Ecology of *Vibrio cholerae* serogroup 01 in aquatic environments[1]

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ABSTRACT *The endemic and seasonal nature of cholera depends upon the survival of* Vibrio cholerae *01 in a viable but not necessarily culturable state in ecologic niches in aquatic environments during interepidemic periods. To understand the ecology of* V. cholerae *it is necessary to know which aquatic ecosystems can harbor it and thus contribute to the endemic presence of cholera in Latin America. This article summarizes knowledge about the ecology of* V. cholerae *01, specifically, the abiotic and biotic factors that are relevant to the microbe's survival in aquatic environments. This pathogen finds favorable conditions in waters characterized by moderate salinity, high nutrient content, warm temperature, neutral or slightly alkaline pH, and the presence of aquatic macrophytes, phytoplankton, zooplankton, fish, mollusks, and crustaceans. These ecologic conditions are typical of estuaries and coastal swamps, and toxigenic* V. cholerae *01 is now considered an autochthonous member of the microbial flora of these environments. The microorganism has also shown the ability to colonize freshwater ecosystems in its viable but not necessarily culturable form, if organic or inorganic substrates that favor its survival are available.*

Vibrio cholerae serogroup 01 consists of the classic and El Tor biotypes, the latter of which is responsible for the seventh cholera pandemic, which is now in progress. These two biotypes comprise the Inaba, Ogawa, and Hikojima serotypes. Epidemic cholera is caused by strains of *V. cholerae* that produce enterotoxin; strains that do not produce the toxin are identified as nonepidemic, although they may cause diarrhea. Vibrios that do not agglutinate serogroup 01 antiserum, known as *V. cholerae* non-01, were previously identified as nonagglutinating or noncholeric vibrios but are now considered *V. cholerae* species. Some non-01 serogroup strains produce toxin and have caused sporadic cases of cholera and small outbreaks of diarrheal disease without causing widespread epidemics (*1*). The recently discovered serogroup 0139 is an exception, because it has caused epidemics in India (*2*) and Bangladesh (*3*).

Until the late 1970s, *V. cholerae* serogroup 01 was believed to be able to survive for only a few hours or days in an aquatic environment *(4).* This idea has been abandoned because it is now known that the presence of the microorganism in aquatic environments does not depend solely on the extent of fecal contamination. In fact, several studies have revealed that there is no correlation between the presence of fecal coliform bacteria and toxigenic and nontoxigenic strains of *V. cholerae* 01 biotype El Tor in aquatic environments (*5–7*). This finding suggests that the pathogenic agent can survive in water that is relatively free of human fecal contamination. Laboratory research (*8, 9*) later confirmed this theory, supporting the hypothesis that the microorganism is an autochthonous member of the microbial flora found in brackish waters typical of estuaries and coastal swamps, as was first suggested by Colwell et al. (*10*). Toxigenic *V. cholerae* 01 biotype El Tor has also been detected for extended periods in freshwater environments where there is no human fecal contamination (*11, 12*).

In response to environmental stress in aquatic environments, such as low concentrations of nutrients and low temperatures, *V. cholerae* 01 and non-

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01 adopt a viable state that enables them to carry out metabolic functions and form colonies without being culturable (*13–16*). If favorable environmental conditions return, *V. cholerae* can become culturable again. *V. cholerae* 01 in a viable but nonculturable state has produced clinical symptoms of cholera in volunteers, which confirms that it maintains its pathogenicity in aquatic environments despite the inability of the cells to be cultured (*16*).

Some experts have suggested that the endemic and seasonal nature of cholera in Bangladesh depends on the presence of the pathogenic agent in a viable but nonculturable state in aquatic ecological niches that serve as reservoirs for the agent between epidemic periods (*13, 16–19*). This article summarizes the ecology of *V. cholerae* 01 on the basis of the abiotic and biotic factors essential for survival of the microbe in aquatic environments.

There is concern that cholera is becoming endemic in Latin America (*20*). According to the Pan American Health Organization, a seasonal type of cholera has emerged in Ecuador, Peru, and various Central American countries, which suggests that the infection is already endemic (*21*). To determine which aquatic ecosystems in Latin America can harbor the microorganism and thus contribute to the endemic nature of this disease, it is essential to understand the ecology of *V. cholerae*.

ECOLOGY OF *VIBRIO CHOLERAE* SEROGROUP 01

Abiotic factors

Water. *V. cholerae*, including its toxigenic strains (*5, 11, 12, 22, 23*), has often been isolated from aquatic environments such as bays (*5*), rivers (*11, 12*), canals (*22*), ditches (*24*), and groundwater (*23*). Cholera transmission takes place primarily through ingestion of water contaminated with the feces or vomitus of patients or, less frequently, with the feces of asymptomatic carriers (*1*). *V. cholerae* serogroup 0139 has also been isolated in aquatic environments (*3, 25, 26*), and the vibrios of this serogroup are believed to be spread primarily by water (*3*).

Nutrients. *V. cholerae* 01 is a facultative anaerobe—it relies on respiration when oxygen is present and fermentation when oxygen is absent. *V. cholerae* can grow in media containing carbohydrates, particularly glucose, as well as nitrogen, sulfur, phosphorus, and sodium (*5, 8, 9*); to obtain such nutrients, it adheres to sediment (*7*). Na^+ is essential for its growth. Two laboratory studies have revealed that toxigenic strains of *V. cholerae* 01 biotype El Tor need Na^+ to survive in the absence of other nutrients and to grow more rapidly in the presence of other nutrients (*9, 27*). It was determined that the need for Na^+ could not be met by substituting the alkali metals Li^+ and K^+ (*27*). However, when Na^+ is available, addition of the alkaline earth metals Ca^{2+} and Mg^{2+} has been found to help prolong the survival of *V. cholerae* (*9*).

Another study found that *V. cholerae* 01 strains of clinical origin (of the classic and El Tor biotypes) could survive up to 12 days in unchlorinated water when ferric oxide (Fe_2O_3) was present (*28*). The microorganism produces vibriobactin, which is a siderophore capable of chelating the iron and then solubilizing it and transporting it to the cell. The authors of the report concluded that iron is an important factor in the epidemiology of cholera.

Salinity. In a laboratory study conducted by Singleton et al. (*8*), it was determined that, in the absence of nutrients, the ideal salinity for growth of toxigenic *V. cholerae* 01 biotype El Tor is 25 parts per 1 000. *V. cholerae* can grow in aquatic environments of high salinity (45 parts per 1 000) if it receives 500 μg or more of tryptone as a substrate. Another laboratory study conducted by the same researchers (*27*) found that the microorganism prefers moderate salinity (from 15 to 25 parts per 1 000) and that it can grow in systems with 1% (weight/volume) tryptone with or without NaCl. This finding indicates that tryptone has sufficient Na^+ to guarantee growth of the microbe. This study supports the hypothesis that toxigenic *V. cholerae* 01 is an autochthonous member of estuarine microbial flora.

Miller et al. (*9*) ascertained that toxigenic *V. cholerae* 01 biotype El Tor is able to survive without nutrients in warm water with a salinity of 2.5 to 30 parts per 1 000 but that the optimal salinity for survival is 20 parts per 1 000. Toxigenic and nontoxigenic strains of *V. cholerae* 01 biotype El Tor have been isolated from brackish waters of estuaries and coastal swamps (*5, 6, 22, 24*). Tamplin and Colwell (*29*) found that, for *V. cholerae* 01 to produce toxins, the optimal salinity ranges between 20 and 25 parts per 1 000. The pathogenic microorganism is nevertheless capable of colonizing freshwater ecosystems. Singleton et al. (*27*) concluded that the presence of *V. cholerae* in aquatic environments is not limited to estuaries, because salinity requirements can be fulfilled through an adequate nutrient concentration in freshwater environments. Miller et al. (*19*) found that the microbe maintained its ability to produce toxins in the laboratory after 64 days of exposure to low salinity. Others have reported that the microbe is able to survive in freshwater environments for extended periods (*11, 12*).

In Queensland, Australia, toxigenic *V. cholerae* 01 biotype El Tor was detected for two months in a river with no human fecal contamination (*11*). The authors of that study concluded that the microorganism is able not only to survive but also to multiply in river water. Another study carried out from 1977 to 1984 (*12*) detected toxigenic *V. cholerae* biotype El Tor in 11 rivers in Queensland, again in the absence of human fecal contamination. The researchers concluded that the microorganism is able to survive and multiply in rivers. Islam et al. (*17*) suggested that freshwater impoundments in an endemic area of Bangladesh could become reservoirs for *V. cholerae* 01.

Temperature. The ideal growth temperature for toxigenic *V. cholerae* 01 biotype El Tor varies between 30 and 37 °C (*5, 9, 27*). In samples collected from three rivers in Queensland between 1977 and 1980, toxigenic *V. cholerae* 01 biotype El Tor was isolated more frequently in the summer than in the winter (*12*).

In a study carried out in Kent, United Kingdom, most environmental isolations of nontoxigenic strains of *V. cholerae* 01 biotype El Tor occurred during the summer (*24*). In the frigid waters (12 °C) of the Peruvian side of Lake Titicaca, which is over 3 000 m above sea level, *V. cholerae* 01 biotype El Tor (of unresearched toxigenicity) was isolated only occasionally and in very low concentrations (*30*).

Acidity. Toxigenic *V. cholerae* 01 biotype El Tor can tolerate alkaline environments and is very sensitive to acidity (*9, 19*). A laboratory study by Miller et al. (*9*) revealed that the optimal pH for survival in 25 °C water is between 7 and 8.5 when salinity is moderate and between 7.5 and 9 when salinity is low.

Sunlight. Because toxigenic *V. cholerae* 01 shows little resistance to ultraviolet radiation, a study has been proposed to research the use of sunlight for disinfecting drinking water in the Andes (*31*).

Biotic factors

Aquatic macrophytes. Toxigenic *V. cholerae* 01 biotype El Tor has been isolated from macrophytes in both seawater and fresh water (*32–34*). In a study carried out in Bangladesh by Spira et al. (*32*), toxigenic *V. cholerae* 01 biotype El Tor was isolated from the roots of *Eichhornia crassipes*, a freshwater macrophyte also known as water hyacinth. The authors observed that adhesion of the microorganism to the plant roots favors its survival.

Under laboratory conditions, Islam et al. (*33*) ascertained that two toxigenic strains of serogroup 01 biotype El Tor survived for longer periods when they were attached to the freshwater macrophyte *Lemna minor* than when they were suspended in the water where the plants had been floating. The pathogenic agent secretes the enzyme mucinase, which is considered to be one of the factors responsible for the virulence of *V. cholerae* (*35*) and which degrades the cellular mucilage of plants, including *L. minor* (*33*). Islam et al. have suggested that aquatic plants could be environmental reservoirs of the microbe through either a nonspecific association or a commensal relationship (*33*).

Phytoplankton and zooplankton. *V. cholerae* serogroup 01 targets species of sea- and freshwater phytoplankton and zooplankton to which it adheres (*13, 36–40*). In a laboratory study, Huq et al. (*39*) researched the ecological association between *V. cholerae* 01 and planktonic copepods from the Chesapeake Bay in the United States of America and from the Buriganga River in Bangladesh. The species of planktonic copepods collected from the Chesapeake Bay were *Acartia tonsa, Eurytemora affinis*, and *Scottolana* spp. In the samples taken from the Buriganga River, the two predominant species of planktonic copepods were not identified. Two vibrio strains were used: one of clinical origin (classic biotype) and one isolated from a river in Dacca, Bangladesh (El Tor biotype). The researchers noted that *V. cholerae* 01 colonized primarily the oral regions and egg sacks of the planktonic copepods. Their results suggest that reproduction of vibrios takes place in the egg sack, the digestive system, and the chitinous exoskeleton of copepods. The pathogenic agent secretes chitinase, an enzyme that enables it to digest chitin and use it as a source of nutrients (*41*). Adhesion to chitinous surfaces provides more resistance to moderate levels of acidity (*42*) and to low temperatures (*43*). Huq et al. concluded that oviposition and expulsion of fecal material by planktonic copepods can expedite dissemination and reproduction of the pathogenic microorganism in aquatic environments (*39*).

A laboratory study by Tamplin et al. (*37*) in Bangladesh indicated that five strains of *V. cholerae* 01 of clinical origin (three of the classic biotype and two of the El Tor biotype) adhered primarily to the exoskeleton of copepods (*Acartia* sp., *Acartia chilkaensis*, *Acartia sewelli*, and *Cyclops* sp.), cladocerans (*Bosmina* sp., *Ceriodaphnia* sp., *Diaphanosoma* sp., and *Bosminopsis* sp.), and rotifers (*Brachionus* sp.). They were also able to attach to two species of green algae (*Volvox* sp. and *Pediastrum simplex*) and to two species of blue-green algae (*Spirulina* sp. and unicellular cyanobacteria).

Islam et al. (*36*) found that toxigenic *V. cholerae* 01 biotype El Tor had a greater tendency to attach to the green alga *Rhizoclonium fontanum* than to the following four freshwater species: a blue-green alga (*Anabaena variabilis*), a green alga (*Cladophora* sp.), an aquatic moss (*Fontinalis antipyretica*), and an aquatic angiosperm (*Elodea canadensis*). It has been suggested that the prolonged survival of toxigenic *V. cholerae* when attached to *R. fontanum* could indicate its ability to derive nutrients from the extracellular products released by this species.

In Bangladesh, toxigenic *V. cholerae* has been isolated in cultures from water samples collected from environmental reservoirs during epidemic periods but not between epidemics (*44*). However, the fluorescent monoclonal antibody method, which is capable of detecting viable but nonculturable vibrios, revealed strains of *V. cholerae* 01 (of unresearched biotype and toxigenicity) in 64% of plankton specimens collected from water at the mouth of the Ganges and Meghna rivers in Bangladesh, even during the interepidemic periods (*13*). In contrast, when culture methods were used, only 0.3% of plankton specimens yielded positive results. That study revealed the presence of *V. cholerae* 01 in the region's aquatic environments throughout the year. Although the surfaces of copepods were the most densely colonized, *V. cholerae* 01 was also found attached to species of rotifers, cladocerans *(Daphnia)*, *Volvox*, and *Desmida*. The authors hypothesized that culturable vibrios in aquatic environments adhere to plankton to withstand the seasonal changes in temperature, salinity, pH, and nutrient concentration and that they enter a nonculturable state for a given period as a way to adapt to such changes. According to this hypothesis, once favorable growth conditions return, *V. cholerae* 01 can again adopt its culturable state and pose the threat of an epidemic if certain plankton blooms contribute to its reproduction.

Undetermined biotypes of *V. cholerae* 01 of unknown toxigenicity have been detected in the viable but nonculturable state in phytoplankton specimens of the species *Anabaena variabilis*, a cyanobacteria collected from a pond in Dacca, Bangladesh. No association has been observed with species of genera such as *Euglena* and *Phacus* (*38*). Islam et al. hypothesized that *V. cholerae* 01 uses extracellular products released from *A. variabilis* for nutrition and that the availability of salts on the mucilaginous surface of this blue-green alga enables *V. cholerae* 01 to survive for prolonged periods in freshwater environments (*45*). They also proposed that photosynthesis by *A. variabilis* supplies oxygen for the aerobic respiration of *V. cholerae* 01 and that the microorganism, in turn, is the source of carbon dioxide for *A. variabilis*. The authors concluded that *V. cholerae* 01 in a viable but nonculturable state is associated with *A. variabilis* and, possibly, with other species of blue-green algae with a mucilaginous surface, which could serve as ecological niches in estuaries and freshwater ecosystems.

Fish, mollusks, and crustaceans. In the United States, toxigenic *V. cholerae* 01 biotype El Tor has been isolated from shrimp and crabs in Louisiana (*22*) as well as from oysters and the intestines of fish caught in Mobile Bay, Alabama (*46*). Nontoxigenic strains of serogroup 01 biotype El Tor have been isolated from oysters (*Crassostrea virginica*) found in estuarine waters in the state of Florida (*6*). In 1991, *V. cholerae* 01 (of unresearched toxigenicity) was detected in fish and mollusks in the coastal waters of Lima, Peru (*30*). The chitinous surface of the crustaceans provides a suitable substrate for reproduction of the pathogenic microbe. Epidemiologic studies (*47–49*) revealed an association between the incidence of cholera and the consumption of fish and other raw or undercooked seafood.

Aquatic birds. In the United States, toxigenic *V. cholerae* 01 biotype El Tor was isolated in 1986 and 1987 from the feces of great blue herons (*Ardea herodias*) in Colorado and Utah but not from water samples collected from the birds' habitat (*50*). The authors concluded that such aquatic birds could be carriers of the pathogenic agent and contribute to its overall dissemination.

CONCLUSIONS

Some experts believe that to properly control cholera it is necessary to prevent humans from coming into contact with the natural reservoirs of toxigenic *V. cholerae* 01 (*51*). This implies the ability to identify aquatic environments where abiotic and biotic ecological conditions enable the microorganism to survive between epidemics.

The optimal conditions for the survival and growth of toxigenic *V. cholerae* 01 are aquatic environments rich in nutrients and with moderate salinity, warm temperatures, and neutral or slightly alkaline pH. Such conditions are typical of estuaries and coastal swamps in equatorial, tropical, and subtropical regions, where many species of phytoplankton and zooplankton, fish, mollusks, and crustaceans thrive (*52, 53*). The ecological niche of *V. cholerae* 01 is currently considered to exist in estuarine waters, where it can survive even in the absence of human fecal contamination.

With the environmental stress that accompanies decreases in nutrient availability and salinity, toxigenic *V. cholerae* 01 assumes a viable state that allows it to perform metabolic functions and to form colonies without being culturable. In this viable but nonculturable state, concentrations of the microorganism adhere to the surfaces of various species of aquatic macrophytes, phytoplankton, and zooplankton. Through such ecological associations, *V. cholerae* can survive between epidemic periods without sacrificing its toxigenicity (*34*), even in freshwater ecosystems. This phenomenon contributes to the endemic nature of cholera.

In general, concentrations of *V. cholerae* 01 in waters without human fecal contamination are considerably lower than the amount required to produce cholera. Nevertheless, it is possible that, during seasonal blooms of phytoplankton and zooplankton, *V. cholerae* 01 concentrations associated with these substrates increase to the point of causing disease (*13, 16, 40*). In the future, additional research will be needed to determine whether *V. cholerae* serogroup 0139 is also associated with species of plankton, macrophytes, and aquatic macrofauna as a survival strategy.

Given the endemic nature of cholera in some Latin American countries, field and laboratory studies are necessary to define and identify the aquatic reservoirs that favor survival of toxigenic *V. cholerae* 01 between epidemic periods. Research is needed to determine whether its association with phytoplankton, zooplankton, and macrophytes of the aquatic ecosystems of this hemisphere is indiscriminate or whether, on the contrary, it exhibits a preference for certain species of blue-green algae and planktonic copepods, as suggested by findings from Bangladesh. It is also necessary to determine both the geographic location of waters that harbor reservoirs of toxigenic *V. cholerae* 01 and the communities that use that water for consumption, bathing, fishing, and other activities.

Finally, elimination of endemic cholera also depends on the progress made in eradicating extreme poverty and, in particular, improving services for drinking water supply, excreta disposal, and wastewater treatment.

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RESUMEN

La ecología de *Vibrio cholerae* serogrupo 01 en ambientes acúaticos

El carácter endémico y estacional del cólera depende de la supervivencia de *Vibrio cholerae* serogrupo 01 en estado viable, pero no necesariamente cultivable, en nichos ecológicos localizados en ambientes acuáticos durante períodos interepidémicos. Para comprender la ecología de *V. cholerae* es preciso conocer los ecosistemas acuáticos que pudieran albergarlo y contribuir a la presencia endémica del cólera en América Latina. El presente artículo tiene por objetivo presentar, en términos resumidos, la ecología de *V. cholerae* 01 organizada según los factores abióticos y bióticos que inciden en la supervivencia del microbio en ambientes acuáticos. Este agente patógeno encuentra condiciones favorables en aguas caracterizadas por niveles moderados de salinidad, un alto contenido de nutrientes, temperaturas cálidas, un pH neutro o ligeramente alcalino y la presencia de macrófitas acuáticas, fitoplancton, zooplancton, peces, moluscos y crustáceos. Estas condiciones ecológicas son propias de los ecosistemas acuáticos de estuarios y pantanos costeros, de cuya flora microbiana *V. cholerae* 01 toxígeno se considera actualmente un miembro autóctono. Este microorganismo también se ha mostrado capaz de colonizar ecosistemas de agua dulce en su forma viable, aunque no necesariamente cultivable, si encuentra sustratos orgánicos e inorgánicos que favorezcan su supervivencia.