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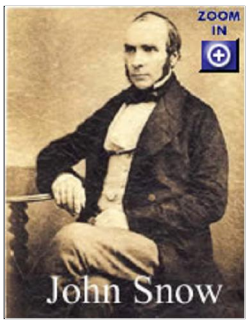
History

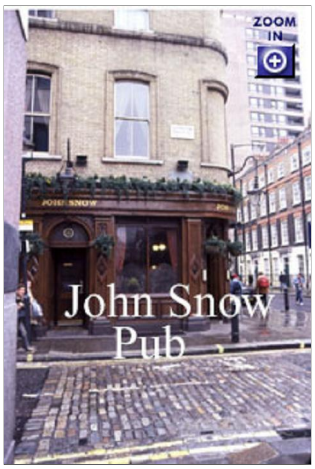
Cholera is one of the most feared clinical entities on earth. Outbreaks in India have been well documented since the early 1800's, in which hundreds of thousands of people became ill. Many of those who got sick went on to die. The organism responsible for this serious diarrheal disease was most likely present in human populations on that subcontinent well before the **British** arrived there. One of the first documented epidemics of cholera occurred in 1817 along the coastal region near the mouth of the Ganges River. Cholera now has a **worldwide presence**, with many people dying each year. Most deaths from cholera can be avoided if adequate medical care were made available.



Cholera spread rapidly throughout the world after the 1817 epidemic, largely due to the inadvertent transport of bilge water, mainly from British ships, but others too, acquired in the Bay of Bengal that contained the organisms. Dumping the contaminated water into their own port cites upon arrival home seeded the local waters with it and insured the eventuality of an outbreak. It then rapidly moved throughout Europe and into Russia. The French were the ones who brought it to the New World, and in 1832, it spread south from Montreal and caused an enormous epidemicin New York City (see: The Cholera Years, by C. E. Rosenberg, The University of Chicago Press, 1987, pp 265). In 1855, a wave of cholera ravaged the citizens of some parts of London. Thousands became ill and died before the medical detective work by ***John Snow*** identified the Broad Street water pump as the single point source of that outbreak. His classical **maps** showing where people who became sick lived convinced him that the only possible source of the infection was the water pump. This landmark study established the **epidemiological view of cholera** that has endured until quite recently. In London on the corner of Broadwick (formerly Broad) stands the ***John Snow pub***, a fitting commemorative honoring the site on which these historic events unfolded. Today, all patrons of the John Snow can enjoy a pint of local ale, and even more importantly, a refreshing glass of crystal clear, pathogen-free water. Since 1961, there have been seven major cholera **pandemics** (The global spread of cholera during the seventh pandemic, 1961-1971 [**source**]), affectingmillions of people living in South America, Africa, Europe, and Asia. To fully appreciate its biology, one must take into account data collected from many different scientific disciplines. Ecology, molecular biology, microbiology, epidemiology, pathology, and long range sensing all have supplied critical pieces of information, which, taken together and integrated, forms a comprehensive body of knowledge as to how cholera enters the human population and what factors regulate its occurrence within the estuary. Thus, cholera is a perfectly suited topic for illustrating the usefulness of the Medical Ecology paradigm.

The Cholera Organism

First isolated, cultured, and characterized by **Robert Koch** in Germany in 1883, the organism is a comma-shaped, flagellated, gram-negative bacterium, ***Vibrio cholerae***. In fact, it was Koch's work on cholera the led the way to firmly establishing the **germ theory of disease**, and helped convince the medical community as to the microbial nature of this devastating clinical condition. For all of his exemplary work, he was the recipient of the Nobel Prize in Medicine in 1905. In the laboratory, it can be easily **grown** at 37°C on blood agar, as well as on selective media such as thio-citrate-bile salt-sucrose. There are many 16 strains of *V. cholerae*, and the **01 and 0139 strains** are the most lethal. While *V. cholerae* is the best characterized of these agents, several other **species of Vibrio** can also cause significant disease.



Pathological strains produce **clinical symptoms and signs**, the most common one by far being a protracted, watery diarrhea. Yet, despite the fact that human populations are routinely infected with it, *V. cholerae*'s natural habitat is not our small intestine, since most infections last for only several days, and the **carrier state** in humans is extremely rare. It was well into the 21st century before its **fundamental niche** was revealed to be the **estuary**, a narrow ecological region known as an **ecotone**. Typically, the first clinical cases of any new outbreak occur in communities situated on or near an estuary. Although this fact was known for at least since the 1800s, it was not considered essential to the natural history of the disease. In fact, its ecological role, once revealed, surprised even those who resolutely suspected that it was essentially an organism that occupied a fundamental niche outside the human host, but could not prove it.

How Do Cholera Epidemics Start?

One of the enigmas related both to its ecology and to human disease was its apparent absence from human populations just prior to epidemics. Besides the fact that extensive clinical research repeatedly failed to identify human carrier states as the source, the bacterium did not form **spores**, so a resting stage could not be demonstrated in the estuarian environment. *V. cholerae* seemed to simply "disappear" at times when cases were not occurring (prior to the arrival of the **monsoons** in South Asia, for example). How does an epidemic get its start if humans are not the source of the initial infection? Could there be reservoir animal species that harbored the organism and occasionally contaminated the human environment, or perhaps there was a stage of the bacterium that was more difficult to find that a spore stage that allowed it to survive in salt water.

Monsoons represent seasonal patterns of precipitation that bring with them changes in both the relative salinity and temperature of the estuaries of major river systems along the entire coast of the Indian subcontinent. These and other related seasonal precipitation events in similar tropical and sub-tropical environments cause dramatic shifts in the aquatic environment, triggering **blooms of phytoplankton**, that in turn serve as the food source for a rich assortment of **zooplankton** grazer species. It has been known from the time of Koch that cholera organisms grow best at a temperatures above 17°C, and in a nutrient broth with a [NaCl] of 5-15 parts per million, well below that of the open ocean, but above that of freshwater. Those conditions are met in the estuaries by episodes of heavy rains in the spring, and appear to be absolutely essential to establishing an ecological setting favoring an outbreak. But where do the organisms come from? After years of intensive laboratory and field studies, it was discovered that many species of **copepods** that comprise the myriad assemblages of zooplankton communities in those estuaries harbor ***V. cholerae*** as an ecto-**symbiont**. Organisms can be found growing on their egg sacks and inside their gut tracts. This discovery opened the way for a more complete description of the ecology of the cholera group of bacteria.

There still, however, remained unanswered questions regarding its epidemic nature. One large missing piece of the puzzle was the fact that zooplankton blooms are not always present in the estuary, and hence cholera cannot routinely be cultured from most brackish water environments. Where did the microorganisms go during quiescent periods in between seasonal rain events? Further laboratory-based research revealed that they could transform into a unique **dormant stage** that was able to survive for months in the sediment of the estuary. This stage was unlike spores of bacteria such as ***Bacillus subtilis***, an aerobic, non-pathogenic, soil-dwelling organism, or its anaerobic cousin, ***Clostridium perfringens***, a soil-dwelling bacterium capable of causing acute gastroenteritis and gas gangrene. These exciting new data enabled investigators to now integrate information regarding the seasonal nature of events surrounding an outbreak in populations living near estuaries with previous data on the physical and chemical requirements for its growth. What has emerged over the last 10 years has quite literally revolutionized the way we look at cholera.

A Modern Synthesis

Seasonally dependent epidemics can now be described in **ecological terms**. Warm, intense episodes of precipitation falling on coastal and nearby inland regions transforms the temperature and salinity profiles of estuaries. These changes create favorable growth conditions for the dormant bacterium, and also, most importantly, for phytoplankton species. The influx of large quantities of freshwater mobilizes stored nutrients in the bottom sediments of the estuary, and gives the cholera bacterium a head start in its growth cycle. Algal blooms in response to higher temperatures, lower salinity, and **nutrient loading** allow for a similar increase in filter feeding zooplankton. Nutrient loading of the estuary typically occurs from a variety of nearby riparian sites (point source and **non-point source** run off), thus serving as the final environmental cue, supplying *V. cholerae* with additional sources of nutrients. This apparently is sufficient enough to permit an increase in bacterial cell numbers to a level that enables the organism to encounter copepods of the right species. Copepod species then pick up the bacteria on their external and internal surfaces. This most likely involves specific bacterial **ligands** and copepod surface **receptor molecules**, yet to be identified and characterized. Once the cholera organism attaches, the crustaceans carry the bacteria along as a normal component of their bodies throughout their life cycle. The bacterium continues to replicate until they completely cover the surface of the copepod's egg sack. When eggs mature, the overall process apparently triggers the cholera bacteria to synthesize, then secrete a **chitinase** that functions to dissolve the outer egg case, facilitating the release of the eggs, dispersing them and the bacteria into the water column. The more dense the population of zooplankton, the more the concentration of free cholera bacteria there will be in the water column. **Filter feeding benthic organisms** (e.g. crabs, clams, and oysters) process large volumes of water, concentrating particulates and the cholera bacteria in their gut tracts. Humans that **harvest** these contaminated food organisms in spring-time, and ingest them raw place themselves and the rest of their local communities at risk from acquiring cholera. A bacterial cell concentration of 10^3/ml of water is necessary to allow an infectious dose of *V. cholerae* to accumulate within mollusks and crustaceans.

Application of Ecological Knowledge to the Control of Cholera

Epidemics

When a single case of diarrheal disease due to *V. cholerae* occurs, it has the potential of spreading into the local water supply, and contaminating entire villages, and sometimes whole coastal regions. **Remote sensing** from a variety of orbiting earth-monitoring **satellites** can simultaneously detect changes in weather patterns and phytoplankton blooms. Other satellites can determine **sea surface temperature** changes. These data, taken in combination, after further refinement, could eventually become the lynch pin in a network of data collection whose sole purpose would be to predict the next cholera epidemic. Early warning of coastal inhabitants based on this premise could result in millions of lives spared from the ravages of this age-old human pathogen.

This new view of cholera was championed by **Rita Colwell** and colleagues. The salient features of its ecology took many years of hard work and insight. The scientific community at-large, bounded by a more traditional (i.e., John Snow/Robert Koch) view of cholera, unofficially encouraged wide spread opposition to many of the then radical hypotheses that, in fact, turned out to be validated in a series of elegantly conducted laboratory and field studies. This attitude resulted in significant delays in getting relevant data into peer-reviewed journals. Unshakable perseverance and a firm belief in the principles of Medical Ecology won the day. Currently, Rita Colwell is the director of the **National Science Foundation**.

More Cholera Mysteries Solved

Another aspect of cholera that was not understood was why its virulence varied greatly from strain to strain. Some strains even failed to produce disease. **Cholera toxin**, an enzyme, was eventually identified as the main virulence factor associated with strains that induced acute diarrhea. Cholera toxin is synthesized and secreted by strains in the 01 and 0139 groups, only. Those lacking this enzyme are far less pathogenic. Its **mode of action** eventually results in prolonged hypersecretion in the small intestine. The diarrhea is so intense

that enterocytes become fragile and begin to sluff off from the basement membrane of the villus soon after symptoms appear.

Cholera toxin attaches at the level of the crypts of Lieberkühn to enterocytes that have surface ganglioside Gm1, a special glycolipid. Internalization of the toxin-ganglioside complex then occurs. The bacterial enzyme catalyses the transfer of ADP ribose from intracellular NAD+ to the s subunit of the trimeric G protein that is normally attached to the cytoplasmic side of the plasma membrane of each enterocyte. ADP ribosylation changes the activity of s subunit so it can no longer hydrolyze its bound GTP substrate, thus deregulating cyclic AMP activity. Hypersecretion immediately ensues. Efflux in chloride and bicarbonate ions into the small intestinal lumen pulls large quantities of water with it by passive osmosis. The process continues until no more toxin is produced, or until the enterocyte is shed into the lumen of the small intestine.

The acute condition caused by cholera toxin is known as "rice water" stool, because the free enterocytes in the almost clear liquid stool give the appearance of rice grains. Oral re-hydration with saline solution is the recommended supportive therapy that has saved countless millions of lives. Antibiotics can reduce the length of time for the diarrheal portion of the illness, but is often in short supply or not available at all. The cholera organism "burns out" on its own within 5-6 days and the patient experiences an uneventful recovery.

The cDNA encoding the toxin has been cloned and sequenced, and the putative protein expressed and characterized. Interestingly, that data clearly showed that cholera toxin was not related to any known protein produced by non-pathogenic strains of Vibrio. Rather, the protein showed large regions of homology and similarity to a family of endotoxins produced by bacteria in a completely different family, the **Enterobacteriaciae** (e.g., *Escherichia coli*, *Shigella spp.* and *Salmonella spp.*). Vibrios are in the family **Vibrionaceae**.

How did Vibrio acquire its enterobacteriaciae-like toxin? Since not all varieties of the cholera bacteria have the toxin, it was hypothesized that the gene encoding it might be the result of a latent **phage** infecting only a few of them (01 and 0139). This suspicion was eventually confirmed, and the phage, as well as the DNA coding region for the toxin, has now been fully described for strains 01 and 0139 by **Mekalanos** [**link2**] and colleagues.

Yet, despite this new insight into the pathogenicity of *Vibrio cholerae*, some important questions still remain unanswered. How and when did these two cholera strains acquire the gene for the toxin molecule? Does the toxin offer selective advantage to the two strains in their estuarine niche or in the human host? Can other species of vibrio acquire the gene encoding the toxin? The answers to these questions await further investigation. In 1999, scientists in New Delhi, India described two new strains of vibrio that are resistant to a variety of standard antibiotics and induce severe diarrhea. They are being referred to as enteropathogenic *Vibrio cholerae*, or *EPVC*. Neither contains the classical cholera toxin molecule, but they apparently have other virulence factors that produce a diarrhea similar to Shigella sp. and toxigenic Escherichia coli.

Within the last few years, marine scientists have come to realize that the open **ocean is teeming with viruses** (1-10 million viral particles/ml) of a surprisingly wide range of types. Not all of these viral particles are infectious, as UV radiation inactivates most of them in the **photic zone**. However, it is likely that at least a small portion of them escape damage long enough to infect numerous species of **microbes**. In the distant past, within the estuary, similar phages most likely gave *V. cholerae* 01 and 0139 a version of E. coli's toxin, which has since that time, evolved somewhat from the parent molecule. The overall process in reminiscent of the mechanisms by which **antibiotic resistance** can and does occur through associations between bacteria and viruses in the gut tract of humans and farm-raised animals, such as chickens, pigs, and cows.

In the meantime, it seems probable that genomic exchanges between dissimilar organisms in estuaries is commonplace, and can rarely result in the emergence of virulent strains of otherwise harmless microbes. The significance of is concept towards explaining the origins of new infectious agents throughout the world cannot be emphasized enough. In order to become more predictive regarding epidemics sharing similar ecological features to that of cholera, we will need to become even more vigilant of the subtle environmental changes in the world's estuaries induced by an ever increasing human population, or suffer the consequences.

Related Links

- **Microbes.info - *The Microbiology***
- **http://www.disasterrelief.org/Disasters/971112cholera/**
- **http://attila.stevens-tech.edu/chembio/ecronenw/final~1.htm**
- **http://www.nbc-med.org/SiteContent/MedRef/OnlineRef/FieldManuals/medman/Cholera.htm**
- **http://www.cdc.gov/ncidod/dbmd/diseaseinfo/cholera_g.htm**

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Levine, M.M. 1994. "Changes in human ecology and behavior in relation to the emergence of diarrheal diseases, including cholera". *Proceedings of the National Academy of Sciences*. Vol. 91. pp. 2390-2394.

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