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The effects of distributed life cycles on the dynamics of viral infections

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ABSTRACT

We explore the role of cellular life cycles for viruses and host cells in an infection process. For this purpose, we derive a generalized version of the basic model of virus dynamics (Nowak, M.A., Bangham, C.R.M., 1996. Population dynamics of immune responses to persistent viruses. Science 272, 74–79) from a mesoscopic description. In its final form the model can be written as a set of Volterra integrodifferential equations. We consider the role of distributed lifespans and a intracellular (eclipse) phase. These processes are implemented by means of probability distribution functions. The basic reproductive ratio R_0 of the infection is properly defined in terms of such distributions by using an analysis of the equilibrium states and their stability. It is concluded that the introduction of distributed delays can strongly modify both the value of R_0 and the predictions for the virus loads, so the effects on the infection dynamics are of major importance. We also show how the model presented here can be applied to some simple situations where direct comparison with experiments is possible. Specifically, phage–bacteria interactions are analyzed. The dynamics of the eclipse phase for phages is characterized analytically, which allows us to compare the performance of three different fittings proposed before for the one-step growth curve.

1. Introduction

The interactions between viruses and cells in an infection process can be seen as an ecological system within the infected host. The mathematical description of these systems has attracted increasing interest in the last years (Wodarz, 2006), especially concerning the characteristics of the immune response after a viral attack. A decade ago, Nowak and Bangham (1996) presented what has been called thereafter the basic model of virus dynamics (BMVD). This model has become quite popular among theorists and experimentalists (see Nowak and May, 2000 or Perelson, 2002). The interplay between the BMVD and the effect of an immune response has proved useful to describe the dynamics of chronic HIV infections (Perelson, 2002). Furthermore, it has provided interesting results concerning topics such as the performance of drug therapies (Bonhoeffer et al., 1997; Wodarz and Nowak, 1999) and lymphocyte exhaustion (Wodarz et al., 1998).

The BMVD describes the time evolution of non-infected cells (X), infected cells (Y) and viruses (V) by the system of equations:

$$\frac{dX}{dt} = \lambda - \delta X - \beta XV$$

$$\frac{dY}{dt} = \beta XV - aY$$

$$\frac{dV}{dt} = kY - \beta XV - uV \qquad (1)$$

The infection process is governed by the parameter β, which determines the rate of successful contacts between the target cells and the viruses. Mortality terms for the three species are considered with constant death rates δ, a and u, respectively. The parameter k measures the rate at which virions are released from a single infected cell. Finally, new target cells are produced by the host at a constant rate λ.

Despite the success achieved by the BMVD, it is clear that the model described in (1) is just a first approximation to the real underlying process. One of the simplifications made in the model is that it assumes that the death rates are exponentially distributed (i.e., mortalities are considered as Markovian random processes) and therefore do not take into account the details of the cellular life cycles. However, delays and structured life cycles are expected to play a very significant role in the dynamics of viral infections. For example, the infection process involves an intracellular phase of the virus, also known as the eclipse phase, which is not explicitly considered in (1). For this reason, in the recent years some works have explored the effects of constant and distributed delays in the BMVD and in the cases where an immune response is considered. Herz et al. (1996) showed for the first time the importance of delays in order to explain the virus loads observed in HIV patients under drug treatment. This delayed

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model was later explored from a more formal point of view by Tam (1999). Similar ideas, with different expressions for the infection term, were considered by Culshaw and Ruan (2000), Fort and Méndez (2002) and Li and Wanbiao (1999). The effect of distributed delays has been explored for different models of virus dynamics by Kirschner and Webb (1997), Mittler et al. (1998), Lloyd (2001), Banks et al. (2003), Nelson et al. (2004) and Rong et al. (2007). Finally, the role of a delayed immune response has been the subject of extensive research. Some examples are the works by Buric et al. (2001), Canabarro et al. (2004), Wang et al. (2007) and the references there in, which focused on the chaotic patterns which can appear in these systems.

The papers mentioned above have helped us to understand how delays can modify the cell–virus and virus–immune system dynamics. However, most of those works focused on the case where only one of the processes (usually the intracellular phase) is delayed. So, they do not considered the possibility of different delays for each process, whose combined contributions could modify the dynamical behavior of the system.

On the other hand, the introduction of delays in the virus dynamics has been usually based on phenomenological (not always rigorous) arguments. Only in Banks et al. (2003), Fort and Méndez (2002) and Wearing et al. (2005) a more formal discussion was provided. We stress that the implementation of delays into dynamical models is sometimes tricky, as memory effects can lead to the breakdown of hypothesis that are well established for Markovian processes. In fact, there is currently a very active research on this subject from the point of view of statistical mechanics (see, for example, Allegrini et al., 2003, 2007; Rebenshtok and Barkai, 2007 and the references therein). Due to the subtleties involved in the formulation of delayed models, we think that a rigorous physical approach is necessary to reach an accurate description of virus dynamics. Here, we propose a system of Volterra integrodifferential equations which is a generalization of the BMVD. This system of equations is derived from a mesoscopic approach where balance equations for each species (X, Y and V) are considered explicitly. Mesoscopic descriptions as that considered here (based on Continuous-Time Random Walk processes) have become common tools for the description of physical and biological processes. At this stage, they have proved useful for the study of heat transport (Emmanuel and Berkowitz, 2007), tumor cell growth (Fedotov and Iomin, 2007), solute transport in porous media (Berkowitz et al., 2000), earthquake dynamics (Helmstetter and Sornette, 2002), financial markets (Masoliver et al., 2006) and many other applications. Here we will explore for the first time their application to the field of virus dynamics.

So, the aim of this paper is to use an integrodifferential approach to show how distributed lifespans and distributed times to viral production can strongly influence the predictions from the BMVD. As a result, we shall show that the value of the basic reproductive ratio R_0 and the virus load can be altered, in accordance with similar conclusions found in (Lloyd, 2001; Nelson et al., 2004; Rong et al., 2007). Furthermore, the advantage of using such a general formalism as the one proposed here is that different situations of interest can be analyzed as particular cases of the model. According to this, we show how our model can be used to fit and characterize the one-step growth curve observed in phage–bacteria interactions. Three fittings proposed before by different authors are compared. We find that, although the three approaches fit the one-step growth curve reasonably well, their predictions concerning the dynamics of the eclipse phase are slightly different.

In the following, we show how a generalized version of the BMVD can be obtained using a mesoscopic description. In Section 2 we present our model, whose formal derivation is given in Appendix A for the sake of clarity. In Section 3 we explore the equilibrium states and their stability, which let us define the basic reproductive ratio R_0. After that, we consider specific situations of special interest in virus dynamics. Specifically, the effects of a constant delay in the phase eclipse (Section 4) and distributed delays in the mortalities of cells and viruses (Section 5) are studied. We also show how the model derived in Section 2 works in the case of phages–bacteria dynamics (Section 6), and we provide some examples using experimental data extracted from the literature. Finally, the main conclusions obtained from our study are summarized in Section 7.

2. The age-structured BMVD

The interest of introducing age-structured effects into a virus dynamics model is not merely academic. Experimentally, it is known that both the phase eclipse and the times to death of cells can exhibit in general complicated time distributions. Specifically, times to death are usually fitted to peaked curves as gamma, Weibull or lognormal functions. So that, we shall propose a model which can capture, in contrast with the classical BMVD, the effects resulting from these life cycles.

The model we consider here is depicted in Fig. 1. It follows the same scheme as the BMVD but some of the random processes (those indicated by the dotted lines) are statistically governed by some probability distribution functions (PDFs). So that, $\varphi_X(t)$ represents the probability that a target cell X dies at age t, with equivalent definitions for $\varphi_Y(t)$ and $\varphi_V(t)$ for infected cells and viruses. Similarly, the function $\phi(t)$ determines the viral production since infection: a cell that becomes infected at time t_0 can release $\phi(t)$ viruses at time t_0+t.

The Volterra integrodifferential equations corresponding to the scheme in Fig. 1 read

$$\frac{dX(t)}{dt} = \lambda - \beta X(t)V(t) - \int_0^t X(t-t')\Psi_X(t')\Omega_X(t-t',t)\,dt'$$

$$\frac{dY(t)}{dt} = \beta X(t)V(t) - \int_0^t Y(t-t')\Psi_Y(t')\,dt'$$

$$\frac{dV(t)}{dt} = -\beta X(t)V(t) + \int_0^t \beta X(t-t')V(t-t')\phi(t')\Phi_Y(t')\,dt' - \int_0^t V(t-t')\Psi_V(t')\Omega_V(t-t',t)\,dt' \quad (2)$$

The formal derivation of this model in terms of a mesoscopic description is provided in Appendix A. Also, to facilitate understanding we provide in Table 1 a summary with all the temporal distributions used in the present paper.

The functions Ψ_X, Ψ_Y, Ψ_V are defined by their Laplace transforms (we denote the Laplace transform of a function by the brackets $[\cdot]_s$ with the conjugate variable s)

$$[\Psi_X]_s \equiv \frac{[\varphi_X]_s}{[\Phi_X]_s}, \quad [\Psi_Y]_s \equiv \frac{[\varphi_Y]_s}{[\Phi_Y]_s}, \quad [\Psi_V]_s \equiv \frac{[\varphi_V]_s}{[\Phi_V]_s} \quad (3)$$

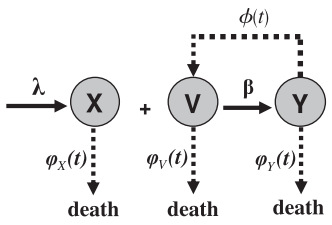

Fig. 1. Scheme of the BMVD model with distributed lifespans and distributed times to viral production.

Table 1
Summary of the notation for the temporal distributions used in the model

PDF	Meaning
$\phi(t)$	Probability of time to virus release after a cell is infected
$\varphi_i(t)$	Time to death of cells of species i
$\Phi_i(t)$	Survival (no-death) probability of cells of species i and age t (see (23))
$\Psi_i(t)$	Instantaneous death rate for cells of age t (see (3))
$\Omega_i(t-t',t)$	Survival (no-infection) probability of a cell of species i from time $t-t'$ to t

where $\Phi_X(t) \equiv \int_t^\infty \varphi_X(t')\,dt'$ is the survival probability for the cells of age t. Analogous definitions hold for Φ_Y and Φ_V. According to (3), the function $\Psi_X(t)$ can be interpreted as the instantaneous death rate for a cell X of age t. Then, the term $\int_0^t X(t-t')\Psi_X(t')\Omega_X(t-t',t)\,dt'$ represents a generalized mortality term with age-distributed death rates. To find the density of cells dying at time t, the density of cells which are present at any previous time $X(t-t')$ is multiplied by the instantaneous rate $\Psi_X(t')$ for cells of age t', and by the function $\Omega_X(t-t',t')$, which is the probability that a cell X has not become infected during the time interval $(t-t',t)$.

Similarly, the term $\int_0^t \beta X(t-t')V(t-t')\phi(t')\Phi_Y(t')\,dt'$ in the equation for $V(t)$ represents the release of new virions from infected cells. The factor $\beta X(t-t')V(t-t')$ gives us the density of cells that became infected at time $t-t'$. This expression is then multiplied by the viral production function $\phi(t')$ and by the survival probability of the infected cells during that period $(\Phi_Y(t'))$.

The system of Eqs. (2)–(3) represents our generalization of the BMVD. An important conclusion from (2) is that the density of infected cells Y does not appear in the equations for $X(t)$ and $V(t)$. It means that the formalism introduced here allows us to reduce the BMVD to a two-species model. We do not need to consider explicitly the density $Y(t)$; the existence of the infected cells is implicitly considered by means of the function Φ_Y appearing in the equation for $V(t)$. Similar results were found in previous works closely related to ours; actually, our model can be seen as a extension of those studied in Nelson et al. (2004) and Rong et al. (2007) to the case of distributed lifespans.

3. Equilibrium states and their stability

The equilibrium states of the model (2) come from the analysis of the fixed points of the system at $t \to \infty$. There are two possible equilibrium states: the first one is the trivial, infection-free state, given by

$$(X_{eq}, Y_{eq}, V_{eq}) = (\lambda\tau_X, 0, 0) \tag{4}$$

where we use $\tau_i = \int_0^\infty \Phi_i(t)\,dt$ to denote the average lifetime of species i, with $i = X, Y, V$. The second state corresponds to the case of a successful infection defined by

$$X_{eq}\int_0^\infty e^{-\beta X_{eq}t}\Phi_V(t)\,dt = \frac{\lambda\tau_X\int_0^\infty e^{-\beta\lambda\tau_X t}\Phi_V(t)\,dt}{R_0}$$

$$Y_{eq} = \lambda\tau_Y\beta V_{eq}\int_0^\infty e^{-\beta V_{eq}t}\Phi_X(t)\,dt$$

$$\int_0^\infty e^{-\beta V_{eq}t}\Phi_X(t)\,dt = \frac{X_{eq}}{\lambda} \tag{5}$$

where Eqs. (27) and (28) have been used, and we have defined

$$R_0 \equiv \beta\lambda\tau_X\left[\int_0^\infty e^{-\beta\lambda\tau_X t}\Phi_V(t)\,dt\right]\left[\int_0^\infty \phi(t)\Phi_Y(t)\,dt\right] \tag{6}$$

As can be seen from (5), it is not possible to give explicit expressions for the equilibrium densities in the infected state. However, it can be proved that this state has biological meaning ($Y_{eq}>0$ and $V_{eq}>0$) only if $R_0>1$. To see this, note that the condition $R_0>1$ applied to the first equation of (5) implies necessarily $X_{eq}<\lambda\tau_X$, which means that the equilibrium density in the infected state is lower than in the infected-free state. Introducing $X_{eq}<\lambda\tau_X$ into the third equation in (5), it follows that only in that case V_{eq} has a positive value. Hence, R_0 can be properly defined as the basic reproductive ratio, which is a key parameter in epidemiology and virus dynamics in order to predict the emergence of an infection (Anderson and May, 1991; Nowak and May, 2000). For $R_0<1$ we have that every single virus generates statistically less than one new virus, so a permanent infection is not possible and the infected state $V_{eq}>0$ does not exist. We also note that the case explored in the present paper, and so the expression (6), is more general than recent estimations for R_0 where the possibility of a distributed intracellular period was also taken into account (Heffernan and Wahl, 2006).

We will now explore the stability of the equilibrium states found. For this purpose, we will use the usual linear-stability analysis, so we introduce $X(t) = X_{eq} + \delta X(t)$ and $V(t) = V_{eq} + \delta V(t)$. Inserting these definitions into (2) and linearizing about the equilibrium state we obtain the following system for the perturbations:

$$\begin{aligned}\frac{d\delta X(t)}{dt} = &-\beta V_{eq}\delta X(t) - \beta X_{eq}\delta V(t)\\ &-\int_0^t \delta X(t-t')\Psi_X(t')\,dt'\\ &+\beta X_{eq}\int_0^t \delta V(t-t')\Psi_X(t')t'\,e^{-\beta V_{eq}t'}\,dt'\end{aligned}$$

$$\begin{aligned}\frac{d\delta V(t)}{dt} = &-\beta X_{eq}\delta V(t) - \beta V_{eq}\delta X(t)\\ &+\beta V_{eq}\int_0^t \delta X(t-t')\phi(t')\Phi_Y(t')\,dt'\\ &+\beta X_{eq}\int_0^t \delta V(t-t')\phi(t')\Phi_Y(t')\,dt'\\ &-\int_0^t \delta V(t-t')\Psi_V(t')\,dt'\\ &+\beta V_{eq}\int_0^t \delta X(t-t')\Psi_V(t')t'\,e^{-\beta X_{eq}t'}\,dt'\end{aligned} \tag{7}$$

Since this system is now linear, we can propose for the perturbations exponential solutions of the form $e^{\mu t}$ to get the characteristic equation

$$\begin{aligned}0 = &(\mu + \beta X_{eq} + [\Psi_X]_\mu)(\mu + \beta X_{eq} - \beta X_{eq}[\phi\Phi_Y]_\mu + [\Psi_X]_\mu)\\ &-\beta^2 X_{eq}V_{eq}\left(1 - \frac{d[\Psi_X]_\mu}{d\mu}\right)\left(1 - [\phi\Phi_Y]_\mu - \frac{d[\Psi_V]_\mu}{d\mu}\right)\end{aligned} \tag{8}$$

where we define $[f]_\mu \equiv \int e^{-\mu t}f(t)\,dt$ in accordance with the notation used above for the Laplace transform.

3.1. Infection-free equilibrium state

First we analyze the stability of the trivial state corresponding to the absence of viruses. Introducing (4) into (8) we obtain

$$1 = \beta X_{eq}[\Phi_V]_{\mu+\beta X_{eq}}[\phi\Phi_Y]_\mu \tag{9}$$

From (9), it is easy to find the necessary condition for the transition from stability to instability. In the BMVD it is known that for values of $R_0<1$ the infected-free state is stable, and becomes unstable otherwise. From (9), it is possible to prove that, in general, this condition holds for any choice of the PDFs. The right-hand side in that equation is a monotonically decreasing

positive function of μ and takes the value R_0 at $\mu = 0$ (see 6). Then, if $R_0 > 1$ Eq. (9) has a solution for a positive value of μ, which is nothing but the sufficient condition for the state to be unstable. If $R_0 < 1$ the solution to (9) will be a negative value of μ. In this case the infection-free equilibrium state is linearly stable and the infection dies out.

3.2. Infected equilibrium state

Using (5), the characteristic equation (8) for the infected state becomes extremely complicated to treat, and it makes impossible to determine analytically the stability of the infected state. However, we can still deduce the characteristics of this state by imposing some conditions to prevent the system from behaving in an unrealistic way. First, we mention again that the infected state does not exist for $R_0 < 1$, so we only need to study the case $R_0 > 1$. Second, we can rewrite the first equation in (5), using (6) and the definition of the Laplace transform, as

$$[\varphi_V]_{\beta X_{eq}} = \frac{[\phi\Phi_Y]_\mu - 1}{[\phi\Phi_Y]_\mu} \tag{10}$$

Then, we conclude that, for any given PDFs, there is only one possible solution for X_{eq}, as the left-hand side of this equation is a monotonically decreasing function of X_{eq}. From that, similar arguments can be applied to the third equation in (5), so it follows that the value for V_{eq} is unique too. As a whole, we have that the infected state is always unique. This, together with the instability of the non-infected state for $R_0 > 1$, allows us to conclude that the infected state cannot be an unstable node or a saddle point, as it would imply that for some initial conditions the system would grow without control towards the state $X \to \infty$ and/or $V \to \infty$. This unbounded behavior is not possible in our system. Then, the only possibility is that the infected state is stable for $R_0 > 1$.

The derivations presented in this section show that the introduction of distributed lifespans and viral production does not modify the stability of the BMVD. Although our mesoscopic model (2) is much more general that the original version (1), we still find that the condition $R_0 = 1$ defines a bifurcation point at which the infection-free state changes its stability and a stable infected state appears. Finally, note that $R_0 > 1$ can also be interpreted as a threshold value for the contact rate

$$\beta > \frac{1}{\lambda\tau_X[\int_0^\infty \mathrm{e}^{-\beta\lambda\tau_X t}\Phi_V(t)\,\mathrm{d}t][\int_0^\infty \phi(t)\Phi_Y(t)\,\mathrm{d}t]} \tag{11}$$

4. The BMVD with a delayed eclipse phase

We have presented a very general model which takes into account age-structured effects for the cellular lifespan and the eclipse phase. However, it can be useful to study some specific and simpler cases which have a special interest for application purposes.

First, we consider here the case when no age-distributed effects are introduced in the death process, i.e., the probability of death is independent of the age of the cells. This corresponds to the situation used in the BMVD, which in our integrodifferential model is recovered by assuming φ_X, φ_Y, φ_V as exponentially decaying functions ($\varphi_X(t) = \delta\,\mathrm{e}^{-\delta t}$, $\varphi_Y(t) = a\,\mathrm{e}^{-at}$, $\varphi_V(t) = u\,\mathrm{e}^{-ut}$). For the eclipse phase, we can assume that when a cell is infected, it takes a fixed constant time τ until the first virion is released and after that, virions are continuously released at a constant rate k. The delay τ is the time necessary to inject the viral core into the cell and make its genetic machinery start the reproduction process. So that, the function $\phi(t)$ in our model will be taken as a step function $\phi(t) = kH(t-\tau)$, where $H()$ is the Heaviside function.

This specific example has been studied by some authors before (Herz et al., 1996; Tam, 1999; Culshaw and Ruan, 2000), so we can compare the predictions from our model with those from previous approaches. Replacing the distribution functions $\varphi_i(t)$, $\phi(t)$ into the general model (2) we obtain

$$\begin{aligned}
\frac{\mathrm{d}X}{\mathrm{d}t} &= \lambda - \delta X - \beta XV \\
\frac{\mathrm{d}Y}{\mathrm{d}t} &= \beta XV - aY \\
\frac{\mathrm{d}V}{\mathrm{d}t} &= \int_\tau^t \beta X(t-t')V(t-t')k\,\mathrm{e}^{-at'}\,\mathrm{d}t' - \beta XV - uV
\end{aligned} \tag{12}$$

In the equation for $V(t)$, the expression $\beta X(t-t')V(t-t')$ represents those cells that became infected at time $t-t'$. So, the new virions appeared are equal to that expression multiplied by the rate k and by the probability $\mathrm{e}^{-at'}$ that the infected cells have survived from time $t-t'$ to t. The expression of R_0 that one obtains for this case, from (6), is

$$R_0 = \frac{\beta\lambda}{\delta u}\left(\frac{k}{a}\,\mathrm{e}^{-a\tau} - 1\right) \tag{13}$$

Note that the system (12) is apparently different to the previous models proposed before for the analysis of a delayed eclipse phase (Herz et al., 1996; Tam, 1999; Culshaw and Ruan, 2000). In those works a delayed term $\beta X(t-\tau)V(t-\tau)$ was introduced ad hoc in the evolution equation for $Y(t)$:

$$\begin{aligned}
\frac{\mathrm{d}X}{\mathrm{d}t} &= \lambda - \delta X - \beta XV \\
\frac{\mathrm{d}Y}{\mathrm{d}t} &= \beta X(t-\tau)V(t-\tau)\,\mathrm{e}^{-a\tau} - aY \\
\frac{\mathrm{d}V}{\mathrm{d}t} &= kY - \beta XV - uV
\end{aligned} \tag{14}$$

However, it is easy to see that the value of R_0 for this model is exactly the expression (13), and the equilibrium states coincide with those found from our model too. Actually, both models represent the same underlying process except for one subtle detail. In the model (14), the fraction of cells $\beta X(t-\tau)V(t-\tau)$ are considered as infected cells only after the time delay τ, which means that Y is taken as the density of productively infected cells. So, from the time of infection $t-\tau$ to the time of first release τ these cells do not belong neither to species Y nor to X, so they are in a transient state not considered explicitly in the model. Instead, in our model the cells become Y cells at time $t-\tau$ and they start releasing the new virions at time t. So that, the only difference between (12) and (14) will be in the solution for $Y(t)$: the value predicted by the model (14) will be always below the one predicted by (12).

5. The effect of age-distributed times for cellular death

Now we study a more realistic case, in accordance to the experimental data available in the literature. We will consider that the eclipse phase follows the same dynamics as that in Section 4, but the death times are now assumed to follow gamma distributions, which are common functions used for fitting experimental data to cellular death times (see for example Hawkins et al., 2007). Hence, in this case we will use

$$\phi(t) = kH(t-\tau), \quad \varphi_i(t) = \frac{t^{\alpha_i - 1}\,\mathrm{e}^{-t/\tau_i^*}}{(\tau_i^*)^{\alpha_i}\Gamma(\alpha_i)} \tag{15}$$

for $i = X, Y, V$, where $\Gamma(\cdot)$ denotes the gamma function. From the characteristic parameters of the gamma distribution α_i and τ_i^* we

can also find the expression for the average lifetimes as $\tau_i = \tau_i^* \alpha_i$ (with arbitrary time units).

Inserting (15) into (6) the basic reproductive ratio R_0 yields

$$R_0 = \frac{(1+\beta\lambda\tau_X\tau_V^*)^{\alpha_V}-1}{(1+\beta\lambda\tau_X\tau_V^*)^{\alpha_V}} k\tau_Y^* \, e^{-\tau/\tau_Y^*} \sum_{j=0}^{\alpha_Y-1} \left[\frac{\alpha_Y - j}{j!} \left(\frac{\tau}{\tau_Y^*} \right)^j \right] \tag{16}$$

for α_Y integer. From (16), it follows that the influence of distributed death ages could be important for the value of R_0 and, in consequence, it modifies the value of the virus load at equilibrium. This effect is represented in Fig. 2, where we have plotted the expression (16) and the equilibrium virus load obtained from (5) as a function of the parameters α (for simplicity we define $\alpha \equiv \alpha_X = \alpha_Y = \alpha_V$) and τ. As seen there, R_0 depends linearly on α, which at the same time yields a power-law dependence of V_{eq} on α. On the contrary, the dependence of these magnitudes on τ does not seem to follow any simple function; specifically, a very sharp behavior for V_{eq} is observed near the critical point $R_0 = 1$ (indicated by dotted lines).

Fig. 3 shows the numerical solution $V(t)$ obtained from the model (2) for different values of the parameter α. For $\alpha = 1$ we recover the situation where lifespans are exponentially distributed, it is, the case of the BMVD. In the three curves shown, the average lifetimes for the three species are the same; it allows us to compare properly the effects of the mortality distributions on the virus load dynamics. Note that the virus loads decrease in time for $t<5$; this is because we have used a value $\tau = 5$ for the eclipse phase, so only after $t = \tau$ the infected cells start to release the first virions, and then the virus load increases drastically. As observed, the values of the virus load for $\alpha = 1$ are lower than in the other two cases for small times. This is because the BMVD assumes unrealistic high probabilities of death for the early stage of the infection, an effect which can be corrected by the gamma-distributed mortalities used here. This point is of great importance concerning the probability of a fast primary immune response to clear the infection. We also find important differences between the maximum virus loads reached at equilibrium; for the parameters used in Fig. 3, the final virus load for $\alpha = 1$ is approximately seven-fold higher than in the case $\alpha = 3$.

Therefore, we conclude that the BMVD underestimates the virus loads in the early stages of the infection and overestimates the peak of the virus load, if compared with the case of distributed mortalities considered here. In consequence, it turns out that we need to know with some detail the life cycle of viruses and cells to obtain an accurate picture of the infection dynamics.

6. Application to phage–bacteria interactions

The interaction between phages and bacteria can be described as two consecutive steps: adsorption and reproduction (McGrath

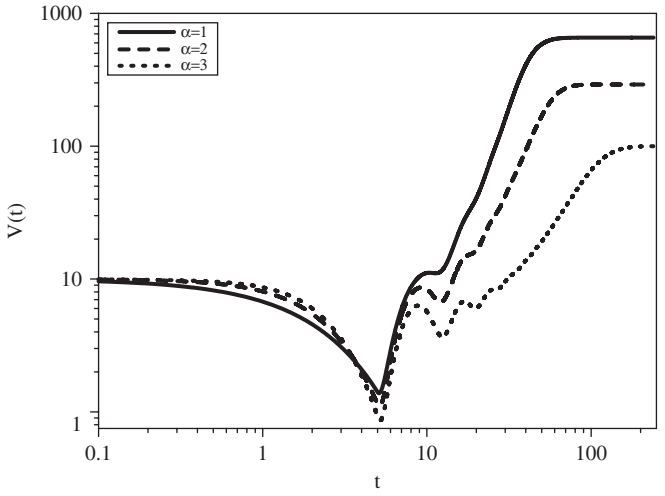

Fig. 3. Virus loads obtained numerically from the general model (2) for the case of gamma-distributed death times. In the legend we show the values of the parameter $\alpha \equiv \alpha_X = \alpha_Y = \alpha_V$ used. For the other parameters in the model, we have used $\lambda = 10$, $\beta = 0.002$, $k = 50$, $\tau = 5$, $\tau_X^* = 3/\alpha$, $\tau_Y^* = 3/\alpha$, $\tau_V^* = 3/\alpha$, with arbitrary time units.

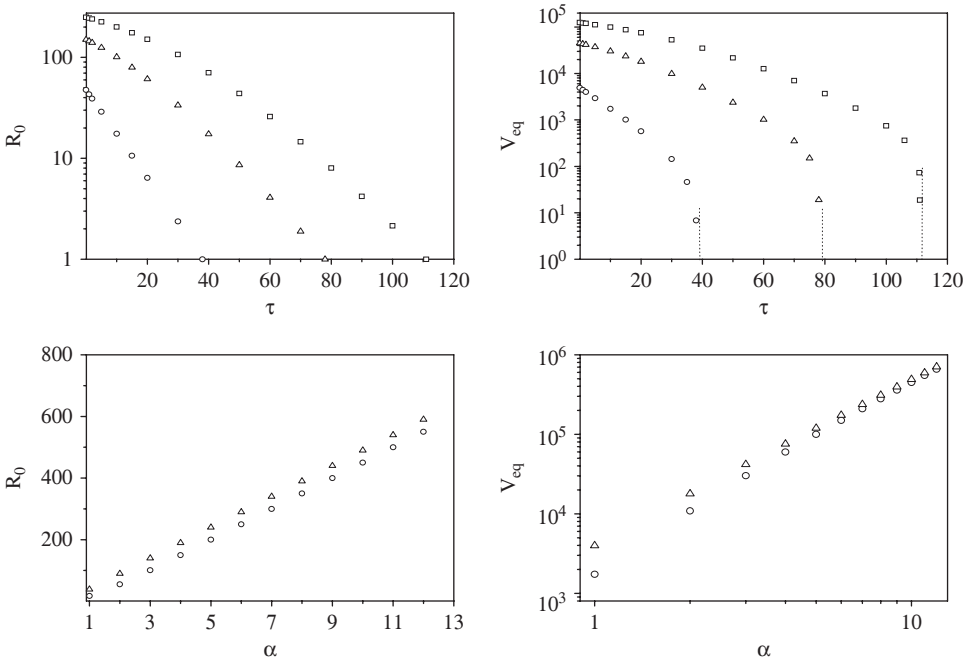

Fig. 2. Values of the basic reproductive ratio R_0 and the equilibrium virus load V_{eq} as a function of the parameters $\alpha \equiv \alpha_X = \alpha_Y = \alpha_V$ and τ, appearing in the mortality and the viral production PDFs, respectively. The dotted lines (graph on the upper right) indicate the bifurcation point at which $R_0 = 1$ and so $V_{eq} = 0$. The other parameters in the model take values $\lambda = 10$, $\beta = 0.02$, $k = 5$, $\tau = 2$, $\tau_X^* = 10$, $\tau_Y^* = 10$, $\tau_V^* = 10$.

and van Sinder, 2007). Adsorption involves a collision between phage and bacteria resulting in a group, called infected bacteria, constituted by the bacteria and the phage attached to its membrane. The second step begins when the phage inoculates its genetic material into the host bacteria and a replication process starts. From this time onwards the number of new viruses increases inside the bacteria, stopping when the bacteria bursts at the end of the latent period. Basically, the main difference between this situation and those explored in the previous sections is that for phages the eclipse phase finishes with a lytic process that involves the death of the infected cell. In terms of the model presented here, this idea can be introduced simply by choosing the appropriate form for the function $\phi(t)$.

Here we deal with the reproduction process, which is known to produce a characteristic one-step growth curve $V(t)$ for virulent phages. Let us consider that at $t=0$ the phage inoculates its genome and all the bacteria become infected instantaneously, with $Y(t=0)=Y(0)$. Then, we can define $J_V(t)=Y(0)\phi(t)$ as the rate of viruses released at time t, following the same notation as in Appendix A (see Eq. (26) and the comments below). As all the cells are assumed to be already infected at $t=0$, the infection process for $t>0$ can be obviated. We can thus take $\Omega_V(t-t',t)=1$ for $0<t'<t$ in Eq. (26) to obtain

$$V(t)=V(0)\Phi_V(t)+\int_0^t Y(0)\phi(t-t')\Phi_V(t')\,\mathrm{d}t' \tag{17}$$

which constitutes our theoretical model for the one-step growth curve. If the one-step growth is known from experiments, the function $\phi(t)$ can be determined by fitting that curve to some function and applying

$$\phi(t)=\frac{1}{Y(0)}\left(\frac{\mathrm{d}V}{\mathrm{d}t}+\int_0^t V(t-t')\Psi_V(t')\,\mathrm{d}t'\right)_{osg} \tag{18}$$

which comes directly from the solution of (17) and the subindex *osg* stands for one-step growth. Expression (18) can only be applied if we know the function Ψ_V, which is related to the mortality distribution φ_V according to (3). At practice, the probability of death for the viruses is usually considered to be very small in the time scale of the experiments, so it can be neglected. In that case, $\Psi_V\approx 0$ and then we find that $\phi(t)$ becomes proportional to the derivative of the one-step growth curve

$$\phi(t)=\frac{1}{Y(0)}\left(\frac{\mathrm{d}V}{\mathrm{d}t}\right)_{osg} \tag{19}$$

For fitting the one-step growth $V(t)$, some authors have considered before different possibilities. A piecewise (or 'segments') function, for example, has been used in (You et al., 2002; Hadas et al., 1997). Continuous functions have been proposed too; for example, a Gaussian function has also been considered for fitting the distribution function $\phi(t)$ (Rabinovitch et al., 1999), or logistic-like functions for the curve $V(t)$ (Fort and Méndez, 2002; Alvarez et al., 2007). For these three cases one finds that the corresponding expressions for $\phi(t)$ are those shown in Table 2. We have written there the functions in terms of three parameters r, τ and V_∞. For the sake of completeness, we also show for each case the relation between these parameters and the eclipse time, the rise rate and the burst size, which are commonly used in experimental works to characterize the one-step growth curve (a proper definition of these is provided in Fig. 4). Note, for example, that in the case of Gaussian function the variance (which is a measure of the width of the one-step growth curve) is $8/(\pi r^2)$.

In Fig. 5 we show the experimental results (symbols) for one-step growth of phage T7 on *Escherichia coli* BL21 grown at different rates (You et al., 2002), while the specific values obtained from the adjustment (lines) in each case are detailed in Table 3. The solid curves in Fig. 5 represent the fitting of the experimental results to the logistic-like function, exhibiting a good agreement. The segments (dotted lines) and the Gaussian function (dashed lines) fittings are also shown in the plot; in the latter, the coincidence with the logistic-like case is so high that the two curves are almost indistinguishable.

From each one of the fittings the corresponding expression for $\phi(t)$ has been estimated. The comparison between them is shown in Fig. 6, where we plot only one of the three cases presented in Fig. 5 for simplicity (the two cases non-shown exhibit a very

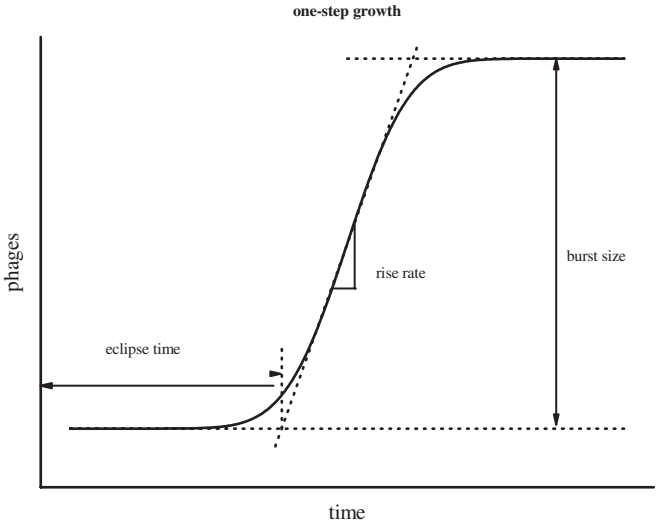

Fig. 4. Definition of the basic parameters that characterize the one-step growth curve. The burst size represents the asymptotic value of the virus load reached for large times. The rise rate measures the value of the maximum slope of the one-step growth. Finally, sketching a tangent line to the curve at the point corresponding to the maximum slope, the crossing with the horizontal axis defines the value of the eclipse time. From these definitions, the expressions shown in Table 2 come straightforward.

Table 2
Characteristics of the three functions proposed for fitting the one-step growth curve, with their explicit expressions for the eclipse time, the rise rate and the burst size

	$V(t)$	$\phi(t)$	Eclipse time	Rise rate	Burst size
Piece-wise	$\begin{cases} 0;\ t<\tau \\ r(t-\tau);\ \tau<t<\tau+\frac{V_\infty}{r} \\ V_\infty;\ t>\tau+\frac{V_\infty}{r} \end{cases}$	$H(t-\tau)$ $-H\left(t-\tau-\frac{V_\infty}{r}\right)$	τ	r	V_∞
Gaussian function	$\frac{V_\infty}{2}\left[1+erf\left(\frac{t-\tau}{4}r\sqrt{\pi}\right)\right]$	$\frac{r}{4}\mathrm{e}^{-r^2\pi(t-\tau)^2/16}$	$\tau-2/r$	$rV_\infty/4$	V_∞
Logistic-like	$\frac{V_\infty}{1+\mathrm{e}^{-r(t-\tau)}}$	$\frac{r\,\mathrm{e}^{-r(t-\tau)}}{[1+\mathrm{e}^{-r(t-\tau)}]^2}$	$\tau-2/r$	$rV_\infty/4$	V_∞

From (19), the estimations for $\phi(t)$ are also shown.

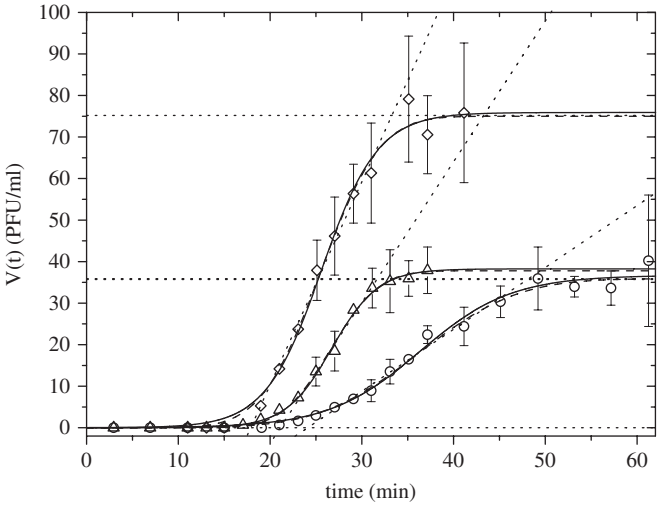

Fig. 5. One-step growth for phage T7 inside *E. coli*. The lines shown represent the fittings from the functions in Table 1 (solid lines represent the logistic-like fitting, dashed lines correspond to the Gaussian function, and the dotted lines the segments fitting). Symbols correspond to experimental results, obtained from You et al. (2002). Circles, triangles and diamonds represent the one-step growth curve for the host growing at 0.7, 1.0 and 1.2 doublings h^{-1}, respectively.

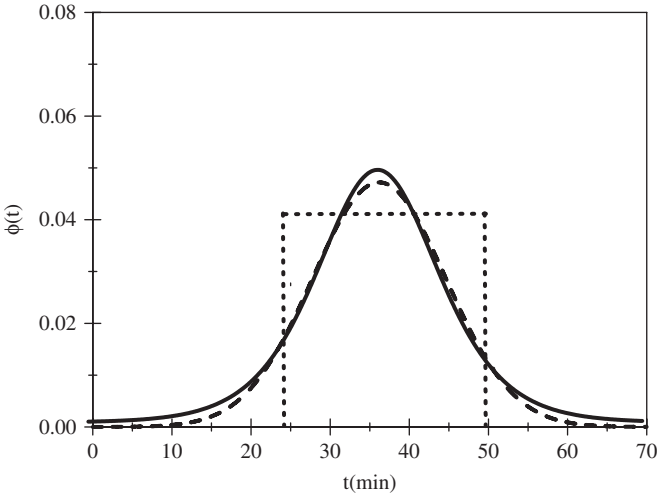

Fig. 6. Comparison between the function $\phi(t)$ predicted from the three different fittings proposed in Table 1. Results shown correspond to the case of growing at 0.7 doublings h^{-1} shown in Fig. 3. The solid, dashed and dotted lines correspond to the predictions from the logistic-like, Gaussian function and segments, respectively.

Table 3
Values of the burst size, the rise rate and the eclipse time obtained from the fittings shown in Fig. 3, using the definitions provided in Table 2

	Three segments	Gaussian function	Logistic-like
Burst size (PFU ml^{-1})	35.9 ± 1.5 (○)	36 ± 1 (○)	37 ± 1 (○)
	35.7 ± 0.9 (△)	37.8 ± 0.6 (△)	38.3 ± 0.6 (△)
	75.2 ± 2.5 (◇)	75 ± 25 (◇)	76 ± 25 (◇)
Rise rate (PFU ml^{-1} min^{-1})	1.5 ± 0.1 (○)	1.7 ± 0.2 (○)	1.8 ± 0.2 (○)
	3.4 ± 0.4 (△)	3.6 ± 0.2 (△)	3.8 ± 0.2 (△)
	4.9 ± 0.2 (◇)	5.9 ± 0.65 (◇)	6.3 ± 0.75 (◇)
Eclipse time (min)	24.1 ± 0.9 (○)	25.6 ± 0.4 (○)	26.3 ± 1.6 (○)
	21.1 ± 0.5 (△)	21.4 ± 0.1 (△)	21.7 ± 0.4 (△)
	17.9 ± 0.3 (◇)	19.1 ± 0.25 (◇)	19.5 ± 0.85 (◇)

The symbols in parenthesis indicate the corresponding curve in Fig. 3.

similar behavior). We observe that for the 'Gaussian' and the 'logistic-like' cases, peaked $\phi(t)$ functions with very similar characteristics are obtained. The 'segments' case, in turn, leads to a discontinuous expression for $\phi(t)$ which slightly differs from the other two. So, we can conclude that the 'segments' fitting gives a poorer estimate for $\phi(t)$ and this can influence the final value of R_0.

We note that in the specific case of phages considered here a new definition for R_0 would be convenient. To this end, we must find the equilibrium states of the system

$$\frac{dX}{dt} = -\beta X(t)V(t)$$
$$\frac{dV}{dt} = -\beta X(t)V(t) + \int_0^t \beta X(t-t')V(t-t')\phi(t')\,dt' \qquad (20)$$

and their stability. Introducing $X(t) = X_{eq} + \delta X(t)$ and $V(t) = V_{eq} + \delta V(t)$ and linearizing about the equilibrium states $(X_{eq}, 0)$ and $(0, V_{eq})$ one can check that the basic reproductive ratio

$$R_0 \equiv \int_0^\infty \phi(t)\,dt \qquad (21)$$

must be higher than 1 for a successful phage growth. Making use of (19)

$$R_0 = \frac{1}{Y(0)}\int_0^\infty \left(\frac{dV}{dt}\right)_{osg} dt = \frac{[V_\infty - V(0)]_{osg}}{Y(0)} \qquad (22)$$

which is nothing but the burst size. This result simply demonstrates that in the case of phage–bacteria interactions the burst size plays the role of a basic reproductive ratio (the infection is successful only for $R_0 > 1$).

7. Conclusions

In the present paper, we have derived a generalization of the BMVD by considering a more accurate life cycle for viruses and cells which includes age-structured effects for mortality and the eclipse phase. As a result, we have shown how the infection dynamics gets modified. Our main motivation here has been to present a rigorous approach to this problem, as many time delays have been introduced in this kind of model just by intuitive or ad hoc arguments. For this reason, we have provided here a mesoscopic derivation based on explicit balance equations that provide a very accurate physical description of the underlying process. In our approach, the life cycle properties are implemented in a probabilistic way by the distribution functions φ_X, φ_Y, φ_V and ϕ (see Fig. 1). Then, although our model requires a more complex formalism, it is advantageous provided that one has the data necessary to evaluate these functions. Anyway, we stress that the BMVD and some possible extensions of it as that presented here are just an oversimplified representation of a real infection process. So, it is unlikely that any quantitative prediction can actually be achieved from these models, whose main value is to provide a qualitative insight into viral dynamics.

We have carried out a formal analysis of the equilibrium states and their stability. Furthermore, we have illustrated how the model works for some simple situations of interest. Specifically, for phage–bacteria interactions we have been able to provide analytical expressions to estimate the function $\phi(t)$ from a one-step growth curve.

In short, the main conclusions obtained from our study are the following:

(i) The mesoscopic formalism presented here allows to reduce the BMVD of three-species to only two species (X and V).

Then, albeit our model requires a more complex mathematical treatment, this simplification can be an interesting advantage.

(ii) We have formally proved that the stability diagram of the BMVD is insensitive to any PDFs considered. It means that the model has an equilibrium infected-free state which becomes unstable for $R_0>1$, which is exactly the same condition necessary for the existence of a stable infected state. This generalizes similar results previously found (Culshaw and Ruan, 2000; Nelson and Perelson, 2002; Wang et al., 2007) that reached the same conclusions for more specific cases.

(iii) The reproductive ratio R_0 and the virus loads can be very sensitive to the distributed mortalities considered. It demonstrates that one needs to know in detail the cellular life cycles, especially for viruses, to describe the infection process. We stress that similar conclusions were already achieved in previous works on age-structured models (Lloyd, 2001; Nelson et al., 2004; Rong et al., 2007).

(iv) For phage–bacteria interactions, we have found that fittings of the one-step growth based on logistic-like and Gaussian functions yield very similar expressions for $\phi(t)$. From the analysis shown here, it is not possible to determine which one of them is more accurate. Anyway, it is clear that both cases give better and more realistic estimates for $\phi(t)$ and R_0 than fittings based on piecewise functions.

In short, we have found that introducing age-distributed processes in the BMVD may modify the dynamics of viral infections. These corrections can be of great interest when the effects of an immune response are also considered in the model. Then, the dynamics of the model is expected to become richer (as happens in the absence of age-structured effects, too) and the role of the cellular life cycles could be more dramatic. Specifically, we expect that age-distributed processes can be able to induce new dynamical patterns as periodicity or chaos, in the line of recent works on this field (Liu, 1997; Buric et al., 2001; Canabarro et al., 2004; Wang et al., 2007). We will address these ideas in a forthcoming paper (currently in preparation).

Acknowledgments

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Appendix A. Derivation of the model

We have introduced $\varphi_X(t)$, $\varphi_Y(t)$ and $\varphi_V(t)$ as the mortality PDFs (see Fig. 1). So that, the probability that a target cell which 'was born' at time $t=0$ has not died yet at time t is given by $\Phi_X(t)$ (hereafter we will refer to it as the 'survival probability') according to

$$\Phi_X(t)=1-\int_0^t \varphi_X(t')\,dt' \tag{23}$$

and analogous arguments hold for $\Phi_Y(t)$ and $\Phi_V(t)$.

Then, we can write the balance equation for the population densities as

$$X(t)=X(0)\Phi_X(t)\Omega_X(0,t)+\int_0^t J_X(t-t')\Phi_X(t')\Omega_X(t-t',t)\,dt' \tag{24}$$

$$Y(t)=Y(0)\Phi_Y(t)+\int_0^t J_Y(t-t')\Phi_Y(t')\,dt' \tag{25}$$

$$V(t)=V(0)\Phi_V(t)\Omega_V(0,t)+\int_0^t J_V(t-t')\Phi_V(t')\Omega_V(t-t',t)\,dt' \tag{26}$$

where $J_X(t)$ represents the density of particles of species X appeared at time t, with equivalent definitions for $J_Y(t)$ and $J_V(t)$. The function $\Omega_X(t-t',t)$ is the probability that a particle X does not become infected during the time interval $(t-t',t)$, while $\Omega_V(t-t',t)$ is the probability that a virus has not been adsorbed by a cell during the same interval. So that, the balance equation (24) simply says that the density of particles X at time t is given by the initial density of particles $X(0)$ not infected yet and still alive, plus those target cells appeared at any time so far, provided they have neither died nor become infected yet. The meaning of Eqs. (25) and (26) can be found using analogous arguments.

Regarding the functions Ω, their explicit form can be found in the following way. For Ω_X we take

$$\Omega_X(t-t',t)=\exp\left[-\int_{t-t'}^t \beta V(t'')\,dt''\right] \tag{27}$$

which corresponds to the solution of the infection equation $dX/dt=-\beta XV$. As the infection is considered independent on the other processes (death and production of new cells by the host), the solution of that ODE within the interval $(t-t',t)$ gives us a proper definition for the probability $\Omega_X(t-t',t)$. Similarly, from $dV/dt=-\beta XV$ we can write

$$\Omega_V(t-t',t)=\exp\left[-\int_{t-t'}^t \beta X(t'')\,dt''\right] \tag{28}$$

The validity of the expressions (27) and (28) can be demonstrated from more rigorous arguments using the age-structured models by Vlad and Ross (2002). In fact, our model (24)–(26) can be seen as a particular case of the very general model by Yadav and Horsthemke (2006), which was in turn based on the original work (Vlad and Ross, 2002). Accordingly, we will follow the formalism in Yadav and Horsthemke (2006) to derive our model.

First, we differentiate the system (24)–(26) with respect to t:

$$\frac{dX}{dt}=-X(0)\Omega_X(0,t)[\varphi_X(t)+\beta V(t)\Phi_X(t)]+J_X(t)-\int_0^t J_X(t-t')\varphi_X(t')\Omega_X(t-t',t)\,dt'-\beta V(t)\int_0^t J_X(t-t')\Phi_X(t')\Omega_X(t-t',t)\,dt' \tag{29}$$

$$\frac{dY}{dt}=-Y(0)\varphi_Y(t)+J_Y(t)-\int_0^t J_Y(t-t')\varphi_Y(t')\,dt' \tag{30}$$

$$\frac{dV}{dt}=-V(0)\Omega_V(0,t)[\varphi_V(t)+\beta X(t)\Phi_V(t)]+J_V(t)-\int_0^t J_V(t-t')\varphi_V(t')\Omega_V(t-t',t)\,dt'-\beta X(t)\int_0^t J_V(t-t')\Phi_V(t')\Omega_V(t-t',t)\,dt' \tag{31}$$

Then, we introduce (24) and (26) into (29) and (31), respectively, so we obtain

$$\frac{dX}{dt}=-X(0)\Omega_X(0,t)\varphi_X(t)+J_X(t)-\beta X(t)V(t)-\int_0^t J_X(t-t')\varphi_X(t')\Omega_X(t-t',t)\,dt' \tag{32}$$

$$\frac{dY}{dt}=-Y(0)\varphi_Y(t)+J_Y(t)-\int_0^t J_Y(t-t')\varphi_Y(t')\,dt' \tag{33}$$

$$\frac{dV}{dt} = -V(0)\Omega_V(0,t)\varphi_V(t) + J_V(t) - \beta X(t)V(t) - \int_0^t J_V(t-t')\varphi_V(t')\Omega_V(t-t',t)\,dt' \quad (34)$$

On the other side, we divide (24) by $\Omega_X(0,t)$ and transform that equation to the Laplace domain (again, we denote the Laplace transform of a function by the brackets $[\cdot]_s$ with the conjugate variable s). After some simple algebra, it can be written as

$$\frac{s[\varphi_X]_s}{1-[\varphi_X]_s}\left[\frac{X}{\Omega_X(0,t)}\right]_s = X(0)[\varphi_X]_s + [\varphi_X]_s[J_X\Omega_X]_s \quad (35)$$

Finally, introducing the inverse Laplace transform of (35) into (32), the evolution equation for the species X reads

$$\frac{dX}{dt} = J_X(t) - \beta XV - \int_0^t X(t-t')\Psi_X(t')\Omega_X(t-t',t)\,dt' \quad (36)$$

where Ψ_X is defined in the Laplace domain by (3). For the species Y and V we can use exactly the same derivation, so that Eqs. (33) and (34) turn into

$$\frac{dY}{dt} = J_Y(t) - \int_0^t Y(t-t')\Psi_Y(t')\,dt' \quad (37)$$

$$\frac{dV}{dt} = J_V(t) - \beta XV - \int_0^t V(t-t')\Psi_V(t')\Omega_V(t-t',t)\,dt' \quad (38)$$

with Ψ_Y, Ψ_V defined implicitly in (3).

Hence, we have obtained the general evolution equations (36)–(38) for the model. However, note that we still need to give expressions for the densities J_i. From Eq. (1), assuming a constant source of target cells, the number of new target cells appearing at any given time can be expressed as

$$J_X(t) = \lambda \quad (39)$$

Similarly, the density of infected cells appearing at time t is given by

$$J_Y(t) = \beta X(t)V(t) \quad (40)$$

Finally, the new viruses appeared at time t are given by the function $\phi(t)$ applied to those cell which were infected at any previous time $t-t'$, provided they have not died yet. This allows us to write

$$J_V(t) = \int_0^t J_Y(t-t')\phi(t')\Phi_Y(t')\,dt' \quad (41)$$

Once we have the explicit expressions for J_X, J_Y and J_V, our model takes the final form (2):

$$\frac{dX}{dt} = \lambda - \beta XV - \int_0^t X(t-t')\Psi_X(t')\Omega_X(t-t',t)\,dt'$$

$$\frac{dY}{dt} = \beta XV - \int_0^t Y(t-t')\Psi_Y(t')\,dt'$$

$$\frac{dV}{dt} = \int_0^t \beta X(t-t')V(t-t')\phi(t')\Phi_Y(t')\,dt' - \beta XV - \int_0^t V(t-t')\Psi_V(t')\Omega_V(t-t',t)\,dt'$$

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