds and wells. They isolated *V. cholerae* 01 biotype El Tor from 21 water samples collected from 17 different tanks. All the positive *V. cholerae* 01 tanks were situated in two small areas at the tail of the delta. It was found that *V. cholerae* 01 have been isolated at one time or another during the year from 17 out of 61 tanks in two areas.

Panja and Ghosh (43) examined 524 water samples for *V. cholerae* collected from Hoogly river in Calcutta, India. They isolated *V. cholerae* 01 and non-01 from 52 water samples and indicated that Hoogly river water may be responsible for cholera infection if used for domestic purposes.

Abu Gareeb (44) carried out a bacteriological survey of the waters of the Hoogly river and the associated canals from December 1958 to August 1959. A total of 89 water samples were examined and *V. cholerae* 01 were isolated from 12 of these samples. The isolation of *V. cholerae* 01 were spread fairly evenly over the whole period of the study which covered both epidemic and non-epidemic periods including the monsoon. This study demonstrated the continuous potential infectivity of the river and canal waters of Calcutta during epidemic and non-epidemic cholera periods.

Nair *et al.* (45) studied the ecology of *V. cholerae* non-01 in freshwater environments of Calcutta, India. They collected water samples from a lake, canal and a pond once in a month during July 1984 to June 1985. *V. cholerae* non-01 was found in all sites during the study period and suggested that *V. cholerae* non-01 is common in freshwater environs of Calcutta.

Rai *et al.* (46) investigated the persistence of *V. cholerae* during interepidemic period in Varanasi, India. They collected 37 water samples from 5 ghats of river Ganges in 1989 and found 18.9% of the samples were positive with *V. cholerae* non-01.

United Kingdom

Lee *et al.* (47) carried out several surveys in various water sources in Kent, UK, to establish the incidence of *V. cholerae* 01 and non-01 in the aquatic environment. Various surface water sources, such as ponds, lakes, ditches, canals, etc. were investigated. They observed that *V. cholerae* 01 and non-01 occurred sporadically in very low numbers in water containing >5 mmol NA^+/litre, during summer. The highest numbers of up to 700 colony forming units/ml appeared regularly during summer in static brackish water containing 25-200 mmol Na^+/litre.

West and Lee (48) investigated the distribution of *V. cholerae* non-01 in a stream and a ditch in Kent, UK, from 1978 to 1980. They observed that *V. cholerae* non-01 occurred in both sites during summer. They also observed that the occurrence of *V. cholerae* non-01 was not related to human faecal contamination.

USA

Kaper *et al.* (49) carried out an investigation in the Chesapeake Bay, U.S.A from October 1976 to January 1978. They examined water, sediments and shellfish samples from 21 stations. Different physical and chemical parameters of water such as temperature, dissolved oxygen, pH, salinity, etc. were also examined. In total 65 *V. cholerae* non-01 were isolated throughout the year during the study period. There was no correlation between *V. cholerae* isolation and faecal coliform counts, whereas isolation of *Salmonella* species correlated with faecal coliform counts. No physical, chemical or microbiological parameters were observed to have a marked association with the incidence of *V. cholerae* non-01. The only striking pattern they observed was the range of salinity. All the strains were isolated from those sampling points where the salinity ranges from 0.4% to 1.7%. The authors concluded that the natural habitat of *V. cholerae* non-01 appears to be natural bodies of water.

The first case in the U.S.A. was detected in 1973, an old man living in Port Lavaca, Texas. *V. cholerae* El Tor Inaba was isolated in the stool sample of this patient. This is the first reported case in U.S.A. since 1911 (50). After 5 years in 1978, another case of a 44 year old man was detected in Lousiana. Then 10 more cases were detected in four additional clusters (51) in the same area surrounding the Gulf of Mexico.

It was found that the only meal which was common to all the infected persons was crab (51). After performing a matched triplet case control study, it was found that the only significant difference in exposure between cases and controls was consumption of crabs.

An attempt was made to isolate *V. cholerae* 01 from seafood and the environment in the sites from which the crabs came along the entire area. *V. cholerae* 01 was isolated from one of the three boiled crabs, from a shrimp caught in a canal and from two Moore swabs from two canals. All the specimens were cultured after enrichment in alkaline peptone water for 6-12 hours. All isolates were *V. cholerae* 01, biotype El Tor serotype Inaba.

It has been hypothesized that *V. cholerae* 01 had been able to survive along the Gulf Coast for years in absence of human disease. The strongest evidence is the fact that the isolates from Texas and all the isolates from Lousiana five years later were of a single phage type unique to the United States. Moreover, the Gulf Coast isolates are haemolytic, and have vibriophage VcA-3 and a unique cholera

toxin gene pattern on Southern-blot analysis (52). These endemic toxigenic *V. cholerae* 01 strains are clearly different from the seventh pandemic El Tor strains isolated worldwide in the past 30 years.

Finally it has been suggested that toxigenic *V. cholerae* 01 can survive and multiply in the environment and persist indefinitely without human faecal contamination.

Colwell *et al.* (53) isolated toxigenic *V. cholerae* 01, serotype Inaba from the Chesapeake Bay and from sewers and bayous in Lousiana. *V. cholerae* 01 from Lousiana and Chesapeake Bay were isolated from some areas which were free from faecal contamination using the presence of *E. coli* as an index. The authors concluded that *V. cholerae* is a component of the autochthonous flora of brackish water, estuaries and salt marshes of coastal areas in the temperate zone.

V. cholerae non-01 were isolated from rivers, creeks, waters, irrigation, canals and ditches in Western Colorado during the summer of 1985. The organism occurred in fresh water having >5 mmol/l of Na^+ as well as in water of higher salinity, approximately 17 mmol of Na^+/l (54).

Incidence of *V. cholerae* 01 and *V. cholerae* non-01 were studied in shellfish, sediment and waters of California, Oregon and Washington during summer of 1984. *V. cholerae* non-01 was found in 23 estuaries and in 44.6% of the 529 samples examined. *V. cholerae* 01 Inaba was isolated from water samples in Morrow Bay, California (55).

Perez-Rosas and Hazen (56) monitored concentrations of *V. cholerae* non-01 and faecal coliform at 12 sites in a rain forest watershed in Puerto Rico for 12 months. Densities of *V. cholerae* non-01 and faecal coliforms were not correlated. High densities of *V. cholerae* non-01 were also found at pristine sites at the highest point in the watershed. This study suggested that *V. cholerae* non-01 strains are indigenous to tropical freshwaters.

Italy

In 1973, outbreaks of cholera were reported simultaneously from three foci in Italy, a coastal town on the Gulf of Naples in the region of Campania, the Adriatic port city of Bari and from Cagliari in Sardinia. Consumption of sea food, particularly mussels, was probably the vehicle of *V. cholerae* infection (57). After 6 years, cholera outbreaks again occurred at Cagliari in Sardinia. All but one of the 12 people with *V. cholerae* 01 infection gave a history of recent consumption of marine bivalves known locally as *Arselle* (pelecypods). *V. cholerae* 01 was also isolated from samples of water and bivalves obtained from a lagoon on the outskirts of the city of Cagliari. *V. cholerae* 01 biotype El Tor, serotype Ogawa, phage type 4 were identified both from environmental and clinical isolates. All the strains isolated in 1979 outbreaks from different sources were of the same phage type as those from the first outbreak in 1973. No evidence of cholera was seen in Cagliari in the years 1973 to 1979. Salmaso *et al.* (58) suggested that the organism may have been maintained in the marine environment.

Australia

In Australia the first case was reported in 1977 from Queensland (59,60). Two persons who shared a caravan in a caravan park excreted toxigenic *V. cholerae* 01, biotype El Tor, serotype Inaba and phage type 2. There was no history of foreign travel by the infected persons. The sanitary facilities were adequate. No cross connection between the septic tank, surface water disposal and reticulated water systems were detected. *V. cholerae* 01 was isolated from the water supply of the caravan park. No *E. coli* was isolated, which indicated that the water supply was not contaminated by sewerage. Investigation revealed that the river which was used as a supplementary source of water supply contained *V. cholerae* 01. At times of increased demand, especially during summer, the river is used as a supplementary source of water supply. The same strain of *V. cholerae* 01 was isolated intermittently from two rivers for 22 months. *V. cholerae* 01 were isolated from one of the rivers persistently for two months. Finally it was suggested that *V. cholerae* 01 not only survive in the river water but also multiply there.

Studies showed that *V. cholerae* 01 were isolated at least once in a year from a river in Australia from 1977 to 1983. There was no indication of human, animal or sewage contamination. No source of importation was also observed. The isolation for a continuous 7 year period from a river surely indicated the long term persistence of *V. cholerae* 01 in the aquatic environment (61).

V. cholerae 01 have also been isolated from water, sediments and plants from eight reverine sites in South East Queensland, Australia. *V. cholerae* 01 was found to be tolerant of low salt levels (62).

Other countries

Bockemuhl *et al.* (63) studied the incidence of *V. cholerae* non-01 at two sites on the Elbe River at Hamburg betwen June 1981 and December 1982. A total of 107 *V. cholerae* non-01 were isolated from 147 water samples during the study period. The vibrio incidence was not related with the faecal coliform counts of the water. It was concluded that *V. cholerae* non-01 are indigenous organisms of the Elbe River.

Venkateswaran *et al.* (64) studied the occurrence of toxigenic vibrios in the freshwater environment of Ohta river in Japan during August through October 1988. They isolated toxigenic *V. cholerae* non-01 from the surface water of this river.

Isa *et al.* (65) investigated an outbreak of cholera in Tumpat, Kelantan, Malaysia. They found that the Kelantan river water was the reservoir and transmitted from the river by river clams. The effort in public health education especially against the use of river water and the consumption of raw clamps

prevented spreading of cholera.

Tamplin & Carrillo (66) studied the occurrence of *V. cholerae* 01 in various environmental water samples collected from March to August, 1991 in Peru. They collected water samples from Pacific coast (Lima), mountain region (Lago Titicaca) and tropical rain forests (Amazon River). They isolated *V. cholerae* 01 by culture and by fluorescent antibody methods. Frequent isolation of *V. cholerae* 01 were observed in seawaters and river water. *V. cholerae* 01 were isolated in high number from the river water (30 °C) in the city of Iquitos, in the upper Amazon basin. *V. cholerae* 01 was found in low numbers and isolated infrequently from the cold (12 °C) waters of Lago Titicaca near Puno, a high altitude city.

Islam *et al.* (67) studied the occurrence of *V. cholerae* 01 and non-01 in pond ecosystems in an endemic area of Bangladesh. They collected a total of 600 samples every 15 days which included surface water, plants, phytoplankton, zooplankton and sediment during May 1988 to April 1989. *V. cholerae* biotype El Tor and serotype Inaba were isolated in the months of February, March, April, September, October and December. Isolation of *V. cholerae* 01 much of the year suggested that freshwater ponds can act as a reservoir of *V. cholerae* 01. *V. cholerae* non-01 were also isolated throughout the year (68).

All these studies are summarised in Table II.

Other epidemiological and ecological studies also indicated aquatic environment as sources of *V. cholerae* in sporadic and epidemic disease outbreaks (69-73).

5. CONCLUSION.

All these environmental isolations of *V. cholerae* 01 and non-01 from a number of places suggest that *V. cholerae* 01 may be a long term inhabitant of some aquatic environments. Aquatic environments consist of different kinds of flora and fauna which may form an infinite number of microenvironments. It would be impossible to examine all these microenvironments for *V. cholerae* and we cannot say with confidence that *V. cholerae* 01 do not reside in the environment. In the case of a human population this constraint does not apply so forcibly because it contains finite number of specimens to be examined. So microbial ecologists are seriously considering a possible ecological niche of *V. cholerae* 01 in the aquatic environment. The interepidemic survival of *V. cholerae* 01 can be explained by such an ecological niche.

It now appears that aquatic environments are the reservoirs of *V. cholerae* 01 but the question remains which particular micro or macro environment or niche in the aquatic ecosystem is the reservoir? Do the *V. cholerae* 01 survive in the aquatic environment as a free-living organism or do they prefer an epibiotic host as a suitable ecological niche? These questions remain to be clarified.

Table II. Isolation of *V. cholerae* from surface water sources in various countries

Source of information	Kinds of water sources	Date	Country
Read and Pandit (1941)	Tanks and jhills in Bengal and Bihar	1939 - 40	India
Venkatraman *et al.* (1941)	Tanks in Madras	1940	India
Panja and Ghosh (1947)	River	1942	India
Abou-Gareeb (1960)	River and canals	1958 - 59	India
Lee *et al.* (1982)	Ponds, ditches, canals, etc. in Kent	1976 - 79	UK
West and Lee (1982)	Streams and ditches in Kent	1978 - 80	UK
Kaper *et al.* (1979)	Chesapeake Bay	1976 - 78	USA
Blake *et al.* (1980)	Canals near Pecan Island and White Lake	1978	USA
Colwell *et al.* (1981)	Chesapeake Bay, sewers and bayous in Louisiana	1977 - 80	USA
Salmaso *et al.* (1980)	Lagoon in Cagliari	1979	Italy
Rogers *et al.* (1980)	Two rivers in Queensland	1977	Australia
Bourke *et al.* (1986)	Four rivers in Queensland	1977 - 85	Australia
Rhodes *et al.* (1986)	River, creaks, canals, ditches, etc.	1985	USA
Bockemuhl *et al.* (1986)	Rivers	1981	Germany
Kaysner *et al.* (1987)	Bay, rivers and canal	1984	USA
Venkateswaran *et al.* (1989)	River	1988	Japan
Perez-Rosas and Hazen (1989)	Watershed	ND	USA
Isa *et al.* (1990)	River	1990	Malaysia
Rai *et al.* (1991)	River	1989	India
Tamplin and Carrillo (1991)	River	1991	Peru
Islam *et al.* (1991)	Ponds	1988 - 89	Bangladesh
Islam *et al.* (1992)	Ponds	1988 - 89	Bangladesh

ND = No data

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