Global Climate and Infectious Disease: The Cholera Paradigm*

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Historically, infectious diseases have had a profound effect on human populations, including their evolution and cultural development. Despite significant advances in medical science, infectious diseases continue to impact human populations in many parts of the world. Emerging diseases are considered to be those infections that either are newly appearing in the population or are rapidly increasing in incidence or expanding in geographic range (*1*). Emergence of disease is not a simple phenomenon, mainly because infectious diseases are dynamic. Most new infections are not caused by truly new pathogens but are microorganisms (viruses, bacteria, fungi, protozoa, and helminths) that find a new way to enter a susceptible host and are newly recognized because of recently developed, sensitive techniques. Human activities drive emergence of disease and a variety of social, economic, political, climatic, technological, and environmental factors can shape the pattern of a disease and influence its emergence into populations. For example, travel affects emergence of disease (*2*), and human migrations have been the main source of epidemics throughout history. Trade caravans, religious pilgrimages, and military campaigns facilitated the spread of plague, smallpox, and cholera. Global travel is a fact of modern life and, equally so, the continued evolution of microorganisms; therefore, new infections will continue to emerge, and known infections will change in distribution, frequency, and severity.

Reports of disease outbreaks have been more frequent during the past few years. For example, two malaria cases were recently reported in New York and traced to local mosquitoes. These cases demonstrate that the potential exists for reintroduction of malaria into areas where it is no longer endemic, such as the United States. Malaria is an old disease with the potential of re-emerging as a new disease, especially in association with climate change.

Tuberculosis (TB), according to the World Health Organization, is now the world's leading killer of adults; 30 million adults are expected to die from TB in the next 10 years. With the spread of HIV, coupled with deterioration of conditions in many cities, not just in developing countries, but throughout the developed world as well, and the explosion in international travel, a resurgence of tuberculosis has occurred in Tokyo, New York, London, and other major cities.

Eastern Europe and the former Soviet Union have been battling a diphtheria epidemic since 1990. More than 25,000 cases have been reported. In October 1995, a hemorrhagic fever of unknown origin swept through Northeast Nicaragua (*2*, *3*). The disease, leptospirosis, characterized by fever and internal bleeding, caused hospitalization of more than 500 Nicaraguans and infected more than 2000 before it was identified by experts at the U.S. Center for Disease Control. Leptospirosis is a bacterial infection transmitted by animal urine or excrement that seeps into food and water supplies. The disease can be treated with antibiotics, and its spread can be curtailed by methods similar to those used for cholera epidemics.

More than 500 cases of Dengue fever were reported in 1995 in the Caribbean region by the Caribbean Epidemiology Center. Dengue, and the more severe Dengue hemorraghic fever, or DHF also raged through Central America between September and November 1995; Dengue reports sharply increased from 23,603 to 46,532; DHF reports rose from 352 to 546. Other countries reporting cases of Dengue or DHF include Belize, British Virgin Islands, Barbados, Dominica, Grenada, Guadeloupe, Guiana, Jamaica, Martinique, Montserrat, Puerto Rico, St. Vincent, Trinidad, and Tobago.

In Columbia, an apparent outbreak of mosquito-borne equine encephalitis killed at least 26 people and forced 13,000 others to seek treatment in September 1995. In November 1995, Labrea black fever, just one of a half-dozen deadly and little understood viral diseases emerging from the rain forests from Latin America began appearing.

Thus, communicable diseases are resurging. Some of the underlying causes are obvious; namely, poverty, which continues to be a huge problem worldwide. Without latrines or indoor plumbing, increasing populations, especially those millions lacking food and housing, create an environment for these diseases to flourish.

An aspect of infectious disease, receiving relatively little attention until recently, is the environment. Malaria, currently claiming about two million victims each year worldwide, could kill an additional million people annually if global temperatures rise, thereby allowing the parasite-bearing mosquito to spread into geographic areas not now affected (*4*). Because most emerging disease agents are not new but are existing pathogens of animals or humans that have been given opportunities to infect new host populations, environmental and social changes—especially those resulting from human activities—which accelerate pathogen traffic need to be defined (*1*).

Cholera as a Paradigm

Cholera offers an excellent example of how information concerning environmental factors permits better understanding of disease—not only virulence, but equally important, transmission and epidemiology. The etymology of the term "cholera" has been in dispute for many years but may provide clues to understanding the disease. Cholera may have been derived from the Greek words, chole (bile) and rein (flow), meaning the flow of bile in that language (*5*). Other investigators suggest the name comes from the Greek word cholera, which means gutter of a roof (*6*). The symptoms of cholera may have suggested to the Greeks the heavy flow of water on roof gutters during thunderstorms. To distinguish the general term, cholera (gutter), from the disease cholera, the word "nousos" or sickness was added to the latter (*5*).

There are descriptions of a disease resembling cholera in Sushruta Samshita from India, written in Sanskrit ~500 to 400 B.C. (*7*). Historical records tracing back 2000 years, in both Greek and Sanskrit, describe diseases similar to cholera (*5*). Thus, from the literature, it is clear that there was cholera before 1817, when the records of the pandemics begin. However, cholera existed on the Indian subcontinent for centuries before the first European arrived, where it was described early in the 16th century, not invading other areas until 1817. When Vasco da Gama landed on the southwestern or Malabar coast of India in 1498, as described by Gaspar Correa, an officer of Vasco da Gama in 1503, about 20,000 men of Calicut died of "a disease which struck them sudden-like in the belly, so that some of them died in 8 hours" (*8*).

The impact of gastrointestinal illnesses, including cholera, on military campaigns has been reviewed by Tramont and Gangarosa (*9*). The battles of Gallipoli, El Ala-

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mein, and other conflicts were influenced and their outcome set by diarrhoeal disease.

The first pandemic of cholera occurred from 1817 to 1823 and was fairly limited in scope and related to the two wars—the Oman War and the war between Persia and Turkey. Before 1817, cholera was most probably a sporadic, summertime illness, perhaps emerging in its violent epidemic form in the early 19th century.

The second pandemic (1829 to 1851) is believed to have begun in Russia, where citizens of Moscow were particularly hard hit. The pandemic spread across the Atlantic Ocean in 1832 to the Americas, initially up the St. Lawrence River and, ultimately, spreading to New York on 23 June 1832. At the time, New York was ripe for a cholera epidemic because of its proximity to the ocean, that is, the rivers flanking Manhattan Island had increased salinity and the city had a bad water and sanitation system (*5*). The disease spread from New York to Philadelphia in 2 weeks and subsequently along the coast to New Orleans.

The second pandemic reappeared in a region of London, close to where Dr. John Snow, physician to Queen Victoria, lived. In the summer of 1849, John Snow determined that the spread of the disease was connected to mixing of drinking water and sewage in Broad Street, Golden Square, and adjoining streets of London. Snow was credited with stopping the Broad Street epidemic by recognizing that the location of deaths from cholera was near the wells on Broad Street and urging the Board of Guardians of St. James Parish, which owned the well and pump, to remove the handle of the pump in September. By then, the epidemic had begun to wane, but this action probably represents the first instance on record of the implementation of an appropriate measure to prevent the transmission of a waterborne disease (*10*). Thus, John Snow is given credit for both stopping the epidemic and proving its connection to drinking water. In reality, Snow never claimed that the removal of the pump handle ended the epidemic in the area of the Broad Street pump. Snow did understand, however, that the disease was spread more easily by contaminated water than by person-to-person contact. He noted that the number of cholera deaths per 10,000 houses from 8 July to 26 August 1854 was 315 for houses whose water was supplied by the Southwark and Vauxhall Company and only 37 for houses supplied by the Lambeth Company. His tracking of the death rate, as a function of water supply, was an important observation in the understanding of the epidemiology of cholera.

A third pandemic, from 1852 to 1859, was followed by the fourth (1863 to 1879), fifth (1881 to 1896), and sixth (1899 to 1923) pandemics. From 1926 to 1960, many believed that cholera would not recur in pandemic form because water supplies had been improved worldwide. Indeed, many parts of the world did become free of cholera. But, nature prevailed and the seventh pandemic began in 1961 and continues to the present on six continents. A new biovar or biotype of *Vibrio cholerae* caused the current pandemic—the El Tor biotype of *V. cholerae* 01, which emerged in Celebes, Indonesia, in 1961. The disease caused by this organism is usually not as severe as that of the classical biotype.

From the pandemics of the 19th century to the recent major epidemics in South America and Africa, cholera left its mark on human history. In Latin America, cholera re-emerged after a 100-year disappearance. Cholera spread throughout parts of Africa in 1991 at a catastrophic rate, killing more people than the epidemic in Latin America. According to the World Health Organization, 45,159 cases and 3488 deaths in 10 African nations were reported up to 23 July 1991. By comparison, 2618 of the 251,553 reported cases in South America were fatal.

Vibrio cholerae non-01 serogroups were not known to cause epidemics of diarrhea; they are known, however, to cause sporadic cases and small outbreaks of diarrheas and extraintestinal infections. However, in October 1992, a dramatic event occurred. An epidemic of cholera-like disease, caused by a *V. cholerae* non-01 serogroup broke out in the southern port city of Madras in southern India. Within a few months, it arose in other southern Indian cities and reached the northeastern city of Calcutta (*11*). By December 1992, there was an outbreak of cholera-like illness in the southern coastal cities of Bangladesh, and the disease eventually spread to the entire country (*12, 13*). The disease affected thousands of people, mainly adults, and caused many deaths in the Indian subcontinent. The causative agent was found to be a new serogroup of *V. cholerae*, defined as 0139, with the synonym Bengal, to indicate that it was first isolated from coastal areas of the Bay of Bengal (*14*).

Since 1993, the serogroup *V. cholerae* 0139 has been reported from India, Bangladesh, Nepal, Burma, Thailand, Malaysia, Saudi Arabia, China, and Pakistan (*14*). The *V. cholerae* 0139 serogroup is nearly identical to *V. cholerae* 01 El Tor but possesses a capsule, and the capsular layer is distinct from the lipopolysaccharide (LPS) antigen. The *V.* cholerae 0139 antigen includes an O-antigen capsule and lipopolysaccharide virulence determinants (*15*). Furthermore, there is a deletion of about 22 kb of DNA from the 01 chromosome in the rfb region and an insertion of a new 35-kb region of DNA that specifies the 0139 LPS and capsules (*16*). The occurrence of epidemics caused by *V. cholerae* 0139 is a significant turning point in the history of cholera because the evidence points to this strain arising as from genetic recombination and horizontal gene transfer, and the acquisition of unique DNA. The 01 antigen has been the relied upon tag for recognition of *V. cholerae* epidemic strains. Now a new serotype was associated with cholera epidemics.

Seroconversion had been reported years ago (*17*), that is, seroconversion between Ogawa and Inaba serotypes of the cholera vibrios possessing specific O antigens. The 0139 strains have been shown to belong to a distinct serogroup, defined by monoclonal antibodies and polyclonal antisera that recognize only the 0139 strains (*18*). In *V. cholerae* 01, the chemical basis for the serogroup-defining antigen lies in the O side chain of LPS. The 0139 LPS differs from 01 LPS in that it has a short O side chain length and different sugar composition (*17*). The evidence further suggests the *V. cholerae* 01 El Tor gave rise to 0139 by acquisition of novel DNA which was inserted into, and replaced part of, the O antigen gene cluster of the recipient strain. From the sequence of the novel DNA, two open reading frames (otn A and otn B) were detected, the products of which showed homology to proteins involved in capsule and O antigen synthesis, respectively. The otn AB DNA determines the distinct antigenic properties of the 0139 cell surface. The otn AB DNA was not detected in 01 strains, but was present in two non-01 *V. cholerae* strains with serotypes 069 and 0141 (*19*).

Antigenic conversion of 01 to non-01, and the reverse, in *V. cholerae* has been demonstrated in the laboratory (*20, 21*). The co-existence of *Vibrio cholerae* 01 and 0139 Bengal in plankton in Bangladesh has also been demonstrated (*22*).

In Bangladesh, the epidemic of *V. cholerae* 0139 started in the chars, the temporary islands off the coast of the Sundarban area in the southwestern coastal districts of Bagerhat. Most of the islands emerge at the end of the monsoon period, and migrant fishermen arrive in October to fish in the Bay of Bengal. The chars are in remote areas, and communication with the mainland is limited. Thus, the 0139 *V. cholerae* epidemic went unnoticed until December 1992, when it was identified in the mainland of Bagerhat; afterward it appeared in five neighboring districts. The epidemic lasted more than 4 months, and involved a total of 46,965 cases and 846 deaths in the six southern districts of Bangladesh. In September 1993, 3 months after its decline in the southern areas, the epidemic moved to

the northern regions of the country. It was reported that epidemic resurgence coincided with the onset of seasonal outbreaks of *Vibrio cholerae* 01 in Bangladesh (*23*).

Vibrio cholerae serogroup 0139 Bengal completely displaced *V. cholerae* serogroup 01 in Calcutta in January 1993, and an epidemic of *V. cholerae* 0139 followed in March to May 1993 (*13*). The organism first caused a large outbreak of cholera-like illness in Madras in October 1992. Initially, cases were clustered in a suburban area 16 km north of the city limit. Similar strains were isolated a month later from other parts of India; for example, Madurai, Vellore, and Calcutta. Interestingly, *V. cholerae* 01 El Tor entered India almost concurrently in Calcutta and Madras in 1964 and spread rapidly over wide areas, outnumbering preexisting classical *V. cholerae* 01 in India. By 1966, El Tor had almost completely replaced classical cholera. In parallel in Calcutta, the 0139 serogroup appeared on or about 20 November 1992 and quickly replaced *V. cholerae* 01 El Tor by December 1992 (*13*).

The data suggest that *V. cholerae* 01 began to be displaced in the southern coastal areas of Bangladesh in 1991 or even earlier. The epidemic that included the coast of southern India and West Bengal (India) arose from a single clone, and the Indian outbreaks were of the same origin (*24*).

Five major rivers of the Indian subcontinent flow through into the Bay of Bengal. These rivers all carry large amounts of agricultural and industrial waste and thus provide nutrients sufficient to convert the coastal waters to eutrophic conditions. Brackish water extends some distance upriver for all rivers.

All *Vibrio* spp. that are pathogenic are adapted to salinities between 5 per mil and 30 per mil. Salinities favorable for growth of *V. cholerae* are found primarily in inland coastal areas and estuaries, but the bacterium thrives in seawater as well. Pathogenic *V. cholerae* grows in water with low salinity if the water temperature is relatively high and organic nutrients are present in high concentrations (*25–27*), that is, high concentrations of organic nutrients can compensate to a degree for lack of salt. Similarly in fresh water, the presence of divalent cations can compensate for Na^+ (*27*). Survival of *V. cholerae* in seawater for more than 50 days has been demonstrated (*28*).

Vibrio cholerae can survive under unfavorable environmental conditions in a dormant state; that is, it is viable but nonculturable (*29*). Representing a spore-like stage, without formation of a true spore coat, dormant cells can survive changes in temperature, salinity, or availability of organic matter, as do the spore-forming bacteria, *Bacillus* spp. (*30*). Viable but nonculturable organisms remain infectious (*31*). *V. cholerae* cells, when viable but nonculturable, are small and spherical (*32*), but apparently can be resuscitated by heat shock (*33*). Viable but nonculturable *V. cholerae* contribute to the occurrence of seasonal epidemics because *V. cholerae* can persist for a long time in the aquatic environment; reintroduction of the organism by infected humans is not necessary. Furthermore, *V. cholerae* is a microbial inhabitant of brackish water and estuarine ecosystems; that is, it is autochthonous, as has been demonstrated by Xu *et al.* (*34*). In addition to elucidation of the salinity requirement and range for *V. cholerae*, many pathogenic *Vibrio* spp. are associated with chitinaceous zooplankton and shellfish, and also can survive on fish and shellfish (*27, 35, 36*).

The association of *V. cholerae* with zooplankton has proven to be a key factor in deciphering the global nature of cholera epidemics. *V. cholerae* preferentially attaches to chitinaceous plankton, for example, copepods, and can be detected in zooplankton in cholera endemic regions. Ocean currents sweeping along coastal areas thereby translocate plankton and their bacterial passengers.

The Origin of Cholera

The history of cholera reveals a remarkably strong association with the sea. The great pandemics followed coastlines of the world oceans. As with acute communicable diseases in general, endemicity of cholera carries the potential of epidemic flare-ups, and pandemicity is always a threat, especially in developing countries having poor sanitation, lack of hygiene, and crowded living conditions. These factors have long been recognized as characteristic of environments in which diarrhoeal diseases flourish.

In historical treatises on cholera, seaborne transportation of cholera provides the prevailing theory of dissemination. Initial cases characteristically occur along coastal areas, among fishermen or boatmen, and outbreaks were commonly ascribed to ships arriving from cholera-epidemic areas (*8*) and, more recently, to discharge of ballast water from ships arriving in a port in Peru from a cholera endemic region. The invasion of *V. cholerae* El Tor, a biotype of cholera, into India was believed "likely to have been carried by the sea-route . . . into Calcutta" (*37*). The early records show an association with bad water, usually taken from rivers or swampy areas, or marshes, where flow of streams was much reduced. All six pandemics of the last century are believed to have started in "Hindoostan," now known as Bangladesh, and to have been caused by the classical biotype *V. cholerae* of the 01 serotype (*38*). The most recent pandemic of 1961 continues today.

The seventh pandemic was different from the six previous ones, in that authorities claimed that it originated in Indonesia and that the cause was *V. cholerae* 01 El Tor. After its appearance in Indonesia in 1961, the disease spread to East Pakistan (Bangladesh) in 1963, India in 1964, the former U.S.S.R. in 1965 to 1966, and Africa in 1970 to 1971. But, the greatest surprise was in 1991 when the seventh pandemic struck South America, first in Peru in the port city of Chancay, 60 km north of Lima. The next day an outbreak was reported from Chimbote, a seaport 400 km north of Chancay. Spread of the outbreak was rapid, and by 7 February 1991, confirmed cases were reported along the Peruvian coast from the Chilean to the Ecuadorean border, ~2000 km distant (*39*). The near simultaneous appearance of cholera along such a great distance of coastline cannot easily or logically be explained by ballast discharge from a single ship in Lima. More likely, the plankton blooms that occurred were triggered by a climatic event, the most logical being El Niño, which brings rain and an influx of nutrients from land and warm sea surface temperatures. These factors have already been associated with initiating plankton blooms. Because phytoplankton blooms can be measured by satellite imagery (*40*) and zooplankton blooms quickly follow phytoplankton blooms (*41, 42*), conditions associated with a cholera outbreak or epidemic can be monitored by satellite. Because a single copepod can carry up to 10^4 cells of *V. cholerae* (*30*), a massive bloom can provide an infectious dose in the brackish water of tidal rivers. An infectious dose has been reported to be 10^3 *V. cholerae* cells, on the basis of human volunteer studies (*31, 43*). It has been shown that several copepods, with *V. cholerae* cells attached to the surface and in the gut (*45*), can carry the requisite infectious dose for clinical cholera. That is, a colonized copepod may contain up to 10^4 cells of *V. cholerae*. During a plankton bloom, several copepods may be ingested in a glass of water, if there is no treatment of the water supply, as is the case in villages in Bangladesh, India, and many other cholera endemic countries (*45*). The chance of consuming this *Vibrio* capsule increases during periods when the concentration of copepods in the water is high, that is, at times of plankton blooms.

Thus, as was the case in the earlier pandemics, spread of the disease was rapid and far flung. In Peru, as early as 12 February 1991, epidemics were reported from communities 50 to 150 km inland, and by 20 February cases were reported in the Andean

highlands. Characteristic of the seventh pandemic, as in earlier pandemics, coastal towns and fishing villages were affected in the Latin America outbreaks during 1990 to 1991.

The disease in Latin America has abated, but remains endemic, as elsewhere in the world where cholera has occurred. Peru, alone, suffered more than 300,000 victims, of which almost 1% died. In 1991, 21 African countries reported a total of 153,367 cases and ~14,000 deaths. In contrast, during 1994, tribal conflicts in the Central African nation of Rwanda claimed more than 500,000 lives and thousands of Rwandans fled to Zaire, Burundi, and Tanzania. About 50,000 Rwandan refugees contracted cholera in the refugee camps, and many thousands died.

Cholera pandemics visited North America (United States and Canada) regularly in the 1800s. The first epidemic broke out in Canada in April 1832, and 2208 died from cholera in Quebec by 2 September 1832 (*38*, *46*).

In Bangladesh and India, many of the cholera outbreaks have been *geographically* localized, demonstrating the occurrence of the disease is typically seasonal (*47*) and correlates with tidal estuaries and riverine systems. Outbreaks in Naples in 1973 and in Portugal in 1974 were traced to uncooked and inadequately cooked seafood, respectively.

The characteristic geographic occurrence of cholera and the speed with which it can be spread were reported more than a century ago (*46*). The implications of the geographical patterns of this disease (with respect to origin of the disease), however, were not pursued until recently, when new methods revolutionized the field of environmental microbiology. Epifluorescent microscopy and hybridoma production of monoclonal antibodies now permit direct detection of *V. cholerae* with the use of fluorescent-labeled monoclonal probes. Gene probes, colony hybridization, and polymerase chain reaction (PCR) methods are highly selective and allow detection of a few cells in water samples (*48*). With the use of monoclonal antibodies, improved fluorescent dyes, epifluorescent microscopy, and equipment for concentration of samples, as few as one to two cells of *V. cholerae* per liter of water can be detected and confirmed by PCR. Fluorescent-labeled RNA probes also provide a sensitive method for detection and enumeration, if used simultaneously with the direct viable count procedure (*49*).

In 1984, Xu *et al.* (*50*) developed an immunofluorescence method for the detection of *V. cholerae* serovar 01 in aquatic samples and enrichment broths. A polyclonal antibody was used in subsequent experiments, and fluorescein-isothiocyanate–conjugated, antirabbit globulin–goat serum and rhodamine-isothiocyanate–conjugated, bovine serum albumin were used as a background stain. Detection of *V. cholerae* 01 with this fluorescent antibody system was significantly more successful than with culture methods.

A field trial of the fluorescent antibody detection was conducted in which 52 water samples were collected in and around Matlab, Bangladesh, during April and May 1982. Only seven samples were positive for *V. cholerae* 01 by conventional culture, after examination of 3431 individual colonies for 01 antigen by slide agglutination (*51*). In contrast, the fluorescent antibody staining method allowed detection of *V. cholerae* 01 in 51 of the 52 samples. The seven samples that were positive by culture were also positive by staining. Surprisingly, recovery by culture of *V. cholerae* 01 was not possible at early stages of enrichment when cells could be detected by fluorescent antibody staining; that is, *V. cholerae* 01 cells could be observed, but overgrowth blocked isolation of those cells in culture. *V. cholerae* 01 was, indeed, present, but not recovered in culture.

Subsequently, a series of microcosm experiments were carried out and the phenomenon described above was discovered, namely, that *V. cholerae* 01 and related human pathogenic bacterial species enter into a viable but nonculturable state, and commonly do so in environmental samples (*30*). Thus, it was now possible to explain why direct viable counts by epifluorescent microscopy consistently were significantly higher than corresponding plate counts. The assumption that all *V. cholerae* 01 cells die off or decay in the environment was no longer valid. Because immunofluorescent microscopy and, subsequently, molecular genetic probes are sensitive in detecting *V. cholerae* 01 in environmental samples, this microorganism can now be readily detected and enumerated in samples where culture methods fail or are inadequate, not to mention time-consuming and expensive.

Viability and pathogenicity of *V. cholerae* in the viable but nonculturable state was initially demonstrated using membrane chambers submerged in semitropical waters at Bimini, Bahamas, and ligated illeal loop assays (*52*). Subsequently, retention of pathogenicity for humans was demonstrated in volunteer feeding experiments, where it was found that from nonculturable vibrios positive cultures could be demonstrated (*31*, *53*), providing evidence that nonculturable *V. cholerae* can maintain pathogenic potential, even after long-term residence in the environment (*53*).

Recently, optimization of the direct fluorescent antibody test in kit form, using a monoclonal antibody, as proposed by Brayton *et al.* (*54*) and Tamplin *et al.* (*55*), has been achieved. The kit provides a simple method for detection of *V. cholerae* within a few minutes and is both inexpensive and convenient for field use, requiring neither refrigeration of the reagents nor incubation of the reaction (*56*, *57*).

For a bacterium capable of attachment to, and colonization of surfaces, surface specificity often is critical. *V. cholerae*, however, offers multiple recognition sites, including not only the intestinal mucosa and brush border cells of the mammalian gut, but also the hindgut mucosa of blue crabs, which contain chitin. Shellfish feeding on planktonic crustaceans are colonized by *V. cholerae* in natural water systems (*58*, *59*). The association of *V. cholerae* with planktonic crustacean copepods is influenced, and likely controlled, by physical and chemical characteristics of the environment. *V. cholerae* may also survive in association with aquatic vegetation; for example, water hyacinths and the blue-green bacterium, *Anabena*, as well as other zooplankton and crustacean invertebrates in the aquatic environment (*60*–*66*).

Seasonal outbreaks of cholera in Bangladesh are geographically related, and the outbreaks are often local (*30*, *67*, *68*). Isolates of *V. cholerae* with diverse seasonal distribution were found to host different phage types (*69*). This evidence indicates that outbreaks lack a common source and likely have a broad distribution as a result of tidal ebb and flow and seasonal flooding.

Recent work on genetic fingerprinting has confirmed that the organism is multiclonal and that some clones are endemic in different geographical regions (*70*).

V. cholerae 01 in Bangladesh, 1987 to 1990

In order to determine more definitely the source and host of *V. cholerae* in the environment, an extensive environmental study was conducted in Bangladesh during 1987 to 1990 (*71*). Samples were collected from 10 fixed stations comprising two river sites and eight ponds in villages surrounding the Matlab area, located 46 km southeast of the capital city of Dhaka, Bangladesh, in the delta formed by the Meghna and Ganges rivers. One of the ponds, a protected pond that was relatively free of human use, was included in the study as a control. Water and plankton samples were collected at the 10 stations every 2 weeks, from February 1987 through January 1990.

Water samples were collected in presterilized glass bottles. Plankton samples were collected by filtering 50 liters of water

through a plastic sampler fitted with a 0.77 mesh net, achieving a 1000-fold final concentration. From the concentrated plankton samples, which were 50 ml in final volume, 10 ml were transferred into each of three different vials. Directly after sampling and while in the field, the samples were preserved in formaldehyde, to a final concentration of 4%. From the remaining 20 ml of each sample, 10 ml were homogenized, using a teflon-tipped, tissue grinder (StedFast Stirrer, Model 300, Fisher Scientific) and enriched by addition of alkaline peptone broth for isolation of *V. cholerae* by conventional culture methods (*51*).

The fluorescent antibody (FA) technique was used to screen formaldehyde-preserved plankton samples for *V. cholerae* 01, as described by Brayton *et al.* (*54*). Temperature, dissolved oxygen (DO), pH, and a variety of chemical parameters were measured at the time of collection, using field instruments (Yellow Springs, Ohio, Model YSI 58 and HACH Chemical Co., Ames, Iowa, Model Hach One). Organisms were identified and grouped as adult copepods, juvenile copepods, nauplii copepods, cladocerans, and "other," in the case of zooplankton. For phytoplankton, the groups included: green algae, diatoms, dinoflagellates, volvox, "other colonial algae," and cyanobacteria. Where possible, each of the above were analyzed to species level.

Monthly means for each station for pH, temperature, iron, salinity, and geometric means of counts of copepods for nauplii, juvenile and adult stages, diatoms, dinoflagellates, as well as percent of samples positive by FA were computed. It was hypothesized that copepods provide a suitable host environment for *V. cholerae*. Therefore, on the basis of earlier data, an association of copepod numbers with presence of *V. cholerae* could be predicted and detectable by fluorescent antibody (FA). For the statistical analysis, the conditional logistic regression model (*72*) was used in which

$$\log \frac{p_t}{1 - p_t} = \beta_0 + \beta_1 (z_{t-1}) + \sum_{i=1}^{7} \gamma_i w_i + \beta_2 [\log(x)] + \beta_3 [\log(x)]^2$$

where p_t is the probability of observing a positive FA reading at time t and z_t is an indicator or lag variable for whether FA was positive for the previous reading at the same site. The terms γ_i are (dichotomous) variables, defined to be 1 if the observation is from the ith pond and 0 otherwise. The control site was arbitrarily defined as pond 0. We allowed x to stand for numbers of adult copepods in some analyses and for nauplii or juveniles in other analyses. We used the logarithm of the untransformed copepod numbers because the distributions were highly skewed. The indicator variable Z_t was included to account for the degree of correlation that may be observed in successive observations at the same location. A quadratic term [that is, $\log(x)^2$] along with a linear term [that is, $\log(x)$] was used to test whether above some concentration of copepods, the probability of a positive FA reading declined. All the models were fit by way of maximum likelihood, using the SAS procedure (PROC Logistic, SAS Institute, Cary, North Carolina).

When the quadratic term was insignificant, we interpreted a positive coefficient (that is, $\beta_2 > 0$) to imply that as x increased the risk of positive FA increased. One overall model was fit to the eight ponds and a separate model was fit to each river site, in part because descriptive statistics indicated that the river sites were distinct from each other and the ponds, but the ponds appeared to be similar. For the river sites, γ_i was omitted from the model. We assessed lack of fit subjectively by fitting the same model to each study site, and also by comparing observed and expected proportions of FA positive examples for different levels of copepods.

A subsequent exploratory analysis, using a stepwise logistic regression model, was used to examine the role of other environmental [air and water temperature, pH, and dissolved oxygen tension (DOT)], chemical (bromine, calcium, carbon dioxide, chloride, NaCl, color, conductivity, copper, fluoride, water hardness, iodine, iron, manganese, phosphorous, NO_3, silicates, sulfates) and biological parameters (blue-greens, chladocerans, colonial algae, diatoms, dinoflagellates, green algae, volvox).

Because of the large number of variables, a preliminary analysis was done in which a Wilcoxon rank sum statistic was computed to compare the distribution of observations with a positive FA reading with those with a negative FA reading. If the variable was significant for either the ponds or one of the two river sites, it was included in the stepwise part of the analysis. A significance level of .05 was used throughout.

The results show that the abundance of *V. cholerae* 01 increases with the abundance of copepods (*71*). This association appears to be the basis of persistence of *V. cholerae* in the environment. Feeding action of many parasitic crustacea, such as copepods, effectively inoculate fish tissues with this pathogen (*73*). These findings, then, led us to examine seasonal distribution of copepods, ocean currents, and cholera epidemiology. The seasonality of cholera epidemics in Bangladesh and of plankton showed interesting correlations. As noted above, results of studies of survival of *V. cholerae* 01 in seawater microcosms revealed that it had the capacity to remain in the culturable state in seawater for a relatively long time, that is, sufficiently long to be carried by ocean currents to widely distant geographical locations (*74*). Other studies showed that, when confronted with high concentrations of carbohydrate, but nitrogen and phosphorous limitation, *V. cholerae* enters the viable but nonculturable state (*75*). Thus, the viable but nonculturable *V. cholerae* could be transported in nutrient poor seawater and, in association with plankton, over several months and thousands of kilometers, depending on currents and tides. Similarly, the organism can persist within a given geographical location for many years, offering an explanation for reappearance of cholera after years of quiescence or seeming absence.

Whether *V. cholerae* is a component of the commensal flora or a symbiont of a given plankton species remains to be determined. There are clues to potential roles of *V. cholerae* 01 in the environment. For example, *V. cholerae* produces chitinase and mucinase (*76–78*) and most strains carry lux genes (*79*). *Vibrio cholerae* strains producing melanin have been isolated. Melanin and its precursors, including homogentistic acid have been implicated in the induction of invertebrate larval settlement and development; for example, of barnacles, oysters and other invertebrates as well as biofilm adhesiveness (*80*). Thus, the autochthonous nature of *V. cholerae* 01 in the aquatic environment takes on greater significance, with respect to function in the natural cycles of aquatic ecosystems. Furthermore, it has been hypothesized that cholera toxin may play a role in the osmoregulation of its environmental host (*30*).

The introduction of filtration sharply reduced the incidence of infectious disease in the United States. From 1900 to 1913, the population served with filtered water increased eightfold, and the typhoid death rate dropped by more than 55% (*10, 81*). In the early years of the 20th century, chlorine, with filtration, virtually eliminated waterborne infectious disease in the United States. The importance of filtration and disinfection in preventing the spread of cholera cannot be overstated, considering the association of *V. cholerae* with plankton in raw water supplies. Filtering water at the time of collection and just before drinking is a successful means of removing cyclops, a planktonic crustacean copepod and vector of the guinea worm, which causes dracunculiasis. The crustacean cyclops-associated worm is removed by filtration with polyester cloth and is now a recommended method of preventing dracunculiasis in Africa (*82*).

During severe flooding, which occurs every year in some areas of Bangladesh, living conditions deteriorate to those of mere survival; building a fire to boil water is simply not possible. Using a filter constructed from either nylon net and one of several different types of sari material, the latter being very inexpensive and readily available in villages in Bangladesh, *V. cholerae* attached to plankton and comprising 99% of the *V. cholerae*, can be removed from water samples (*83*). From the results of extensive experiments using *V. cholerae* 01 and 0139 strains isolated from cholera victims of epidemics in Bangladesh, Brazil, India, and Mexico, it was found that this simple filtration procedure, involving the use of domestic sari cloth, can reduce significantly the number of cholera vibrios in raw water from ponds and rivers commonly used for drinking (*83*). Whether the number of cholera cases can be reduced by introducing this simple, low technology approach is currently under study.

Global Climate, Global Change, and Human Health

As already mentioned, the latest outbreak of cholera began in Peru in 1991 and spread quickly to nearly all neighboring countries (*84*). The disease evolved in explosive epidemics, the largest recorded since the beginning of the seventh pandemic in Sulawesi (the Celebes), Indonesia, in 1961. The epidemics behaved differently in the nations of Latin America affected by cholera, according to prevailing levels of poverty, health education, sanitation, and risk factors (*84*).

In Peru, cholera appeared in January 1991, and at the end of the summer, Chancay, Chimbote, Piura, Lima, Trujello, and other localities were affected in succession or simultaneously along 1200 km of the Pacific Coast (*85*). In 3 weeks, the epidemic covered >2000 km of coastal areas and caused 30,000 cases and 114 deaths in the first 7 days.

Cholera reached Ecuador 6 weeks after the outbreak in Peru, and spread throughout the country within 2 months; however, the intensity of the epidemic was less than in Peru. A milder outbreak followed in Columbia. The epidemic in Brazil appeared at the border of Columbia and Peru, in the Amazon, São Paolo, and Rio de Janeiro basins, in July to September. Eight months later, the disease reached Bolivia.

All South American countries were affected in 1991 except Argentina and Paraguay, the latter having some cases in 1992. Uruguay was fortunate in being relatively free of cholera cases. Mexico was hit on 13 June 1991; subsequently outbreaks occurred in Guatemala in July, in El Salvador in August, and then in Honduras. Nicaragua reported cholera early in 1992, and even worse epidemics occurred in 1993. Chile had its first case confirmed on 12 April 1991 in Santiago, 1700 km south of Peru. By 1992, there were 99 cases. In Costa Rica, the first case appeared on 5 January 1991. More than 1.5% of the Peruvian population was estimated to have come down with cholera during the first 3 months of 1991. The sixth pandemic, seventh pandemic, and U.S. Gulf Coast isolates were concluded to be three clones, apparently evolving independently from environmental, nontoxigenic, non-01 El Tor organisms (*70*). The 0139 isolates are concluded to have evolved from seventh pandemic isolates of *V. cholerae* 01 El Tor.

El Niño Events

The trade winds blowing westward across the central Pacific force warm surface water from the seas near Peru toward Tahiti. Thus, cold currents, rich in nutrients and phytoplankton, circulate up from the ocean bottom off the Peruvian coast to replace the warm water moving west. El Niño is a warming of surface waters in the Central Pacific of 1°C greater than normal.

Coincidental to the cholera outbreak in Peru was a warm event related to El Niño in the tropical Pacific from 1990 to June 1995 and is the longest on record since 1882. It occurred in the context of a tendency for more frequent El Niño events and fewer La Niña events since the late 1970s (*86*). Returning every 4 years on average and usually lasting approximately a year, El Niño, an unusual warming in the central Pacific Ocean, creates storms and disrupts wind patterns (*87*). The surprise during 1991 to 1995 was that the El Niño lasted for more than 3 years, the longest time period since monitoring began in the 1870s.

Recent interannual changes in the strength and seasonal evolution of the surface level southwest monsoon winds have been related to variations in summer phytoplankton blooms of the northwestern Arabian Sea and also the Bay of Bengal. In the Bay of Bengal, synthesis of satellite remote sensing with analysis of in situ hydrographic and meteorological data sets, and cholera case data for Bangladesh, has provided evidence that cholera cases occur following a rise in ocean surface temperatures (*88*) (Fig. 1).

From 1979 to 1981, monsoon phytoplankton blooms in the northwest Arabian sea peaked during August and September, and appeared to lag the development of open-sea upwelling by at least 1 month. Coastal upwelling, from May to September, yielded the most extreme concentrations of phytoplankton biomass. Phytoplankton biomass on the Omani continental shelf increased during both the early and late phases of the 1980 southwest monsoon, because of stronger coastal upwelling. The Somali current in the Arabian Sea has much the same directional flow as currents in the Bay of Bengal (*89*).

Kiorboe and Neilsen (*42*) studied seasonal distributions of biomass, egg production, and production rates of pelagic copepod communities. Copepod production was found to be episodic and occurring in bursts associated with phytoplankton blooms. The seasonal distribution of copepod biomass was unimodal; concentrations peaked in June and July in Denmark, where the studies were done. A spring production burst was observed, and egg production rates varied significantly with concentrations of chlorophyll and total microplankton biomass, but only weakly with the abundance of dinoflagellates, nanoflagellates, ciliates, and copepod nauplii. Significant copepod egg production occurred only when concentrations of diatoms and other large phytoplankters were high. The conclusion is that copepod pro-

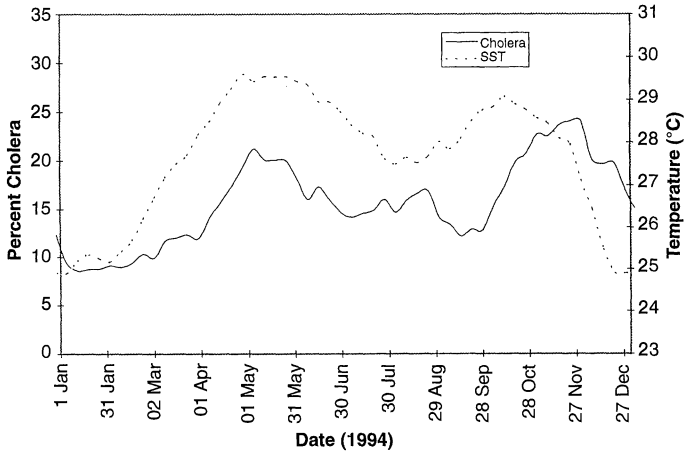

Fig. 1. Relationship between sea surface temperature and cholera case data in Bangladesh from January to December 1994.

duction depends on episodic phytoplankton blooms.

From all of this evidence, it is now possible to utilize remote sensing and computer processing to integrate ecological, epidemiological, and remotely sensed spatial data for the purpose of developing predictive models of cholera outbreaks (*40*). The ability to predict conditions conducive to pandemics of cholera should allow public health measures to be taken prospectively, rather than retrospectively.

In this case study of cholera, the interdisciplinary cross-cut of oceanography, ecology, microbiology, marine biology, epidemiology, medicine, and satellite imagery (space science) will allow a new conceptualization and understanding of this historic scourge of humankind and, ultimately, prevention of global pandemics of this disease.

REFERENCES AND NOTES

1. S. Morse, *Emerging Infect. Dis.* **1**, 7 (1995).
2. Center for Disease Control and Prevention, Atlanta, GA, 1994, "Addressing emerging infectious disease threats: A prevention strategy for the United States," (U.S. Department of Health and Human Services, Public Health Service).
3. *The Japan Times*, Nov. 8, 1995, p. 1.
4. J. A. Patz, P. R. Epstein, T. A. Burke, J. M. Balbus, *J. Am. Med. Assoc.* **275**, 217 (1996).
5. S. W. Lacey, *Clin. Infect. Dis.* **20**, 409 (1995).
6. D. Barua and W. B. Greenough, Eds., in *Current Topics in Infectious Disease* (Plenum, New York, 1991), p. 372.
7. K. Bishagratna, *An English Translation of the Sushruta Samhita* (Chowkhamba Sanskrit Series Office, Varanasi, India, 1963), pp. 352–356.
8. J. C. Peters, "Early history of Asiatic cholera, in India as known to Europeans A.D. 1503–1800," *Asiatic Cholera*, E. C. Wendt, Ed. (William Wood, New York, 1885).
9. E. C. Tramont and E. J. Gangarosa, in *Infections of the Gastrointestinal Tract*, M. J. Blaser, P. D. Smith, J. I. Ravdin, H. B. Greenberg, R. L. Guerrant, Eds. (Raven, New York, 1995).
10. D. A. Okun, *J. Environ. Engin.* **122**, 453 (1996).
11. T. Ramamurthy *et al.*, *Lancet* **341**, 703 (1993).
12. Cholera Working Group, *ibid.* **342**, 387 (1993).
13. G. Nair *et al.*, *J. Infect. Dis.* **169**, 1029 (1994).
14. T. Shimada *et al.*, *Lancet* **341**, 1347 (1993).
15. M. K. Waldor, R. Colwell, J. J. Mekalanos, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 11388 (1994).
16. L. E. Comstock, J. A. Johnson, J. M. Michalski, J. G. Morris Jr., J. B. Kaper, *Mol. Microbiol.* **19**, 815 (1996).
17. R. B. Sack and C. E. Miller, *J. Bacteriol.* **99**, 688 (1969).
18. G. Surabhi *et al.*, *FEMS Immunol Med. Microbiol.* **8**, 293 (1994).
19. E. M. Bik, A. E. Bunschoten, R. D. Gouw, F. R. Mooi, *EMBO J.* **14**, 209 (1995).
20. P. Brayton, D. Roszak-Macdonnel, R. R. Colwell, *XIV Int. Cong. Microbiol. Univ. Manchester, England* (1986).
21. R. R. Colwell, A. Huq, M. A. R. Chowdhury, P. Brayton, B. Xu, *Can. J. Microbiol.* **41**, 946 (1995).
22. A. Huq *et al.*, *Lancet* **345**,1249 (1995).
23. A. K. Siddique *et al.*, *Trop. Geog. Med.* **46**, 147 (1994).
24. S. Faruque *et al.*, *J. Clin. Microbiol.* **31**, 2513 (1994).
25. F. L. Singleton, R. W. Attwell, M. S. Jangi, R. R. Colwell, *Appl. Environ. Microbiol.* **43**, 1080 (1982).
26. ———, *ibid.* **44**, 1047 (1982).
27. A. Huq, P. A. West, E. B. Small, M. I. Huq, R. R. Colwell, *ibid.* **48**, 420 (1984).
28. P. M. Munro and R. R. Colwell, *Water Res.* **30**, 47 (1994).
29. D. B. Roszak and R. R. Colwell, *Microbiol. Rev.* **51**, 365 (1987).
30. R. R. Colwell and A. Huq, in Vibrio cholerae *and Cholera: Molecular to Global Perspectives*, I. K. Wachsmuth, O. Olsvik, P. A. Blake, Eds. (American Society for Microbiology, Washington, DC, 1994), pp. 117–133.
31. R. R. Colwell *et al.*, *World J. Microbiol. Biotechnol.* **12**, 28 (1996).
32. K. A. Kondo, A. Takade, K. Amako, *FEMS Microbiol. Lett.* **123**, 179 (1994).
33. S. N. Wai, T. Moriya, K. Kondo, H. Misumi, K. Amako, *ibid.* **136**, 187 (1996).
34. H. Xu *et al.*, *Microb. Ecol.* **8**, 213 (1983).
35. T. Kaneko and R. R. Colwell, *J. Bacteriol.* **113**, 24 (1973).
36. J. D. Oliver, R. A. Warner, D. R. Cleland, *Appl. Environ. Microbiol.* **45**, 985 (1983).
37. D. Barua, in Vibrio cholerae *and Cholera*, Y. Takeda, Ed. (KTK Scientific Publishers, Tokyo, 1988), pp. 9–32.
38. E. C. Wendt, Ed., *A Treatise on Asiatic Cholera* (William Wood, New York, 1885), p. 403.
39. "Cholera–Western Hemisphere" *Morb. Mortal. Wkly. Rep. Upd.* **42**, 89 (1992).
40. L. Beck, B. Wood, S. Dister, *Geo. Info. Syst.* **Sept.**, 32 (1995).
41. A. J. Southward, S. J. Hawkins, M. T. Burrows, *J. Therm. Biol.* **20**, 127 (1995).
42. T. Kiorboe and T. J. Neilson, *Copepods. Limnol. Oceanog.* **39**, 493 (1994).
43. M. M. Levine *et al.*, in *Acute Enteric Infections in Children: New Prospects for Treatment and Prevention*, T. Holme, J. Holmgren, M. H. Meison, R. Möllby, Eds. (Elsevier/North-Holland, New York, 1981), chap. 26.
44. M. R. Sochard, D. F. Wilson, B. Austin, R. R. Colwell, *Appl. Environ. Microbiol.* **37**, 7 (1979).
45. A. Huq, E. Small, P. West, R. R. Colwell, in *Vibrios in the Environment*, R. R. Colwell, Ed. (Wiley, New York, 1984), p. 521.
46. R. Pollitzer, *Cholera* (World Health Organization, Geneva, 1959), p. 1019.
47. R. I. Glass, M. Claeson, P. A. Blake, R. J. Waldman, N. F. Pierce, *Lancet* **338**, 791 (1991).
48. I. K. Wachsmuth, P. A. Blake, O. Olsvik, Vibrio cholerae *and Cholera: Molecular to Global Perspectives* (American Society for Microbiology, Washington, DC, 1994).
49. M. Nishimura, K. Kita-Tsukamoto, K. Kogure, K. Ohwada, *Bull. Jap. Soc. Microbiol. Ecol.* **7**, 43 (1992).
50. H. S. Xu *et al.*, *J. Microbiol. Methods* **2**, 221 (1984).
51. A. Huq *et al.*, *Appl. Environ. Microbiol.* **56**, 2370 (1990).
52. J. D. Grimes and R. R. Colwell, *FEMS Microbiol. Lett.* **34**, 161 (1986).
53. R. R. Colwell *et al.*, in *Advances in Research on Cholera and Related Diarrhoeas*, R. B. Sack and Y. Zinnaka, Eds. (KTK Scientific Publishers, Tokyo, ed. 7, 1990) pp. 327–343.
54. P. Brayton, M. Tamplin, A. Huq, R. R. Colwell, *Appl. Environ. Microbiol.* **53**, 2862 (1987).
55. M. L. Tamplin, A. L. Gauzens, A. Huq, D. A. Sack, R. R. Colwell, *ibid.* **56**, 1977 (1990).
56. J. A. K. Hasan, A. Huq, M. L. Tamplin, R. Siebeling, R. R. Colwell, *J. Clin. Microbiol.* **32**, 249 (1994).
57. J. A. K. Hasan *et al.*, *FEMS Microbiol. Lett.* **120**,143 (1994).
58. M. A. Hood and G. E. Ness, *Appl. Environ. Microbiol.* **43**, 578 (1982).
59. ———, G. E. Rodrick, N. J. Blake, *Microb. Ecol.* **9**, 65 (1983).
60. J. Kaper, H. Lockman, R. R. Colwell, S. W. Joseph, *Appl. Environ. Microbiol.* **37**, 91 (1979).
61. D. R. Nalin, V. Daya, A. Ried, M. M. Levine, L. Cisneros, *Int. Immun.* **25**,768 (1979).
62. W. M. Spira, A. Huq, Q. S. Ahmad, Y. A. Saeed, *Appl. Environ. Microbiol.* **42**, 550 (1981).
63. M. S. Islam, B. S. Drasar, D. J. Bradley, *J. Trop. Med. Hyg.* **92**, 396 (1989).
64. ———,*Trans. R. Soc. Trop. Med. Hyg.* **84**, 422 (1990).
65. ———, *ibid.* **93**,133 (1990).
66. M. S. Islam, M. A. Miah, M. K. Hasan, R. B. Sack, M. J. Albert, *ibid.* **88**, 298 (1994).
67. R. Glass *et al.*, *Am. J. Epidemiol.* **116**, 959 (1982).
68. W. M. McCormack, M. S. Islam, M. Fahimuddin, W. H. Mosley, *ibid.* **89**, 393 (1969).
69. R. I. Glass *et al.*, *J. Infect. Dis.* **151**, 236 (1985).
70. D. K. R. Karaolis, R. Lan, P. R. Reeves, *J. Bacteriol.* **177**, 3191 (1995).
71. R. R. Colwell, A. Huq, E. Russek-Cohen, D. Jacobs, in preparation.
72. D. Hosmer and S. Lemeshow, *The Applied Logistics Regression Analysis* (Wiley Interscience, New York, 1987).
73. J. Field and L. Owens, *Aust. Microbiol.* **15**, 138 (1994).
74. P. M. Munro and R. R. Colwell, *Water Res.* **30**, 47 (1996).
75. T. Shiba, R. T. Hill, W. Straube, R. R. Colwell, *Appl. Environ. Microbiol.* **61**, 2583 (1995).
76. R. R. Colwell, *J. Bacteriol.* **104**, 410 (1970).
77. R. V. Citarella and R. R. Colwell, *ibid.*, p. 434 (1970).
78. B. A. Kay, C. A. Bopp, J. G. Wells, Vibrio cholerae *and Cholera: Molecular to Global Perspectives*, I. K. Wachsmuth, O. Olsvik, P. A. Blake, Eds. (American Society for Microbiology, Washington, DC, 1994), chap. 1.
79. L. Palmer and R. R. Colwell, *Appl. Environ. Microbiol.* **57**, 1286 (1991).
80. S. I. Kotob, S. L. Coon, E. J. Quintero, R. M. Weiner, *ibid.* **61**, p. 000 (1995).
81. J. W. Ellms, *Water Purification* (McGraw-Hill, New York, 1928).
82. D. Yohalem and S. Fry, Eds., *Orientation to Guinea Worm Disease: A Guide for Use in Pre-Service and In-Service Training*, Field Rep. 320 (Water and Sanitation Health, Arlington, VA, 1991).
83. A. Huq *et al.*, *Appl. Environ. Microbiol.* **62**, 2508 (1996).
84. L. Mata, *Ann. N.Y. Acad. Sci.* **740**, 55 (1994).
85. L. Seminario, A. López, E. Vásquez, M. Rodriguez, *Rev. Per Epidemiol.* (Perú) **4**, 8 (1991).
86. K. E. Trenberth and T. J. Hoar, *Geophys. Res. Lett.* **23**, 57 (1996).
87. J. Tibbetts, *Bioscience* **46**, 566 (1996).
88. L. Beck, B. Wood, A. Huq, R. Colwell, unpublished data.
89. J. C. Brock, and C. R. McClain, *J. Geophys. Res.* **97**, 733 (1992).

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