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Infectious disease and environment: cholera as a paradigm for waterborne disease

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Introduction: a global context

The current international awareness of the importance of combating infectious diseases can provide the opportunity for a multidisciplinary approach that joins medicine with many other scientific and technological capabilities, with broad implications for the quality of daily life. Science and technology are major forces that have the potential to balance the world's inequities. The scientific community and world leaders must therefore work together to use knowledge and its applications to improve the condition of the planet. The connection between cholera and the environment provides a paradigm for this perspective.

A global context indisputably frames all human health issues in the twenty-first century. This context is based on several realities: the worldwide movement of people and goods, the recognition that earth processes operate on a global scale, and a dynamic international scientific enterprise. Health issues are no longer simply a personal matter between patient and physician, if they ever were. Nowadays they encompass an individual's complex relationship with the global environment. As Gro Harlem Brundtland—former director of the World Health Organization (WHO)—said, "[i]n the modern world, bacteria and viruses travel almost as fast as money. With globalization, a single microbial sea washes all of humankind. There are no health sanctuaries." [Brundtland GH, Lecture at the World Economic Forum, Davos, 29 Jan. 2001, available at http://www.who.int/ director-general/speeches /2001 /english/20010129_davosunequaldistr.en.html]. According to the WHO, infectious diseases account for about one quarter of all deaths worldwide (and these do not include cancer, cardiovascular and respiratory diseases, many of which we currently know to be triggered by infections). Several of them, including diarrheal diseases, tuberculosis and malaria, are classical infectious killers, whereas a new emerging infectious disease—AIDS—has become the second leading cause of death in just two decades. In children, infectious diseases are responsible for 63% of deaths, and diarrheal diseases, which until recently ranked first, are still the second most frequent cause of death for children under age five.

Throughout the past half century, international travel has skyrocketed; there are now approximately 500 million international arrivals per year. The greatest increases have taken place since the mid-1990s. International arrivals increased in every region of the earth, but in Africa and the Middle East they jumped by almost half. The world has become integrated and global; consequently, the notion that it is possible to successfully eradicate a disease from the face of the planet has become simplistic. Infectious disease is a moving target, and climate shifts will affect any disease that has an environmentally sensitive stage or vector. Recognizing signals from climate models and incorporating them into health measures can thus provide new opportunities for proactive—rather than reactive—approaches to public health. Ecosystems do not respond linearly to environmental changes, nor do the pathogens that live in them.

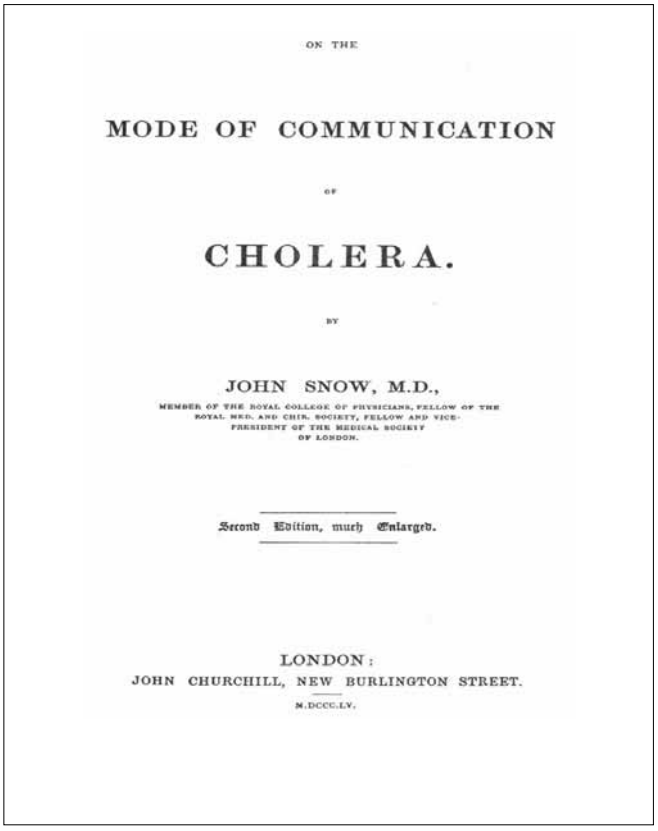
This article is based on the lecture that the author delivered at the Institute for Catalan Studies, Barcelona, Spain on October 26, 2004.

The spiral of complexity begins to unfurl at the most minute scale, that of the atom, and curves up through successive levels of life, through the cell, the organism, the community, the ecosystem. This view of biological systems, which I term biocomplexity, roots epidemiology firmly in ecology.

Cholera

Over the last few decades, our understanding of cholera has evolved from a linear reductionist model involving a waterborne bacterium and a human host to a much more complex picture. The current characterization of the ecology of cholera includes global weather patterns, aquatic reservoirs, bacteriophages, zooplankton, the collective behavior of surface-attached cells, an adaptable genome, and the deep sea, together with the bacterium and its host.

Where did cholera come from? The recorded history of diarrheal disease in India goes back 2,500 years. Ancient Sanskrit texts describe a severe illness that we speculate was cholera. Until the early nineteenth century, this disease was primarily confined to the Indian subcontinent, but it then spread to Europe and the Americas. Since 1817, western medical history has described seven global cholera pandemics, each spreading illness and death around the world. The second pandemic reached the United States in 1832, traveling from New York to Philadelphia in two weeks and then migrating along the coast all the way to the Gulf of Mexico. This pandemic continued for another 19 years, making its most notorious appearance in London in 1849. There, John Snow (1813-1858) observed a correlation between the disease and where it spread, and with the source of public water. In a speech he delivered in 1853 at the 8th anniversary of the Medical Society of London, Snow laid out a theory that recognized a distinct and specific cause for each communicable disease [9]. In 1855, he published *On the Mode of Communication of Cholera* (Fig. 1). This treatise was a milestone in public health as it correctly identified the fecal-oral route of human infection and offered powerful arguments for the germ theory.


ON THE

MODE OF COMMUNICATION

OF

CHOLERA.

BY

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Fig. 1. *On the Mode of Communication of Cholera*, by John Snow, published in 1855.

Ecology of *Vibrio cholerae*

The requirement of *Vibrio cholerae* for salt to grow led microbiologists to suggest that its ancestral home is the sea, perhaps a deep-sea vent. Genomics has helped to substantiate this theory. In 1999, during dives by the submersibles *Alvin* and *Nautile*, sulfide chimneys samples were collected from undersea hydrothermal vents on the East Pacific Rise. *Vibrio* species isolated from the chimneys were identified that bore significant similarity to *Vibrio cholerae*, suggesting that this species is autochthonous to the deep sea. The discovery that *V. cholerae* had two chromosomes, one large and one small, was of great interest, because all bacteria were presumed to have only a single chromosome. The two chromosomes, both of which are necessary for metabolism and replication, were sequenced in 2000.

V. cholerae toxin genes, of which there are fifty, reside on the large chromosome. The sequencing data confirmed that *V. cholerae* is a versatile organism, able to live in several habitat types and to infect the human gastrointestinal tract. In 2002, Andrew Camilli and colleagues reported that, when *V. cholerae* passes through the human gut, certain genes appear to greatly accelerate their activity—, so that the bacterium is 700 times more infectious than the control strain [8]. In addition, lateral transfer of genetic material clearly occurs in this organism. Virulence genes are distributed in environmental strains of *V. cholerae* from various serogroups, providing an environmental reservoir of such genes. For example, it is known that *Vibrio* O139 serovar acquired novel DNA from other cholera strains in its environment [3]. This underscores the versatility of cholera and gives even greater credence to

the significance of the environment in understanding the complexity of this disease. However, it is difficult to isolate *V. cholerae* O1 from the environment, where it competes with some 250 other serovars.

Bacterial viruses have been found to play a major role in the microbial ecology of aquatic ecosystems. Lysogeny occurs more frequently in *V. cholerae* O1 El Tor and O139 strains than in O1 classical strains and is believed to impart antibiotic resistance to the host bacterium. Phage infection may give rise to new toxigenic variants, and polylysogeny can occur [1,2,5,6]. We observed Classical and El Tor strains of cholera infected with a temperate phage, and at least three prophages (inserted DNA) were found to exist in one strain of El Tor. Should such a mechanism operate in the wild, genetic material, such as toxin genes, could be transmitted in the environment via multiple temperate phages. A major insight acquired over the past few decades is that pathogens such as *V. cholerae* can exist in a viable state even though they cannot be cultured. The pathogens in which the "viable but non-culturable" phenomenon has been studied include *Aeromonas* spp., *Campylobacter coli*, *Campylobacter jejuni*, *Escherichia coli*, *Helicobacter pylori*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Pseudomonas putida*, *Salmonella enteritidis*, *Salmonella enterica* serovar Typhimurium, *Shigella sonnei*, *Shigella flexneri*, *Shigella dysenteriae*, *Vibrio cholerae* O1, *Vibrio cholerae* O139, and *Pasteurella piscida*.

Vibrio cholerae's interactions with its environment are another major line of inquiry. The ecological relationship of cholera and planktonic copepods was first established in 1983, when Anwar Huq and I showed that *V. cholerae* attach to live copepods in the Chesapeake Bay and around Bangladesh [5]. This strongly suggests that cholera is probably not an eradicable disease, because its causative organism lives naturally in riverine, brackish and estuarine ecosystems. Currently, we are investigating whether the seasonality of cholera in endemic areas is associated purely with temperature and salinity, or whether *V. cholerae's* interactions with given species of hosts—namely these copepods—also affect its seasonal abundance. At present, we are studying whether two epidemic variants of *V. cholerae*, O1 and O139 serovars, preferentially attach to specific genera of copepods.

Vibrio cholerae's environmental capabilities

Not all bacteria compete well for space on surfaces. Among *Vibrio* species, those that are pathogenic attach the best. This is further evidence of the autochthonous aquatic nature of these bacteria. Among the copepods to which *Vibrio* species attach, *Eurytemora* is more prevalent in the upper Chesapeake Bay and *Acartia* in the lower Bay. There are also seasonal fluctuations in the prevalence of these species. Further research in various environments is necessary in order to determine whether the selectivity of copepod species —for example, the ability of serovar O1 to attach better to a particular copepod species—is linked to a more severe cholera epidemic when that particular copepod is more abundant. Adhesive ability is an important attribute of *V. cholerae*, whether in the environment or in the human gut. The "environmental" capabilities of *V. cholerae* include its ability to secrete a powerful chitinase, which assists its growth on chitin surfaces. Besides colonizing copepods, *V. cholerae* is also present in shellfish. Our hypothesis is that cholera originally evolved commensally with marine animals, such as copepods, which provided them with a surface to grow on, nutrition and perhaps other mutual benefits.

In the ocean ecosystem, the ability of bacteria to break down chitin is of major ecological importance. Another interesting capability is the production by *V. cholerae* of a mucinase that enables the bacterium to penetrate the mucus barrier that covers the gastrointestinal epithelium. Carla Pruzzo, of the Università Politecnica delle Marche, Ancona, Italy, has reported that serum from the hemolymph of the Mediterranean mussel *Mytilus galloprovincialis* increases attachment to intestinal epithelial cells [10]. The upshot is that when *Vibrio* is ingested with seafood, the bacterium acquires "bridging molecules" that make it very adhesive in the intestine. Both virulence and infectivity depend on bacterial properties and environmental factors.

The formation of a bacterial biofilm also enhances colonization by *V. cholerae*. This protective clustering behavior is another mechanism in the bacterium's life cycle in aquatic environments that serves to also protect it from stomach acid when ingested by humans. Biofilm formation is one of several bacterial processes—including the production of virulence factors and bioluminescence—that are regulated by a special form of bacterial communication called quorum sensing. Other environmental attributes of *V. cholerae* are its salinity and temperature tolerances. Table 1 gives a broad overview of the bacterium's temperature and salinity ranges in various experimental settings. The salinity most favorable for *V. cholerae* is between 2 and 14 g/l. In Chesapeake Bay, *V. cholerae* concentrations are higher in the northern part of the bay, where salinity is low, and when the weather is warmer. In fact, the combined temperature and salinity conditions —both of which display seasonal patterns—predict the presence of *V. cholerae* with an accuracy ranging from 75.5 to 88.5% [7].

Table 1. Ranges of temperature and salinity tolerance of *Vibrio cholerae* in various experimental settings (adapted from [7])

Experimental setting	Temp (°C) (study range)	Salinity (g/l) (study range)
Laboratory microcosm		5, 10
Laboratory microcosm		15
Laboratory microcosm	20, 25	15, 25
Chesapeake Bay (estuary)	15–20	4–12
Chesapeake Bay (estuary)	>17[a]	4–17
Southern California (coastal areas)	No preference	1–10
Louisiana (coastal areas)	Depended on salinity (18–30)	<1
Florida (estuary) and laboratory microcosm	20–35	12–25, 10–25
England (river and marsh ditch)	>9[b]	3–12
Japan (rivers and coastal areas)	ca. 21[c]	0.4–32[d]

[a]*V. cholerae* was detected at temperatures above 10°C.
[b]Highest temperatures occur in August.
[c]*V. cholerae* was detected at temperatures above 7°C.
[d]The detection range was 0.4–32.5 g/l.

El Niño and cholera outbreaks

In the Southern Hemisphere, a study of the coast of Peru has added new insights to the cholera story. Here, cholera surfaced in 1991 after a century of absence in Latin America. Cholera has recurred in Peru since then, following a seasonal pattern, with the greatest number of cases in summer (June-March) in Lima and other major cities along the coast. Erin Lipp and others studied *V. cholerae* O1 and *ctxA*. The detection of *V. cholerae* followed ambient temperature increases and coincided with or preceded annual outbreaks of cholera in summer [6]. Off the Peruvian coast, there was a significant correlation between cholera incidence and elevated sea surface temperature from October 1997-June 2000, which included the 1997-1998 El Niño event [4]. This link suggests that an early-warning system for cholera risk could be established for Peru and neighboring countries. Although not related directly, both El Niño events and cholera outbreaks have increased since the 1970s. This pattern has emerged in both Peruvian waters and the Bay of Bengal. Sea surface temperature and height, as well as plankton blooms, can be remotely sensed and thus used to forecast outbreaks of cholera.

The contributions of other disciplines to the study of infectious disease

Many concentric circles formed by the perspectives of other disciplines frame our study of infectious disease, whether cholera or others, thereby providing us with new and deeper insights. Seemingly unrelated disciplines may have direct implications for epidemiology, illuminating the intricate and subtle routes that pathogens can take. For example, human beings and the "concentric circle" called social science, which we use to study ourselves, are very much a part of cholera's complex story. In addition to laboratory results and satellite studies, social science has been used to find a practical tool for removing cholera from the drinking water in Bangladesh. This tool—available even in the poorest household—is the sari cloth. Folded eight to ten times, the cloth becomes a 20-mm mesh filter, as we measured by electron microscopy. Straining water through several layers of sari cloth may be enough to prevent ingestion of infectious levels of cholera bacteria. For this purpose, old sari cloth, not new, is preferred, because its holes are smaller and better able to trap plankton. Laboratory studies showed that old sari cloth folded at least eight times filtered out more than 99% of the *V. cholerae* attached to plankton. We carried out a three-year study in 65 villages in Matlab, Bangladesh, comprising a total study population of about 133,000 people; the incidence of cholera was roughly half among those who used sari filters compared to the control. The severity of disease also appears to have been reduced in villages that filtered, but this finding awaits confirmation [1].

Theorists of mathematical networks have come up with intriguing results in the past few years that can be applied to study biological systems. Whereas older models classified networks as either organized or random, Steven Strogatz and Duncan Watts proposed a third type of network as being more realistic. They examined three diverse real-world systems: the

nervous system of *Caenorhabditis elegans*, the hundreds of thousands of actors in the Internet Movie Database, and the power grid of the western United States. They found that a third model, the small-world network—which included "shortcuts" to increase connectedness—best depicted these three systems. Small-world networks among human beings have short connections and many clusters. Whereas shortcuts can be beneficial in many settings, in a social context they can be devastating, enabling infectious diseases to spread more easily. Such shortcuts have been created, in fact, by air travel, which has generated routes for diseases to spread across the world in a matter of hours.

Today, in a world where people, pathogens and invading pests travel around the world in unintended, often unpredictable ways, through both natural and human-made means, we can no longer circumscribe the dynamics of an infectious disease within a neat and orderly framework and thus expect to contain and understand its complexity. In a world of ever-more-rapid change, understanding the patterns of infectious disease and developing appropriate treatment that can be readily implemented requires not only knowledge of the biological, physical, and social sciences, but also the ability to integrate this information into an effective response.

References

1. Colwell RR, Huq A, Islam MS, Aziz KMA, Yunus M, Huda Khan N, Mahmud A, Bradley Sack R, Nair GB, Chakraborty J, Sack DA, Russek-Cohen E (2003) Reduction of cholera in Bangladeshi villages by simple filtration. Proc Nat Acad Sci USA 100:1051-1055
2. Espeland EM, Lipp EK, Huq A, Colwell R (2004) Polylysogeny and prophage induction in secondary infection in *Vibrio cholerae*. Environ Microbiol 6:760-763
3. Faruque SM, Nair GB (2002) Molecular ecology of toxigenic *Vibrio cholerae*. Microbiol Immunol 46:59-66
4. Gil AI, Louis VR, Rivera IN, Lipp E, Huq A, Lanata CF, Taylor DN, Russek-Cohen E, Choopun N, Sack RB, Colwell RR (2004) Occurrence and distribution of *Vibrio cholerae* in the coastal environment of Peru. Environ Microbiol 6:699-706
5. Huq A, Small EB, West PA, Huq MI, Rahman R, Colwell RR (1983) Ecological relationships between *Vibrio cholerae* and planktonic crustacean copepods. Appl Environ Microbiol 45:275-283
6. Lipp EC, Rivera ING, Gil AI, Espeland EM, Choopun N, Louis VR, Russek-Cohen E, Huq A, Colwell RR (2003) Direct detection of *Vibrio cholerae* and *ctxA* in Peruvian coastal water and plankton by PCR. Appl Environ Microbiol 69:3676-3680
7. Louis VR, Russek-Cohen E, Choopun N, Rivera ING, Gangle B, Jiang SC, Rubin A, Patz JA, Huq A, Colwell RR (2003) Predictability of *Vibrio cholerae* in Chesapeake Bay. Appl Environ Microbiol 69:2773-2785
8. Merrell DS, Butler SM, Qadri F, Dolganov NA, Alam A, Cohen MB, Calderwood SB, Schoolnik GK, Camilli A (2002) Host-induced epidemic spread of the cholera bacterium. Nature 417:642-645
9. Winkelstein W Jr (1995) A new perspective on John Snow's communicable disease theory. Am J Epidemiol 142:S3-S9
10. Zampini M, Canesi L, Betti M, Ciacci C, Tarsi R, Gallo G, Pruzzo C (2003) Role for mannose-sensitive hemagglutinin in promoting interactions between *Vibrio cholerae* El Tor and mussel hemolymph. Appl Environ Microbiol 69:5711-5715