

# Environmental factors associated with emergence of disease with special reference to cholera

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العوامل البيئية المرتبطة بظهور الأمراض ولاسيما الكوليرا  
أنوار الحق وريتا ر. كولويل

خلاصة ظهرت مؤخراً على الصعيد العالمي عدة أمراض وخيمة ذات صلة بعوامل بيئية . وتتطرق هذه المقالة إلى تلك العوامل . ونولي اهتماماً خاصاً بنمط مصلي من صمات الكوليرا ( الهیضة ) تم التعرف عليه مؤخراً ، ونجمت عنه أوبئة انتشرت في الهند وبنغلاديش .

**ABSTRACT** Many serious diseases related to environmental factors have recently emerged worldwide. This paper examines such factors, giving particular attention to a newly recognized serotype of *Vibrio cholerae*, which has caused epidemics in India and Bangladesh.

**Les facteurs environnementaux associés à l'apparition des maladies, s'agissant plus particulièrement du choléra**

**RESUME** De nombreuses maladies graves liées à des facteurs environnementaux sont apparues récemment dans le monde entier. Le présent article examine ces facteurs, en s'attachant plus particulièrement au sérotype nouvellement identifié de *Vibrio cholerae* qui a provoqué des épidémies en Inde et au Bangladesh.

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## Introduction

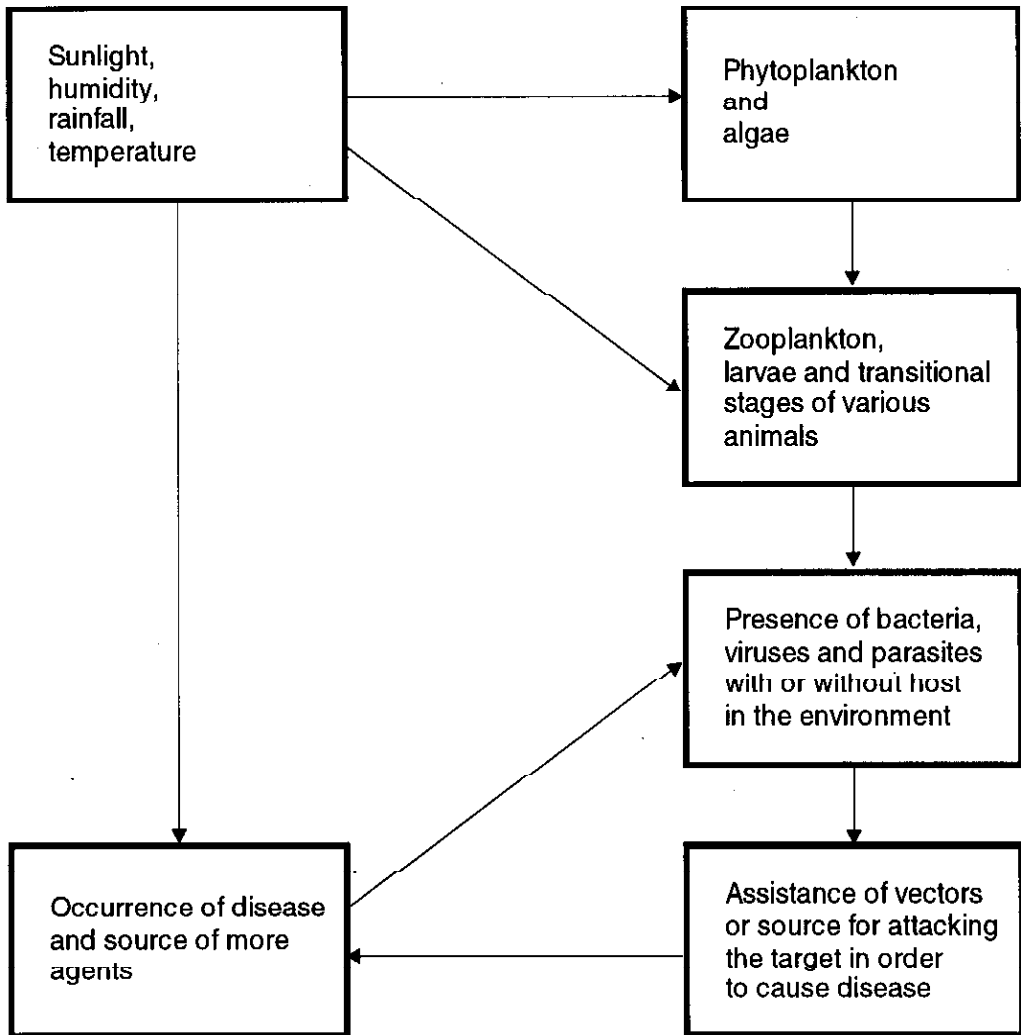
Infectious diseases, once expected to be eliminated as a significant public health problem, remain the leading cause of death in the world [1]. Many factors have contributed to the persistence and increase in the occurrence of infectious diseases, such as societal changes, health care, food production, human behaviour, environmental change, public health infrastructure and microbial adaptation [2]. Many diseases related to environmental factors have recently emerged worldwide and are of a degree to raise serious concerns.

The focus of this paper is on the environmental factors associated with emergence of disease. Pathogens, such as bacteria, viruses and parasites, that cause disease in humans and animals may depend partially or entirely for their existence on other physical, chemical, or biological factors. Many are strictly vector-dependent while others are not. For both types, environmental factors can affect directly, or indirectly, survival, persistence and ability to produce disease. Temperature is a key factor (Figure 1). Sunlight can affect the persistence and spread of a pathogen if it is associated with phytoplankton and/or algae. For example, when algae and phytoplankton increase in biomass, zooplankton blooms rapidly follow. Bacteria associated with zooplankton also increase. Also, humidity resulting from evaporation due to elevation of temperature may play an important role in the occurrence of many diseases. With appropriate humidity and moisture, most bacteria survive longer than they would in less humid or dry areas. There are many diseases common to tropical climates that are linked to water transmission. If transmission between hosts does not involve vectors, then water, or at least humid conditions, can be involved in transmission [3]. For example, in warm and

humid regions, where water is available as a transmitting medium, *Vibrio cholerae* may proliferate rapidly to the level of an infective dose. In general, it is impossible to separate environmental factors from biological factors, as can be seen from interrelationships in nature that play a significant role in the emergence of infectious diseases.

## Effect of bacterial attachments on their growth

Bacteria often require a substrate to attach themselves to before they can multiply, and the attachment can be host specific or site specific. Davis et al. [4] emphasized the importance of attachment or colonization in defining the pathogenicity of bacteria: "the term virulence ... is the degree of pathogenicity used to encompass two features of a pathogenic organism, a) its infectivity, the ability to colonize a host, and b) the severity of the disease produced". The surface characteristics of various materials, as well as their chemical and organic origin, when suspended in water, can influence colonization by bacteria [5]. More than half a century ago, Heukelekian and Heller [6] showed that at low nutrient concentrations substrate plays an important role in bacterial multiplication. Suspended particle-associated microorganisms are abundant in the aquatic environment. The range of these surfaces is diverse and multiple in origin. Some are man-made (for example, created by ocean dumping), the surfaces of which may differ from one location to another. However, substrates in the vast natural environment, controlled by climate, may be even more diversified. Climate change in the environment is usually not rapid, allowing cells to acclimatize. Zobell [7] reported that bacteria attached themselves to inert particles and



**Figure 1 A model demonstrating possible interactions of environmental factors influencing disease or disease outbreaks**

Source: Yasuno M, Whitton BA, eds. Biological monitoring of environment pollution. Tokyo, Tokai University Press, 1988

hypothesized that adsorption was beneficial for the growth of bacteria. The factors that influence attachment include temperature, pH and nutrient concentration [8].

Phytoplankton, zooplankton and the eggs of several invertebrate species, when

suspended in the water column for several months, afford an excellent surface rich with nutrients to attract bacteria [9,10]. These biological components themselves are directly affected by the physical and chemical parameters of the environment.

Southward et al. [11] published a report of changes in the distribution and abundance of zooplankton and intertidal organisms in the western English Channel as sea temperature rose. The authors observed extensive changes in marine communities off the coast of south-west Britain and the western English Channel during the past 70 years, a period of time during which there was climate warming from the early 1920s, then cooling to the early 1980s, with recent resumption of warming. The change in annual mean temperature was approximately  $\pm 0.5$  °C. The authors observed marked changes in the plankton community structure as well as the distribution of plankton and intertidal organisms, an increase or decrease of two or three orders of magnitude. It was interesting to note that there was an increase in the abundance and range of warm-water species during periods of warming, with a decrease for cold water species. The reverse occurred during the period of cooling. From climate models, it is predicted that a rise of 2 °C mean temperature in the next 50 years will result in 200–400 latitudinal shifts in the distribution of fish and benthos, including extensive restructuring for planktonic, pelagic and benthic communities.

### **The role of temperature and humidity on disease occurrence**

Higher animals are very sensitive to climate change. Terrestrial vertebrate hosts are more likely to be affected by changes in environmental temperature (and humidity) compared to those animals living in an aquatic environment [12].

In aquatic environments, higher temperature means more evaporation, causing increased humidity, an increased concentration

of nutrients and a general change in ecology. The physics and chemistry of the ocean change with climate, altering functional relationships in the marine food web [13]. The initial effects are observed in the lower trophic levels, with significant changes in phytoplankton biomass and shifts in species dominance. For example, phytoplankton blooms and red tides have been known since ancient times, causing disease among fish and shellfish [13].

Diseases such as malaria and eastern encephalitis are transmitted via the mosquito, the life cycle of which is dependent on temperature and precipitation. Similarly, rodent-associated diseases are also correlated with climate. A good example is the emergence of hantavirus in the United States. In 1993, hantavirus pulmonary infection was confirmed among 94 persons, involving 20 states, with 48% mortality. In the southern United States, after six years of drought, heavy rains in 1992–93 caused grasshoppers to thrive and pine nuts to increase in abundance, resulting in a ten-fold increase in deer mice [14], which are the vectors of hantavirus. Komar and Spielman [15] concluded that recent landscape and faunal changes had caused zoonotic eastern encephalitis to re-emerge in Massachusetts after having been dormant for 100 years.

### **The aquatic environment and *Vibrio cholerae***

One emerging health problem is the spread of a newly recognized serotype of *Vibrio cholerae*, O139, which has caused epidemic cholera in Bangladesh and India. Serotype O139 of *V. cholerae* was first identified in India and a few months later in part of coastal Bangladesh, gradually moving inland.

The association of *V. cholerae*, the causative agent of cholera, and its host, the copepod, has been under study for more than 25 years. It is now well established that *V. cholerae* is autochthonous to the aquatic environment and closely associated with crustacea. Cockburn and Cassanos in 1960 first addressed the association of *V. cholerae* with plankton, observing a correlation between the incidence of cholera and presence of increased numbers of blue-green algae in the water [16]. This correlation was explained as resulting from photosynthesis of the algae, resulting in increased dissolved oxygen in the water and an elevated pH, supporting growth of *V. cholerae*. In addition, *V. cholerae* releases an enzyme, mucinase, which actively degrades mucin and mucin-like substances present in plant cells. Several investigators have tested the hypothesis of Cockburn and Cassanos, and suggested a possible relationship between *V. cholerae* and phytoplankton [17].

Silvery and Roach [18] found that when blue-green algae begin to disintegrate after the peak bloom, the Gram-negative heterotrophic bacterial population immediately begins to increase. The authors further noted the presence of high concentrations of Gram-negative bacilli in the gelatinous cover of the blue-green filaments. By using a direct detection method, *V. cholerae* was observed to be associated with the cyanobacterium *Anabaena variabilis*, persisting in the mucilaginous sheath for around 15 months [19]. However, the specificity of the association of *V. cholerae* with phytoplankton was not clear, only inferential, especially since several peaks of algae bloom are observed in a given season. In addition, amplification of *V. cholerae* O1 has not been correlated with algae. Silvery and Roach [18] observed only one big peak of an algae bloom occurring during an annu-

al cycle, suggesting that once an algae bloom has disintegrated, the bacterial cells require another mechanism of survival and multiplication to cause an epidemic.

Association of *V. cholerae* with aquatic plants in laboratory microcosm experiments has been demonstrated by Spira et al. [20]. In this study, *V. cholerae* was found to concentrate on the surface of the water hyacinth, *Eichhornia crassipes*, the most abundant aquatic plant in Bangladesh. They postulated this mechanism accounted for enhanced survival and offered a potential reservoir for *V. cholerae*. Other floating plants, such as *Lemna minor*, a common duckweed found in freshwater environments, have also been reported to harbour *V. cholerae* [21]. However, these plants were also found to be colonized by *Aeromonas* spp. [22], indicating nonspecific bacterial attachment to aquatic vegetation. The specificity of the association of *V. cholerae* with phytoplankton has not been demonstrated, and the role of aquatic vegetation, other than as a passive carrier, in the epidemiology of *V. cholerae* is doubtful.

In laboratory microcosm experiments, *V. cholerae* O1 and the newly recognized serogroup O139 of *V. cholerae* were also observed to attach to chitin particles [23]. This has led Nalin et al. [24] to hypothesize that *V. cholerae* O1 attached to chitin particles would be protected from the acid environment of the stomach. In a recent field investigation in Bangladesh, one of twelve plankton samples was positive for *V. cholerae* by culture, whereas four were positive by direct fluorescent antibody (DFA) staining [25]. When examined for *V. cholerae* O139 Bengal, two out of 12 plankton samples were positive by DFA and none by culture. One of the plankton samples carried both *V. cholerae* O1 and O139, determined by DFA staining [25].

Enhanced survival of *V. cholerae* O1 could be demonstrated in laboratory experiments [10] when live copepods were present. *V. cholerae* O1 associated with planktonic living copepods survived significantly longer in laboratory microcosms than *V. cholerae* O1 attached to dead copepods, with or without *Pseudoisochrysis* spp., a blue-green upon which the copepods feed. Copepod egg cases were found to have significant attachment by *V. cholerae* when examined by scanning electron microscopy [10]. Other organisms, such as *Pseudomonas* spp. and *Escherichia coli*, showed nonspecific attachment [10].

There are two distinct seasons (one major and the other minor) for cholera epidemics in Bangladesh, spring and autumn [26]. During the years 1964–80, the major cholera peak was during September and November [26]. The minor peak, however, was longer, between January and April. According to Oppenheimer et al. [27], zooplankton populations decrease during the monsoon season, because of less nutrient availability, then increase significantly during August and September, once the post monsoon phytoplankton blooms occur. An important report by Kiorboe and Neilsen [28] indicates that there are two distinct seasons for several species of copepod egg production. One is between February and April and the other is during August and September. This fits very well into our hypothesis of copepods' role in the survival, multiplication and transmission of *V. cholerae* in the natural aquatic environment [10]. The findings of Kiorboe and Neilsen [28] on the production of copepod eggs fit well because production occurs just before the cholera season in Bangladesh; this is particularly important because free-swimming cells of *V. cholerae*, even in the nonculturable state, can attach to copepod eggs and multiply rapidly [10].

The number of *V. cholerae* O1 cells ingested must be high enough to constitute an infective dose— $10^4$  to  $10^6$  cells, depending on the state of health of the victim [29]. There are enough *V. cholerae* O1 occurring on copepods to cause cholera if ingested while bathing or swimming or by drinking untreated water from ponds, rivers and lakes of cholera-endemic countries, notably Bangladesh. The number in a glass of water of 150–200 millilitres would be enough to cause cholera, i.e., trigger an infection or even an epidemic.

### Viable but nonculturable *V. cholerae*

Isolating *V. cholerae* from environment samples throughout the year, especially between epidemics, has not been consistently achievable until recently [30]. In fact, an explanation was sought for the "mysterious" disappearance of *V. cholerae* O1 from the environment during interepidemic periods, notably in cholera-endemic countries like Bangladesh. Furthermore, cholera was not recognized in Latin America before the current pandemic for at least two reasons: lack of recognition of the existence of an aquatic reservoir of *V. cholerae* in Latin America and lack of routine investigation of O1 and non-O1 cholera vibrios [31]. However, the discoveries of the past decade have revealed the existence of a dormant, i.e., viable but nonculturable state, which *V. cholerae* O1 enters in response to nutrient deprivation and other environmental conditions [32] as well as the persistence of *V. cholerae* in estuarine, riverine and coastal environments as a natural habitat.

The use of the term "viable but nonculturable" has increased steadily during the past 15 years. Counts of bacteria obtained

by direct microscopy were often 200 to 5000 times more than the number of colonies on plates. Zobel in 1946 reconfirmed earlier findings that only a small percentage of bacterial cells actually present in a given water sample are enumerated by plate count [7]. Furthermore, it was assumed until recently that when bacterial cells were unable to grow on culture plates they were dead [33]. However, these so called "dead cells" can be shown to be viable, but non-culturable, using direct viable count methods [34]. Very recently, reactivation of viable but nonculturable *V. cholerae* O1 has been reported [35]. Significant changes in cell morphology have been observed by electron microscopy during the process of conversion of culturable cells to the nonculturable state [36]. Adverse nutritional conditions and fluctuation of environmental parameters, such as temperature, can cause bacteria to become nonculturable. The time required for different species of bacterial cell may vary from several hours to months. It is speculated that there may be one or more factors in the environment responsible for converting a normal culturable cell to the viable but nonculturable state [23]. The viable but nonculturable state reported for *V. cholerae* is now recognized to be a common phenomenon in many species of bacteria [37].

### Serotype conversion of *V. cholerae*

Another important and well studied phenomenon is serotype or biotype conversion, which has been reported by many investigators. For cholera, *V. cholerae* serotype O1 is considered to be the most virulent and the epidemic serotype. All other serotypes, known as non O1, are usually but not al-

ways nontoxigenic and are not considered to be of major epidemic relevance. Recent outbreaks of cholera in India, Bangladesh and several other countries have been found to be caused by a non-O1 *V. cholerae*, assigned to serotype O139 [38]. This serotype is now considered to have been derived from the environment. For the first time, serious concerns have been raised about other potentially pathogenic serotypes that may be present in the environment. In laboratory microcosm experiments, seroconversion of *V. cholerae* non-O1 to O1 and vice versa have been demonstrated [39]. Seroconversion and/or changes in cell surface properties may occur naturally in the environment [40]. Seroconversion has been observed in *V. cholerae* under different temperature and salinity conditions, suggesting that the phenomenon may occur more commonly than known before, in brackish, estuarine or sea water throughout the year. Clearly, environmental factors have a direct influence on the pathogenicity of *V. cholerae*.

In conclusion, *V. cholerae*, an environmental (autochthonous) inhabitant of brackish, estuarine, and marine ecosystems represents an important agent of disease that can be dramatically influenced by environmental changes, including global environmental change.

### Acknowledgement

The work was partially supported, in part, by the Thrasher Research Fund, grant UM 930720-8011, the National Oceanic and Atmospheric Administration through a University of Maryland Sea Grant, SG3527614, and National Institutes of Health grant 5 R01 A/19716-13, subcontract from the University of Maryland at Baltimore.

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