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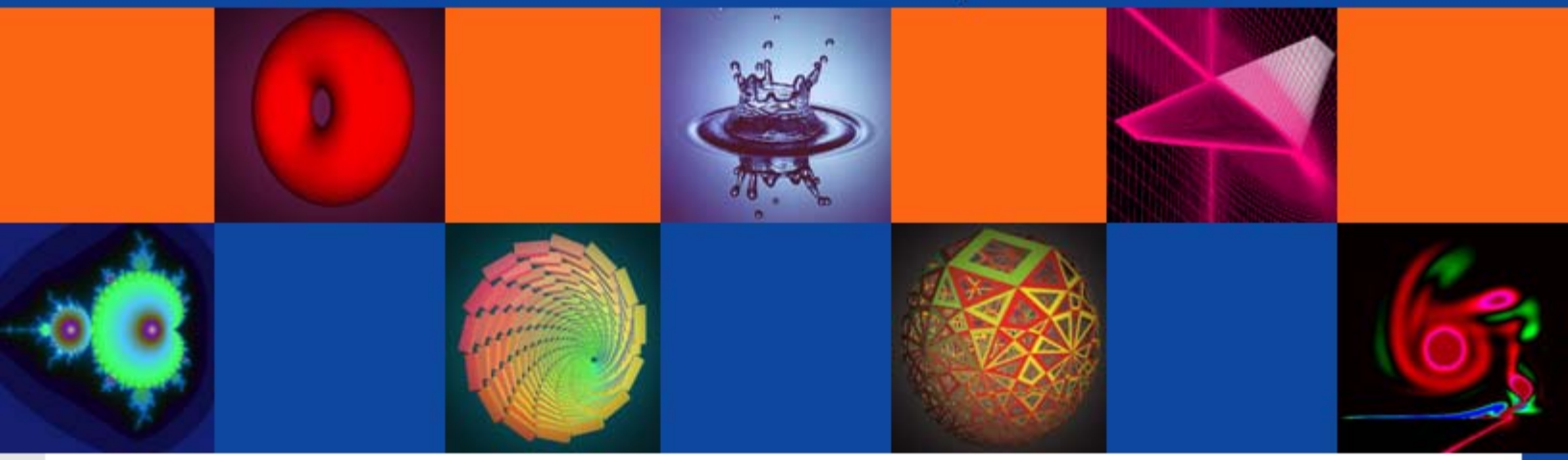

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Technical Report 2014-06

http://www.uta.edu/math/preprint/

A theoretical model of coinfection dynamics: Modeling competition dynamics between *Borrelia burgdorferi* and *Anaplasma phagocytophilum* within a human host.[*]

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Though many mathematical models have been used in the field of epidemiology, few of them aim to predict the outcome of competitive coinfection dynamics within humans. This study created a theoretical model to analyze the competition dynamics between two the infectious bacteria, *Borrelia burgdorferi* and *Anaplasma phagocytophilum*, within a human host. Interactions between two bacterial species in the human body are complex due to both microorganisms competing for the same resource and eliciting the body's innate immune response at different rates. The goal of this study was to identify whether resource limitation or increased immune responses would be more efficient in clearing this specific coinfection scenario. Iron was the resource chosen for this study, and the populations of neutrophils and macrophages within our model represented the innate immune system. Literature values were used to parameterize the interactions for numerical simulations. Results showed that a 61% decrease in iron availability below baseline parameters would clear the coinfection, while a 137% increase in neutrophils would produce the same results. Applying these two methods simultaneously showed improved results, where only a 45% reduction in iron along with a 45% amplification of neutrophils was equally efficient at clearing the coinfection. The results showed that these changes have the potential to be artificially induced in humans as an alternative treatment method to antibiotics for coinfection scenarios.

INTRODUCTION

For humans, the first line of defense against most pathogens is our skin. The skin works as an effective physical, biochemical, and adaptive immunological barrier between our 'inner' and 'outer' environments [16]. It is not impenetrable though, and one way pathogens can penetrate this barrier is through injuries such as cuts. A second way for pathogens to enter our system is through contact with non-intact skin, such as the mucosa linings of the eyes or mouth [20].

If a pathogen penetrates our skin barrier and enters our bloodstream, the body may become vulnerable to that pathogen's disease. If there is more than one pathogen existing within a host

[*] This research was supported by an NSF UBM-Institutional grant DUE#0827136 as part of the UTTER Program at UT Arlington (http://www.uta.edu/math/utter/).
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simultaneously, the scenario is referred to as a coinfection, or polymicrobial infection. Coexisting pathogen interactions can be either direct or indirect, depending on how strongly they elicit innate immune responses and how intensely they compete for resources. Eventually, there will be three possible outcomes to most coinfection scenarios: either both species survive, one species persists and the other one is eradicated, or both species are eradicated. The resulting scenario will depend on how well the two species can evade phagocytes and consume essential resources.

The innate immune system is an evolutionary and ancient part a host's defense mechanism. Our innate immune system is fixed within our genome, and includes the recognition of pathogen-associated molecular patterns and most of our inflammatory responses. During an inflammatory response, the immune system's macrophages, dendritic cells, mast cells, neutrophils, eosinophils, and natural killer cells are all activated. The activation of these innate immune system cells are almost always a sign of infection by a pathogen, and their main role is to get rid of the infection [9]. A vertebrate's innate immunity is largely dependent upon myeloid cells, which engulf and destroy pathogens. Myeloids include cells such as macrophages and neutrophils. Macrophages are distributed throughout the body of the host and are capable of engulfing and killing microbes, as well as initiating the adaptive immune response to most pathogens. Neutrophils on the other-hand are said to be short-lived, specialized killers with a lifespan of about 6 hours [1]. In this study's model, macrophage and neutrophil populations were chosen to represent the host's innate immune system.

In order to further understand the dynamics of a coinfection, we created a theoretical model to research the possible outcomes between two specific bacteria from a realistic biological scenario. The two species of bacteria chosen for our model were *Borrelia burgdorferi* and *Anaplasma phagocytophilum* because they can both be found living within the gut of a specific tick. The *Ixodes* tick, which can be found in both North America and Europe, serves as a vector for many bacteria including *B. burgdorferi* and *A. phagocytophilum*. These ticks are prone to coinfection with multiple bacteria in their gut, due to their feeding habits, and thus have the ability to transmit more than one bacterium to any given host. In addition, because both *B. burgdorferi* and *A. phagocytophilum* are being transmitted via an arthropod, they have an initial advantage over a host's immune system. Arthropod saliva often exhibits immunosuppressive characteristics, which in turn gives the bacteria the ability to survive in the presence of the immune system's initial response [20].

Borrelia burgdorferi is a gram-negative bacterium that causes Lyme disease in humans. *B. burgdorferi* is transmitted to humans and other mammals through tick bites, however, only about 1% of recognized tick bites actually result in Lyme disease [6]. This small percentage of infection is due to the fact that it takes a minimum of twenty-four to forty-eight hours of attachment for an infected tick to effectively spread the bacteria. Lyme disease is one of the most well known tick-borne diseases, and is characterized by the classic erythema migrans rash and

flulike symptoms caused during its early stages of infection. The disease itself though, as well as its symptoms, can be easily treated through the use of antibiotics. If left untreated, however, infection by the bacteria can spread to the joints, heart, and the nervous system, and cause extensive damage. This is because *B. burgdorferi* travels in the blood stream and establishes itself in the body's tissues, having a preference for tendons, brain cells, and the endothelial cells of blood vessels [20]. Unlike most bacterial species, *B. burgdorferi* is unique in the fact that it has evolved to replicate on limited amounts of iron, and elements such as Manganese (Mn), Zinc (Zn), and Magnesium (Mg), play a stronger role in the growth of *B. burgdorferi.* This is not to say, however, that this bacterium does not need iron at all. Instead, is used mainly for detoxification purposes rather than for storage [22].

The second bacterium in this system is called *Anaplasma phagocytophilum,* which was formerly known as *Ehrlichia phagocytophila* [19]. *A. phagocytophilum* is also a gram-negative bacterium, transmittable to humans via the *Ixodes* tick, and causes a disease known as human granulocytic anaplasmosis (HGA). *A. phagocytophilum* is unique because once inside a human host, it will infect a neutrophil cell and begin to multiply without inducing a respiratory burst. A respiratory burst is the innate killing mechanism of a neutrophil, and although the invasion does not induce the neutrophil's respiratory burst, it also does not completely suppress it. The bacterium enhances the infected neutrophil's secretion of a signaling protein, IL-8, to recruit more neutrophils to the site of infection. This allows the nearby bacteria to continue invading the migrating neutrophils. This disease was first described in 1994 and was considered potentially fatal for the elderly and immunocompromised individuals [24]. Some symptoms of this HGA include high-grade fever, rigors, and a general feeling of discomfort or illness. It is often accompanied by thrombocytopenia, a deficiency of platelets in the blood causing bleeding into the tissues, bruising, and slow blood clotting after injury, as well as leucopenia, which is a reduction in the number of white blood cells. In order to treat this disease, the antibiotic Doxycycline is recommended and, like many other illnesses, if left untreated HGA can become life threatening. Like most bacteria, *A. phagocytophilum*'s requires iron to survive and replicate.

Lastly, one of the most important aspects to mention of this study is resource availability. Of about thirty micronutrients, iron has a very particular role in mediating host-pathogen interactions [4]. Iron has many different roles within the human body, mainly in relation to hemoglobin, myoglobin, and in reactions that produce energy. About 65 to 75 percent of the body's iron is found in the form of hemoglobin, and the rest is used for other crucial processes. Very little iron is freely accessible since any excess is stored in the body as a reserve. The link to host-pathogen interactions lies in the fact that most pathogens need a sufficient amount of iron for growth and reproduction, and this is where competition for this limited resource begins. When the body recognizes an infectious threat, it reduces its iron levels even further through a defense mechanism mediated by a regulatory hormone called hepcidin [4]. This form of iron sequestration plays a crucial role in the body's ability to clear an infection by starving the

bacteria. Due to iron's importance in both humans and pathogens, as well as its crucial role in host-pathogen interactions, iron will represent the common resource in this study's model.

The purpose throughout this paper is to find, and model, whether an increase in immune response or a reduction in resource availability would be a more efficient method for the body to clear a coinfection of *B. burgdorferi* and *A. phagocytophilum*. Resource availability will be represented by iron reserves, and neutrophil and macrophage populations will represent the human host's innate immune response. Resource availability and immune response are two important elements that can drastically alter competition dynamics of a coinfection depending on their availability and their interactions with the bacteria present.

FLOW CHART AND EQUATIONS

A flow chart was formed as a graphical representation of the interactions occurring within this complex coinfection system. Five populations are interacting with one another, and have been labeled as R, for the common resource, N_a, for the *A. phagocytophilum* population, N_b, for the *B. burgdorferi* population, P_n, for the host's neutrophil population, and P_m, for the host's macrophage population.

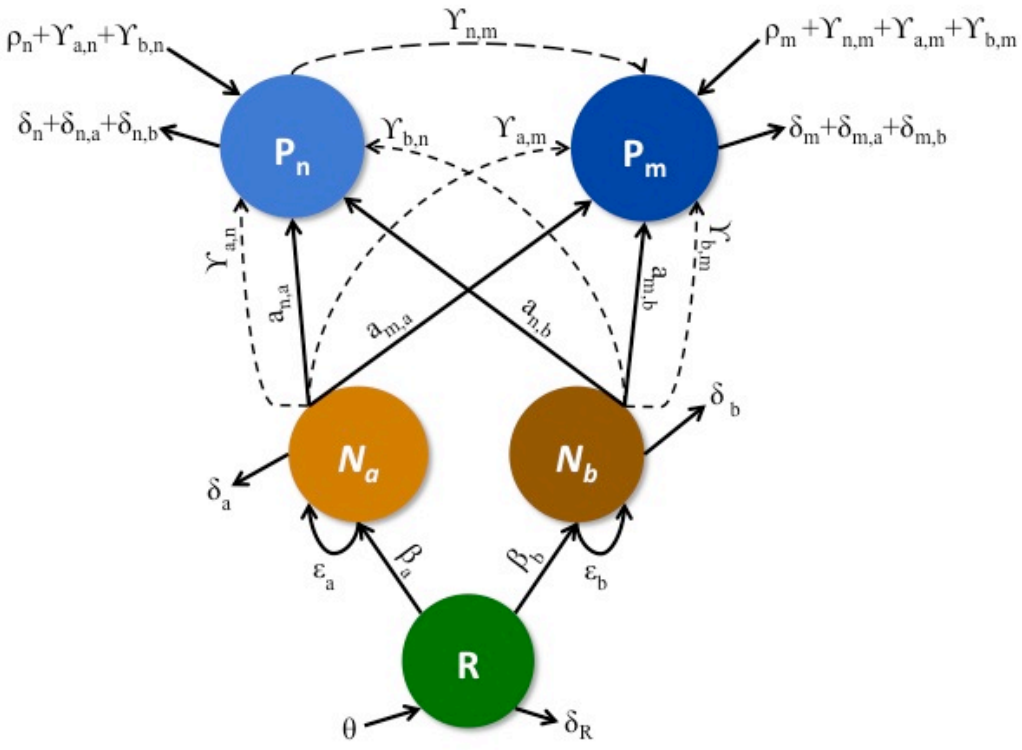

Figure 1 This flow-chart is a graphical representation of interactions between *B. burgdorferi* and *A. phagocytophilum* during a coinfection scenario within a human host. R represents iron, N_a represents *A. phagocytophilum*, N_b represents *B. burgdorferi*, P_n represents neutrophils, and P_m represents macrophages.

Since most pathogenic bacteria use iron as a resource for their proliferation, anemia usually presents itself in a host with a bacterial infection. In this model, the incoming background rate R is represented by two constants θ_D represents the amount of iron from the host's diet, and θ_T represents the amount of iron put into the system from tissue breakdown. These two constants have been combined as θ in the model for simplicity. Iron has three outgoing rates, one is its natural outgoing rate from the host's own usage, $\delta_R R$, and two are the outgoing rates from bacterial species' iron usage, $\beta_a R N_a$ and $\beta_b R N_b$. β_a represents the affinity constants for iron uptake of *A. phagocytophilum* and β_b is for *B. burgdorferi*. These rates and constants form the first differential equation representing the change in iron in the host's system:

$$\frac{dR}{dt} = \theta - R(\beta_a N_a + \beta_b N_b + \delta_R)$$

The two bacterial populations incoming rates are related to the amount of iron that they take in and how efficiently they convert that iron into new bacterial cells. This increase is represented by $\beta_i \varepsilon_i R N_i$, where $i = a, b$, defining each bacterial species. Throughout the rest of this paper, a subscript of "a" will represent *A. phagocytophilum* and a subscript of "b" will represent *B. burgdorferi*. Like before, β_i is the per capita growth rate for each of the bacterial species, and ε_i is the efficiency constant, which represents the conversion of resource uptake to replicated bacterium. The outgoing rates for each bacterium are contributed to by the host's immune cells and by their natural death rates. Each immune cell, represented by the neutrophil and macrophage populations, has an attack rate for each bacteria, $a_{i,j}$, where $i = a, b$, the bacteria species being attacked, and $j = n, m$, the immune cell that is at work. Throughout the rest of this paper, a subscript of "n" will represent neutrophil cells and a subscript of "m" will represent macrophage cells. Altogether, the outgoing rate contributed from the immune system on a bacterial species would be $a_{i,j} N_i P_j$, where $i = a, b$ and $j = n, m$. The natural outgoing rate is represented by $\delta_i N_i$, where $i = a, b$ for each bacterial species. These rates and constants formed the following two differential equations representing the changes in the bacterial populations within the host's system:

$$\frac{dN_a}{dt} = N_a\left(\beta_a \varepsilon_a R - a_{a,n} P_n - a_{a,m} P_m - \delta_a\right)$$

$$\frac{dN_b}{dt} = N_b\left(\beta_b \varepsilon_b R - a_{b,n} P_n - a_{b,m} P_m - \delta_b\right)$$

Our immune cell's population incoming rates are made up of constant background rates referred to as the body's natural production of these cells, and elicitation rates, which is the body's increased production of these cells in response to a bacteria's presence within the system. The background production per capita rates are represented by ρ_j, where $j = n, m$. The elicitation constants are represented by $\gamma_{i,j}$, where $i = a, b$, defining which bacterial species is

eliciting the immune cell, and $j = n, m$, defining which immune cell is being amplified. Each bacterium elicits both neutrophils and macrophages at different rates. The outgoing rates of our immune cell populations are contributed to by a number of natural per capita death rates: δ_j, where $j = n, m$, represents the natural per capita rate at which the immune cells usually leave the system in no response to an infection, and $\delta_{j,i}$, where $j = n, m$, and where $i = a, b$, represents the rate at which the immune cell undergoes apoptosis upon phagocytosis of each bacterium. For the macrophage differential equation, there is a specific parameter in addition to the ones formerly discussed. As an infection proliferates, neutrophils are unable to clear it on their own, and once it becomes serious, the body recognizes the need to track the invading species for future recognition. The body produces antibodies, an act carried out by macrophages who are alerted by neutrophils to migrate to the site of infection. This added elicitation rate is represented by $\gamma_{n,m}$. These rates and constants form the differential equations that represent the changes in the immune cell populations in the host's system:

$$\frac{dP_n}{dt} = \left(\rho_n + \gamma_{a,n}N_a + \gamma_{b,n}N_b\right) - P_n\left(\delta_n + \delta_{a,n}N_a + \delta_{b,n}N_b\right)$$

$$\frac{dP_m}{dt} = \left(\rho_m + \gamma_{n,m}P_n + \gamma_{a,m}N_a + \gamma_{b,m}N_b\right) - P_m\left(\delta_m + \delta_{m,a}N_a + \delta_{m,b}N_b\right)$$

PARAMETERS

Table 1 Table of parameters involved in the system along the values used in producing the different simulations. The baseline parameter values on the list were gathered from credible literary sources. Recall that subscript "a" represents the species *A. phagocytophilum*, subscript "b" represents *B. burgdorferi*, subscript "n" represents neutrophils, and subscript "m" represents macrophages.

Parameter	Parameter description	Value/Range (unit)
θ [10]	Inflow of common resource ≈ Iron;	$\theta_{diet} = 334\ ng \cdot ml^{-1} \cdot day^{-1}$ $\theta_{tissues} = 8333\ ng \cdot ml^{-1} \cdot day^{-1}$
B_a/ B_b [14] [24] [10]	Affinity constants for iron uptake of bacterial species a, b	K for B_a and $B_b \approx 1000\ \frac{ng}{mL}$ B_a = max growth/K = $2.7 \cdot 10^{-8}$ $ml \cdot ng^{-1} \cdot day^{-1}$ B_b = max growth/K = $2.85 \cdot 10^{-8}$ $ml \cdot ng^{-1} \cdot day^{-1}$
ε_a/ ε_b [14] [24]	Conversion constant of resource uptake to replicated bacterium	$\varepsilon_a = 6.26 \cdot 10^6\ cells \cdot ng\ of\ iron^{-1}$ $\varepsilon_b = 8.00 \cdot 10^6\ cells \cdot ng\ of\ iron^{-1}$
$a_{a,n}$/ $a_{b,n}$ [12]	Attack rates of neutrophils on species a, b	$a_{a,n} = 5.0 \cdot 10^{-5}\ ml \cdot cell^{-1} \cdot day^{-1}$ $a_{b,n} = 8.1 \cdot 10^{-5}\ ml \cdot cell^{-1} \cdot day^{-1}$
$a_{a,m}$ / $a_{b,m}$ [12]	Attack rates of macrophages on species a, b	$a_{a,m} = a_{b,m} = 4.05 \cdot 10^{-5}\ ml \cdot cell^{-1} \cdot day^{-1}$

$\gamma_{a,n}$/ $\gamma_{b,n}$ [16]	Elicitation rates of species a, b on neutrophils	$\gamma_{a,n} = \gamma_{b,n} = 8.0 \cdot 10^{-1}\ neutrophils \cdot bacteria^{-1} \cdot day^{-1}$
$\gamma_{a,m}$ / $\gamma_{b,m}$ [16]	Elicitation rates of species a, b on macrophages	$\gamma_{a,m} = \gamma_{b,m} = 2.0 \cdot 10^{1}\ macrophages \cdot bacteria^{-1} \cdot day^{-1}$
$\gamma_{n,m}$ [16] [12]	Elicitation rates of neutrophils on macrophages	$\gamma_{n,m} = 0.04$ $macrophages \cdot neutrophils^{-1} \cdot day^{-1}$
δ_n /δ_m [22] [10]	Natural death rates for neutrophils, macrophages	$\delta_n \approx 2.0\ day^{-1}$ $\delta_m \approx 0.33\ day^{-1}$
δ_R [10] [2] [8]	Natural turnover rate of iron	Range between 0.24 – 0.33 day^{-1} Value chosen for $\delta_R = 0.30\ day^{-1}$
δ_a/δ_b	Natural death rates of bacterial species a, b	$\delta_a = \delta_b = 1.0 \cdot 10^{-9}\ day^{-1}$
$\delta_{n,a}$/$\delta_{n,b}$ [12]	Apoptosis rates of neutrophils upon uptake of species a, b	$\delta_{n,a} = \delta_{n,b} = 1.8 \cdot 10^{-8}\ ml \cdot cell^{-1} \cdot day^{-1}$
$\delta_{m,a}$/ $\delta_{m,b}$ [3]	Apoptosis rates of macrophages upon uptake of species a, b	$\delta_{m,a} = \delta_{m,b} = 3.2 \cdot 10^{-6}\ ml \cdot cell^{-1} \cdot day^{-1}$
ρ_n /ρ_m [20] [22]	Production constant of neutrophils, macrophages in the system	$\rho_n = 1.67 \cdot 10^{7}\ cell \cdot ml^{-1} \cdot day^{-1}$ $\rho_m = 3.13 \cdot 10^{5}\ cell \cdot ml^{-1} \cdot day^{-1}$
Total serum volume	Total serum volume found in men and women	Males = 3196 +/- 402 ml Females = 2280 +/- 342 ml Estimated average= 3000 ml

Research on parameter values

The incoming background rate for iron was calculated from literary values found in a paper that focused on the regulation of iron in the body by hepcidin, an antimicrobial-like peptide hormone. From this, we gathered that about 1mg of iron is typically absorbed per day from a person's diet, while about 25 mg per day is recycled into the serum by senescent red blood cells. These numbers were then converted into the units the system was modeled after, and assuming that the average person has about 3000 ml of serum, it was calculated to be about 334 ng of iron per milliliter of serum, per day from the host's diet alone [10]. The amount of iron recycled into the serum was calculated to be about 8333 ng of iron per milliliter of serum, per day, an amount that is significantly more than the diet's input.

The per capita growth rate for *B. burgdorferi* was calculated from literary values found in a paper that describes the growth kinetics of *B. burgdorferi* in BSK II medium. They recorded a maximum increase in bacterial cells from $2 \cdot 10^8$ cells per ml of medium to $3 \cdot 10^9$ cells per ml in 10 days. From this, it was calculated that the maximum growth rate of this bacteria is 0.27 per

day. Because both *A. phagocytophilum* and *B. burgdorferi* have similar characteristics of very slow growth rates, this number was used for both species [14]. Although it was stated that both species were not very dependent on iron to supplement their growth, in comparison to other pathogenic bacteria, *B. burgdorferi* was more efficient in its uptake of iron than *A. phagocytophilum,* therefore ε_i was different for each species. To calculate ε_i, it was first found that in a standard BSK II medium there is about 1.6 μM of iron. From this, the amount of iron available to the bacteria grown in the experiment was used calculated the maximum populations produced in the course of the experiment. $3.13 \cdot 10^7$ cells were produced per ng of iron for *A. phagocytophilum* and $8.00 \cdot 10^6$ cells produced per ng of iron for *B. burgdorferi* [24]. In addition to the maximum growth rate, a half saturation constant for each bacterial species, K, was needed where β_i would be the maximum growth rate divided by K. This would give an accurate per capita growth rate. The K values for the specific species in this model were not readily available in the literature, so the K value for *Staphylococcus aureus*, a pathogenic bacterium often involved in coinfections, was used [10]. This half saturation constant was used for both bacteria. With these two numbers, the maximal growth rate and K, the final β_i for both bacteria was calculated to be $2.7 \cdot 10^{-11} ml \cdot ng^{-1} \cdot day^{-1}$.

The attack rates of neutrophils on the bacterial species were calculated from a literature values that defined the movement of flagellates, $0.81 \cdot 10^{-10}$ m^3 per cell per day. This was used as an overestimate for the attack rate for the neutrophils, which could also be defined as the rate at which neutrophils migrate to the site of infection, assuming all neutrophils are at work on the pathogens once they reach the site. Also, assuming the average person holds about 3000 ml of serum volume, this estimate was converted to $8.1 \cdot 10^{-5}$ ml per cell per day. A significant characteristic of *A. phagocytophilum* though, is its ability to evade neutrophils by invading them and using them as a host to replicate in. In this process, they manipulate the neutrophil's intracellular processes, further evading any intracellular damage the neutrophil could inflict on them. Knowing *A. phagocytophilum* has this advantage over *B. burgdorferi,* there was a need to represent it in the parameter values by slightly decreasing the attack rates of neutrophils on *A. phagocytophilum* to $5.0 \cdot 10^{-5}$ ml per cell per day. In calculating the attack rates of the macrophages on the bacterial species, there was no need to differentiate them because neither species had any uncharacteristic interactions with them, concluding that the macrophage attack rates should be of equal magnitude for both species. Indirectly, macrophages would play more of a role in attacking *A. phagocytophilum* because of its evasive characteristics in its interactions with neutrophils. Values for the migration rates on macrophages were not readily available in the literature, but the rate should be significantly less than the neutrophil value. Macrophages are much greater in size than neutrophils, and therefore move much slower when migrating to a site of infection. In addition to this, macrophages are often not recruited to a site unless there is a certain degree of infection present, one that cannot be cleared by neutrophils alone. Given these two factors, the migration rate of the macrophages was set at half the rate for neutrophils, and calculated an attack rate of $4.05 \cdot 10^{-5}$ ml per cell per day [12].

The elicitation rates of the bacterial species on the macrophages were calculated from a literature source that measured macrophage and neutrophil concentrations in rainbow trout infected with *Yersinia ruckeri,* which usually clears in about 4 days. From this, it was gathered that a healthy individual has about $4 \cdot 10^5$ macrophages circulating per ml per day [16]. In the infection with the rainbow trout, there were about 10^4 bacterial cells at a given site during an infection, and assuming that about half of the macrophages, $2 \cdot 10^5$, would be available to respond to an infection, the elicitation rate was calculated at about 20 macrophages per bacteria per day. Comparing the concentration of neutrophils and macrophages within the same infection allowed for the estimation of elicitation rates of the neutrophils on the macrophages. Before we assumed that about half of the available macrophages would respond to the infection directly. We then assumed the other half would respond to the infection indirectly by responding to the concentration of neutrophils gathering at the site of infection. Assuming $2 \cdot 10^5$ macrophages per ml per day are responding to neutrophils, given there is a concentration of $5 \cdot 10^6$ neutrophils per ml, the elicitation rate of neutrophils on macrophages was calculated to be 0.04 macrophages per neutrophil per day. The elicitation rates of the bacterial species on the neutrophils were calculated from the same paper, where the information gathered indicated there were 10^6 neutrophils per ml per day, and given there were about 10^4 bacterial cells at a given site during an infection, the elicitation rate of neutrophils was calculated to be about 100 neutrophils per bacteria per day [16]. Due to the interactions between *A. phagocytophilum*'s with neutrophils, $\gamma_{a,n}$ needed to be less than $\gamma_{b,n}$; therefore $\gamma_{a,n}$ was decreased from 100 neutrophils per bacteria per day to 0.3 neutrophils per bacteria per day and *B. burgdorferi* was decreased to 0.8 neutrophils per bacteria per day, to better represent their fitness within the human system.

The natural death rates of the neutrophils were calculated from a literature value that listed the average lifespan of a neutrophil was 6 to 12 hours [22]. This range was converted into 2 to 4 neutrophils per day, and the lower value within the range was used in running simulations. The natural death rates of the macrophages was calculated in the same way from another literary source, which stated the average lifespan of a macrophage was about 71 hours, supporting the fact that macrophages are far more durable than neutrophils [10]. These values were calculated to be about 2.0 per day for neutrophils and 0.33 per day for macrophages.

The natural turnover rate of iron was calculated from an original literary value that stated the total iron secretion per day for males was about 1.18 mg per day, and 1.66 mg per day for women [8]. Another source stated that the total iron content in the body was an average of 4 to 5 mg, while the iron content in the serum was about 3 to 4 mg of this [2]. Using these values, the natural death rate of iron was calculated to be within a range of 0.24 to 0.33 per day.

The natural death rate of the bacterial species was estimated as a value between each bacteria species' maximum growth rate and zero. It was found, through literary searches, that these two

species are very efficient within a host, and are not likely to die off at a significant rate naturally, so the value for their natural death rate should be closer to zero than to the maximal growth rate. This also allowed for δ_i to be treated more as a migration rate out of a certain area of the body rather than a real death rate. After running simulations with several values within this range, 0.10 per day was a suitable value because it produced dynamics likely to take place in a coinfection of this type.

The apoptotic rates of the neutrophils were calculated from a literature value where the rate of apoptosis in neutrophils was about 0.3 per day. When adding this rate into the model, it was converted to be about $3 \cdot 10^{-5}$ ml per bacterial cell per day. To further represent *A. phagocytophilum's* increased fitness within the system we decreased $\delta_{n,a}$ by one order of magnitude. This specifically accounts for *A. phagocytophilum's* ability to slow the death rate a neutrophil once it has invaded it. This allows *A. phagocytophilum* to replicate at larger amounts without having the host's naturally high turnover rate to prevent its proliferation. The apoptotic rates of the macrophages was calculated from a literary value that measured the apoptotic rates of macrophages in a stressful environment, and found that most macrophages undergo apoptosis in these conditions in about 24 hours. In adding this to the model, it was converted to about $4.167 \cdot 10^{-6}$ ml per bacterial cell per day, which was kept the same for the competing microbial species.

The incoming background rate of neutrophils was calculated from literary values that stated that there are about 10^9 neutrophils circulating per kilogram of body weight per day. Assuming the average weight of our host is about 50 kg, ρ_n was calculated to be about $1.67 \cdot 10^7$ cells per ml per day. The incoming background rate of macrophages was calculated from a literary value that stated the total output of monocytes per day in an average person was about $9.4 \cdot 10^8$ cells [22]. From here, it was calculated that this was about $3.13 \cdot 10^5$ cells per ml of serum per day.

Calibration of parameter values

A number of parameter values were calibrated upon running simulations with this theoretical model. With baseline parameter values, biologically viable outcomes were not being produced, thus, these values were adjusted to predict more realistic outcomes, which could be applicable to a real coinfection with these two species. First, it was noticed that the bacteria in this system seemed to have unlimited growth, so θ_T was adjusted to 1000 ng of iron per milliliter of serum, per day, a significant decrease from the aforementioned number, but still applicable. In addition, the maximum growth rate for both bacteria was decreased, from $2.7 \cdot 10^{-4}$ per day to $2.15 \cdot 10^{-6}$ per day for *B. burgdorferi* and $2.2 \cdot 10^{-6}$ per day for *A. phagocytophilum.* This resulted in a new set of β_i, where β_b was now $2.15 \cdot 10^{-9}$ per day and β_a was now $2.2 \cdot 10^{-9}$per day. The lower background rate produced more realistic growth for the bacteria in our model, while the differing β_i now produced dynamics of competition between the species, which is

expected in a coinfection of this sort, and was therefore a better baseline value to start at. In running simulations, it was noticed one bacterium was dying off much quicker than the other, at a rate that wouldn't be realistic when dealing with two efficient species. In response, both the attack and elicitation rates were calibrated to simulate a more biologically significant scenario, where both species would be able to coinfect the same system simultaneously. The elicitation rates were changed to lower values, where $\gamma_{a,n}$ was decreased to 0.2 neutrophils per bacteria per day, $\gamma_{a,m}$ was decreased to 1.0 macrophage per bacteria per day, $\gamma_{b,n}$ was decreased to 3.0 neutrophils per bacteria per day, and $\gamma_{b,m}$ decreased to 1.0 macrophage per bacteria per day. Like before, the $\gamma_{a,n}$ value was significantly less than the $\gamma_{b,n}$ value, representing the differing characteristics of the two species within our system. Knowing that the attack rates for both species should differ in response to *A. phagocytophilum*'s unique interactions with neutrophils, the attack rate of neutrophils on *B. burgdorferi*, $a_{b,n}$, was decreased from $8.1 \cdot 10^{-5}$ ml per cell per day to $8.5 \cdot 10^{-5}$ ml per cell per day. In the simulations, the host's defenses were amplifying quicker than they would normally, so it was believed that there had been an overestimation of the incoming background rates for the immune cells. There seemed to be a limitless amount of neutrophils and macrophages to combat both species, and because this is unrealistic, the numbers were calibrated to lower values. Thus ρ_n, was decreased from $1.67 \cdot 10^7$ cells per ml per day to $1.67 \cdot 10^7$ cells per ml per day and ρ_m we decreased left at $3.13 \cdot 10^5$ cells per ml per day.

MODEL EQUILIBRIUM AND STABILITY

There were four different scenarios that were analyzed for stability and for equilibrium values. These four scenarios were when neither species of bacteria was present (also called a trivial equilibrium), when one species was present and the other one was not (this represents two scenarios), and when both of them were part of the system (the co-infection scenario).

Trivial Equilibrium

The first scenario was the trivial equilibrium, where none of the two bacterial species are present. In the case of *A. phagocytophilum* and *B. burgdorferi,* the two bacterial species are absent when N_a and $N_b = 0$. When these two values were set equal to zero, the following equilibrium values were obtained:

$$R = \frac{\theta}{\delta_R} \qquad P_n = \frac{\rho_n}{\delta_n} \qquad P_m = \frac{\rho_m + \gamma_{n,m}(\frac{\rho_n}{\delta_n})}{\delta_m}$$

These values were then inserted into the differential equations, and the equations were analyzed for the trivial equilibrium using a Jacobian matrix. The following is what the Jacobian matrix for the Jacobian matrix looked like:

$$\begin{matrix}
-(\delta_R) & -\left(\frac{\theta}{\delta_R}\right)\beta_a & -\left(\frac{\theta}{\delta_R}\right)\beta_b & 0 & 0 \\
0 & \beta_a\varepsilon_a\left(\frac{\theta}{\delta_R}\right) - a_{a,n}\left(\frac{\rho_n}{\delta_n}\right) - a_{a,m}\left(\frac{\rho_m + \gamma_{n,m}(\frac{\rho_n}{\delta_n})}{\delta_m}\right) - \delta_a & 0 & 0 & 0 \\
0 & 0 & \beta_b\varepsilon_b\left(\frac{\theta}{\delta_R}\right) - a_{b,n}\left(\frac{\rho_n}{\delta_n}\right) - a_{b,m}\left(\frac{\rho_m + \gamma_{n,m}(\frac{\rho_n}{\delta_n})}{\delta_m}\right) - \delta_b & 0 & 0 \\
0 & \gamma_{a,n} - \left(\frac{\rho_n}{\delta_n}\right)\delta_{a,n} & \gamma_{b,n} - \left(\frac{\rho_n}{\delta_n}\right)\delta_{b,n} & -(\delta_n) & 0 \\
0 & \gamma_{a,m} - \left(\frac{\rho_m + \gamma_{n,m}(\frac{\rho_n}{\delta_n})}{\delta_m}\right)\delta_{a,m} & \gamma_{b,m} - \left(\frac{\rho_m + \gamma_{n,m}(\frac{\rho_n}{\delta_n})}{\delta_m}\right)\delta_{b,m} & \gamma_{m,n} & -(\delta_m)
\end{matrix}$$

Using a system of pivoting the column of zeros, the eigenvalues would be the five diagonal values: $-\delta_R, -\delta_m, -\delta_n, \beta_a\varepsilon_a R - a_{a,n}P_n - a_{a,m}P_m - \delta_a$, and $\beta_b\varepsilon_b R - a_{b,n}P_n - a_{b,m}P_m - \delta_b$.

Although the equilibria for the other three scenarios were analyzed numerically for stability, at this time, due to their complexity and length, we are unable to interpret. Instead, we analyzed these scenarios using a value called R_0.

R_0 Analysis

Any infective agent has something called an R_0, a mathematical ratio created to gauge how 'infectious' an agent is. The number stands for how many new infections are created from one. For example, influenza has an R_0 of 2-3, so if a person infected with influenza where placed in an otherwise healthy population, we would expect him to infect around 2 to 3 people, where each of them would also infect 2 to 3 people, if left to run rampant among the population without medical treatment. If this person were infected with smallpox rather than influenza, we would expect him to infect around 5 to 7 people in the population, since smallpox has a greater R_0 of 5-7. Smallpox is notably more infectious and serious within a population than influenza, and this well-known fact is reflected in the Smallpox agent's R_0 value. From here, you can calculate how quickly the entire population will be completely overtaken by the agent.

When comparing infections and diseases, the R_0 value is often looked at because it gives the researcher an indication of how 'fit' the agent is to infiltrate our populations and our systems. We chose to look at our theoretical bacteria's R_0 for this reason, while our model looks at the outcome of our bacterial species in only one system, this analysis shows us how the species 'infectivity' changes the outcome of our model on a microscopic, single system, scale.

Traditionally, if an agent's R_0 is less than 1, we can say the species, along with its infection, will die out of the population, or in our case, our human's system. If an agent's R_0 is greater than 1, we can say that the species will proliferate within the population, and if not dealt with, will eventually overtake the entire population, or in our case, will overtake the system and

probably kill the human. To solve for our N_a's R_0, named R_a, and N_b's R_0, named R_b, we took their corresponding equations:

$$\frac{dN_a}{dt} = N_a\left(\beta_a \varepsilon_a R - a_{a,n}P_n - a_{a,m}P_m - \delta_a\right)$$

$$\frac{dN_b}{dt} = N_b\left(\beta_b \varepsilon_b R - a_{b,n}P_n - a_{b,m}P_m - \delta_b\right)$$

Ratios were then created of their incoming rates over their outgoing rates. By this logic, should the outgoing rates have a greater magnitude than the incoming rates, it will have an $R_0 < 1$, and our species will die out of the population. Should the incoming rates calculate to a value greater than the outgoing rates, we expect the opposite to occur, with the $R_0 > 1$.

$$R_a = \frac{\beta_a \varepsilon_a \left(\frac{\theta}{\delta_R}\right)}{a_{n,a}\left(\frac{\rho_n}{\delta_n}\right) + a_{m,a}\left(\frac{\rho_m + \gamma_{n,m}\left(\frac{\rho_n}{\delta_n}\right)}{\delta_m}\right) + \delta_a}$$

$$R_b = \frac{\beta_b \varepsilon_b \left(\frac{\theta}{\delta_R}\right)}{a_{n,b}\left(\frac{\rho_n}{\delta_n}\right) + a_{m,b}\left(\frac{\rho_m + \gamma_{n,m}\left(\frac{\rho_n}{\delta_n}\right)}{\delta_m}\right) + \delta_b}$$

We included these R_0 equations in our model through our bacteria's *beta* parameter:

$$\beta_a = R_a \frac{\delta_R\left(\frac{a_{m,a}\delta_n\rho_m + a_{n,a}\delta_m\rho_n + a_{m,a}\gamma_{n,m}\rho_n}{\delta_m\delta_n} + \delta_a\right)}{\theta\varepsilon_a}$$

$$\beta_b = R_b \frac{\delta_R\left(\frac{a_{m,b}\delta_n\rho_m + a_{n,b}\delta_m\rho_n + a_{m,b}\gamma_{n,m}\rho_n}{\delta_m\delta_n} + \delta_b\right)}{\theta\varepsilon_b}$$

With this, we could vary this new parameter and see how the outcome of our system changed. We ran simulations in a mathematical program, with the parameters in Table 1 defining the parameters in our model, therefore also defining both R_a and R_b, where we increased R_a and R_b by very small increments over time ($R_a/R_b + 0.05$), and witnessed the outcome of the competition within the human system.

Out of 3721 runs of simulations:

1606 runs (43.1%) had an outcome where N_a overtook the system.

1421 runs (38.1%) had an outcome where N_b overtook the system.

682 runs (18.3%) had an outcome where neither N_a nor N_b overtook the system, and both species were driven out.

12 runs (0.3%) had an outcome where both N_a and N_b overtook the system, creating a coinfection within the system.

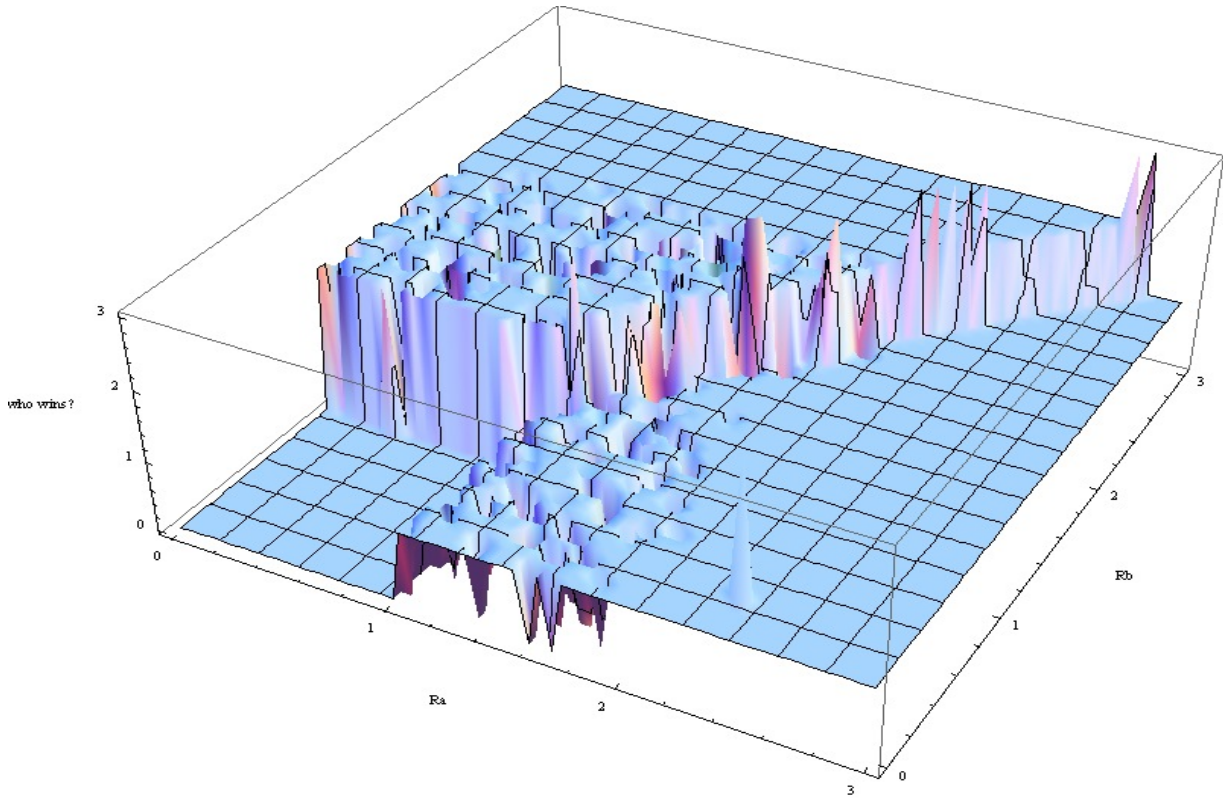

Figure 2 3D graph of 3721 runs of R_0 Loop; 43.1% of the runs 'A won', 38.1% of the runs 'B won', 18.3% of the runs 'neither A nor B won', 0.3% of the runs 'A and B won'.

RESULTS

Mathematical Analysis

The R_0 analysis showed that with our current set of parameter values, N_a, *A. phagocytophilum*, is more likely to drive N_b, *B. burgdorferi*, out of the system, and successfully overtake the system itself. This outcome occurred 43.1% of the time, owing to the difference in parameters for N_a and Nb. This is not the most ideal outcome we would want for our human, but it's also not the worst. With our current parameter set, our analysis shows us that our bacterial species are different

enough, in a way that induces competition, where only one bacterium is likely to survive within the system. So, if a human were to develop a coinfection with *A. phagocytophilum* and *B. burgdorferi*, this coinfection would naturally convert itself into a monomicrobial infection, just based on the differences in fitness between the two species, leaving only one species to be dealt with artificially. This prevents excessive antibiotic usage and excessive strain on our human's system, increasing their chances at survival.

Ideally, we would like to see a natural competition develop between the two species, where they would be unable to coexist, and eventually drive each other out of the system, clearing the coinfection altogether without need for excessive medical intervention. This ideal outcome occurred only 18.3% of the time, so although it is possible, it's unlikely. The worst outcome that could occur would be that both species are able to coexist within the system, and develop into a coinfection that would become detrimental for our human. This outcome occurred only 0.3% of the time, a very unlikely possibility, which in our human's case, is very good, as they would be less likely to survive the coinfection.

Model Simulations

After running simulations, several different dynamics developed. In order to decide the outcome of the system as a whole, we needed to define threshold values that would either sustain our host or declare our host as deceased. For the common resource, iron, the threshold value was found to be 334.05 ng per ml per day. This was calculated from iron levels with a recently discovered prevalence for deep vein thrombosis, an often fatal complication of complex illnesses [29]. Should the iron concentration in our simulations decrease below this value, we would assume our host with fatal iron levels, and would define the outcome of the scenario.

The threshold values for our bacterial concentrations were defined as well, where once the concentration decreased below 10^{-4} cells per milliliter, we considered the species to be cleared from the system, since 10^{-4} cells per milliliter equates to 0.3 of a bacterial cell in the system. We are assuming 0.3 of a bacterial cell would prove unviable and impossible to replicate further. With this assumption in mind, any mathematical artifacts that should present after our bacterial concentrations decrease below their threshold value would be disregarded as they are considered a product of the equations and the program, and not applicable to our biological system.

Another important parameter to consider was the neutrophil values. Neutropenia is the presences of abnormally few neutrophils in the blood, leading to an increased susceptibility of infection to our host. Because neutrophils usually make up 50-70% of the white blood cells, and they are the primary defense against infections. Patients with neutropenia are more susceptible to bacterial infections, so it was essentially that the model reflected healthy values of neutrophils to sustain the life of the human host.

Healthy System

Our first set of simulations was modeled after our trivial equilibrium where neither bacterial species were present. Our simulations showed the iron concentration normalizes to healthy amounts, where the phagocyte concentrations, shows this dynamic as well, as the neutrophil and macrophage concentrations normalize to healthy amounts. In this scenario, our bacterial concentrations are set to zero, as described in our trivial equilibrium.

Coinfection

Our simulations begin with a coinfection within a healthy human system, where two different species of bacteria with different bacterial parameters are able to simultaneously infect the same host, drawing on the same resource and eliciting the same predators, neutrophils and macrophages. Our simulations showed the iron concentrations remained at sustainable levels, well above the threshold value for our host. Figure 4 shows the phagocyte concentrations. Figure 3 shows the bacterial concentrations where N_a and N_b rapidly proliferate within the system. N_b proliferates to greater concentrations than N_a, and although N_a is present at high enough concentrations to be considered in this coinfection, it continues to naturally die out of the system over the two-week infection period.

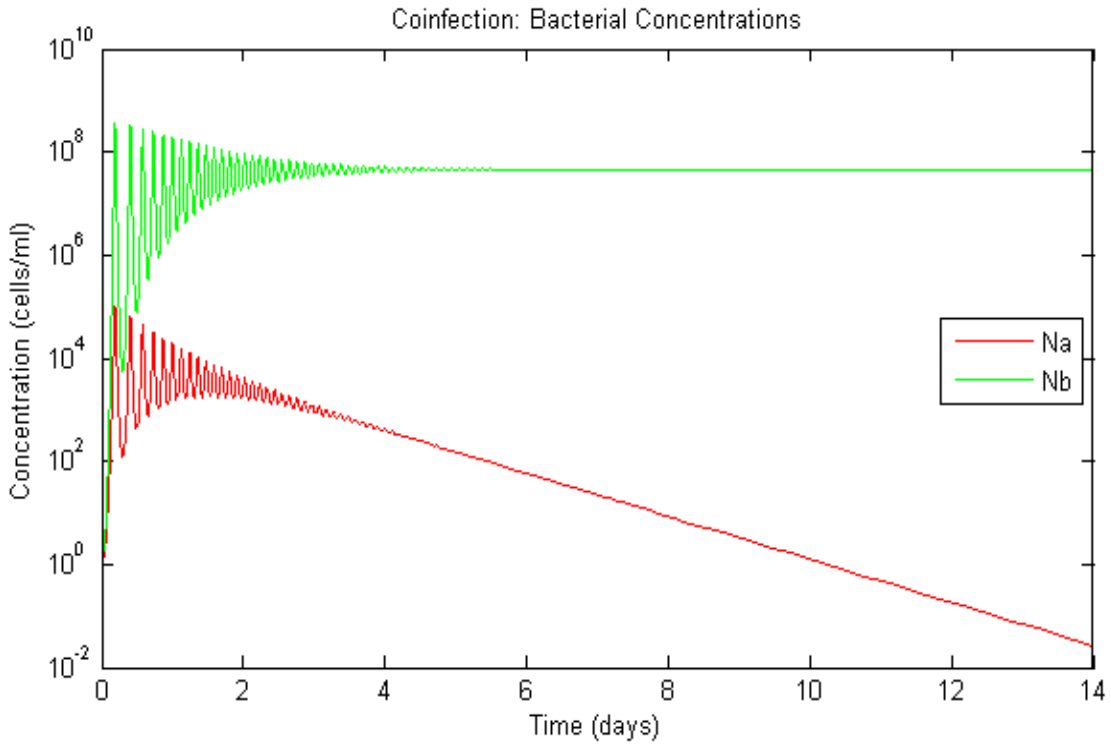

Figure 3 Coinfection equilibrium. N_a concentrations normalize ~10^8 cells/ml and N_b concentrations never normalize and eventually die out of the system after 14 days.

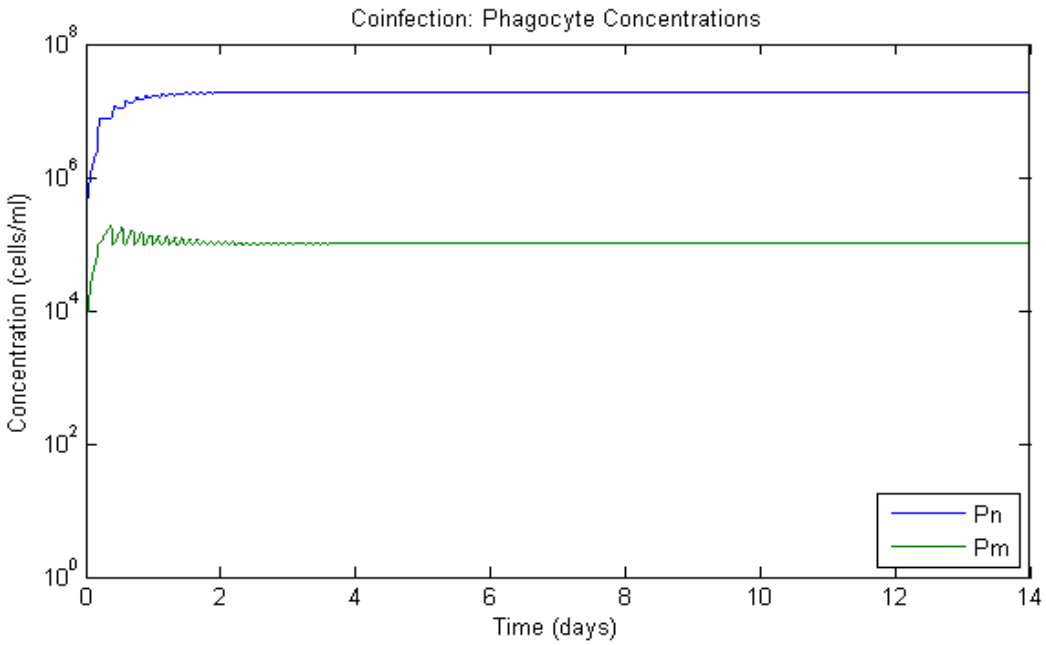

Figure 4 Coinfection equilibrium. Both bacteria are present in the system. Neutrophil concentrations normalize to ~10^7 cells/ml and macrophage concentrations normalize to ~10^5 cells/ml.

Resource Reduction

The next set of simulations aimed to model what changes occurred when the iron resource was voluntarily reduced as an uncharacteristic treatment method to starve the proliferating bacteria in the host. Decreasing the background production rate of iron from ~8700 nanograms per milliliter per day to 2500 nanograms per milliliter per day produced competition between the two species, and both N_a and N_b were driven out of the system in less than 1.5 days, as shown in Figure 5. For this scenario, the neutrophils concentrations normalized to $8.5 \cdot 10^6$ cells/ml and the macrophage concentrations normalized to $2.0 \cdot 10^6$ cells/ml. The iron concentrations normalized to 8200 ng/ml, healthy concentrations for our host.

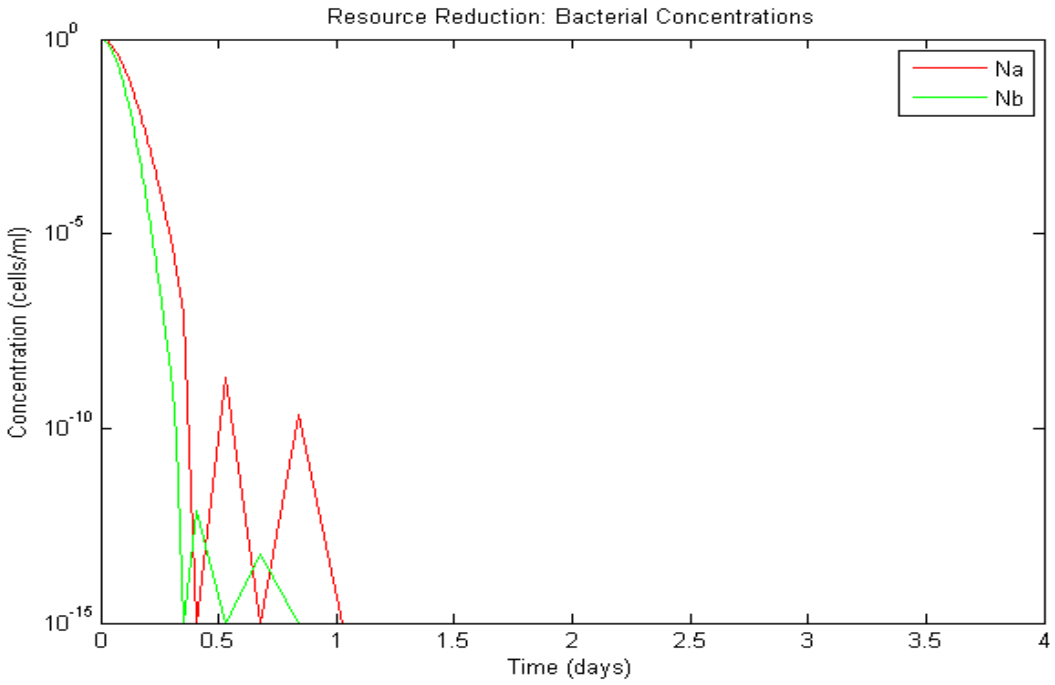

Figure 5 Resource reduction where θ was reduced to ~2500 ng/ml day. Both the Na and Nb decreased to unviable concentrations, and were considered cleared from the system in less than 1.5 days.

Neutrophil Amplification

The next set of simulations aimed to model what changes occurred when the neutrophil concentrations were amplified as an uncharacteristic treatment method to overwhelm the proliferating bacteria with an increased population of predators in the host. Increasing the background production rate of neutrophils in the body from ~$1.67 \cdot 10^7$ cells per milliliter per day to ~$4.50 \cdot 10^7$ cells per milliliter per day produced competition between the two species, where N_a was cleared from the system in less than 0.5 days and N_b needed almost 10 days to clear, as shown in Figure 6. For both of these scenarios, the neutrophil concentrations normalized to $2.25 \cdot 10^7$ cells/ml, the macrophage concentrations normalized to $2.5 \cdot 10^6$ cells/ml. The iron concentrations normalized to about 3750 ng/ml, above our threshold value, but now no longer a healthy concentration for our human.

Macrophage Amplification

Amplification of the macrophage concentrations proved to be impractical, as there needed to be an increase of at least 1000% in order to change the dynamics within the system. Therefore, these simulations were disregarded.

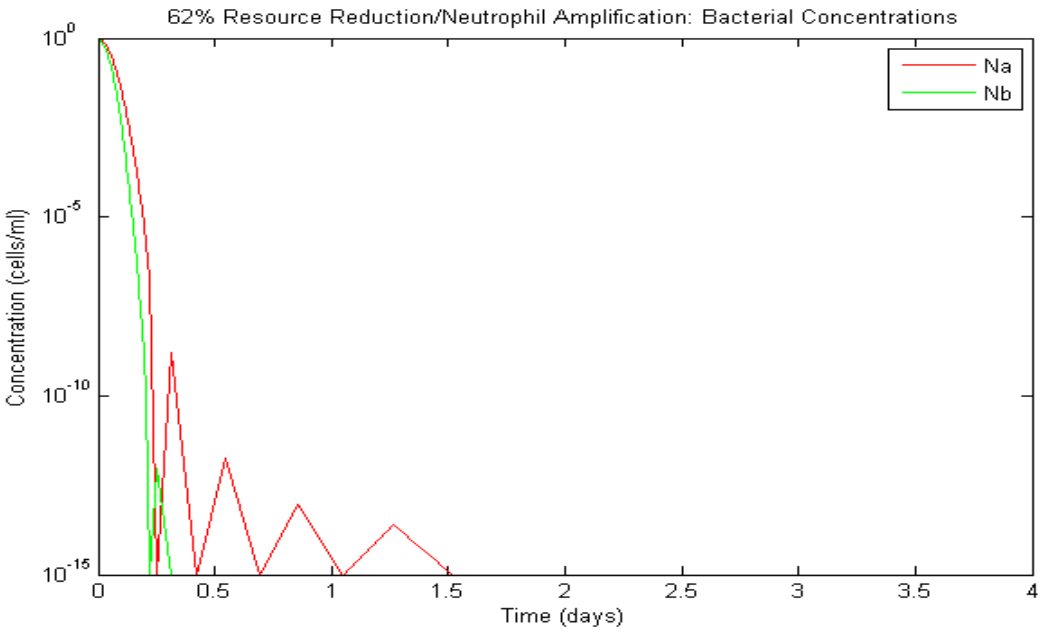

Figure 7 Resource reduction and neutrophil amplification simulation, where ρ_n was increased to $2.7 \cdot 10^7$ cells/ml·day and θ was reduced to ~3300 ng/ml·day (62% difference). Both the N_a and N_b decreased to unviable concentrations, and were considered cleared from the system, in under 0.5 days.

Resource Reduction and Neutrophil Amplification

The next set of simulations aimed to model what changes would occur should the iron resource be reduced at the same time the neutrophil concentrations were amplified. These percentages of decrease in the iron background rate and the increase in the neutrophil background rate were done symmetrically. It wasn't until a 62% reduction in iron and amplification in neutrophil concentrations that the dynamic changed, where this was enough to clear the coinfection in less than 2 days, as shown in Figure 7. The neutrophil concentrations normalized to $1.4 \cdot 10^7$ cells/ml and the macrophage concentrations normalized to $2.5 \cdot 10^6$ cells/ml. The iron concentrations normalized to 11000 ng/ml, the highest resource concentration we witnessed in our simulations.

DISCUSSION

This theoretical model was created as a representation of possible scenarios to the coinfection model that has been designed throughout the past four semesters. It was improved by redesigning the flowchart, and thus the set of equations, to represent more realistic interactions, as well as an updated set of parameter values. In the previous model, before specific bacteria were chosen, the

parameters values were based on generic average values for the bacterial population. The model is designed to represent the dynamics that develop when two bacterial species infect one human host, where both the species rely on one common resource and are combated by the host's innate immune system, made up of neutrophils and macrophages. The common resource used is one that most bacteria need to proliferate within our system, iron. The bacteria would take up the resource at varying rates and also elicit the immune cells at rates different from the other.

Modifications were made to the original, generic flowchart, where previously only four significant populations were considered, and now there are five: the resource population, the two bacterial species, the neutrophil population, and the macrophage population. This flowchart formed five differential equations representing the rate of change of each population based on the natural changes within the population themselves, and their interactions with the other populations. Each population has unique characteristics that change its concentration within a human host, which were represented with parameter values from reliable literary sources. These baseline values were integrated into the set of equations and were run in the computer program MATLAB, which, when analyzed, produced unrealistic biological results. The parameter values were then calibrated and set to produce applicable and realistic results for the coinfection scenario. The simulations that produced meaningful results were translated into percentages for easy comparison and to pinpoint which action for treatment was most efficient. In any simulation where the host's resource concentrations were drained to fatal levels, the amplifications and reductions were not considered as viable treatment methods. However, when our host was able to sustain itself, based on its iron levels, then these simulations were considered in drawing our conclusions.

Treating a proliferating coinfection between two uncharacteristic bacterial species is not a straightforward decision. Heavy antibiotic usage is now discouraged, as it can increase selective pressures within bacterial species and encourage antibiotic resistance to develop. Manipulating the body's resource availability and immune responses could be a viable option in treating bacterial infections without significant amounts of antibiotics. This is specifically what this theoretical model hoped to answer: which natural process could be amplified or reduced to most efficiently clear a coinfection in a human host.

In general, the lower the percentage of amplification or reduction of any concentration would be preferable to a drastic change, which can cause other harmful side effects to the host that can outweigh the benefit of it clearing the preexisting coinfection. With this in mind, amplifying the macrophage concentrations is not a probable option for our system. There needed to be at least a 1000% increase in order to change the dynamics within the system. This significant increase is impractical and arguably out of reach. This population does not seem to influence the dynamics of the system strongly enough to consider it as a treatment option. Increasing the neutrophil concentration was far more efficient, yet still a risk for the host, needing a 169% increase to clear the coinfection. Reducing the iron concentration was even more efficient, needing about a 71%

to clear the coinfection. In comparing a 169% increase in neutrophil concentrations to a 71% decrease in iron concentrations, a reduction in resource availability would be the better option for our host. The harmful side effects from such amplifications in neutrophil concentrations, such as severe tissue inflammation, outweigh the risks of decreasing the host's iron reserves. Combining the decrease in iron concentration and the increase in neutrophil concentration was the most efficient way to rid the system of both bacterial species. A 62% reduction in iron concentrations and a 62% amplification in neutrophil concentrations proved to be effective at driving both species out of the system. This should greatly minimize the side effects from both treatment methods, proving this natural manipulation of the body's resources and defenses to be an applicable treatment method as an alternative to the overuse of antibiotics.

In continuing with this model, there are several places where improvements could be made. To more accurately describe how a proliferating infection affects the body, there would need to be some representation of the immune system relying on the common iron resource as well. As the bacteria continue to consume the nutrients in the body, the host becomes more deprived of them and more unfit, which is usually reflected in the immune system's effectiveness in responding to an infection. The current model assumes that the phagocyte populations would be one hundred percent efficient throughout the entire infection, which is not an accurate representation of our body's natural defenses. Our immune system wanes as an infection begins to dominate our system, and this is another source of fatality for the host that is not currently represented by this model. Currently, only iron levels define our host's fatality within this system.

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