Mitigating the future impact of Cholera Epidemics

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Abstract

Cholera epidemics have been analysed using epidemiological models that depend on human-to-environment-to-human spread of toxigenic *Vibrio cholerae* bacteria (Koelle *et al.* 2004, Koelle *et al.* 2005). Within such a framework the vulnerable human population is the key driving variable, and mitigation of cholera outbreaks lies in both the human-to-environment domain (sanitation) and in the environment-to-human domain (water supply, immunisation). Previous international research on cholera identified a potential environmental (i.e. aquatic) reservoir of the bacteria (Colwell & Huq 1994). CSIR research in central Mozambique demonstrated the presence of cholera bacteria in the rivers at the onset of the rainy season while no cholera cases were reported among the local population. This demonstrates that cholera epidemics are not linked to the mere presence of the bacterium, but also to its virulence. The research then focused on the seasonal recurrence of epidemics in Beira between 1999 and 2005 and tested the possibility that environmental factors such as temperature, rainfall, humidity, sea-surface temperatures and ocean chlorophyll (plankton production) might determine *Vibrio cholerae* virulence. Rainfall emerged as the key driver of both inter-annual and intra-annual variability in the number of reported cholera cases. The Beira results suggest that an endemic reservoir of *Vibrio cholerae* bacteria is amplified by environmental variables independently of the human-to-environment link. Under these circumstances, water supply and not sanitation is the key to mitigating an epidemic. The cholera epidemic that occurred in KwaZulu-Natal, South Africa, between 2000 and 2001 was also analysed using the approach developed in the Beira study. The results demonstrate that the scale of the epidemic in KwaZulu-Natal was also controlled by rainfall, but cholera epidemics in KwaZulu-Natal are not an annual occurrence, despite a fairly regular rainfall regime. Further testing is required to determine if the *Vibrio cholerae* bacteria is endemic in KwaZulu-Natal as it is presumed to be in Beira. If it is not endemic then mitigation must focus on preventing human-to-environment spread and the emphasis must be on sanitation. However, at the onset of any future epidemic the mitigation measures should emphasise the environment-to-human link via water supply and immunisation.

1. Introduction

Vibrio cholerae is a highly contagious waterborne bacterium that causes severe diarrhea epidemics. It occurs in many regions, but epidemics in the developing world have notable adverse impacts on social and economic development. In 2005, 52 countries reported cholera cases of which 95% occurred on the African continent (WHO 2006, WER 81). Here mortality rates ranged between 0% and more than 5% of the number of infections reported. In South Africa the KwaZulu-Natal Province is the most affected region, registering 92% of the national reported cases and deaths in the 2000/2001 epidemic. In addition to the cost of mitigation measures such as the provision of purified water, improved sanitation and vaccinations, the debilitating effects of the disease have an economic impact on productivity through absence of sick patients (the recovery period is up to 3 weeks). In a study done before the 2000/2001 epidemic the annual cost of diarrhea related illness in South Africa (of which cholera is only a part) was estimated at R 3.375 billion or about 1% of GDP (Pegram *et al.* 1998).

Attempts to mitigate the impacts require an understanding of *V. cholerae* ecology. Despite much research (Tamplin *et al*, 1990; Colwell and Huq, 1994; Islam *et al*, 1994a; Colwell, 1996; Jiang, 2000) more research is needed to fully understand the ecology and the environmental drivers of the bacterium in the natural context. Two approaches have been developed to explain the timing and magnitude of epidemics: The first is an epidemiological approach in which person-to-environment-to-person infections are modeled, and

the second is an environmental approach in which a change in the environment triggers the amplification of the bacteria virulence or concentration. The different approaches are not necessarily exclusive of one another, but in different situations one system will dominate over the other. Since the different models advocate different mitigation measures, it is important from the perspective of governance that the appropriate intervention be identified.

An epidemiological model proposed by Koelle *et al.* (2004) attributes the year-to-year variability in cholera cases in the Matlab region of Bangladesh to immune responses of the population to different *V. cholerae* biotypes. In epidemiological approaches the ability for *V. cholerae* to reproduce is captured in the term R_o. This term has a number of loose definitions, but here we use the stricter definition, that is the number of new infections per existing infection. Implicit in this is a rate for explosive nature of an epidemic. Low values for R_o imply that one infected individual will infect a small number of additional individuals in the vulnerable population within the viable lifetime of the bacteria. Accordingly higher values imply more explosive epidemics. The value of R_o is typically treated as a constant which means that epidemiological models are poor at resolving the variability of case data at sub-annual frequencies. This is exacerbated when case census data is presented in annual or monthly time-series despite the fact that epidemiological models typically include terms that describe high frequency variables: the rate of recovery from infection (5 days), the death rate of vibrios in the environment (30 days) and the possible role of hyperinfectious state (5 hours) (figures taken from Hartley *et al.* 2006).

The second approach to understanding *V. cholerae* ecology rests on the fact that cholera epidemics are an annual occurrence in certain areas. Numerous studies have investigated the seasonal-, inter- and intra-annual cholera case data and the potential link with climate variability. Environmental variables such as sea surface temperature (SST), sea surface height, rainfall, humidity and air temperature (Colwell 1996, Jiang *et al.* 2000, Rodó *et al.* 2002, Huq *et al.* 2005, Jensen *et al.* 2006) could explain the seasonal behaviour of the disease. Complexity is added when these variables influence pH, salinity, availability of nutrients and plankton (zoo and phytoplankton) community structures, all of which are ecologically linked to the bacteria (Huq *et al*. 1983, Islam *et al*. 1994, Colwell 1996, Colwell 2004). In most of these models oceanic proxies are strongly correlated with cholera cases suggesting that a marine-based reservoir is responsible for large-scale epidemics (Colwell 1996, Lobitz *et al.* 2000, Codeço 2001). Models linking environmental drivers with cholera cases have been developed for Bangladesh (Lobitz *et al.* 2000, Longini *et al.* 2002, Rodó *et al.* 2002, Koelle & Pascual 2004, Huq *et al.* 2005, Koelle *et al.* 2005, Jensen *et al.* 2006), Indonesia (Simanjuntak *et al.* 2001), the America's (Salazar-Lindo *et al.* 1997, Pascual *et al.* 2000, Codeço 2001, Jiang & Fu 2001, Gil *et al.* 2004) and Ghana (De Magny *et al.* 2006).

Where the inter-annual variability in the magnitude of cholera epidemics is considered, global variables such as ENSO (El Nińo/Southern Oscillation) have been invoked to provide early warning systems for epidemics (Lipp *et al*. 2002; Rodo *et al.* 2002; Cazelles *et al.* 2005, Pascual and Dobson 2005). However, standard parametric and linear techniques often find weak associations between environmental variables and cholera amplification (Pascual and Dobson, 2005).

This research explores the extent to which environmental factors influence cholera epidemics in southern Africa. The main shortcoming in previous applications of the approach is the inherent correlation when comparing any annual cycle and annual outbreaks of cholera. We call this "the seasonal correlation problem" and it can be illustrated by an absurd comparison between cholera cases and annual electricity consumption cycle that increases in winter and decreases in summer. Both signals are dominated by a cyclical signal but any correlation between the two does not mean that there is a causal link. This study presents results from a new approach that overcomes this problem. The distinction is in the emphasis that is placed on apparent spikes and troughs that seem to occur in the cholera case data during the course of an epidemic. This sub-epidemic variability has a frequency of 2-6 weeks, and in traditional epidemiological approaches has been treated as "noise" in the case-data. Indeed the concept underlying the term R_o does not allow infection rates to increase and decrease in order to produce the spikes and troughs in the case-data. Our approach assumes that the high frequency variability represents the real dynamics of cholera epidemics. This research demonstrates that the

"noise" is strongly correlated with environmental drivers and that cholera amplification in KwaZulu-Natal, South Africa, and Beira, Mozambique is independent of population dynamics.

2. Data and methods

Three sources of cholera case data exist for the city of Beira, Mozambique: Cholera Treatment Centre – CTC, Direcção Provincial de Saúde – DPS, and Saúde da Cidade. We use the Saúde da Cidade data because of the rigorous definition of cholera cases (Williams *et al.* 2005). Cholera case data are presented as the total number of cholera cases recorded in each week (referred to as epidemiological weeks) between 1999 and 2005. The data appears to indicate a lack of cholera cases in 2000/2001, a period of major epidemics in South Africa, and it is unclear if data for this year is accurate. Although this short time-series data set does not allow for a reasonable assessment of long-term drivers such as population immunity, it does indicate variability with periods as low as 3-4 weeks.

A number of potential environmental drivers from the area were considered. Air temperature, precipitation and humidity records were obtained from the Beira airport (Beira, meteorological office), and an inland precipitation record was obtained for the town of Chimoio (Mozambique meteorological office, Maputo). Remote sensing provided sea surface temperature (SST) and chlorophyll-*a* concentration for a large part of the Mozambique Channel. These data were derived from 8-day composite SeaWiFS images and interpolated onto the same time-scale as the cholera case data.

In order to determine the environmental variables that best correlated with the cholera cases a wavelet approach was adopted (Hudgins *et al.* 2003). This excludes the "seasonal correlation problem" because the seasonal (1-year frequency) component of each dataset is analysed separately from the sub-annual variability. In order to use the wavelet approach, all data were rendered on comparable scales and were normalised by subtraction of the mean and division by the standard deviation. The analysis accommodates lag-times (Melice & Servain 2003) and is based on the optimisation of the variance between the cholera dataset and the environmental drivers in the >1 cycle per year frequency domain. The result

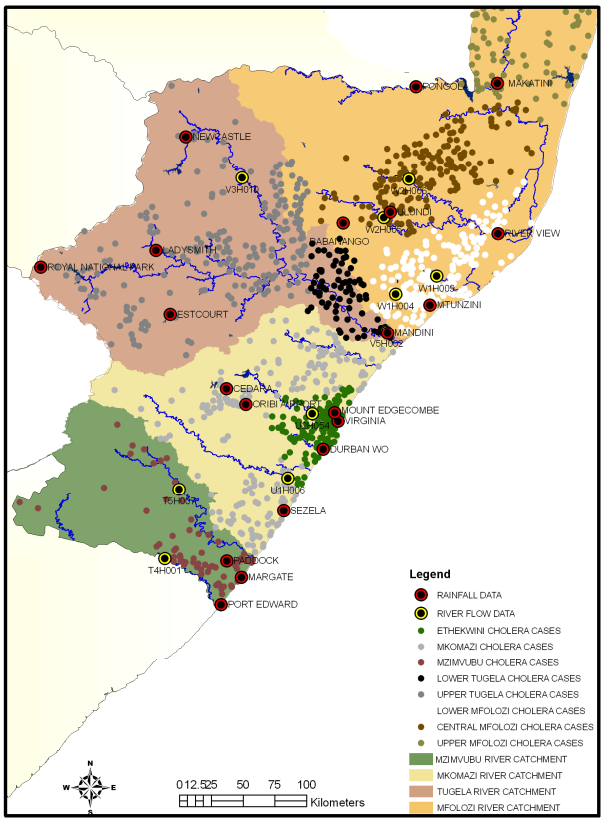

Figure 1. Cholera case-data fro KwaZulu-Natal was dominated by the 2000/2001 epidemic, and in the absence of multi-year datasets each catchment was analysed separately using the "Pienaar Model" (van den Bergh *et al.* 2008). (Source Pienaar *et al.* 2008)

is a "best fit" between the cholera case data and the environmental variables that may drive the epidemics. The method has been employed to understand cholera epidemics (van den Bergh *et al.* 2008) and we now use the term the *Pienaar Model* to refer to the result.

Cholera case-data for KwaZulu-Natal obtained from Dr. Hemson of the Human Sciences Research Council covers the period 2000/2001 and 2001/2002 and is based on the Department of Health KwaZulu-Natal Database. Since only one epidemic is represented in the case-data the modeling approach that was adopted for KwaZulu-Natal differs slightly from the Beira methodology. Instead of a multi-year case-data comparison with environmental variability from a single location, the case-data for KwaZulu-Natal was separated into

spatially distinct regions, and the climate-cholera model was tested in each area separately.

The Hemson database was geo-referenced by cross-referencing the place names at which cholera cases were reported. This was done using the 1:50 000 scale maps of South Africa (www.madmappers.com). Of the 1 474 place names, approximately 1 115 could be identified without ambiguity accounting for 111 897 of the total number of 148 600 cases of cholera. The geo-referenced case data was plotted with a GIS watershed overlay to provide a first approximation of the spatial analytical units. After considering patterns in rainfall, temperature and the case data, some of the catchments were further subdivided. Altogether eight spatial units were determined. The resulting spatial analysis units are (see figure 1):

1. eThekweni which is a subset of the Mkomazi catchment
2. The Mzimvubu River catchment
3. The upper Tugela River catchment
4. The lower Tugela River catchment
5. The upper Mfolozi River catchment
6. The central Mfolozi catchment
7. The lower Mfolozi catchment

The spatial and temporal scale of analysis is very important to uncover seasonal drivers of infectious diseases. The study done by Sultan *et al.* (2005) showed that the climate-disease link only becomes apparent at large spatial scales as the correlations between seasonal variations and climate variables are weak at small scales. This is the result of a combination of other factors such as demographics and acquired immunity that play a local role and act as “noise”.

3. Cholera in Beira, Mozambique

Water samples inland from Beira along the Pungwe River, ponds and in wells were monitored for the presence of *V. cholerae* bacteria at the start (October 2006) and towards the end (January 2007) of the rainy season. This was a period when no significant cholera outbreaks were reported. Sediment samples from the Pungwe River near Chitengo tested positive on both occasions that they were sampled, and a single sample taken from the Chikamba Dam tested positive. Although many other samples tested negative in this period the positive tests indicate that individuals in the population are being exposed to *V. cholerae*, but this did not necessarily lead to epidemics.

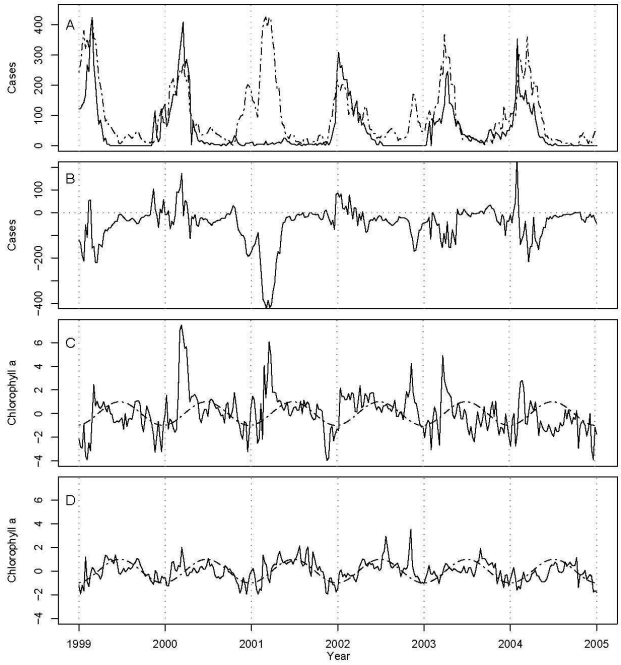

Figure 2. Modeled cholera case-data for Beira (A stippled line) reasonably approximates the actual case data (A solid line). The residuals between the modeled and observed cases (B) show that most of the variability in cholera cases is well represented by the model. The Chlorophyll-a variation off the mouth of the Pungwe River (C solid line) has peaks that correspond to high cholera case incidence, but has the same fundamental seasonal signal (C & D stippled line) as the offshore chlorophyll-a variability (C solid line).

The monitoring data, although sparse, make a very important point. Under conditions of continuous low-level exposure it might be anticipated that epidemics should break out at random times, and yet they only occur at specific times of the year (see figure 2A solid line). The monitoring data shows that *V. cholerae* may be present but it does not necessarily lead to a cholera outbreak. Conversely there must be a season-specific trigger that initiates the annual epidemics. This strongly suggests that the amplification of the environmental reservoir of cholera is driven by environmental factors. The link to oceanic chlorophyll-a (figure 2 D) is not as convincing as in other parts of the world except for the chlorophyll-a in the Pungwe River mouth (figure 2C) and a link to runoff is suggested. The *Pienaar Model* suggests that the accumulative effect of rainfall, which is also

intuitively linked to runoff, is the environmental variable that best correlates with cholera cases. According to the *Pienaar Model* the number of cholera cases reported for Beira can be approximated using the equation:

$$Cases = 0.32 \int_{-8}^{-1} RB.\delta t + 0.57 \int_{-4}^{-1} RM.\delta t - 4.17$$

Where RB and RM are the rainfall in Beira and Chimoio respectively, and the integration period is in weeks (figure 2A stippled line).

In the same way that Koelle *et al.* (2005) use the residual between their epidemiological model and the observed case data to factor out the effect of environmental drivers of cholera, we consider the residuals between the *Pienaar Model* and the observed case data to indicate the intrinsic drivers of the system. The amplitude of variability in the residual (figure 2B) is substantially less than that of the model, suggesting that the dominant factor in driving cholera epidemics in the Beira area is environmental amplification.

4. Cholera in KwaZulu-Natal

A recent analysis of the 2000/2001 cholera epidemic in KwaZulu-Natal (Mendelsohn & Dawson 2008) follows a very similar approach to that adopted in the Beira study in that cholera case-data is compared with almost exactly the same range of environmental variables. The conclusion is also that rainfall is the dominant environmental variable that drives cholera in the province. Although this is reassuring, the study suffers from many of the problems associated with such environmental comparisons, and in particular the dataset comprises a single epidemic resolved monthly. This will almost certainly present the "seasonal correlation problem". By using the wavelet approach employed in the *Pienaar Model* for Beira it is possible to determine if accumulative rainfall is an accurate predictor of the cholera epidemic.

The results of the Pienaar Model used to predict the KwaZulu-Natal epidemic of 2000/2001 are presented in figure 3 for the various catchments (blue lines represent the case-data and the red line is the model). The timing and magnitude of the peaks and troughs in the case-data throughout the epidemic appear to be accurately reflected in the

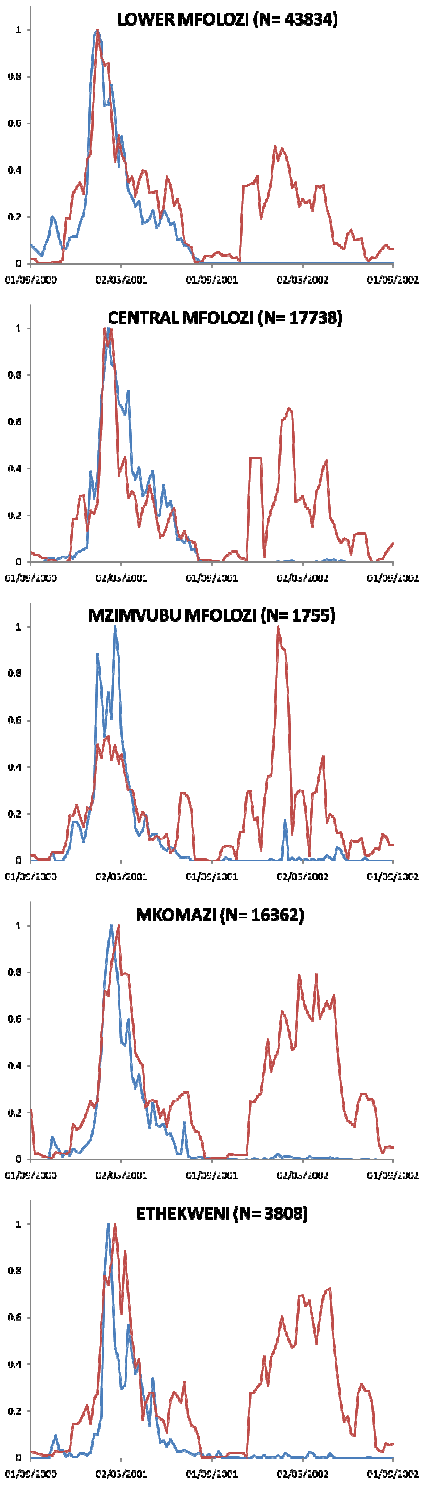

Figure 3. Comparison between cholera case-data (blue) and modelled cholera cases using an accumulative rainfall model (red) for selected catchments in KwaZulu-Natal

model. An important distinction between this result and that obtained for Beira is that different rainfall accumulation and lag times are required to generate the “best fit” model. Whereas the Beira model was optimised on an eight-week accumulation of rainfall, the KwaZulu-Natal models use a rainfall accumulation of between 4 and 5 weeks, and a lag-time of 7 to 9 weeks. Because the KwaZulu-Natal cholera cases have a different model from the Beira model it is likely that the physical relationship between accumulated rainfall and cholera cases is catchment-specific. It is not yet clear what the cause of this relationship is, but gradient or river flow rates are likely to link with water supply (rainfall but also dam management) to influence the cholera distribution. Accordingly the model was independently formulated for each catchment and slightly different results were obtained in each (Pienaar *et al.* 2008).

An important observation about the modeled cholera cases for KwaZulu-Natal is the fact that the *Pienaar Model,* using slight variations in the accumulation and lag parameters from that used in Beira, provides an accurate reflection of the epidemic year only. The case-data for the following year, 2001/2002, does not match the model at all except that the very subdued peaks in the case-data occur at the same time that peaks were forecast by the model. This has been attributed to endemism (Pienaar *et al.* 2008). In Mozambique the *V. cholerae* bacteria are presumed to be endemic, and hence they are present year-round in the environment. It is only when environmental conditions favour the amplification of the reservoir that cholera epidemics will occur. In contrast the scenario in KwaZulu-Natal is presumed to be based on a lack of endemism. This means that *V. cholerae* bacteria are not present year-round in the environment, and epidemics will only occur when the bacteria is introduced from an outside source. This scenario is therefore a hybrid between the epidemiological, human-to-environment-to-human rationalisation, and the environmental, environment-to-human rationalisation. The onset of the cholera epidemic is brought about when the bacterium is introduced into the aquatic system, presumably by humans. If this occurs during favorable environmental conditions the bacteria will flourish and the ensuing epidemic will follow an environmentally controlled trajectory.

5. Discussion and conclusions

The relationship between cholera cases and accumulative rainfall in Central Mozambique and in KwaZulu-Natal is interesting and it leads to a number of questions. The initial debate around the environmental vs. human role in epidemics is important because it directs policy makers in the direction of appropriate mitigation. The issue is partially addressed under the assumption of endemism. In Beira, cholera cases seem to correlate well with environmental drivers, namely rainfall, whereas in KwaZulu-Natal this is only true during an epidemic. The same rainfall in previous and subsequent years did not necessarily lead to epidemics. This is assumed to imply that *V. cholerae* is not endemic in KwaZulu-Natal. It is clear that *V. cholerae* is contagious in that it has spread through the world in a very systematic way (Todar 2005), and it is likely that repeated epidemics will ultimately lead to endemism. It is important that the lack of endemism in South Africa is established through background sample testing, because it is not possible to eradicate the bacteria after it has naturalised. Where it has naturalized, mitigation must emphasise water supply instead of sanitation.

A further implication of the correlation between cholera case-data and accumulative rainfall is the re-affirmation of the poor knowledge we have of *V. cholerae* ecology. In order to translate the inviting correlation into a strong argument for causation, it will be necessary to link the physical changes associated with accumulated rainfall to the biology of the bacteria. The extent of inundation, in particular, will vary over periods as short as the duration of a storm, and the hydrological lead time on runoff will vary from days to weeks depending on the size and gradient of the catchment. Since the initial onset of a cholera epidemic is through environmental amplification, it seems likely that this process continues even after the epidemic has started and humans are clearly contributing to the environmental reservoir through poor sanitary conditions. The *Pienaar Model* is completely independent of terms such as the number of infections or the size of the vulnerable population, suggesting that environmental amplification is the primary driver even when human re-infection is at its extreme.

The precise causal mechanism for bacterial amplification is not yet clear as increased runoff has an effect on nutrients, salinity and turbidity in both the rivers and the sea while inundation has an effect on the use of the landscape for transport, subsistence and domestic water, which introduces the possibility of social adaptive mechanisms also playing a role. Future research must focus on determining the causal mechanism behind this observation.

South Africa has been fortunate that cholera epidemics such as that of 2000/2001 have not recurred in recent years, but there will be another in the future. If the current assumption of a lack of endemism in South Africa stands up to microbiological testing, then the mitigation of future epidemics lies in the prevention of human-to-environment transmission. The emphasis has to be on sanitation. At the onset of an epidemic any advantage gained in the investment in sanitation becomes irrelevant and the environmental feedback will amplify the epidemic. At this point the mitigation strategy changes to one focused on the supply of clean water and breaking the environment-to-human exposure pathway.

The link between rainfall and cholera also provides a useful set of mitigation tools. The first is based on the lead-time between rainfall and cholera epidemics. In Beira this is of use because it is almost certain that an epidemic will occur each year. In KwaZulu-Natal this is not quite as useful because the criteria for starting an epidemic are not environmentally controlled (at least not by the variable assessed in this and other studies), but at the first sign of an epidemic its magnitude can be forecast for the purposes of resource deployment.

A second area in which the *Pienaar Model* is of use is in mitigating the impact of climate change. Global climate models (GCMs) appear to be relatively consistent that climate change scenarios for eastern and south-eastern Africa will bring about increases in precipitation (Hewitson *et al.* 2005). This implies that epidemics will become bigger in the future, and probably more frequent. There is a danger that *V. cholerae* will naturalise and become endemic in some areas, and epidemics will become an annual event as they are in Mozambique. While investment in water supply and sanitation is a social imperative in South Africa, it is also mitigation against the future impact of waterborne diseases such as cholera. The eThekwini strategic response to climate change includes consideration of infectious diseases, and cholera was selected as the case study (Pienaar *et al.* 2008). The current cost to the country of infrastructural investment in water supply and sanitation can be offset against the cost of treating cholera victims, particularly in the light of the high probability that cholera epidemics will increase in magnitude and frequency in the future.

6. References

CAZELLES, B., CHAVEZ, M., MCMICHAEL, A.J. & HALES, S. 2005. Nonstationary influence of El Nino on the synchronous dengue epidemics in Thailand. *PLoS Medicine*. 2: e106.

CODECO, C.T. 2001. Endemic and epidemic dynamics of cholera: The role of the aquatic reservoir. *BMC Infectious Diseases*. **1**.

COLWELL R.R. & HUQ A. 1994. Environmental reservoir of *Vibrio cholerae*, the causative agent of cholera. *Annals of the New York Academy Society* 740**:** 44–54.

COLWELL, R.R. 1996. Global climate and infectious disease: the cholera paradigm. *Science* 274: 2025–2031.

COLWELL, R.R. 2004. Infectious disease and environment: cholera as a paradigm for waterborne disease. *International Microbiology*. 7: 285 – 289.

DE MAGNY, G.C., CAZELLES, B. & GUEGAN, J.-F. 2006. Cholera Threat to Humans in Ghana Is Influenced by Both Global and Regional Climatic Variability. *Ecohelath* 3: 223-231.

GIL, A.I., LOUIS, V.R., RIVERA, I.N.G., LIPP, E., HUQ, A., LANATA, C.F., TAYLOR, D.N., RUSSEK-COHEN, E., CHOOPUN, N., SACK, R.B. & COLWELL, R.T. 2004. Occurrence and distribution of Vibrio cholerae in the coastal environment of Peru. *Environmental Microbiology* 6: 699-706.

HARTLEY, D.M., MORRIS, J.G. Jr., SMITH, D.L. 2006 Hyperinfectivity: A critical element in the ability of *V. cholerae* to cause epidemics? *PLoS Med* 3(1): e7.

HEWITSON, B., TADROSS, M. & JACK, C. 2005. Potential Impacts and Vulnerabilities of Climate

Change on Hydrological Responses in Southern Africa. (ed. R.E. SCHULZE) Chapter 3, 3-1 to 3-5 (WRC Report 1430/1/05, Pretoria).

HUDGINS, L., FRIEJE, C.A. & MAYER, M.E. 1993. Wavelet transform and atmospheric turbulence. *Physical Review letters* 71: 3279-3282.

HUQ, A., SMALL E.B., WEST P.A., HUQ, M.I., RAHMAN, R. & COLWELL, RR. 1983. Ecological relationships between *Vibrio cholerae* and planktonic crustacean copepods. *Applied Environmental Microbiology* 45: 275–83.

HUQ, A., SACK. R.B., NIZAM, A., LONGINI, I.M., NAIR, G.B., ALI, A., MORRIS, J.G., KHAN, M.N.H., ALBERT, M.J., SACK, D.A & COLWELL, R.R. 2005. Critical Factors Influencing the Occurrence of Vibrio cholerae in the Environment of Bangladesh. *Applied and Environmental Microbiology.* 71: 4645-4654.

ISLAM MS, DRASAR S, SACK RB. 1994a. The aquatic environment as reservoir of *Vibrio cholerae*: a review. *Journal Diarrhoeal Disease Research* 11: 197-206.

ISLAM, M.S., MIAH, M.A., HASAN, M.K., SACK R.B. & ALBERT, M.J. 1994. Detection of non-culturable *Vibrio cholerae* O1 associated with a cyanobacterium from an aquatic environment in Bangladesh. Transac R. Soc. *Trop. Med Hyg*. 88: 298-299

JENSEN, M.A., FARUQUE, S.M., MELKALANOS, J.J. & LEVIN, B.R. 2006. Modeling the role of bacteriophage in the control of cholera outbreaks. *Proceedings of the National Academy of Sciences of the United States of America (PNAS).* 103: 4652-4657.

JIANG, S.C., MATTE, M., MATTE, G., HUQ, A. & COLWELL, R.R. 2000. Genetic Diversity of Clinical and Environmental Isolates of Vibrio cholerae Determined by Amplified Fragment Length Polymorphism Fingerprinting. *Applied and Environmental Microbiology.* 66: 148-153.

JIANG, S.C. & FU, W. 2001. Seasonal Abundance and Distribution of Vibrio cholerae in Coastal Waters Quantified by a 16S-23S Intergenic Spacer Probe. *Microbial Ecology.* 42: 540-548.

JIANG SC. 2000. *Vibrio cholerae* in coastal waters of southern California: abundance, distribution and relationship to environmental conditions. *Hydrobiologia* 460: 157-164.

KOELLE, K, RODO, X, PASCUAL, M, YUNUS, M & MOSTAFA, G. 2005. Refractory periods and climate forcing in cholera dynamics. *Nature* 436(4): 696-700 doi:10.1038/nature03820

KOELLE, K. & PASCUAL, M. 2004. Disentangling Extrinsic from Intrinsic Factors in Disease Dynamics: A Nonlinear Time Series Approach with an Application to Cholera. *The American Naturalist.* 163(6): 910-913.

LIPP, E.K., HUQ, A. & COLWELL, R.R. 2002. Effects of Global Climate on Infectious Disease: The cholera model. *Clinical Microbiology Reviews.* 15: 757-770.

LOBITZ, B., BECK, L., HUQ, A., WOOD, B., FUCHS, G., FARUQUE, A.S.G. & COLWELL, R.R. 2000. Climate and infectious disease: use of remote sensing for detection of Vibrio cholerae by indirect measurement. *Proceedings of the National Academy of Sciences of the United States of America (PNAS).* 97: 1438–1443.

LONGINI, I.M., YUNUS, M., ZAMAN, K., SIDDIQUE, A.K., SACK, R.B. & NIZAM, A. 2002. Epidemic and Endemic Cholera Trends over a 33-Year Period in Bangladesh. The *Journal of Infectious Diseases.* 186: 246-251.

MELICE, J-L. & SERVAIN, J. 2003. The tropical Atlantic meridional SST gradient index and its relationships with SOI, NAO and Southern Ocean. *Clim. Dyn.* 20 : 447-464

MENDELSOHN, J & DAWSON, T. 2008. Climate and cholera in KwaZulu-Natal, South Africa: The role of environmental factors and implications for epidemic preparedness. *International Journal of Hygiene and Environmental Health* 211:156-162

PASCUAL, M. & DOBSON, A. 2005. Seasonal Patterns of Infectious Diseases. *PLoS Medicine,* 2: 0018-0020.

PASCUAL, M., BOUMA, M.J. & DOBSON, A.P. 2002. Cholera and climate: revisiting the

quantitative evidence. *Microbes and Infection*. 4: 237–245.

PEGRAM, G.C., ROLLINS, N. & ESPEY, Q. 1998. "Estimating the costs of diarrhoea and epidemic dysentery in Kwazulu-Natal and South Africa." *Water SA* 24(1**):** 11-20.

PIENAAR, M, WOODBORNE, S & VAN DER MERWE, M. 2008. Global change scenarios for the occurrence of cholera epidemics in eThikweni. CSIR client report 161647.

RODO, X., PASCUAL, M., FUCHS, G. & FARUQUE, A.S.G. 2002. ENSO and cholera: A nonstationary link related to climate change? *Proceedings of the National Academy of Sciences of the United States of America (PNAS).* 99**:** 12901-12906.

SALAZAR-LINDO, E., PINELL-SALLES, P., MARUY, A. & CHEA-W00, E. 1997. El Nino and diarrhoea and dehydration in Lima, Peru. *The Lancet.* 350**:**1597-1598.

SIMANJUNTAK, C.H., LARASATI, W., ARJOSO, S., PUTRI, M., LESMANA, M., OYOFO, B.A., SUKRI, N., NURDIN, D., KUSUMANINGRUM, R.P., PUNJABI, N.H., SUBEKI, D., DJELANTIK, S., SUKARMA, SRIWATI, MUZAHAR, LUBIS, A., SIRENGAR, H., MAS'UD, B., ABDI, M., SUMARDIATI, A., WIBISANA, S., HENDARWANTO, SETIAWAN, B., SANTOSO, W., PUTRA, E., SARUMPAET, S., MA'ANI, H., LEBRON, C., SOEPARMANTO, S.A., CAMPBELL, J.R. & CORWIN, A.L. 2001. Cholera in Indonesia in 1993–1999. *American Journal of Tropical Medicine and Hygiene* 65**:** 788–797.

SULTAN, B., KARIMA LABADI, K., JEAN-FRANCOIS GUEGAN, J-F & JANICOT, S. 2005. Climate Drives the Meningitis Epidemics Onset in West Africa. *PLoS Medicine,* 2**:** 0043-0049.

TAMPLIN, M.L., GAUZENS, A.L., HUQ,A., SACK, D.A. & COLWELL, R.R. 1990. COLWELL. Attachment of *Vibrio cholerae* Serogroup 01 to Zooplankton and Phytoplankton of Bangladesh Waters. *Applied and Environmental Microbiology* 56**:** 1977-1980

TODAR, K. 2005. *Vibrio cholerae* and Asiatic Cholera In: Todar's Online textbook of bacteriology. http://www.textbookofbacteriology.net (Accessed 29 August, 2008)

VAN DEN BERGH, F., HOLLOWAY, J., PIENAAR, M., KOEN, R., ELPHINSTONE, C. & WOODBORNE, S. 2008. A Comparison of various Modelling Approaches applied to Cholera Case Data. *ORiON* 24**:** 17-36.

WILLIAMS, L., CHAEM IDR TEAM & COLLINS, A. 2005. The history of cholera data at Beira. Draft report submitted as part of the Infectious Disease Risk Management (IDRM) Programme in Mozambique and Bangladesh

WORLD HEALTH ORGANIZATION. 2006. Weekly epidemiological record. 81**:** 297-308.

Endnote:

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MP-managed the data and modeling
SW & MP– wrote the text
MVDM – completed the editing and referencing

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