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Seasonal prevalence of cholera pathogen *(Vibrio cholerae)* in water and plankton samples in the southern coastal part of Bangladesh

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Abstract

In the present study seasonal prevalence of *Vibrio cholerae*, serogroup O1 and O139 (VC O1 and O139) were investigated in several water bodies of southern coastal area adjacent to the Bay of Bengal. The study revealed that the gross total counts of both VC O1 and O139 in water were 2.2×10^7 cfu/L and 5.0×10^6 cfu/L which were the highest followed by zooplankton sample with 7.0×10^5 cfu/L and 4.6×10^5 cfu/L and phytoplankton sample with 4.0×10^5 cfu/L and 3.4×10^5 cfu/L, respectively. It was also revealed that VC O1 counts were appeared to be higher in all types of samples than that of VC O139 throughout the investigation period and summer counts were found to be higher than that of monsoon and winter counts.

Keywords: Bangladesh, Cholera, *Vibrio cholerae*, phytoplankton, zooplankton, Public health, Water

Introduction

The bacterium *Vibrio cholerae* belongs to the family Vibrionaceae (Baumann *et al.,* 1984). Out of around 200 serogroups, only the *Vibrio cholerae* O1 and O139 serogroups are the potential pathogen for causing sporadic, epidemic and pandemic cholera (Brayton *et al.*, 1986). Within the O1 serogroup, the ability to produce cholera toxin (CT) is an essential determinant of virulence. *V. cholerae* O1 strains that do not produce CT may be isolated from patients with sporadic diarrhoeal intestinal sites, environmental specimens or foods but have not yet demonstrated the potential to cause epidemic cholera (Johnston *et al.,* 1983, Morris *et al.,* 1984; Minami *et al.,* 1991). *V. cholerae* are the normal inhabitants of aquatic environment (Sakazaki & Shimada, 1977). Toxigenic *V. cholerae* O1 and O139 are the main causative agents of cholera (Albert *et al.,* 1993; Islam *et al.,* 1995; Sack *et al.,* 1980), acute dehydrating diarrhoea, which occurs in epidemic (Glass *et al.,* 1982; Longini *et al.,* 2002) and pandemic (Kaper *et al.,* 1992) forms. *V. cholerae* O1, the etiological cholera agent, is responsible for the morbidity and mortality in numerous areas in Asia, Africa and Latin America (Swerdlow *et al.,* 1992; Lipp *et al.,* 2003).

Cholera is a major public health problem in developing countries, which is caused by infection of the intestine with toxigenic *V. cholerae* (Islam *et al.,* 1995). Cholera is endemic in Bangladesh for many years and maintains a regular seasonal pattern (Glass *et al.,* 1982). In Bangladesh, cholera epidemics occur twice every year, the highest peak during post monsoon (September-January) and second smaller peak during pre-monsoon (March-May). During inter-epidemic period *V. cholerae* cannot be cultured from the surface water, whereas in epidemic season it can be isolated from the patients' body as well as from surface water (Alam *et al.*, 2006). *V. cholerae* O139 have been identified as an etiological agent of cholera since 1993 (Albert *et al*., 1993). The rest of the non-O1 and non-O139 serogroups of *V. cholerae* do not cause sporadic or small outbreaks of diarrhoea and extra intestinal infections (Joseph *et al.*, 1965). When *V. cholerae* is not wrecking havoc in the human intestine, it may be found in diverse aquatic environments such as estuaries, rivers, ponds etc. (Islam *et al.*, 1995; Lipp *et al.*, 2003). *V. cholerae* can survive either as free living planktonic organisms in the water column or associated with phytoplankton and zooplankton (Huq *et al.*, 1984 and Islam *et al.*, 1995). In the natural habitat, *V. cholerae* remains attached to *Anabaena* sp and *V. cholerae* O1 was found to be persistent in the mucilaginous sheath of a blue green alga, *A. variabilis* in the artificial aquatic environments. Some investigators studied viability of *V. cholerae* O1 in different aquatic environments (Islam *et al*., 1995). Ecology, epidemiology, microbiology and remote sensing *V. cholerae*, a serious pathogen for humans, has been the subject of intense study for more than a century, yet the discovery that this bacterium is a natural inhabitant in the riverine, estuarine, and coastal waters throughout temperate and tropical regions of the world (Colwell, 1996).

The study on count of *V. cholerae* O1 and O139 in plankton and water sample are scarce in Bangladesh. For the ecological study, it is necessary to know the variation of *V. cholerae* O1 and O139 count in water and other micro-flora and fauna as they are the most pathogenic cholera pathogen across the world. Such study will help to manage cholera pathogen in an integrated approach keeping in mind the interest of *V. cholerae* O1 and O139, plankton and humans.Therefore, the current study was undertaken to explore the abundance and ecology of *V. cholerae* during epidemic and interepidemic periodsthat will also pave the way of better understanding and further research in connection with epidemiological perspective of cholera.

Materials and methods

The study selected several coastal ponds in different areas of Mathbaria Upazila under Pirojpur district (Bangladesh) and were surveyed. Finally three pristine ponds were chosen. The chosen ponds were designated as: pond 1(site-1), pond 2 (site-2) and pond 3 (site-3) and those ponds are learnt to be the potential reservoir of *V. cholerae* round the year. Sampling was carried out for a period of 36 months starting from October 2006 to September 2009 (October to January were treated as winter, February to May as summer and June to September as monsoon for convenience).

A total of 324 samples comprising each of 108 water, phytoplankton and zooplankton samples were analyzed for consecutive 36 months (3 years). In each round, one 5 liter sampling bottle was filled with water for 20 times from different areas of each pond and the same were filtered through 64-µm and 20-µm-pore-sized nylon nets (Millipore Corp., Bedford, , *Massachusetts* 01870, USA). The 64-µm-pore-sized net was placed sequentially in front of the 20-µm mesh-sized nylon net, with each having a collecting bucket at the base of the net. In this way, 100 liters of water was filtered from each pond in each round in order to get the final concentration of 50 ml separately from two nets with a view to analyzing phyto- and zooplankton respectively along with their possible attachment with *V. cholerae*. The nets were hung high while the plankton-free water was filtering out from where 200 ml water sample was collected into another bottle. So, there were three types of samples, viz, 50 ml phytoplankton sample, 50 ml zooplankton sample and 200 ml plankton-free water sample in the separately labeled vials from each pond and accordingly 9 types of samples were collected from three ponds in each turn. The same trend of sample collection was continued for consecutive 36 months totaling the annual number of samples as 108 and sum total of samples for three consecutive years were 324 (108 water samples, 108 zooplankton sample and 108 phytoplankton samples). All samples were collected by using aseptic technique in sterile dark Nalgene bottles (Nalgene Nunc International, St. Louis, Mo. *Missouri*, USA) placed in an insulated plastic box and were transported at ambient air temperature from the site of collection to the central laboratory of the International Center for Diarrhoeal Disease Research, Bangladesh (ICDDR, B), in Dhaka. Both the samples from 64-µm mesh sized and 20-µm mesh sized plankton nets were further concentrated in the laboratory to a final volume of 5 ml by filtering through a 0.22-µm-pore-size bacteriological membrane filter (Millipore). All samples were processed the following day, with approximately 20 hours of elapsing between sample collections in the field and processing in the laboratory.

Samples were enriched in alkaline peptone water referred as APW (Difco, Detroit, MI) and incubated at 37 °C for 6 to 8 hours. Serological tests were performed using polyvalent and monoclonal antibodies specific for *V. cholerae* O1 and O139. Samples were pre-incubated overnight in the dark with 0.025% yeast extract (Difco) and 0.002% nalidixic acid (Sigma-Aldrich, St. Louis, MO, USA). The samples were then centrifuged and the pellet was stained with cholera DFA reagents like fluorescein isothio cyanate-labelled antiserum specific for O1 or O139 (New Horizon Diagnostics, Columbia, MD). Fluorescent stained cells were observed and counted under UV light by using an epifluorescence microscope (Olympus B×51) and were recorded with the help of a digital camera attached with the same microscope (Olympus DP20).

Results and discussion

In the present study, the total frequency of *V. cholerae* O1 was found to be the highest (2.2×10^7 cfu/L) in water samples and lowest (4.0×10^5 cfu/L) in phytoplankton samples. Out of all sites, the maximum frequency of *V. cholerae* O1 (2.5×10^6 cfu/L) was obtained from water sample of site 2 in summer'07 and minimum (4.0×10^4 cfu/L) from site 2 in winter'08. In site 1, maximum *V. cholerae* O1 (1.9×10^6 cfu/L) was obtained from water sample in both summer'07 and summer'09 and minimum (1.4×10^5 cfu/L) in winter'07.

The frequency of *V. cholerae* in association with zooplankton was highest ($3.4\text{x}10^4$ cfu/L, $3.1\text{x}10^4$ cfu/L and $6.8\text{x}10^4$ cfu/L) in site 1, 2 and 3, respectively throughout the summer seasons of the studied period and minimum association was in winter seasons (4.9×10^3, 5.5×10^5 and 2.6×10^4 in site 1, 2 and 3, respectively). Site 2 and 3 showed highest total *V. cholerae* O1 (2.5×10^6 cfu/L and 1.9×10^6 cfu/L) in summer'07 and summer'09 respectively and lowest in winter'08 (4.0×10^4 cfu/L) and winter'07 (5.1×10^4 cfu/L), respectively (Table 1). In all

Table 1. Seasonal frequency of V. cholerae O1 in water, zooplankton and phytoplankton samples in three sites.

Site	Year	Season	Water (cfu/L)	Zooplankton (cfu/L)	Phytoplankton (cfu/L)	Total
1	2006-2007	Winter	1.1×10^5	2.7×10^4	8.6×10^3	1.5×10^5
		Summer	1.9×10^6	3.4×10^4	1.2×10^4	1.9×10^6
		Monsoon	4.3×10^5	1.7×10^4	6.9×10^3	4.3×10^5
	2007-2008	Winter	5.5×10^4	4.9×10^3	3.0×10^3	1.3×10^5
		Summer	2.4×10^5	2.4×10^4	2.2×10^4	1.9×10^6
		Monsoon	9.8×10^4	1.3×10^4	1.6×10^4	4.2×10^5
	2008-2009	Winter	1.4×10^5	8.2×10^3	5.1×10^3	1.4×10^5
		Summer	1.9×10^6	1.7×10^4	1.3×10^4	1.9×10^6
		Monsoon	2.8×10^5	1.2×10^4	8.6×10^3	4.3×10^5
2	2006-2007	Winter	3.9×10^5	2.1×10^4	1.4×10^4	4.2×10^5
		Summer	2.5×10^6	3.1×10^4	2.6×10^4	2.5×10^6
		Monsoon	5.3×10^5	1.7×10^4	9.2×10^3	5.5×10^5
	2007-2008	Winter	1.4×10^5	5.5×10^3	2.1×10^3	4.0×10^5
		Summer	5.9×10^5	1.9×10^4	1.6×10^4	2.3×10^5
		Monsoon	2.3×10^5	1.0×10^4	1.1×10^4	5.0×10^5
	2008-2009	Winter	1.9×10^4	1.7×10^4	4.2×10^3	4.1×10^4
		Summer	2.1×10^6	2.5×10^4	1.4×10^4	2.4×10^6
		Monsoon	2.2×10^5	1.0×10^4	1.3×10^4	5.2×10^5
3	2006-2007	Winter	8.5×10^5	3.3×10^4	1.6×10^4	8.9×10^5
		Summer	1.9×10^6	6.2×10^4	3.3×10^4	1.9×10^6
		Monsoon	9.0×10^5	4.4×10^4	2.1×10^4	9.7×10^5
	2007-2008	Winter	4.6×10^5	3.3×10^4	1.5×10^4	8.7×10^5
		Summer	1.7×10^6	6.8×10^4	3.4×10^4	1.8×10^6
		Monsoon	9.0×10^5	4.8×10^4	1.9×10^4	9.6×10^5
	2008-2009	Winter	5.3×10^5	2.6×10^4	1.2×10^4	8.8×10^5
		Summer	2.3×10^6	6.4×10^4	3.1×10^4	1.9×10^6
		Monsoon	8.0×10^5	5.6×10^3	1.6×10^4	9.7×10^5
Total			2.2×10^7	7.0×10^5	4.0×10^5	2.3×10^7
Percentage (%)			95%	3%	2%	100%

(Winter = October to January, Summer = February to May, Monsoon = June to September)

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cases, a seasonal prevalence of *V. cholerae* O1 was detected where the pathogen was found to be flourished more in summer and declined significantly in winter.

The frequency of *V. cholerae* O139 showed more or less similar pattern of findings like *V. cholerae* O1 and its highest count was with water samples (5.0×10^6 cfu/L) and lowest in phytoplankton samples (3.4×10^5 cfu/L) out of the sum total of 5.8×10^6 cfu/L during study periods. In all sites, the maximum frequency of *V. cholerae* O139 was obtained from site 3 in summer'07 (4.4×10^5 cfu/L) and minimum from site 2 in winter'06 (9.9×10^4 cfu/L). In site 1, *V. cholerae* O139 was found to be maximum (3.2×10^5 cfu/L) in summer'07 and minimum (1.0×10^5 cfu/L) in winter'06, winter'07 and winter'08 throughout the investigation period. In site 2, the highest *V. cholerae* O139 (3.3×10^5 cfu/L) was found in summer'07 and lowest ($1.0\text{o}10^5$) in each of winter'07 and winter'08. In site 3, the maximum *V. cholerae* O139 count (4.4×10^5 cfu/L) was in summer'07 and lowest (1.2×10^5 cfu/L) in winter'07 and winter'08 (1.2×10^5 cfu/L), respectively. Zooplankton had highest (2.2×10^4 cfu/L) individual attachment with *V. cholerae* O139 in site 1 in summer'09 and (2.5×10^4 cfu/L) site 2 in summer'07 and lowest in winter (6.6×10^3 cfu/L, 1.0×10^4 cfu/L and 1.1×10^4 cfu/L) in site 1, 2 and 3, respectively (Table 2). In all cases, a seasonal prevalence of *V. cholerae* O139 was detected where the organism has been found to flourish in summer months and decline in winter months.

Moreover, comparative analysis showed that the grand total count of VC O1 was higher in water, phyto and zooplankton samples than that of VC O139 throughout the study period. This indicates the prevalence of VC O1 over VC O139 in all the studied period. The summer season had highest count of both VC O1 and VC O139 followed by monsoon and winter in three years study period (Table 1 and 2). This indicates that the summer temperature, salinity and other water parameters might have some favor for the replication of both groups of pathogenic *V. cholerae* and such coincidence might have linked with cholera epidemics in Bangladesh.

In the present study, the total counts of VCO1 in water, zooplankton and phytoplankton samples were 2.2×10^7 cfu/L, 7.0×10^5 cfu/L and 4.0×10^5 cfu/L respectively and the total counts of VCO139 were 5.0×10^6 cfu/L, 4.6×10^5 cfu/L and 3.4×10^5 cfu/L, respectively. However, Ahmed *et al.* (2007) found an association of *V. cholerae* with plankton especially cyanobacteria in relation to some physicochemical parameters in the river Buriganga, Dhaka, Bangladesh. Monthly abundance of phytoplankton and zooplankton varied from 457 to 14166 and from 169 to 1655 individual in L^{-1}, respectively. Monthly average of fecal coliform in water, zooplankton and phytoplankton samples were 3.99×10^9, 4.54×10^3 and 4.28×10^2 (CFU/L), respectively.

Table 2. Seasonal frequency of V. cholerae O139 in water, zooplankton and phytoplankton samples of three studied sites.

Sites	Year	Study period	Water (cfu/L)	Zooplankton (cfu/L)	Phytoplankton (cfu/L)	Total
1	2006-2007	Winter	9.7×10^4	6.6×10^3	5.4×10^3	1.0×10^5
		Summer	2.6×10^5	1.9×10^4	1.4×10^4	3.2×10^5
		Monsoon	1.9×10^5	1.3×10^4	8.4×10^3	2.1×10^5
	2007-2008	Winter	9.5×10^4	3.9×10^4	3.5×10^3	1.0×10^5
		Summer	2.6×10^5	2.0×10^4	1.6×10^4	3.0×10^5
		Monsoon	2.0×10^5	9.0×10^3	8.3×10^3	2.0×10^5
	2008-2009	Winter	1.0×10^5	1.0×10^4	7.4×10^3	1.0×10^5
		Summer	2.8×10^5	2.2×10^4	1.6×10^4	3.1×10^5
		Monsoon	1.7×10^5	1.3×10^4	1.0×10^4	2.1×10^5
2	2006-2007	Winter	9.6×10^4	1.2×10^4	7.5×10^3	1.1×10^5
		Summer	2.9×10^5	2.5×10^4	2.1×10^4	3.3×10^5
		Monsoon	1.6×10^5	1.8×10^4	1.4×10^4	2.2×10^5
	2007-2008	Winter	9.2×10^4	1.0×10^4	1.8×10^3	1.0×10^5
		Summer	2.5×10^5	1.9×10^4	9.1×10^3	3.1×10^5
		Monsoon	2.1×10^5	1.1×10^4	5.7×10^3	2.1×10^5
	2008-2009	Winter	9.6×10^4	1.0×10^4	1.9×10^3	1.0×10^5
		Summer	2.9×10^5	1.9×10^4	9.8×10^3	3.1×10^5
		Monsoon	1.9×10^5	1.1×10^4	6.4×10^3	2.1×10^5
3	2006-2007	Winter	1.2×10^5	1.4×10^4	1.0×10^4	1.3×10^5
		Summer	3.9×10^5	3.1×10^4	2.5×10^4	4.4×10^5
		Monsoon	2.1×10^5	2.1×10^4	1.7×10^4	2.5×10^5
	2007-2008	Winter	1.7×10^5	1.1×10^4	8.7×10^3	1.2×10^5
		Summer	2.5×10^5	3.4×10^4	3.0×10^4	4.3×10^5
		Monsoon	1.5×10^5	1.7×10^4	1.7×10^4	2.4×10^5
	2008-2009	Winter	9.1×10^4	1.5×10^4	7.9×10^3	1.2×10^5
		Summer	2.2×10^5	3.1×10^4	3.1×10^4	4.2×10^5
		Monsoon	1.4×10^5	1.8×10^4	1.6×10^4	2.5×10^5
Total			5.0×10^6	4.6×10^5	3.4×10^5	5.8×10^6
Percentage (%)			86%	8%	6%	100%

(Winter = October to January, Summer = February to May, Monsoon = June to September)

During epidemics, toxigenic *V. cholerae* O1 and O139 were isolated from the surface water and plankton

samples. It was also observed that cholera toxin (ctx) positive in water and phytoplankton samples of the river. A bloom of *Oscillatoria* sp. (1.6×10^4 individual L^{-1}) occurred in the upper reaches of the river Buriganga.

In the current research, the highest and lowest counts of VC O1 were 2.5×10^6 cfu/L and 1.3×10^5 cfu/L respectively, whereas, the highest and lowest counts for VCO139 were 4.4×10^5 cfu/L and 1.0×10^5 cfu/L, respectively. These findings are relatively more than that of the findings of Ganesh *et al.* (2009) who proved that *V. cholerae* populations in the sediment to be high at 2.3×10^7 cfu/g after 150 days of culture (DOC) and low in 1.9×10^7 cfu/g at 25th DOC. *V. parahaemolyticus* in sediments was high (5.5×10^7 cfug) at 150th DOC and low (1.3×10^7 cfu/g) at 25th DOC.

The reports of the present study supplement the findings of Alam *et al*. (2006) to consider the aquatic environment of Mathbaria zone to be the potential reservoir of VC O1 and O139 Bengal. The aquatic environment of Mathbaria (Bangladesh) was found to be the reservoir for *V. cholerae* O1 and O139 Bengal (Alam *et al*., 2006). He also observed that significant clumping of the bacteria during the inter-epidemic periods and the fluorescent micrographs revealed large numbers of *V. cholerae* O1 in thin films of exopolysaccharides (biofilms) of planktons.

The findings of present study strongly support the findings of Huq *et al*. (1990). The present study not only found VC O1 using fluorescent antibody method but also VC O139 from water, phyto- and zooplankton samples. Both the authors' data are potential to study the ecology of *V. cholerae*. In the current study, out of grand total count, the highest VC O1 was found in water sample (95%) followed by zooplankton sample (3%) and phytoplankton sample (2%), but in case of VC O139 in the water sample scored 86% which was the highest followed by zooplankton sample (8%) and phytoplankton sample (6%). Huq *et al.* (1990) showed that *V. cholerae* O1 could be detected by direct immunofluorescence microscopy throughout the year in water samples even when the organism could not be isolated i.e., cultured, from water. They also reported for the first time that the presence of *V. cholerae* O1 throughout the year by using a fluorescent antibody detection method to detect organisms in >63% of plankton specimens where <1% of the sample were positive for culturable *V. cholerae* O1.

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