Endemic and epidemic dynamics of cholera: the role of the aquatic reservoir

ABSTRACT
Further development on cholera modeling requires a better understanding of V. cholerae ecology and epidemiology. We need estimates of the prevalence of V. cholerae infection in endemic populations as well as a better description of the relationship between dose and virulence.

INTRODUCTION
Background Since Snow's seminal work (1855), when cholera epidemics were first associated with a contaminated water supply, this disease has been the focus of intense study. Cholera is a waterborne disease characterized by severe diarrhea. The etiological agent is V. cholerae O1 (more recently also V. cholerae O139), which colonizes the small intestine and produces an enterotoxin responsible for a watery diarrhea. Without prompt treatment, a person with cholera may die of dehydration in a matter of hours after infection. Cholera outbreaks are generally associated to contaminated food and water supplies. Appropriate sanitation and safe water are the main weapons against this disease. Until the 19th century, cholera was confined to the Indian sub-continent. There, cholera outbreaks are seasonal with one or two peaks per year. From this region, cholera has spread throughout the world seven times since 1817. The last pandemics began in 1961 in Indonesia, spread through the Asian continent during the 60's, reached Africa in 1970 and Latin America in 1991. The occurrence of successive cholera outbreaks throughout Africa and Latin America during the 90's raised the concern that cholera had established itself in these regions as an endemic disease. Once cholera arrives into a new region, either carried by an infected person or by contaminated water and food, we may expect one of three possible outcomes: no outbreak, an outbreak possibly followed by few waves; or a cholera outbreak followed by subsequent outbreaks that may assume a persistent seasonal pattern. I call these, respectively, cholera-free; epidemic and endemic patterns. What demographic, environmental and sociological factors drive these outcomes? Studies suggest that the number of susceptibles; exposure to untreated water and sewage; and the presence of an aquatic reservoir of V. cholerae are important factors. How important they are and how they interact with each other and with other variables to drive cholera epidemiology, are open questions. Recently, it was found that toxigenic V. cholerae can survive in some aquatic environments for months to years, in association with zooplankton and other aquatic organisms. Under stress, this pathogen assumes a viable but not culturable state, becoming undetectable to traditional bacteriological techniques. These findings suggest that the aquatic environment may be a reservoir of toxigenic V. cholerae in endemic regions. What is the role of the aquatic reservoir in promoting epidemic and endemic cholera? What regions are more likely to maintain endemic cholera? What are the best approaches to prevent and control cholera outbreaks? What are the best predictors of the fate of a community after the introduction of cholera? Here, I start to address these questions by proposing a mathematical model that incorporates what are considered important determinants of cholera dynamics. Despite the great effort towards understanding cholera dynamics, the mechanisms driving V. cholerae dynamics in water is still poorly known. Thus, it is appropriate to start a modeling exercise with a simple model (with few variables) and then add complexity as needed. Following this methodology, I started with a basic Susceptible-Infective-Recovered model coupled to an aquatic

population of V. cholerae. The objective is to explore the role of the aquatic reservoir on the persistence of endemic cholera as well as to define minimum conditions for the development of epidemic and endemic cholera. Mathematical models are powerful tools for the development of epidemiological theories. They can synthesize the current empirical knowledge about the disease into a coherent mechanistic framework. These models may help us to infer causal relationships and to suggest experimental designs to test alternative hypotheses. This work is structured in three parts. First, the mathematical model for epidemic and endemic cholera is presented. Secondly, the proposed model is applied to three hypothetical communities to simulate cholera-free, epidemic and endemic situations. Thirdly, I consider possible causes of endemic oscillations. I finish this work discussing insights brought from model analyses into cholera dynamics. Mathematical Model The model proposed here is an extension of Capasso's model, used to describe the 1973's cholera epidemics in Italy. In Capasso's version, two equations describe the dynamics of infected people in the community and the dynamics of the aquatic population of pathogenic bacteria. In our formulation, the dynamics of the susceptible population is included since I wish to study long term dynamics. The mathematical model is: Symbols are listed in Table 1 and a diagrammatic representation of the model is shown in Figure 1. Equation 1a describes the dynamics of susceptibles in a community of constant size H. Susceptible individuals are renewed at a rate n. Renewal may occur as result of birth, immigration and/or loss of acquired immunity (cholera apparently does not confer life-long immunity). Susceptible people becomes infected at a rate a λ (B), where a is the rate of contact with untreated water and λ (B) is the probability of such person to catch cholera. Probability of catching cholera depends on the concentration of V. cholerae in the consumed water. Experimental studies suggest that it is necessary a heavy inoculum of V. cholerae in order to develop cholera. Here, this dependence is represented by a logistic dose response curve (Figure 2) where K is the concentration of V. cholerae in water that yields 50% chance of catching cholera. I assume that the only route for infection is the ingestion of contaminated water from non-treated sources. Equation 1b describes the dynamics of infected people in the community. This category includes not only cholera cases but also those with asymptomatic and mild infections. In reality, only 1 to 30% of V. cholerae infections actually develops into severe cholera cases. By combining all infection types into a single model compartment, I am assuming that they follow the same dynamics, i.e., that the case-to-infection ratio remains constant through the epidemics. This is reasonable if infection virulence is strongly determined by host factors (sensitivity to cholera toxin, blood type, etc) and bacterial factors (biotype, etc), that are not expected to change during the period of interest. A potential problem with this assumption is that inoculum size may affect the case-to-infection ratio. It is not clear, however, if inoculum size is directly related to virulence or if it just increases the chance of intestine colonization. Here, I assumed that inoculum size affects the per capita infection rate (i.e., the probability of colonization), but not the severity of symptoms. Equation 1b states that the infected population increases as susceptibles become infected (first term in the equation) and decreases as they recover from the disease or die. The third equation describes the dynamics of pathogenic V. cholerae in the aquatic reservoir, in this case, the set of untreated waters consumed by the population. Environmental V. cholerae is found in ponds, wells, rivers, estuaries and coast waters. These environments have quite distinct physical, chemical and biological characteristics and V. cholerae dynamics is probably controlled by different factors in each of them. Classical studies on the survival of culturable V. cholerae in aquatic environments suggest that V. cholerae cannot maintain

a stable population in the environment. Observed rates of population extinction in microcosms are variable, ranging from 0.02 day-1 to >3 day-1. Islam, however, showed that population decay does not necessarily imply death, but also the transition towards a non-culturable state. Using fluorescent antibody techniques, he found V. cholerae surviving for more than 15 months within the mucilagenous sheaths of a filamentous alga (extinction rate <0.002). Here, equation 1c states that bacterial density in the water results from the balance between local birth and death processes and the inflow of contaminated sewage. The parameter e defines the average contribution of each infected person to the aquatic population of V. cholerae. At last, equations (2) specify the initial conditions (all individuals are initially susceptible). Model predictions The model predicts three qualitative outcomes after the introduction of few cholera infectives into a susceptible population: cholera does not spread; epidemic cholera and endemic cholera. Here I describe each dynamics separately and illustrate them using three hypothetical communities, whose parameters are shown in Table 2. First case: cholera-free population Consider a community that does not experience cholera for generations. All individuals are susceptibles. There are neither infective or immune individuals nor toxigenic bacteria in the water. Superscript * indicates equilibrium quantities. The first question of interest is: What will happen to this community if a small number of infectives comes in? Stability analysis of equation system 1 (see additional material: Appendix) indicates that if the number of susceptibles in this population is greater than a critical number SC, an outbreak will occur. Otherwise, the number of cases will decrease and return to zero (Figure 3). This threshold is given by: The Sc threshold increases proportionally to the barriers to cholera infection (K), and recovery (r) as well as the net mortality rate of V. cholerae in the water. It decreases, on the other hand, as contamination of water supplies as well as contact with these waters increase. In other words, the better water quality and sewage treatment are, the greater must be the pool of susceptibles in order to trigger a cholera outbreak. Rearranging equation 5, we find the maximum degree of contamination each infected person may cause to the water reservoir without causing public health hazards: Asymptomatic cases of V. cholerae infection yield 102-105V. cholerae per gram of feces. In severe cases, yield increases to 106-109 cells/ml of rice-water stool. If we know the proportion of infections that are asymptomatic, mild and severe, we could obtain a rough estimation of e as Consider a hypothetical community with 10,000 members sharing the same water source. Suppose that the water reservoir is directly contaminated with sewage. If a = 1, cholera outbreak in this community will develop only if individual contribution to water contamination exceeds ca. 7 cells/ml day-1. Now, consider that each infected person yields 1 × 104 cells person-1 day-1. This water will remain infective unless it is diluted to a value less than 7 cells/ml. Such dilution requires 4 × 104/7 = ca. 5700 liters of water per infected person per day. A real pond, however, is not perfectly mixed as this model assumes. If water used for consumption is taken from a point close to the excretion site, the risk of acquiring the infection will be higher even if the pond is large. Moreover, V. cholerae is found associated to phytoplankton, macrophyte, zooplankton, crustacea and other aquatic organisms. On the surface of these organisms, density of bacteria may be 100 to 1000 times greater than in the aquatic medium. These organisms are not evenly distributed within the water body. Phytoplankton tends to concentrate on the water surface, zooplankton migrates along the water column on a daily basis. If water is taken from phyto- or zooplankton-rich patches, risk of catching cholera will increase, enhancing the probability of triggering an outbreak in the community. This model is too simple to provide quantitative predictions on cholera dynamics. Nevertheless, its

qualitative results verifies known alternative approaches to the prevention of cholera outbreaks: 1. Minimize water contamination as well as consumption of untreated waters: This is the classical approach to cholera control. Good sanitation reduces the parameter e and water treatment reduces parameter a. The smaller these parameters are, the larger must be the susceptible pool in order to a cholera outbreak to develop (equation 5). 2. Dilute cholera diarrhea with large amounts of water to make water uninfective. Second case: Epidemic cholera Now, consider a community whose susceptible pool exceeds the threshold SC. The introduction of infectives into this community will trigger a cholera outbreak. Figure 4 shows the dynamics of infectives, susceptibles and bacteria in community 2 (Table 2) after the introduction of a few infectives. Cholera outbreak follows a traditional epidemiological curve. The initial reproduction rate of the disease is positively affected by the degree of contamination of the water supply (e) as well as the frequency of contact with these waters (a): Together with the outbreak curve, we observe a bacterial bloom in the water. Since growth rate is negative, bacterial population will eventually decline when the susceptible pool decreases below the critical size SC (Figure 4b). From this point on, bacterial extinction rate exceeds human excretion and the environmental V. cholerae population eventually goes extinct. Community 2, then, returns to the cholera-free steady state. Third case: Endemic cholera Consider our hypothetical community 3 (Table 2). Its parameters are identical to those set for community 2, except that n is greater. Since S0 >SC, the introduction of infectives in community 3 starts a cholera outbreak. In this case, however, cholera does not vanish after the first peak. It actually returns in successive waves and eventually converges to a positive endemic equilibrium (Figure 5). Setting the derivatives of equation system 1 to zero and solving it algebraically, we obtain the endemic equilibrium: The equilibrium number of infected individuals in the population is a fraction ϕ of the surplus population (H-SC), where ϕ is Fraction ϕ increases as the susceptible turnover rate n increases and tends to a at large values of n. This fraction is a function of human parameters, only. Although in theory any community with S0 >SC and n > 0 is expected to become endemic, this is not likely to occur in practice. To maintain an endemic state, I* must be much greater than 1, otherwise infection will fade-out due to stochastic processes. Stability analysis (see additional material: Appendix) indicate that the endemic equilibrium is stable to small perturbations if SC >S0 and mb >nb. The route to the endemic equilibrium involves dumped oscillations (Figure 5). Cholera waves arise when the susceptible population grows above the threshold SC, triggering a new outbreak. Eventually, the number of infectives stabilizes into a fixed fraction of the population. Bacterial dynamics in the water is also oscillatory and follows the human excretion pattern. In summary, our model predicts that cholera outbreaks should occur in communities whose susceptible pool is greater than the susceptible threshold (SC). The magnitude of the threshold depends on a combination of environmental, sociological and strain-specific factors. The environmental reservoir reduces the susceptible threshold. In the presence of a permanent reservoir of V. cholerae (nb tending to mb), the threshold tend to zero, and community of any size in contact with the reservoir should be subject to an outbreak. Environmental triggers of cholera outbreaks In endemic regions, cholera outbreaks are often associated to climatic events. In some parts of Africa, outbreaks occur during the dry season or right after heavy rainfalls . In the Americas, disasters caused by the El Niño preceded large cholera outbreaks. In Bangladesh, cholera season coincides with the post-monsoon period. Flooding and drought are likely to affect cholera dynamics in a complex way. Flooding of streets and cities washes contaminated feces and sewage into the rivers. It may also disrupt water distribution service and

aggravate hygiene conditions. Drought, on the other hand, reduces the availability of potable water; aggravates hygiene conditions; increases the number of people sharing the same water supply; and may increase per capita water contamination. All these factors contribute to a greater rate of contact with unsafe waters (parameter a) and increased (floods) or reduced (floods or droughts) dilution of the per capita bacterial yield (parameter e). Besides weather changes, seasonal variations of water temperature has been also associated to cholera outbreaks. Cholera outbreaks in Peru and Bangladesh are preceded by periods of warm waters, during which toxigenic V. cholerae is detected in the environment. Colwell explains this pattern by an association between zooplankton seasonal growth (that generally peaks during the warm season) and V. cholerae dynamics. Floods, droughts, temperature and biotic interactions have potentially quite different impacts on cholera seasonality. To untangle the potential roles of these factors, I conducted a series of simulations of equation system 1 where the following parameters are assumed to vary seasonally: 1) the contact rate with contaminated water (simulating seasonal variations in water quality); 2) the per capita excretion rate (simulating seasonal variations in per capita water contamination); 3) the growth rate of V. cholerae (simulating seasonal variations in autochtonous V. cholerae abundance in the water). In all simulations, a sine function with period equal to 365 days was used to simulate the seasonal oscillations. Scenario 1. Seasonal oscillation of the contact rate (a) Here, I substituted the parameter a in equation 1 by the sin function: The forced equation system was numerically solved (using the software ModelMaker) using parameters for the hypothetical community 3 (Table 2). To test the sensitivity of the obtained results to parameter change, I also carried out a sensitivity analysis where the model was simulated 42 times, keeping all parameters at their default values (Table 2) and changing only one (Table 3). All 42 simulations resulted in periodic dynamics with an annual peak of cholera (Figure 6). In general, peaks tended to occur earlier in larger populations with high water contamination. Low values of K and long bacterial residence time also contributed to the early onset of cholera outbreak. In most simulations (except those with the minimal values of e, H, and n), cholera outbreak was followed by a period of variable length during which infection incidence remained ca. constant. This "plateau" eventually vanished and a period with no human infection was noted until the next annual outbreak. Figure 6 shows the effect of population size on the infection dynamics. The greater the population size (H) or the susceptible turnover rate (n), the shorter is the period without any infection. Scenario 2. Seasonal oscillation of the per capita contamination rate (e) In this scenario, the parameter e in equation system 1 was replaced by the periodic function: Simulations were performed using the same parameter values listed for the first scenario (now a is constant). Seasonal variation of e forces an annual outbreak whose peak occurs from 3 months earlier up to one month later the e(t) peak. The greater the population size and the susceptible turnover rate are, the earlier the outbreak starts. Low a and low e are the main factors delaying the cholera onset. A period with no infections precedes each annual big outbreak (Figure 7). A prevalence "plateau" often follows the annual cholera outbreak. The dynamics is very similar to that observed for the scenario 1. For some parameter combinations, however, this plateau rises to form a second outbreak of minor intensity. Conditions favoring the occurrence of this second peak include high K, high extinction rate and fast recover rate. Scenario 3. Seasonal oscillation of V. cholerae extinction rate (nb-mb) In this last scenario, the term netnb = (nb-mb) in equation system 1 was replaced by the periodic function: The goal is to simulate, in a phenomenological way, the seasonal variation of V. cholerae abundance in the environment due to

autochtonous growth. Seasonal growth of V. cholerae in the environment has been linked to factors as temperature, copepod abundance and chlorophyll. However, the mechanistic relationships between these variables are still not clearly defined and I opted for not including them explicitly in the model. Forty-two simulations were again performed. All simulations showed annual outbreaks of V. cholerae infection 2 to 4 months after the onset of V. cholerae growth in the water. Small populations, and low rate of contact with contaminated water helped to delay cholera outbreak. Differently from the previous two scenarios, the number of infected people never dropped to zero in large populations (Figure 8).

CONCLUSION
This work is an effort towards the formulation of a cholera theory that incorporates a V. cholerae environmental reservoir. The model proposed is very simple and does not include many features of this complex system. Nonetheless, this study brings some new insights into cholera epidemiology. Most studies on cholera epidemiology concentrate on either social or environmental factors. This work, however, shows that the reproduction rate of cholera is a function of social and environmental factors. It is necessary to determine the relative weights of each one of these components in order to develop appropriate control strategies. Further developments on cholera modeling require a better understanding of V. cholerae ecology and epidemiology. We need estimates of the prevalence of V. cholerae infection in endemic populations. We also need better estimates of the required infection dose as well as a better description of the relationship between dose and virulence.