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The impact of cell regeneration on the dynamics of viral coinfection

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Many mathematical models of respiratory viral infections do not include regeneration of cells within the respiratory tract, arguing that the infection is resolved before there is significant cellular regeneration. However, recent studies have found that $\sim 40\%$ of patients hospitalized with influenza-like illness are infected with at least two different viruses, which could potentially lead to longer-lasting infections. In these longer infections, cell regeneration might affect the infection dynamics, in particular, allowing for the possibility of chronic coinfections. Several mathematical models have been used to describe cell regeneration in infection models, though the effect of model choice on the predicted time course of viral coinfections is not clear. We investigate four mathematical models incorporating different mechanisms of cell regeneration during respiratory viral coinfection to determine the effect of cell regeneration on infection dynamics. We perform linear stability analysis for each of the models and find the steady states analytically. The analysis suggests that chronic illness is possible but only with one viral species; chronic coexistence of two different viral species is not possible with the regeneration models considered here. *Published by AIP Publishing.* [<http://dx.doi.org/10.1063/1.4985276>]

Respiratory virus infections are a leading cause of mortality worldwide. Studies have found that $\sim 40\%$ of patients hospitalized with influenza-like illness are infected with more than one virus, sometimes leading to longer duration of hospitalization and higher probability of admission to the intensive care unit compared to single viral infection. Mathematical models have been used to examine respiratory viral infection dynamics; however, many of them do not include regeneration of cells within the respiratory tract since the time course of these infections is shorter than any significant cellular regeneration. For longer infections, however, cell regeneration might influence the infection dynamics. We investigate four mathematical models incorporating cell regeneration during respiratory viral coinfection to determine the effect of cell regeneration on infection dynamics. By performing stability analysis of the models, our investigation suggests that cell regeneration can lead to chronic disease but with only one viral species.

I. INTRODUCTION

Respiratory diseases are the third leading cause of death in the world.¹ One of the major causes for these diseases is viral infections of the respiratory tract. These infections can be severe enough to cause chronic diseases such as asthma, pneumonia or bronchiolitis especially in immunocompromised patients like the elderly and children.²⁻⁴ With the recent development of molecular biology techniques such as multiplex polymerase chain reaction, not only a larger number of viruses have been detected but also more than one virus has been detected in the same respiratory specimen.^{5,6} Infections with multiple respiratory viruses in the same patient have now been reported in many studies.⁷⁻²³ Several

investigations reported that respiratory tract infections with more than one virus are found in 70% (Ref. 7) of the hospitalized patients who are suffering from severe bronchiolitis, although other studies show that the prevalence rates may be lower, varying from 15% to 39%.^{7,19,24} The resistive ability of the host to the viruses depends on the nature of the participating viruses and is significantly different in single and multiple respiratory viral infection.¹⁹

Many of the viruses responsible for respiratory infections co-circulate around the same time of the year, mainly during winter months, and target the epithelial cells in the respiratory tract.¹⁰ They share not only the same epidemic season but also the same replication site,²⁰ making it possible to simultaneously infect the same host. Under these circumstances, the growth of one virus can affect that of other viruses in some way.^{25,26} The clinical impacts of coinfection in patients are not clear and need to be better understood. There is evidence that disease severity due to coinfection is as severe,^{12,13,15} less severe,^{11,12} or more severe^{8,9} than single virus infections. Detailed studies of the time course of these infections have not yet been done even though understanding the dynamics of coinfection is important, as treating one infection might affect the other infection during coinfection.

The use of mathematical modeling as a tool to study many areas of the sciences is growing. Models are essential for finding answers where laboratory experiments are impossible, impractical, or expensive. They also make it possible to identify the most important processes that govern behavior of a biological system. In viral disease modeling, models are used as quantitative tools to explain biological mechanisms that cause changes in the viral load during viral infections and have contributed significantly to the understanding of viral dynamics.²⁷ Mathematical modeling of viral dynamics for human immunodeficiency virus (HIV),^{28,29} hepatitis B virus (HBV),^{30,31} hepatitis C virus (HCV),³² and

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influenza^{33,34} infections have played a role in disease control and drug treatment strategies.

Our previous work³⁵ was the first to investigate infection dynamics of two viruses in the human respiratory tract. We found that the faster growing virus would suppress growth of the other virus during a coinfection. The basic coinfection model predicted that typical respiratory coinfections would last between 6 and 10 days, which is not any longer than the typical duration of single virus infections. Part of the reason for this result is that cell regeneration was not included in the model, so once the initial target cell population was depleted, the infection resolved. The lack of cell regeneration also precludes the possibility of chronic coinfections. Unfortunately, some studies have noted that days of hospital stay are longer for patients who are infected by more than one virus than those of single virus infected patients.^{19,21} In order to more accurately model these longer-lasting infections, we extend the coinfection model to include cell regeneration.

Epithelial cells are regenerated normally in the body according to homeostatic mechanisms. Though some epithelial cells show a high rate of constant cell regeneration, respiratory tract epithelial cells regenerate slowly during normal adult homeostasis.³⁶ Once infected, however, these epithelial cells have the ability to regenerate new cells rapidly.³⁷ An infection in the respiratory tract may cause a decline in cell counts as these infections are responsible for cell damage. During viral infections, the respiratory system can initiate self renewal of epithelial cells in the lung by stimulating not only progenitor cells near dead cells but also through cell replacement by proliferation of the remaining undamaged cells.³⁸ Investigations that incorporate cell regeneration into the model are mostly used for long-lasting diseases such as HIV^{39,40} or HCV,^{41,42} but regeneration is typically neglected for respiratory infections since initiation of epithelial cell regeneration time is long compared to the duration of the viral infection.⁴³ Since coinfections can last longer than single infections,²¹ however, cell regeneration might play an important role in the dynamics of coinfections.

In this study, we extend our previous model and analyze a more biologically realistic mathematical model of coinfection that incorporates cell regeneration of the host compared to the basic model from our previous work.³⁵ Here, we examine four different models for cell regeneration and compare the predicted outcomes by presenting stability analysis. We calculate the infection-free equilibrium and boundary equilibria, which are chronic infections with only a single virus, along with the basic reproductive numbers. We also determine stability criteria for the infection-free equilibrium and boundary equilibria. Finally, computer simulations are presented to support the analytical results from the stability analysis. Our results show that the addition of cell regeneration allows chronic infections, although with only a single virus; chronic coinfections are not possible even with more resources for the viruses.

II. METHODS

A. Basic model

We extend the simple compartmental ordinary differential equation model for coinfection which we proposed in previous work³⁵

$$\begin{aligned} \dot{T} &= -\beta_1TV_1 - \beta_2TV_2, \\ \dot{E}_1 &= \beta_1TV_1 - k_1E_1, \\ \dot{E}_2 &= \beta_2TV_2 - k_2E_2, \\ \dot{I}_1 &= k_1E_1 - \delta_1I_1, \\ \dot{I}_2 &= k_2E_2 - \delta_2I_2, \\ \dot{D}_1 &= \delta_1I_1, \\ \dot{D}_2 &= \delta_2I_2, \\ \dot{V}_1 &= p_1I_1 - c_1V_1, \\ \dot{V}_2 &= p_2I_2 - c_2V_2. \end{aligned}$$

In this model (Fig. 1), infection is initiated by two viruses, V_1 and V_2 , by infecting the available target cells (susceptible cells), T , at corresponding infection rates of the two viruses β_1 and β_2 . Here, each cell is infected by only one type of virus. Once infected, cells enter an eclipse phase, E_1 or E_2 , where these newly infected cells are involved in the intracellular process of viral replication before actually producing virus particles. After average transition times $\frac{1}{k_1}$ and $\frac{1}{k_2}$, the cells become productively infectious cells, I_1 and I_2 , which produce viruses at rates p_1 and p_2 . Thus, successive cycles of cell infections quickly result in an exponential growth of viruses of both kinds, V_1 and V_2 . Virus replication continues over the life span of these infected cells, respectively, $\frac{1}{\delta_1}$ and $\frac{1}{\delta_2}$, after which the infectious cells die and are

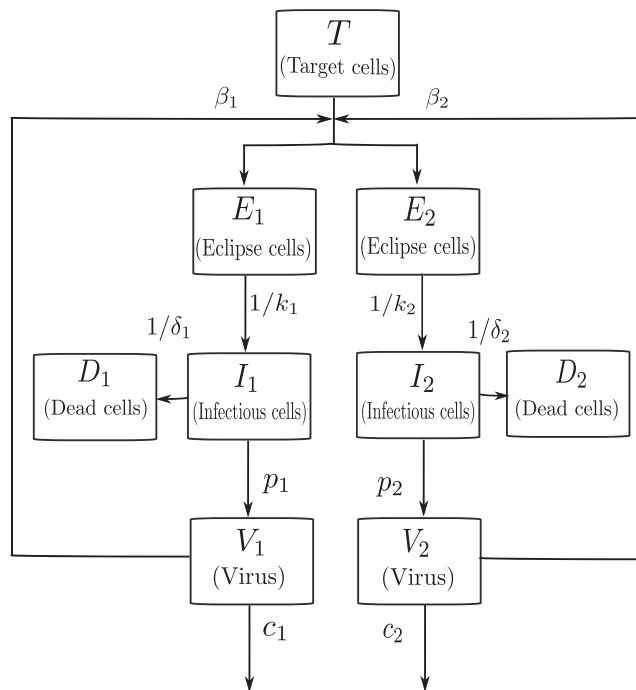


FIG. 1. Compartmental model diagram of coinfection by two viruses. The viruses, V_1 and V_2 , infect the same type of target cell population, T , but do not infect the same cell. Then, they enter into eclipse phases, E_1 and E_2 , where they take time of $\frac{1}{k_1}$ and $\frac{1}{k_2}$ before they go into the actively producing viral phases of I_1 and I_2 , where they produce viruses at rates of p_1 and p_2 . The newly produced viruses go on to infect other target cells. Free viruses are cleared at clearance rates of c_1 and c_2 . Infected cells die at rates of $\frac{1}{\delta_1}$ and $\frac{1}{\delta_2}$ and are counted as dead cells, D_1 and D_2 .

counted as dead cells, D_1 and D_2 . Some of the newly produced free viruses get cleared at rates c_1 or c_2 . Model parameters and variables are defined in Table I. In this basic model, cell regeneration is not considered because epithelial cells regenerate in 5–7 days and might take up to one month to completely grow^{33,37} while uncomplicated viral infections are short compared to the time it takes for cells to regenerate in the respiratory tract.

We also ignore several other factors to simplify the biological complexity of the real system, avoiding estimation of the values of the extra parameters which would be necessary to explain those factors. For example, the immune response is not considered in this model since little quantitative information is available about the interaction between host’s immune response and respiratory infectious viruses. Although there have been some attempts to incorporate the immune response into mathematical models of infection, for acute infections, experimental immune data are often too sparse to build accurate models.⁴⁴ Here, two different viruses infect the same type of target cells but not the same cell simultaneously which will not necessarily always be the case.^{45–47} Finally, exponential distributions for eclipse and infectious transition times are considered to simplify the computation even though it is known to be biologically unrealistic (a cell is not able to produce virus as soon as it is infected).^{48,49}

B. Different regeneration models

Since the results from our previous work³⁵ seem to depend on the fact that the viruses are competing for a limited cell population, the model is expanded here to include cell regeneration in different forms. Regeneration of

TABLE I. Definition of model variables and parameters.

Variable	Definition			Units
T	Number of uninfected target cells			Relative cell counts
E	Number of cells in eclipse phase			Relative cell counts
I	Number of infectious cells			Relative cell counts
D	Number of dead cells			Relative cell counts
V	Infectious viral titer			PFU/ml
Parameter	Definition	IAV ^a	RSV ^a	Units
T_0	Initial target cells	1.0	1.0	Relative cell counts
V_0	Initial viral titer	1.0	1.0	PFU/ml
β	Viral infection rate	82.73×10^{-7}	0.03	(PFU/ml) ⁻¹ day ⁻¹
k	Transition rate from E to I	4.20	1.27	day
δ	Death rate of infectious cells	4.20	1.27	day
p	Viral production rate	0.12×10^9	76.45×10^2	(PFU/ml) day ⁻¹
c	Viral clearance rate	4.03	1.27	day ⁻¹
r	Target cell regeneration rate	0.03 ^b	0.03 ^b	day ⁻¹
R_0	Basic reproduction number	58.6	142.19	

^aParameter values are taken from Ref. 35.

^bParameter value from Refs. 33 and 37.

epithelial cells in the respiratory tract can be modeled in several ways. Here, we examine four different ways to model cell regeneration in the human respiratory tract.

1. Model 1: Constant growth

Constant growth rate has been considered in many studies^{39,40,50,51} to model population growth. In this model, we assume that the target cell population are produced at a constant rate, r , in the human respiratory tract, modeling the scenario that cells are produced from sources within the living organism of the host to maintain homeostasis. This creates an unlimited supply of cells for the two viruses to promote infection. Keeping all other system variables the same as that of the basic model, the target cell equation now becomes

$$\dot{T} = -\beta_1TV_1 - \beta_2TV_2 + r.$$

This is the simplest way one can model cell regeneration.

2. Model 2: Target cell replication

Model 2 refers to cell growth that is proportional to the available target cells in the respiratory tract. Here, new cells are a result of reproduction of the available target cells. This type of regeneration model has been used previously to model influenza infection dynamics.⁵² Keeping all other system variables the same as that of the basic model, the target cell equation becomes

$$\dot{T} = -\beta_1TV_1 - \beta_2TV_2 + rT.$$

3. Model 3: Replacement of dead cells

Model 3 considers cell regeneration in the form of cell growth proportional to dead cells. There is evidence that cell destruction stimulates target cell reproduction,³⁷ so that when there is more cell death, the remaining target cells will reproduce at a faster rate. Handel *et al.* and Bauer *et al.* used this type of regeneration in their models of influenza virus.^{53,54} Keeping all other system variables the same as that of the basic model, the target cell and dead cell equations become

$$\begin{aligned} \dot{T} &= -\beta_1TV_1 - \beta_2TV_2 + r(D_1 + D_2), \\ \dot{D}_1 &= \delta_1I_1 - rD_1, \\ \dot{D}_2 &= \delta_2I_2 - rD_2. \end{aligned}$$

4. Model 4: Logistic growth

Logistic growth is another popular way to incorporate cell regeneration into mathematical models.^{42,50,51,55–57} The logistic function gives a density dependent growth rate which causes cell regeneration to slow down when target cell counts get high. In this model, we assume logistic growth of target cells with a carrying capacity of 1. So keeping all other system variables the same as that of the basic model, the target cell equation becomes

$$\dot{T} = -\beta_1TV_1 - \beta_2TV_2 + rT(1 - T - E_1 - I_1 - E_2 - I_2).$$

C. Computer simulation

For numerical simulations, we consider influenza A virus (IAV) and respiratory syncytial virus (RSV) coinfection in the human respiratory tract, with parameter values for both viruses taken from fits to experimental data as detailed in Fig. 3 of our previous work.³⁵ For both viruses, the data consist of viral load only, so the units of cell measurements are unspecified. We assume the initial number of target cells is 1, so the units of the remaining cell variables are in cell counts relative to the initial number of target cells. Viral units are determined by the experimental data and are in plaque forming units (PFU) per milliliter. The values are also given in Table I. Simulations are implemented using the *lsode* function in Octave 3.6.4 to solve the system of differential equations.

III. RESULTS

Our primary interest is in investigating whether the addition of cell regeneration will allow for the existence of chronic coinfection. Thus, when we determine the steady states of each of the models, we are looking for steady states where both V_1 and V_2 are non-zero. The existence of this steady state is not enough, however, to conclude that chronic coinfections might be observed in patients since unstable steady states are not likely to be observed in practice. We also need to examine the stability of the chronic coinfection to determine whether there are parameter values for which chronic coinfection is a stable steady state.

A. Stability analysis of the basic model

We first examine the possible dynamics of the coinfection model without cell regeneration. Let $Q = (T^*, V_1^*, V_2^*, E_1^*, E_2^*, I_1^*, I_2^*, D_1^*, D_2^*)$ be the non-negative steady state solution of the model. The steady states of this model give one infection free equilibrium, showing no coexistence of two viral infections.

1. Infection free steady state

$$Q_{0T} = (T^* = T, \quad V_1^* = 0, \quad V_2^* = 0, \quad E_1^* = 0, \quad E_2^* = 0, \\ I_1^* = 0, \quad I_2^* = 0).$$

This model has a one-parameter family (parameterized by the non-negative values of T) of steady states. Since there are no viruses of either type ($V_1^* = 0$ and $V_2^* = 0$), the steady states are considered infection free states. If virus is inoculated into the healthy cells (target cells), the system will approach one of these steady states depending on the parameter values and initial conditions of the system variables.

a. Eigenvalues. Linearising the model near the equilibrium, the eigenvalues of these steady states, Q_{0T} , are

- $\lambda_1 = 0$
- $\lambda_2 = 0$
- $\lambda_3 = 0$
- $\lambda_{4,5,6} =$ Roots of the function $F_1(\lambda)$ and
- $\lambda_{7,8,9} =$ Roots of the function $F_2(\lambda)$

where $F_i(\lambda)$ is given by

$$F_i(\lambda) = \lambda^3 + \lambda^2(c_i + \delta_i + k_i) + \lambda(c_i\delta_i + \delta_i k_i + c_i k_i) \\ + (c_i\delta_i k_i - \beta_i p_i k_i T),$$

with $i = 1, 2$.

Using the Routh-Hurwitz criterion of stability, we obtain the following necessary conditions for local asymptotic stability of this state. As the constant coefficients $(c_i + \delta_i + k_i)$ and $(c_i\delta_i + \delta_i k_i + c_i k_i)$ are always positive, the non-trivial conditions are as follows:

$$(c_i\delta_i k_i - \beta_i p_i k_i T) > 0 \\ (c_i + \delta_i + k_i)(c_i\delta_i + \delta_i k_i + c_i k_i) > (c_i\delta_i k_i - \beta_i p_i k_i T).$$

The first condition can be re-written in terms of the basic reproductive number for single virus infection, $R_{0i} = \frac{\beta_i p_i T}{c_i \delta_i}$, giving the condition that $R_{0i} < 1$. This first condition states the stability of a single virus infection if satisfied. The second condition can also be re-written in this way giving a lower bound

$$R_{0i} > 1 - (c_i + \delta_i + k_i) \left(\frac{1}{c_i} + \frac{1}{\delta_i} + \frac{1}{k_i} \right).$$

The eigenvalue $\lambda_1 = 0$ is the eigenvalue for the eigenvector that lies along the target cell axis in the phase space of the model, which means that there is no growth or decay of small perturbations along this axis. Since all values of T are possible fixed points, the system shows no preference for a particular value of T .

Eigenvalues given by the functions $F_1(\lambda)$ and $F_2(\lambda)$ determine viral infection due to a particular virus. For example, if the roots of $F_1(\lambda)$ are positive, then an initial inoculum of virus V_1 will grow and an acute infection will occur. If the roots of $F_1(\lambda)$ are negative, then an initial inoculum of virus V_1 decays with time and there will not be an infection. Infection dynamics due to the second virus, V_2 , are determined by the roots of $F_2(\lambda)$ in the same manner. It is noteworthy that chronic infection as well as chronic coinfection is not possible with either of the viruses in the absence of cell regeneration. We could, however, see acute infections with either virus or an acute coinfection if the roots of both $F_1(\lambda)$ and $F_2(\lambda)$ are positive.

In the case of an acute infection, the system will end up in the trivial steady state where $T = 0$. While this seems biologically unrealistic, such target-cell limited models are commonly used to model within host infections.^{33,58} In reality, the trivial steady state corresponds to death of all target cells, but not all cells in the respiratory tract are target cells for infectious viruses. Respiratory viruses show a preference for certain cell types within the respiratory tract determined by the cell surface receptors to which they bind.^{46,59,60} There are also some target cells that will be protected by the immune response as the infection progresses^{44,61} (not explicitly included in our model) and will remain at the end of the infection. Finally, there is evidence that respiratory infections do cause massive cell death in the respiratory tract,⁶²⁻⁶⁴ but most patients recover from

this. For these reasons, the trivial steady state is biologically plausible.

b. Basic reproductive number. Overall growth of the coinfection is determined by the value of the basic reproductive number, R_0 , of the model. R_0 can be found from the spectral radius (largest eigenvalue) of the next generation matrix, i.e., (FV^{-1}) , where F is the transmission matrix and V is the transition matrix supplied by the system of equations.⁶⁵ A detailed calculation is shown in the appendix. R_0 for this model is given by

$$R_0 = \max(R_{01}, R_{02}),$$

where R_{01} is the basic reproductive number for the first virus, V_1 , and R_{02} is that for the second virus, V_2 . A threshold condition for the growth of infection is given by $R_0 = 1$. So if the value of R_0 is greater than 1, the corresponding steady state is unbounded, which means growth of infection with either of the two viruses in the respiratory tract. If R_0 is less than 1 or R_{01} and R_{02} are individually less than 1, there will be no growth of infection due to either of the viruses. Thus, the infection free steady state, Q_{0T} , is locally asymptotically stable if $R_0 < 1$ and unstable if $R_0 > 1$.

2. Infection free steady state when $T^* = 0$

$$Q_0 = (T^* = 0, \quad V_1^* = 0, \quad V_2^* = 0, \quad E_1^* = 0, \quad E_2^* = 0, \\ I_1^* = 0, \quad I_2^* = 0).$$

a. Eigenvalues. This trivial steady state is an infection free equilibrium where all the state variables are zero. This steady state arises after an acute illness as both of the viruses become zero after consuming all the susceptible target cells within the host.

Linearizing the system of equations near the state, Q_0 , the eigenvalues are

$$\begin{aligned} \lambda_1 &= 0, \\ \lambda_2 &= 0, \\ \lambda_3 &= 0, \\ \lambda_4 &= -c_1, \\ \lambda_5 &= -c_2, \\ \lambda_6 &= -\delta_1, \\ \lambda_7 &= -\delta_2, \\ \lambda_8 &= -k_1, \\ \lambda_9 &= -k_2. \end{aligned}$$

This steady state is globally stable since all the eigenvalues are negative or zero. If the other steady state ($T^* = T$) is not stable, the system ends up in this equilibrium.

An example of the dynamics of coinfection without regeneration is shown in Fig. 2 for influenza A virus (IAV) and respiratory syncytial virus (RSV). The replication of one

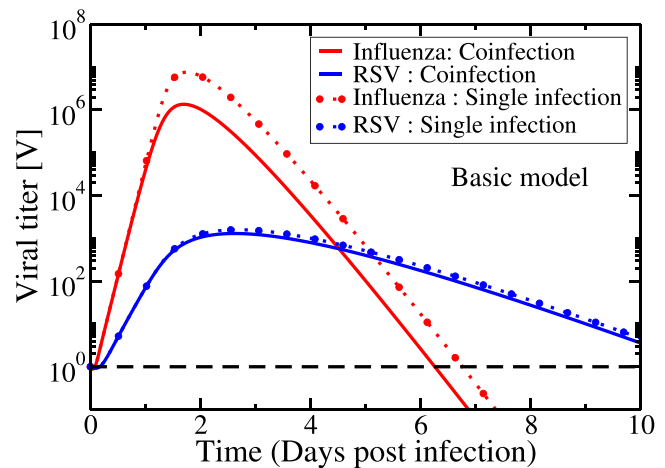


FIG. 2. Coinfection time course of RSV and influenza A virus for the basic model (no cell regeneration) with single infection in dotted line.

virus (RSV) is reduced, as compared to RSV single infection, by the presence of the other virus (IAV), while replication of the other virus (IAV) is largely unaffected. In this case, the infection resolves itself due to the consumption of all target cells, leaving the two viruses without new cells to infect. Here, the values of R_{01} and R_{02} corresponding to IAV and RSV, respectively, are found to be $R_{01} = 58.65$ and $R_{02} = 142.19$.

B. Stability analysis of cell regeneration models

Models incorporating cell regeneration into the disease dynamics give several possible steady state solutions: trivial equilibrium where all the variables become zero, Q_0 ; infection free equilibrium, Q_{0T} where all the variables go to zero except target cells, T ; and two boundary equilibria. In this paper, the term boundary equilibrium refers to a chronic single viral infection where either only the first virus, V_1 , exists (V_2 free state), Q_1 , or where only the second virus, V_2 , exists (V_1 free state), Q_2 . Interestingly, we find no steady state with co-existence of two viruses. In Sec. II B 1–II B 4, the steady states of the four models incorporating cell regeneration are presented along with their respective eigenvalues and stability conditions.

1. Model 1: Constant growth

For this model, an unlimited supply of target cells is included by adding a constant rate of cell birth, r , to the equation of target cells, T . Note that we have not included dead cells in the stability analysis of this model since they do not play a role in the dynamics of the infection. This model results in chronic infection with only one of the viruses, so there are two possible steady states.

a. Chronic infection states. There are two boundary equilibria where only one of the viruses will turn into a chronic infection,

$$\begin{aligned} Q_1 &= (T^*, V_1^*, V_2^* = 0, E_1^*, E_2^* = 0, I_1^*, I_2^* = 0), \\ Q_2 &= (T^*, V_1^* = 0, V_2^*, E_1^* = 0, E_2^*, I_1^* = 0, I_2^*), \end{aligned}$$

where

$$T^* = \frac{c_i \delta_i}{p_i \beta_i}, \quad V_i^* = \frac{p_i r}{c_i \delta_i},$$

$$E_i^* = \frac{r}{k_i}, \quad I_i^* = \frac{r}{\delta_i},$$

for $i = 1, 2$. As the constant parameters are positive, these equilibria exist and are biologically realistic.

Eigenvalues: The first three eigenvalues are given by the roots of the characteristic equation that consists of the functions, $P_{ij}(\lambda)$ and $R_i(\lambda)$. The function $P_{ij}(\lambda)$ is given by the equation

$$P_{ij}(\lambda) = \frac{1}{\beta_i c_i \delta_i p_i} (\lambda^3 + \lambda^2 A + \lambda B + C),$$

where $i, j = 1, 2$ and

$$A = \beta_i c_i c_j \delta_i p_i + \beta_i c_i \delta_i \delta_j p_i + \beta_i c_i \delta_i k_j p_i,$$

$$B = \beta_i^2 c_i^2 c_j \delta_i^2 \delta_j p_i^2 + \beta_i^2 c_i^2 c_j \delta_i^2 k_j p_i^2 + \beta_i^2 c_i^2 \delta_i^2 \delta_j k_j p_i^2,$$

$$C = \beta_i^2 c_i^4 c_j \delta_i^4 \delta_j k_j p_i^2 (R_{0i} - R_{0j}).$$

The remaining four eigenvalues are given by the roots of the function, $R_i(\lambda)$ which is given by the equation

$$R_i(\lambda) = \frac{1}{\beta_i c_i \delta_i p_i} (\lambda^4 + \lambda^3 D + \lambda^2 E + \lambda F + G),$$

where $i = 1, 2$ and

$$D = \beta_i c_i^2 \delta_i p_i + \beta_i c_i \delta_i^2 p_i + \beta_i c_i \delta_i k_i p_i + \beta_i^2 r p_i^2,$$

$$E = \beta_i^2 c_i^3 \delta_i^3 p_i^2 + \beta_i^2 c_i^3 \delta_i^2 k_i p_i^2 + \beta_i^2 c_i^2 \delta_i^3 k_i p_i^2 + \beta_i^3 c_i^2 \delta_i r p_i^3$$

$$+ \beta_i^3 c_i \delta_i^2 \gamma p_i^3 + \beta_i^3 c_i \delta_i k_i r p_i^3,$$

$$F = \beta_i^4 c_i^3 \delta_i^3 \gamma p_i^4 + \beta_i^4 c_i^3 \delta_i^2 k_i r p_i^4 + \beta_i^4 c_i^2 \delta_i^3 k_i r p_i^4,$$

$$G = \beta_i^5 c_i^4 \delta_i^4 k_i r p_i^5.$$

Using the Routh-Hurwitz stability criterion, Since A, B, D, E, F, and G are always positive, the only condition derived from the Routh-Hurwitz stability criterion that is not automatically satisfied for the first steady state, Q_1 , is

$$\frac{\beta_1 c_2 \delta_2 p_1}{\beta_2 c_1 \delta_1 p_2} > 0,$$

$$\frac{R_{01}}{R_{02}} > 0.$$

Also for the second steady state, Q_2 , is

$$\frac{\beta_2 c_1 \delta_1 p_2}{\beta_1 c_2 \delta_2 p_1} > 0,$$

$$\frac{R_{02}}{R_{01}} > 0.$$

The system will end in a chronic infection with V_1 if the basic reproductive number for V_1 , R_{01} , is larger than the basic reproductive number for V_2 , R_{02} , or it will end in a

chronic infection with V_2 if the basic reproductive number for V_2 is larger than the basic reproductive number for V_1 .

2. Model 2: Target cell replication

In this model, cell growth is implemented as proportional to the availability of susceptible target cells, T . This model has only one steady state.

a. Infection free steady state. The steady state is the trivial infection free state, similar to that of the basic model

$$Q_0 = (T^* = 0, \quad V_1^* = 0, \quad V_2^* = 0, \quad E_1^* = 0, \quad E_2^* = 0,$$

$$I_1^* = 0, \quad I_2^* = 0).$$

Here, again target cells go to zero signifying an acute illness that ends up consuming all the available susceptible target cells during the coinfection.

Eigenvalues: The eigenvalues of this steady state are

$$\lambda_1 = 0,$$

$$\lambda_2 = 0,$$

$$\lambda_3 = -c_1,$$

$$\lambda_4 = -c_2,$$

$$\lambda_5 = -\delta_1,$$

$$\lambda_6 = -\delta_2,$$

$$\lambda_7 = -k_1,$$

$$\lambda_8 = -k_2,$$

$$\lambda_9 = r.$$

This trivial steady state is unstable as one of the eigenvalues is positive which is in contrast with the basic model with no cell regeneration where we found stable acute infection. The instability can be explained by the term rT which provides the single positive eigenvalue (λ_9). This causes cells to re-grow without bound, which is uninteresting in the absence of virus, but when virus is present, this can lead to an oscillatory response with cycles of acute infections. Introduction of a small amount of virus will lead to an acute infection which consumes almost all the available target cells. The few remaining target cells will start to regenerate the epithelium while virus is slowly cleared. If the clearance rate of virus is slower than the regeneration rate of target cells, there will be a fresh supply of target cells before all the virus is cleared from the system. This can then start another acute infection.

3. Model 3: Replacement of dead cells

This model considers cell regrowth stimulated by cell death in the respiratory tract. Here, three equilibria are found including the infection free equilibrium, Q_{0T} and boundary equilibria, Q_1 and Q_2 .

a. Infection free steady state. The first possible steady state is the infection free state where target cells, T , can have any value

$$Q_{0r} = (T^* = T, \quad V_1^* = 0, \quad V_2^* = 0, \quad E_1^* = 0, \\ E_2^* = 0, \quad I_1^* = 0, \quad I_2^* = 0, \quad D_1^* = 0, \quad D_2^* = 0).$$

Eigenvalues: Eigenvalues for this steady state are

- $\lambda_1 = 0$
- $\lambda_2 = -r$
- $\lambda_3 = -r$
- $\lambda_{4,5,6} = \text{Roots of the function } F_1(\lambda) \text{ and}$
- $\lambda_{7,8,9} = \text{Roots of the function } F_2(\lambda)$

The function $F_i(\lambda)$ is given by

$$F_i(\lambda) = \lambda^3 + \lambda^2(c_i + \delta_i + k_i) + \lambda(c_i\delta_i + \delta_i k_i + c_i k_i) \\ + (c_i\delta_i k_i - \beta_i p_i k_i T),$$

with $i = 1, 2$.

Using the Routh-Hurwitz criterion, we obtain the following conditions for the local asymptotic stability of this state

$$(c_i\delta_i k_i - \beta_i p_i k_i T) > 0, \\ R_{0i} < 1,$$

and

$$(c_i + \delta_i + k_i)(c_i\delta_i + \delta_i k_i + c_i k_i) > (c_i\delta_i k_i - \beta_i p_i k_i T), \\ R_{0i} > 1 - (c_i + \delta_i + k_i) \left(\frac{1}{c_i} + \frac{1}{\delta_i} + \frac{1}{k_i} \right).$$

The eigenvalues and stability criteria for this state are identical to those of the infection free state of the basic model with no cell regeneration.

Basic reproductive number: The associated R_0 for this steady state is given by the equation

$$R_0 = \max(R_{01}, R_{02}),$$

where

$$R_{0i} = \frac{\beta_i p_i T}{c_i \delta_i},$$

for $i = 1, 2$.

The basic reproductive number is also found to be identical to that of no cell regeneration model.

b. Chronic infection states. This model also has two possible chronic infection states

$$Q_1 = (T^*, V_1^*, V_2^* = 0, E_1^*, E_2^* = 0, I_1^*, I_2^* = 0, D_1^*, D_2^* = 0), \\ Q_2 = (T^*, V_1^* = 0, V_2^*, E_1^* = 0, E_2^*, I_1^* = 0, I_2^*, D_1^* = 0, D_2^*),$$

where

$$T^* = \frac{c_i \delta_i}{p_i \beta_i}, \quad E_i^* = \frac{c_i \delta_i V_i^*}{k_i p_i}, \\ I_i^* = \frac{c_i V_i^*}{p_i}, \quad D_i^* = \frac{c_i \delta_i V_i^*}{r p_i},$$

with $i = 1, 2$.

Eigenvalues: Eigenvalues of these steady states are

- $\lambda_1 = 0$
- $\lambda_2 = 0$
- $\lambda_{3,4,5} = \text{Roots of the function } P_{ij}(\lambda) \text{ and}$
- $\lambda_{6,7,8,9} = \text{Roots of the function } R_i(\lambda).$

Here, $P_{ij}(\lambda)$ is given by

$$P_{ij}(\lambda) = \frac{1}{\beta_i p_i} (\lambda^3 + \lambda^2 A + \lambda B + C),$$

where $i, j = 1, 2$ and

$$A = \beta_i c_j p_i + \beta_i \delta_j p_i + \beta_i k_j p_i, \\ B = \beta_i^2 c_j \delta_j p_i^2 + \beta_i^2 c_j k_j p_i^2 + \beta_i^2 \delta_j k_j p_i^2, \\ C = \beta_i^2 p_i^2 k_j c_j \delta_j c_i \delta_i (R_{0i} - R_{0j}).$$

$R_i(\lambda)$ is given by

$$R_i(\lambda) = \frac{1}{\beta_i p_i} (\lambda^4 + \lambda^3 D + \lambda^2 E + \lambda F + G),$$

where

$$D = \beta_i p_i (c_i + \delta_i + k_i - r) + \beta_i^2 p_i V_i^*, \\ E = \beta_i^2 p_i^2 \{ (c_i \delta_i + \delta_i k_i + k_i c_i) - r(c_i + \delta_i + k_i) \} \\ + \beta_i^3 c_i p_i^2 V_i^* + \beta_i^3 \delta_i p_i^2 V_i^* + \beta_i^3 k_i p_i^2 V_i^*, \\ F = \beta_i^3 p_i^3 \{ \beta_i V_i^* (c_i \delta_i + \delta_i k_i + k_i c_i) - r(c_i \delta_i + \delta_i k_i + k_i c_i) \}, \\ G = \beta_i^5 c_i \delta_i k_i p_i^4 V_i^*.$$

The conditions for local asymptotic stability of this state are determined by the inequalities

$$c_i + \delta_i + k_i > r, \\ \frac{c_i \delta_i + \delta_i k_i + k_i c_i}{c_i + \delta_i + k_i} > r, \\ \beta_i V_i^* > r.$$

The first criterion states that the boundary state is stable if the sum of transition rate from eclipse phase (k_i), viral decay rate (c_i), and infected cell death rate (δ_i) is greater than the regeneration rate. The quantities on the left all describe processes within the viral lifecycle, so this condition suggests that the replication process must be faster than cell regeneration. Additionally, the third condition states that the rate of cell regeneration has to be smaller than the rate of new infections ($\beta_i V_i^*$) by the virus, V_i , to maintain the chronic infection with V_i .

4. Model 4: Logistic growth

When the model assumes a logistic growth function, we find two kinds of infection free steady states.

a. Infection free steady state. The infection free steady state is where other variables remain zero and the target cells are equal to the initial value of $T_0 = 1$. This suggests two

possible scenarios. The first is that there was no infection by either of the viruses. A second option is that there was an acute infection leading to destruction of all cells, followed by the re-growth of all cells

$$Q_{01} = (T^* = 1, V_1^* = 0, V_2^* = 0, E_1^* = 0, E_2^* = 0, I_1^* = 0, I_2^* = 0).$$

Eigenvalues: Eigenvalues of this steady state are

- $\lambda_1 = 0$
- $\lambda_2 = 0$
- $\lambda_3 = -r$
- $\lambda_{4,5,6} =$ Roots of the function $F_1(\lambda)$ and
- $\lambda_{7,8,9} =$ Roots of the function $F_2(\lambda)$.

The function $F_1(\lambda)$ and $F_2(\lambda)$ are given by the equation

$$F_i(\lambda) = \lambda^3 + \lambda^2(c_i + \delta_i + k_i) + \lambda(c_i\delta_i + \delta_i k_i + c_i k_i) + (c_i\delta_i k_i - \beta_i p_i k_i),$$

where $i = 1, 2$.

Using the Routh-Hurwitz criterion, we obtain the following conditions for the local asymptotic stability of this state. As $(c_i + \delta_i + k_i)$ and $(c_i\delta_i + \delta_i k_i + c_i k_i)$ are always positive, the non-trivial conditions are

$$R_{0i} < 0, \\ R_{0i} > 1 - (c_i + \delta_i + k_i) \left(\frac{1}{c_i} + \frac{1}{\delta_i} + \frac{1}{k_i} \right),$$

where $i = 1, 2$. The eigenvalues and stability criteria for this state are identical to those of the infection free steady states of the basic model with no cell regeneration except that the number of target cells will always return to the maximum value.

Basic reproductive number: The associated R_0 for this steady state is given by the equation

$$R_0 = R_{01} + R_{02} - R_{01}R_{02},$$

as seen for other infection free equilibria.

b. Infection free steady state when $T^ = 0$.* The trivial infection free steady state is

$$Q_0 = (T^* = 0, V_1^* = 0, V_2^* = 0, E_1^* = 0, E_2^* = 0, I_1^* = 0, I_2^* = 0).$$

Eigenvalues: The eigenvalues are

$$\lambda_1 = 0, \\ \lambda_2 = 0, \\ \lambda_3 = -c_1, \\ \lambda_4 = -c_2, \\ \lambda_5 = -\delta_1, \\ \lambda_6 = -\delta_2,$$

$$\lambda_7 = -k_1, \\ \lambda_8 = -k_2, \\ \lambda_9 = r.$$

Again, the eigenvalues for this state are identical to those of the infection free steady state ($T^* = 0$) of model 2. This trivial steady state is unstable as one of the eigenvalues is positive.

We see that implementation of different types of cell regeneration leads to different dynamics. Our results are summarized in Table II. While two of the regeneration models investigated here allow chronic infections to develop, none of the models allow for chronic coinfections.

C. Application to RSV and influenza A virus

In this section, we present numerical simulations of the viral coinfection time course for each of the models. We take two of the more common respiratory viruses, IAV and RSV, to investigate the impact of cell regeneration on the dynamics of simultaneous infection in the human respiratory tract. Clinically, IAV-RSV is a common coinfection pair that is documented in several studies^{10,15,21} and are reported to have an impact on each other's transmission dynamics due to their seasonal co-circulation.²⁶ Parameter values of IAV and RSV are taken from our previous study³⁵ and are given in Table I. The results of the computer simulations are illustrated in Fig. 3 where both of the infections are initiated with the same initial amount of viruses and also at the same time. In the simultaneous infection with IAV and RSV, IAV causes an acute infection; however, it dies out quickly, while the slow growing RSV infection persists for more than two weeks, indicating a chronic infection with RSV alone for models of constant cell growth (model 1) and cell growth proportional to dead cells (model 3). Models of cell growth proportional to target cell (model 2) and logistic cell growth (model 4) predict a similar infection growth profile to the basic model of no cell regeneration. Since cell regeneration is proportional to available target cells in model 2 and target cell density regulates the cell regeneration rate in the logistic growth model (model 4), it is obvious that the abundance of target cells is restricted by the consumption of viruses, thus showing no possibility of chronic disease in simultaneous infection. Finally, no model shows coexistence of two viral infections even though there is an unlimited resource of target cells. These results coincide with the theoretical results of the stability analysis given in Table II. The boundary equilibria, Q_1 and Q_2 , of the models with constant growth (model 1) and growth proportional to dead cells (model 3) give

TABLE II. Dynamics of coinfection models.

Model	Steady states
0. No cell regeneration	Infection free equilibrium
1. Constant growth	Chronic single infection
2. Target cell replication	Trivial equilibrium
3. Replacement of dead cells	Infection free equilibrium, chronic single infection
4. Logistic growth of target cells	Infection free equilibrium

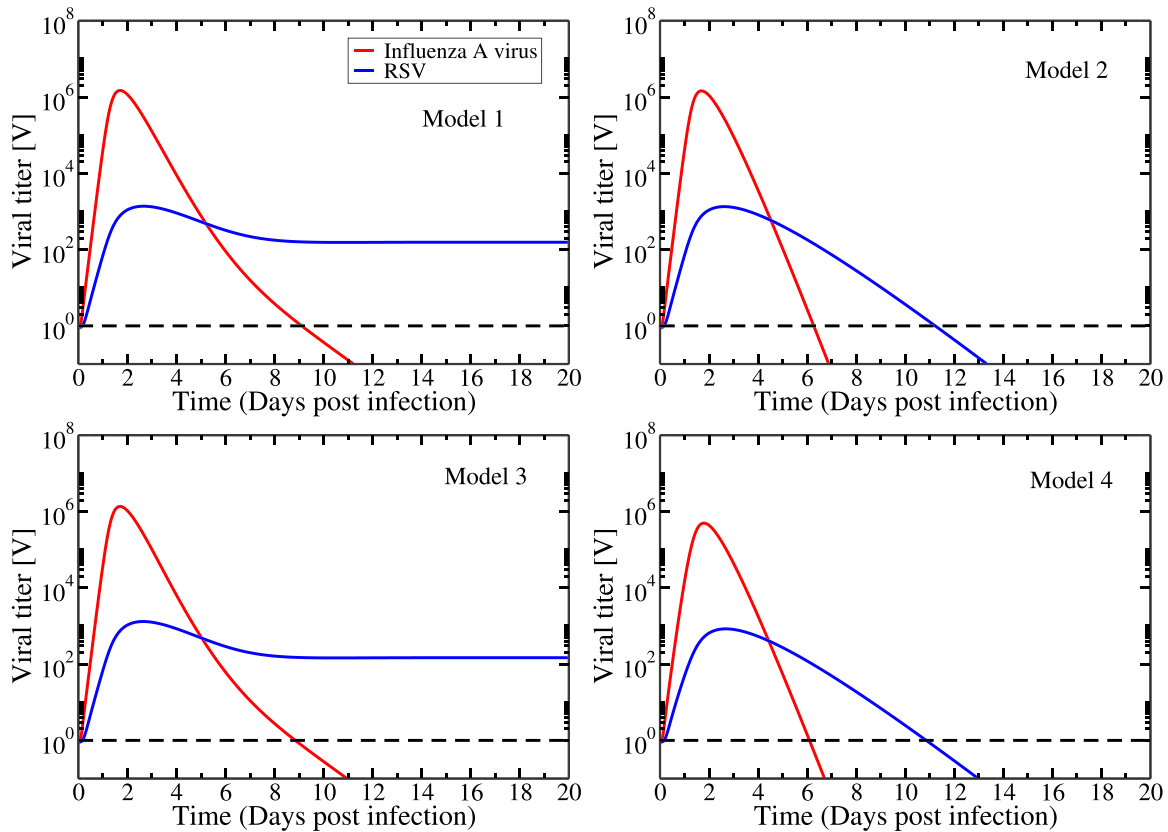


FIG. 3. Coinfection time course of RSV and influenza A virus for different cell regeneration models with regeneration rate, r equal to 0.0333 per day.

chronic infection with only one virus from RSV and IAV while in models of growth proportional to target cell (model 2) and logistic cell growth (model 4), acute infection with IAV persists for a few days and dies within two weeks along with the mild RSV infection.

We also calculated the duration of coinfection for each of the models, as shown in Fig. 4. Here, coinfection duration is defined as the time during which both viral concentrations are found above the detection limit of 1.0 PFU/ml or 1.0 TCID₅₀/ml. As earlier, models of cell growth proportional to target cell (model 2) and logistic cell growth (model 4) give the same coinfection duration as that of the basic model of

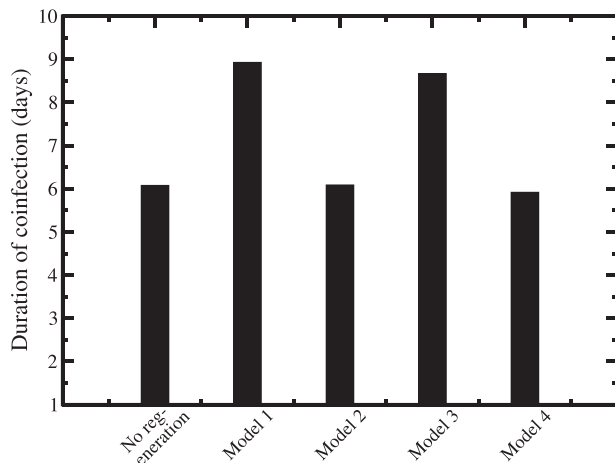


FIG. 4. Coinfection duration predicted by each model of cell regeneration in simultaneous infection with influenza and RSV having the regeneration rate, r , equal to 0.0333 per day.

around 6 days, while the models of constant growth (model 1) and growth proportional to dead cells (model 3) give coinfection durations almost equal to 9 days.

Figure 4 shows the coinfection duration for a specific regeneration rate, but the coinfection duration might depend on the exact value of the regeneration rate. To explore this dependence, coinfection durations are plotted as a function of cell regeneration rate in Fig. 5. For models constant growth (model 1) and growth proportional to dead cells (model 3), we see that the coinfection duration becomes longer with the increase in cell regeneration rate. If the cell regenerates at a constant rate (model 1) or regeneration is proportional to dead cells (model 3), duration of coinfections increases gradually if cells start to regenerate at a rate greater than 100/day, otherwise coinfection lasts for 6 days only. When regeneration is dependent on available target cells (model 2), coinfection duration is constant at 6 days for regeneration rate less than 1/day and then increases slightly beyond this point. For the model assuming logistic growth, coinfection duration is again constant for small regeneration rate but then decreases for regeneration rate larger than ~ 0.1 /day.

IV. DISCUSSION

In this paper, we have shown the existence of different possible infection outcomes when different cell regeneration mechanisms are considered. The steady states that were found are the infection free equilibrium where both viruses become extinct, and the two boundary equilibria where the first virus outcompetes the second virus or the second virus outcompetes the first virus. In the previously published basic model of coinfection,³⁵ viruses compete for the limited

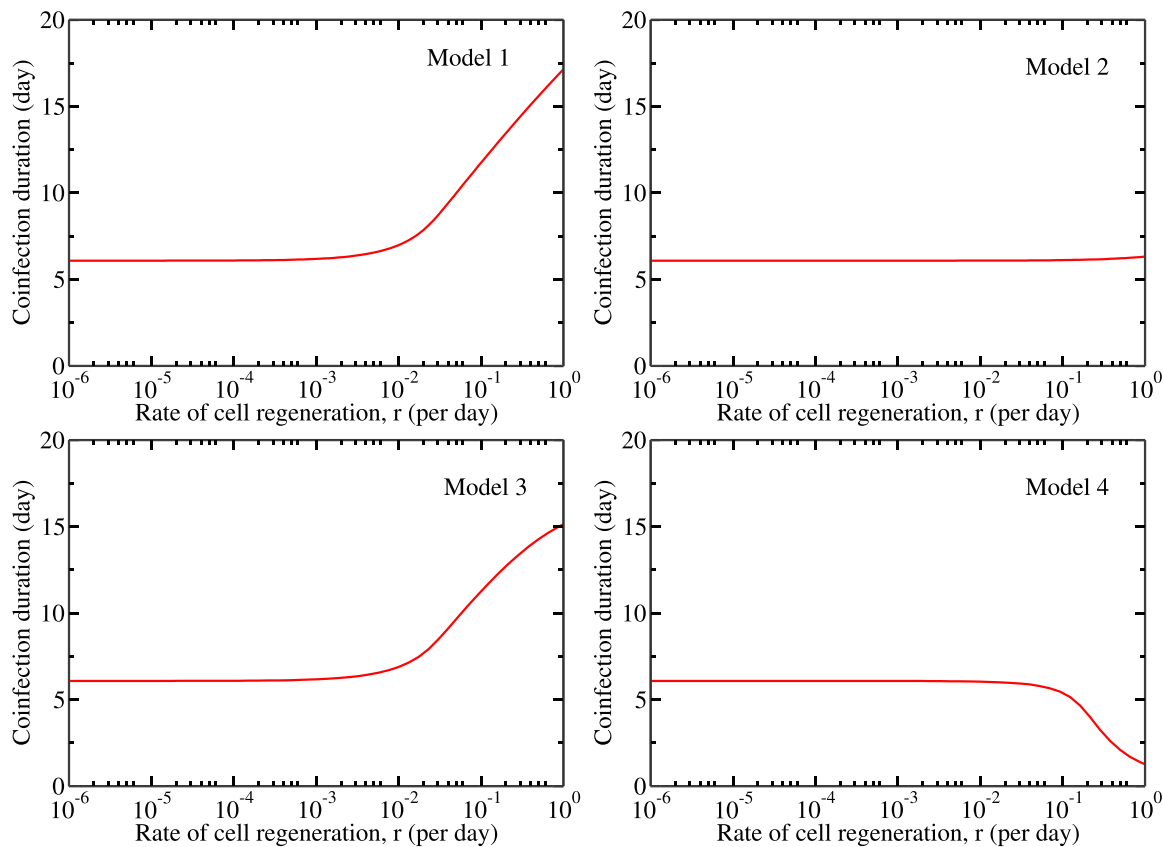


FIG. 5. Coinfection duration of simultaneous infection with RSV and influenza A virus for different cell regeneration models as a function of regeneration rate, r .

resource of target cells and in this case, it was shown that the virus with the faster growth rate infects more target cells than the other. Eventually, however, both viruses die out due to lack of target cells. When these viruses get a renewable pool of target cells or a source which is proportional to dead cells as in the case of models 1 and 3, they keep infecting more target cells, prompting the infection to become chronic, but with only one virus. In models 2 and 4, where cell regeneration is proportional to the available target cells or logistic growth, respectively, the infection growth profile is found to be similar to that of the model with no cell regeneration. Depending on the exact value of regeneration rate, we found that coinfection duration can be more than two weeks for models 1 and 3.

Our primary finding that there cannot be chronic coexistence supports the idea of the competitive exclusion principle first suggested by Volterra.⁶⁶ The competitive exclusion principle states that if n number of species depend on fewer than n number of resources, it is impossible to have indefinite coexistence of n species under some assumptions. It is, however, important to keep in mind that withdrawing some of the model assumptions may allow different coinfection dynamics such as coexistence of two viruses. Our model still assumes spatial homogeneity for target cell density. This assumption may not be realistic as propagation of virus is a localized process and viral infectivity is found to be largely dependent on cell concentrations.^{52,67} Bauer *et al.* suggested that if the cell regeneration rate is not only a function of number of available target cells or number of dead cells but also their location, viral dynamics more closely resembled

experimental results,⁵⁴ for single infections and so could also better model coinfection dynamics.

The parameters of our model are time-invariant, similar to the assumptions of Lotka-Volterra's competitive exclusion model where coexistence is not possible.⁶⁸ Ecologists^{69–73} have shown that coexistence of two species on one resource is possible only if the environment is time-variant, spatially inhomogeneous and/or follows a nonlinear growth rate for the resource. McGehee and Armstrong⁷⁴ proved that coexistence of two species on one resource is possible if the growth rate is nonlinear and the rest of the limiting factors stay unchanged. However, they found the coexistence to be a periodic orbit rather than an equilibrium point.

Another assumption that might lead to coexistence if relaxed is that a single cell is infected by only one type of respiratory infectious virus. Studies show, however, that different viruses can attach to the same receptor and therefore infect the same cell.⁷⁵ Allowing both viruses to infect the same cell may ease some of the resource competition in our model and could change coinfection dynamics by producing viruses at different rates than singly infected cells.

Finally, our model has neglected the role of the immune response. Viruses may themselves produce factors that can influence cells of the immune system which will also affect a second virus. It is reported that chronic infections have the potential to inhibit epithelial cell repair which further aids secondary viral infections to take place easily due to the damage and the immunological changes in the respiratory tract environment.³⁸ Interplay through the immune responses is very likely to alter coinfection dynamics.⁷⁶

Coinfections are found to be affected by the availability of target cells as replication of viruses during infection depends on resource dynamics. Here, cellular regeneration acts as a continuous available resource for the viruses to grow for a longer time compared to having no cell regrowth at all. Thus, in the presence of a continuous resource of cell, previously observed coinfections turn into chronic coinfection with one virus. However, under the model assumptions of spatial homogeneity of cellular concentration, time independent parameters, linear growth rates of target cells, absence of superinfection of the same cell by two different viruses, and consideration of exponential distribution of number of eclipse and infected cells, no cell regeneration models of this study support coexistence of two viral infections during coinfection in the human respiratory tract.

APPENDIX: COMPUTATION OF R_0

The characteristic equation for the infection free steady state ($T^* = T$) of the basic model is

$$\lambda * \{ \lambda^3 + \lambda^2(c_1 + \delta_1 + k_1) + \lambda(c_1\delta_1 + \delta_1k_1 + c_1k_1) + (c_1\delta_1k_1 - \beta_1p_1k_1T) \} * \{ \lambda^3 + \lambda^2(c_2 + \delta_2 + k_2) + \lambda(c_2\delta_2 + \delta_2k_2 + c_2k_2) + (c_2\delta_2k_2 - \beta_2p_2k_2T) \} = 0.$$

R_0 can be found from the spectral radius (largest eigenvalue) of the next generation matrix, i.e., (FV^{-1}), where F is the transmission matrix and V is the transition matrix supplied by the system of equations⁶⁵

$$F = \begin{pmatrix} 0 & 0 & 0 & 0 & \beta_1T & 0 \\ 0 & 0 & 0 & 0 & 0 & \beta_2T \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & p_1 & 0 & 0 & 0 \\ 0 & 0 & 0 & p_2 & 0 & 0 \end{pmatrix},$$

$$V = \begin{pmatrix} -k_1 & 0 & 0 & 0 & 0 & 0 \\ 0 & -k_2 & 0 & 0 & 0 & 0 \\ k_1 & 0 & -\delta_1 & 0 & 0 & 0 \\ 0 & k_2 & 0 & -\delta_2 & 0 & 0 \\ 0 & 0 & 0 & 0 & -c_1 & 0 \\ 0 & 0 & 0 & 0 & 0 & -c_2 \end{pmatrix},$$

$$FV^{-1} = \begin{pmatrix} 0 & 0 & 0 & 0 & -\frac{\beta_1T}{c_1} & 0 \\ 0 & 0 & 0 & 0 & 0 & -\frac{\beta_2T}{c_2} \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ -\frac{p_1}{\delta_1} & 0 & -\frac{p_1}{\delta_1} & 0 & 0 & 0 \\ 0 & -\frac{p_2}{\delta_2} & 0 & -\frac{p_2}{\delta_2} & 0 & 0 \end{pmatrix}.$$

The dominant eigenvalue of the matrix FV^{-1} is equal to R_0 , where

$$R_0 = \max(R_{01}, R_{02}).$$

The R_0 calculation is the same for models 2 and 4.

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