Phage in the Time of Cholera

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Bacteriophage (bacterial viruses) were heralded as revolutionary therapeutic agents soon after the discovery by Félix d'Herelle in 1917 of an "invisible microbe" capable of lysing bacteria[1]. Bacteriophage appeared to be efficient killers of their bacterial hosts – we now know that their life history is far more complex than first assumed[2] – and so the effort to use phage as curatives or prophylaxis spread quickly to research institutes in Europe, North America, and Asia[3]. d'Herelle himself spearheaded many of these efforts, the most famous of which was the initiation of an extensive campaign to use phage in the treatment and prevention of cholera in colonial India. The authors of one such study conclude by noting that "the results establish sufficient probability in favour of a significant effect of the administration of bacteriophage to form a basis of practical policy in the treatment and prevention of cholera in villages"[4]. The early hopes never fulfilled expectations, for both clinical and political reasons[3], and the eventual development of broad spectrum antibiotics provided a more reliable, effective means of controlling bacterial infections. The rise of antibiotic resistance has, in turn, revived interest in bacteriophage therapy despite concerns and uncertainties as to its effectiveness[5]. We consider here an alternative approach to modern bacteriophage therapy, by revisiting the idea of inoculating bacteriophage directly into the environment.

Most tests, theories, and proposals to implement bacteriophage therapy regard the human body as the potential site for intervention[6,7]. But for many bacterial diseases affecting human health, the pool of infecting bacteria comes from water, soils, food, and other host organisms; some of these potential sources of infection do not possess a complex immune system capable of selectively eliminating foreign agents. In contrast to agricultural settings where environmental application of phage as biocontrol is already being considered[8], we believe there exists an as yet overlooked opportunity to reduce the severity, extent, and persistence of some bacterial epidemics by developing ecological-based cures for human disease.

A suitable target disease is cholera. Recent studies have demonstrated a significant correlation between the increase in density of cholera-specific phage and the decrease in density of *Vibrio cholerae* (in both water sources and fecal matter from infected patients)[9,10]. The reasons are apparently simple: presence of *V. cholerae* provides an opportunity for the spread and increase of phage which leads to decreasing host density, which in turns leads to the washout/death of phage. A comprehensive description of cholera disease dynamics involves many factors including environmental seasonality[11], long-distance dispersal mediated by alternative hosts[12], as well as life-history modalities that enable *V. cholerae* to respond to stressful conditions[13]. Without diminishing the importance of these and other factors, in the case of cholera it is apparent that phage and bacteria go through alternating boom-and-bust cycles. What are the practical steps of intervention so as to minimize the likelihood of devastating epidemic booms of *V. cholerae*?

Briefly, the peak of phage lags behind the peak of bacteria. Growing up O1 and/or O139 serogroup-specific phage in the lab, therapy by the flask as it were, and then adding phage to at-risk water sources may augment the ability of phage to keep pace with the dynamics of its host and suppress the spread of an epidemic. In a sense, we are suggesting altering the "natural course"[10] of host-phage population dynamics with an artificial injection of phage. The utility and effectiveness of any such ecological inoculation depend on careful balancing of environmental connectivity of infected areas, risks to human populations, as well as the life-history and parameterization of the biocontrol agent themselves. Ultimately, limiting and/or eliminating an undesirable bacterial population constitutes a problem in coevolutionary biological control. Recent theoretical work on coevolutionary dynamics of bacteria and bacteriophage in simple aquatic environments demonstrates that coevolution-induced outcomes, e.g. eradication of phage and host, sequential strain replacement, or host-phage diversification, depend on characterizing (and possibly manipulating) rates of mutagenesis, host growth rate and strain-specific adsorption rates, and host-range characteristics of mutants[14]. However, the ecology of natural environments is far more complex. Likely sites for intervention include sources of drinking water, wells, and sewage systems so as to minimize the flow of bacterial agents into water used for drinking and bathing. Assessments of the lifetime of phage in local habitats would be necessary as conditions (*e.g.*, temperature, salinity, pH) change over the course of intervention. In addition, the ecohydrology of the affected region may be important, as intervention strategies will depend on whether disease outbreaks are localized to isolated sites, linked to seasonal flooding, or occur along riverine corridors.

These concerns notwithstanding, cholera-specific phage are already found in natural environments and there exists strong evidence to suggest that their presence leads to the decline of cholera epidemics[10]. The risks associated with ecological bacteriophage therapy should be mitigated by the use of virulent, in contrast to temperate, strains of phage. In this regard, the previously identified lytic phages JSF1 and/or JSF5 specific to *V. cholerae* serogroup O1 seem ideal candidates for initial studies[10]. If the origins of seasonal cholera epidemics are harbored within environmental pools, then efforts should be made to seek out the most effective means of adding bacteriophage to eliminate the incubation and growth of *V. cholerae* populations when they are at their most vulnerable. Diminishing the density of *V. cholerae* would also be important to impeding the spread of disease, since the infectious dose is generally considered to be on the order of 10^4 bacterial cells. Thus far, the spread of cholera has been mitigated by improvements in water quality, low-cost preventative measures in at-risk regions, *e.g.,* filtering water through sari cloth[15], as well as by improvements in post-infection treatment, *e.g.*, single-dose antibiotic therapy[16], though the global cholera pandemic has not abated. Bacteriophage could become an additional tool in the public health struggle against cholera. The initiation of controlled experiments that incorporate recent advances in the genetics and evolutionary ecology of phage may offer hope that d'Herelle's early mission to eradicate cholera in the Indian subcontinent need not have been in vain.

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