The Effects of the Immune System on Bacteriophage Therapy of Cholera

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Abstract

Cholera is an infection of the small intestine which results in diarrhea and vomiting, resulting in excessive water loss. Individuals who consume water contaminated with virulent strains of the *Vibrio cholera* bacterium become infected with this disease. Countries such as Bangladesh and Dhaka have frequent cholera outbreaks and in order to limit the severity of cholera outbreaks, phage treatment could be considered. In order to understand the immune response to the bacteriophage a mathematical model that combined the dynamics and interactions of the bacteria, bacteriophage and the immune system was analyzed.

Of the three equilibria found and analyzed, only one that included all populations thriving was a stable equilibrium. However this stable endemic equilibrium was numerically intractable and had to be sought after with specific parameter values in *Mathematica* or direct simulation results from *Matlab*. While a direct correlation between the production rate of the immune system components and the bacteriophage population was observed, further analysis is required in order to make stronger supported claims in lieu of the several simplifications made for the sake of answering the research question at this level.

Introduction

The availability of clean water in many underdeveloped nations has been the central issue around numerous political, economic, and sociological campaigns. Cholera infections are prevalent in communities where the consumptions of contaminated water is commonplace. Cholera stems from the infection of *V. cholera*, Gram-negative bacteria, in the small intestine. Victims of the infections suffer from severe dehydration and electrolyte loss via stool, causing deaths in regions where adequate resources such as antibiotics or rehydration solutions are not available for treatment. While the immune system is designed to try to combat against the Cholera infection, traditionally susceptible victims such as children and elderly may have weaker immune systems due to malnutrition, stress, and natural causes. These factors cause the victim to lack the physiological tools needed to effectively combat the Cholera infection.

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The introduction of a bacteriophage, a virus whose host of replication is a bacterium, has been a treatment plan of considerable interest in some applications of epidemiology. A problem that arises in phage treatment, however, is the possibility that while the immunological response may be limited against the bacterial populations, it may be potent enough against the viral particles such that phage treatment does not get an opportunity to eradicate the \textit{V. cholera} bacteria. This study seeks to use mathematical modeling to investigate the dynamics of phage, bacteria, and immunological components in specific patients whose immune system is not strong enough to combat \textit{V. cholera}, but is strong enough to combat the phage particles. Having the latter in mind, we sought to investigate the question, “\textit{Can the immune system mitigate phage treatment in V. Cholera?}”

**Model**

Using methods outlined in Biologist’s Guide to Mathematical Modeling in Ecology and Evolution, a flow chart was constructed showing the interactions in the system. B(t) is for the cholera bacteria population, L(t) is for cholera in the latent bacteria, V(t) is for free-floating bacteriophage and A(t) represents the population of the immune system components. Assumptions made about the model are that when cholera is infected with bacteriophage it goes into a latent stage for some time before free-floating virions burst out of the cholera, the immune system will only attack the bacteriophage and bacteriophage is marked for consumption by macrophages via antibodies.

**Assumptions**

In the pursuit of a tangible answer to our research questions, a few assumptions about the construction of the model and the starting conditions had to be implemented. The largest of these lies in the ambiguity of the representation of the immune systems response. Through the paper, the term \textit{immune components} will be designated to describing the “active” population combating the bacteriophage. It is known that the immune system works in a far more complex manner involving antibodies, macrophages and T-cells, but all of these components are simplified into one population for the purpose of the research question.
Lastly, we address the reader. The scope of this report is strictly to use a simply modified set of logistic-based differential equations to model an infection being treated with bacteriophage therapy in order to investigate the population dynamics of the bacteria in question, the inoculated bacteriophage and the immune systems components. The specific use of cholera bacteria is arbitrary. Sufficient data about cholera could be found and used purposefully for the scope of the report and that is precisely what was done.

Flow Chart

In the construction of our model, each population is measured by the amount of particles present per mL of substance. The free floating bacteria (B (t)) as well as the latent bacteria (L (t) – Population of bacteria infected with virions) both have logistic growth rates governed by a carrying capacity pre-determined to be an amount appropriate for an infected human intestine. Both populations in our model are presumed to have roughly the same lifespan and so they both share an identical death rate. The loss experienced by each population through death does however differ based on the size of their population.
Based on the law of mass action, cholera bacteria come into contact with virions at some rate \( g \) and are successfully infected at a probability of latency value. The latent bacteria then harbor growing virions that burst forth from them after some period of time. The time this takes is represented by \( \phi \) in the flow chart above. A key difference to note at this junction is that the quantity of latent bacteria dying is not the same quantity joining the free floating virion population. A burst size term must be used to account for the fact that about fifty to two hundred virions are created in one cholera bacteria. This is depicted in the break flow by the term \( \beta \) in the second arrow indicating the population joining the free floating virions.

Virions \((V(t))\) in the model die at a rate that differs from that of either bacteria population. Loss of virions in our system also occurs by another law of mass action term that overlooks the interaction between immune system components and the virions themselves. This law of mass action term is similar to that of the bacteria-virion interaction but differs in the following way: The encountering of immune system components and virions ensures the death of both particles. This also occurs based on a ratio of virions to immune cells \((\alpha)\). The term \((x)\) depicts the contact rate between virions and immune system components and the term \((y)\) represents the probability of death of both particles.

Lastly, the immune system components \((A(t))\) are created at some rate \( f \) that the body dictates necessary to battle the infection it detects in the intestine of the infected and they too die off at a rate that differs from that of either bacteria or virions. The set of four differential equations derived from the above flow chart is simply put together by adding each term that signified growth of the population, such as law of mass action or logistic birth rate, as a positive term in its respective population equation. Likewise, any terms that depicted a loss of population, such as a death rate or a different law of mass action term, was subtracted from the appropriate equation. Based on the above flow diagram, four differential equations were derived for the system.

\[
\frac{dB}{dt} = r_b B(t) \left( 1 - \frac{B}{K} - \frac{L}{K} \right) - g\delta B(t)V(t) - dB(t)
\]

\[
\frac{dL}{dt} = r_l L(t) \left( 1 - \frac{B}{K} - \frac{L}{K} \right) + g\delta B(t)V(t) - \phi L(t) - dL(t)
\]

\[
\frac{dV}{dt} = \beta \phi L(t) - d_2 V(t) - xyV(t)A(t)
\]

\[
\frac{dA}{dt} = fV(t) - d_3 A(t) - \alpha xyV(t)A(t)
\]
Based on the constructed flowchart, a table of parameters where made that represent the many components of the system. Most parameters are taken from sources that were relevant to our study; however, it was not possible to find applicable values for every single parameter. In those cases, educated guesses were made based upon our knowledge of the system we were modeling.

### Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Meaning</th>
<th>Dimensions</th>
<th>Values Used</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>(d)</td>
<td>Death rate of bacteria</td>
<td>1/ Day</td>
<td>0.4</td>
<td>5</td>
</tr>
<tr>
<td>(d_2)</td>
<td>Death rate of Bacteriophage.</td>
<td>1/ Day</td>
<td>0.068</td>
<td>4</td>
</tr>
<tr>
<td>(d_3)</td>
<td>Death rate of immune response components.</td>
<td>1/ Day</td>
<td>0.15</td>
<td>3</td>
</tr>
<tr>
<td>(a)</td>
<td>Ratio of number of virions to immune cells</td>
<td>[ \frac{\text{# of virions}}{\text{ml}} \div \frac{\text{# of Immune cells}}{\text{ml}} ]</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>(r_b)</td>
<td>Reproductive rate of bacteria</td>
<td>1/ Day</td>
<td>1.88</td>
<td>2</td>
</tr>
<tr>
<td>(r_1)</td>
<td>Reproductive rate of bacteria with latent phage</td>
<td>1/ Day</td>
<td>Educated Guess 1.37</td>
<td>2</td>
</tr>
<tr>
<td>(\phi)</td>
<td>Rate of bacteriophage population growth from latent to free floating phase.</td>
<td>1/ Day</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>(f)</td>
<td>Number of macrophages produce in response to a number of virions</td>
<td>[ \frac{\text{# of immune components}}{\text{# of virions}} \div \frac{1}{\text{day}} ]</td>
<td>Varied</td>
<td>1</td>
</tr>
</tbody>
</table>
The number of collisions between bacteria cells and virions per unit of time.

\[ \frac{1}{\text{# of virions/ml} \cdot \text{day}} \]

**Educated Guess**

1.44*10^-6

**δ** Probability of latency

Unit less

0.6

**β** Burst rate of virions from bacteria

\[ \frac{\text{# of virions}}{\text{# of bacteria}} \]

100

7

The number of collisions between virions and immune cells per unit of time.

\[ \frac{1}{\text{# of immune cells/ml} \cdot \text{day}} \]

**Educated Guess**

2.3*10^-6

**y** Probability of macrophage consuming virion

Unit less

0.6

**K** Max amount of bacteria present in intestine

Bacteria per milliliter

10^9

2

**Methods**

After constructing the model shown earlier, Mathematica was used to solve all four equations simultaneously to achieve equilibria. Of the seven sets of four-population equilibria, only four were all positive real populations number and only those were explored further. A general Jacobian matrix for the model was then made and used to make a specific Jacobian for each of the four pre-stated equilibria. The eigenvalues of each of these Jacobians was then found and based on the accepted interpretation of eigenvalues in the scope of our research, it was discovered that a single completely non-trivial stable equilibrium existed.

To further explore the mathematical model of interest, both Mathematica and Matlab were used to make graphical interpretations of each population over a certain period of time. Parameters found in research or educational guesses to the best of current knowledge were used in the model to solve the system starting with an initial value for each population. In further analyses of the death and birth rate parameter of each population, other parameters were fixed and the ones in question were varied to see the results. This same method was assumed for the analysis of other parameters in the model particularly the burst size of latent bacteria lysis and the encounter rates of each interacting population.
In understanding the roles of these parameters tested, one could possibly derive meaningful biological information and hopefully highlight valid truths in the model as well as potential areas for improvement.

Analysis

The varying of the “f” parameter is the larger subject of concern in the scope of our research question. While the changes in population dynamics brought on specifically by changes in other parameters are notable, the varying effects in the final populations of virion particles and immune components can arguably be correlated directly to an increase or decrease in “f”. This is reasonable because f is the production rate of the immune components and is directly dependent on the population of virions.

Because of the structure of our model and the limitations of MatLab, choosing values of “f” below ≈120 yielded negative population values and consequential error messages. However f values greater than 120 yield perfectly analyzable results. As can be seen in the graphs below, after a large drop in the virion population, typically one large oscillation can be observed within about 2.4 – 24 hours of inoculation and the larger the f value, the lower the amplitude of the oscillation is. Coupled with this is the slight increase in the immune component population which is to be expected. What can also be seen in the same graphs is the dwindling of the cholera bacteria population into numbers significantly lower than an amount necessary for an infection.

For f values that are arguable exceedingly large, (magnitudes within the upper range of $10^2$ approaching $10^3$), in our there is observation of a large rise in the bacteria population after a large drop. This seems to be a direct observation of the issue that brought about the research question for this report itself: An excessive production of immune components causing a hindrance of bacteriophage growth which in turn induces a resurge of the bacteria population.

Lastly for f values approximately larger than 150, much after 100 days, a dampening oscillation of every population is also observed. The graphs below depict the change in population dynamics based on an increase in the f value used by 50 parts per mL starting from f = 150 till f = 350 and a special look at f = 500.
What is notable is the large increase in immune components coupled with a not so large decrease in virions. However upon a closer look at bacteria population when $f = 500$, we see regrowth of the bacteria population to amounts that could suggest reinfection. The first graph shows the results of this from afar, the second properly reveals the increase of bacteria as well as the oscillations of each population. Note that the new resting population for the bacteria is close to x-axis.
Jacobian and Stability

To investigate stability, the standard method of constructing a Jacobian and finding corresponding eigenvalues for each equilibrium expression in our system of four equations was implemented. Due to the complexity of several of the seven equilibria found in Mathematica, only the Jacobian of the trivial and semi-trivial equilibria each were analyzed.

The trivial equilibrium was evaluated in the general Jacobian matrix first. The resulting matrix, since it is lower triangular, reveals its eigenvalues to be the elements along the diagonal. Since to ensure a growing population it was postulated that the growth rate of the cholera bacteria \((r_b)\) be larger than its death rate, the term \((-d + r_b)\) is accepted to be positive. The presence of this positive eigenvalue assures that the trivial equilibria is unstable because not all real parts of each eigenvalue is negative as seen by the example just stated.

The eigenvalues of the semi-trivial equilibria evaluated Jacobian required some further analysis. As can be seen below, the last two eigenvalues have conditions that depict possibly complex values. The restriction \(\frac{r_b - d}{d + \theta r_l} > \frac{d^2 r_b}{\delta \phi g k}\), suggests that when one of the last two eigenvalues is positive, its counterpart is positive. This then lets us assume that one of those eigenvalues is positive and, consequentially, that the semi-trivial equilibrium is also unstable.
General Jacobian Matrix

\[
\begin{pmatrix}
-d - \frac{Br_b}{K} + r_b \left(1 - \frac{B}{K} - \frac{L}{K}\right) - g\delta V & -\frac{Br_b}{K} & -B\delta V & 0 \\
-\frac{Lr_l}{K} + g\delta V & -d - \varnothing - \frac{Lr_l}{K} + r_l \left(1 - \frac{B}{K} - \frac{L}{K}\right) & B\delta V & 0 \\
0 & \beta\varnothing & -d_2 - A\varphi & -V\varphi \\
0 & 0 & f & -d_3 - axyV \\
\end{pmatrix}
\]

Jacobian at trivial equilibrium

\[
\begin{pmatrix}
-d + r_b & 0 & 0 & 0 \\
0 & -d - \varnothing - r_l & 0 & 0 \\
0 & \beta\varnothing & -d_2 & 0 \\
0 & 0 & f & -d_3 \\
\end{pmatrix}
\]

Stable if trace is negative and determinant is positive (these conditions lead to negative real eigenvalues).

- Particular trace is \(-2d - d_2 - d_3 - \varnothing + r_b + r_l\)
- Particular determinant is \(d_2 d_3 (r_b - d)(r_l - \varnothing - d)\)

Conditions for negative trace:
1. \(d_3 > d_2\)  \((\text{Not valid for our parameter found in literature})\)
2. \(d_2 > d\)
3. \(\varnothing > r_b + r_l\)

Conditions for positive determinant:
1. \(d \geq r_l\)
2. \(d \geq rb\)

Or

1. \(rb > d\)
2. \(d < r_l - \varnothing\)  \((\text{not valid for our set of parameters, but if our educated guess is 0.04 units or greater in magnitude than our old educated guess, this condition is valid})\)
The failed trace condition above however means that the trivial equilibrium is not currently feasible for our parameter values.

Jacobian at semi-trivial equilibrium

\[
\begin{pmatrix}
-d + r_b & -r_b \left(1 - \frac{d}{r_b}\right) & -\delta dK \left(1 - \frac{d}{r_b}\right) & 0 \\
0 & -d - \phi - \frac{dr_l}{r_b} & \delta dK \left(1 - \frac{d}{r_b}\right) & 0 \\
0 & \beta \phi & -d_2 & 0 \\
0 & 0 & f & -d_3
\end{pmatrix}
\]

Stable if trace is negative and determinant is positive (these conditions lead to negative real eigenvalues).

- Particular trace is \(-\left(d_2 + d_3 + \phi + r_b\right) + \frac{dr_l}{r_b}\)
- Particular determinant is \(-\frac{d_3 (d - r_b) \beta g \phi \delta ((d - r_b)) + d_2 (\phi r_b + d (r_b - r_l))}{r_b}\)

Conditions for negative trace:

1. \(d < \frac{r_b (d_2 + d_3 + \phi + r_b)}{r_l}\)

Conditions for positive determinant:

1. \(\beta > -\frac{-d_3 (\phi r_b + d (r_b - r_l))}{g K \phi \delta (d - r_b)}\)

Because we assumed \(r_b > r_l\), the numerator remains negative. Due to all parameters defined as positive as to remain biologically meaningful, denominator must be negative. This leads us to the second condition for a positive determinant:

2. \(d < r_b\)
Future Directions

In order to improve this model, one would need to find more biologically accurate values for parameters that were gotten from educated guesses. While these educated guesses provided a decent ground on which to analyze the model, in some sense the accuracy of the model itself can still be brought to question. We could also consider collecting the appropriate parameters necessary for the population analysis of entirely different bacterial infections. Using the same model on vastly different other bacterial infections could provide insight into bacteriophage treatment, the likes that may not easily be perceived when considering the variations of just one kind of infection.

Secondly, we could improve of the terms used to describe the interaction of virions and bacteria could be implemented. According to an article in the virology blog (viriology.ws), the term MOI (Multiplicity of Infection) is often used to account for the number of virions added per cell during an infection. In our model, the law of mass action term accounts for the quantity of latent bacteria. Then, at some rate ($\phi$) the latent bacteria dies and based on the burst size ($\beta$) a number of virions consequentially burst forth. This whole mechanism could be replaced by simply using exclusively an MOI term such as a Poisson distribution function exemplified in the blog pre-stated.

Lastly, to eliminate the worry of negative values recurring, perhaps different simulation software could be used. The reason why “f” values less than 120 yield negative data being ignored in Matlab is to be further investigated but it is probable that limitations in the software and the model itself are to blame.

Conclusion

This model of a bacterial infection has its limitations but it provides some insight to the dynamics of a bacteriophage treatment and gives foundation substance to the scope of larger studies that can further investigate the effects of the immune system in the application of bacteriophage therapy. Several more questions can be asked based on the results found: Is this concept of a latent infection merely a result of the model and not a reflection of a practical scenario?

Infections are often known to be eliminated after they reach a certain level of low population density and re-growing infections are typically associated with long term infections rather than short-term ones. This hints at the formation of our model indiscriminately forcing the dynamics of the infection to be long term since cholera is known to be short-term. Whether that is true or not, the system does in its own stride give substantial answer to if a bacteriophage treatment can be affected by the strength of the immune system.
References

1. Felsenfeld, O. (1965). Immune cell counts and risks of respiratory infections among infants exposed pre- and postnatally to organochlorine compounds: a prospective study


5. Notes on Food, Beverages and Fomites Contaminated with Vibrio Cholerae


