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THE ROLE OF DELAYS IN INNATE AND ADAPTIVE IMMUNITY TO INTRACELLULAR BACTERIAL INFECTION

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Abstract. The immune response in humans is complex and multi-fold. Initially an innate response attempts to clear any invasion by microbes. If it fails to clear or contain the pathogen, an adaptive response follows that is specific for the microbe and in most cases is successful at eliminating the pathogen. In previous work we developed a delay differential equations (DDEs) model of the innate and adaptive immune response to intracellular bacteria infection. We addressed the relevance of known delays in each of these responses by exploring different kernel and delay functions and tested how each affected infection outcome. Our results indicated how local stability properties for the two infection outcomes, namely a boundary equilibrium and an interior positive equilibrium, were completely dependent on the delays for innate immunity and independent of the delays for adaptive immunity. In the present work we have three goals. The first is to extend the previous model to account for direct bacterial killing by adaptive immunity. This reflects, for example, active killing by a class of cells known as macrophages, and will allow us to determine the relevance of delays for adaptive immunity. We present analytical results in this setting. Second, we implement a heuristic argument to investigate the existence of stability switches for the positive equilibrium in the manifold defined by the two delays. Third, we apply a novel analysis in the setting of DDEs known as uncertainty and sensitivity analysis. This allows us to evaluate completely the role of all parameters in the model. This includes identifying effects of stability switch parameters on infection outcome.

1. **Introduction.** Immunity refers to the ability of a host to resist infection by microbes (bacteria, viruses, etc.) that would otherwise cause infection. Immunity has many facets, but it is typically divided into two categories: adaptive (aquired immunity) and innate (natural immunity or innate resistance). Innate immunity refers to nonspecific defense mechanisms that attempt to clear microbes within hours of their appearance in the body. These mechanisms include physical barriers

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such as skin, chemicals in the blood, and immune system cells that attack foreign (nonself) cells in the body [41]. This innate response is activated by properties of pieces of microbes known as antigens [40]. Adaptive immunity refers to an antigen-specific response. Antigens first must be processed and recognized, then immune cells of the adaptive response are generated that are designed to attack that specific antigen (for a review see [26]). Adaptive immunity also includes a memory response that allows future responses against a specific antigen to be more efficient [26]. Many diseases that afflict mankind are now thought to be the result of dysfunctional of innate or adaptive immune responses, or both [8]. Dysfunction here can simply refer to a failure of the innate immune system to effectively discriminate self from nonself or it could be a more complex problem involving failure of a well tuned regulation of innate-adaptive connections rather than recognition itself. Timing of the response can also represent a measure of efficiency of host immunity against microbial infection. Essential to the successful removal of pathogens is the early recognition of microbes by components of the innate immune system [24]. Recognition of microbial infection and initiation of host defenses are controlled by a variety of mechanisms. These components include the complement system [12], specialized receptors expressed on natural killer (NK) cells [50] and the family of Toll-like receptors (TLRs) that are expressed on myeloid as well as lymphoid cells and that recognize specific microbial-derived molecular structures. Toll-like receptors have recently emerged as key components of the innate immune system that detect microbial infection and trigger antimicrobial host defense responses [14]. Successful engagement of these pathways can lead to a successful immune response allowing clearance of pathogens. A well orchestrated innate and adaptive immune response will lead to pathogen eradication and host immunity. Failure to efficiently discriminate self from nonself in innate as well as adaptive immunity can lead to pathogen proliferation and ultimately sepsis and may also be the cause for development and maintenance of autoimmune and chronic inflammatory diseases and of allergies.

In this framework, elucidating the complex immunoregulatory system of innate and adaptive responses is decisive in understanding disease progression and can help in preventing and treating many infectious diseases by designing more efficient therapies and vaccines. Many examples of the application of delay differential equation systems to immunology can be found in the literature (see [45] and [5] for reviews). Recent work has been devoted to delay differential equation models of specific infectious pathogens, such as HIV [16, 17, 46, 47] and LCMV [10]. Here we address the more general immunological question of how timing and memory in innate and adaptive responses can affect host immunity versus intracellular bacteria infection. In previous work [7] we developed a delay differential model of the innate and adaptive immune response to intracellular bacterial infection (the baseline model). We specifically addressed timing of immune responses and the role of delays, as well as how different kernels and delay functions can affect infection outcome. Our results indicated that local stability properties of both the chronic and latent infection states did not depend on delays in the adaptive response. In the present work we extend the baseline model to include a term that directly allows for bacteria killing by adaptive immunity. This would capture, for example, the role of antibodies in clearing bacteria, activated macrophage killing of bacteria, or complement-mediated bacterial clearance and define a more comprehensive role for delays in both innate and adaptive responses. We derive local stability results

under different delay kernels (uniform for innate response and positive exponential for adaptive response), considering only the case for the most biologically relevant delay function, namely where both responses depend solely on the past levels of bacterial load. We critically analyze our conclusions addressing parameter uncertainty and how it might affect infection outcome. The existence of stability switches in parameter space is investigated using a comprehensive sampling method known as Latin hypercube sampling (LHS). The new delay differential model is described in Section 2, illustrating new terms, delay functions, delay kernels and how we address uncertainty and sensitivity analyses. Analytical results and local stability analysis are shown respectively in sections 3 and 4. In section 4 we also describe the numerical DDE solver implementation (*dde23*) and how we apply uncertainty and sensitivity analysis in a DDE setting. Section 5 shows numerical results and section 6 illustrates numerical and biological remarks and conclusions.

2. **Mathematical models and analysis of dynamics.** Our baseline model consisted of five variables: uninfected target cells (X_U), infected cells (X_I), bacteria (B), and phenomenological variables capturing innate (I_R) and adaptive (A_R) immunity. The modified model equations are shown below.

$$\begin{cases} \frac{d}{dt}X_U(t) = s_U - \alpha_1 X_U(t)B(t) - \mu_{X_U}X_U(t) \\ \frac{d}{dt}X_I(t) = \alpha_1 X_U(t)B(t) - \alpha_2 X_I(t)A_R(t) - \mu_{X_I}X_I(t) \\ \frac{d}{dt}B(t) = \alpha_{20}B(t)\left(1 - \frac{B(t)}{\sigma}\right) - \alpha_3 B(t)I_R(t) - \alpha_4 \mathbf{B(t)A_R(t)} \\ \frac{d}{dt}I_R(t) = s_{I_R} + \int_{-\tau_1}^{0} w_1(s)f_1(x(t+s))ds - \mu_{I_R}I_R(t) \\ \frac{d}{dt}A_R(t) = s_{A_R} + \int_{-\tau_2}^{0} w_2(s)f_2(x(t+s))ds - \mu_{A_R}A_R(t) \end{cases} \tag{1}$$

where

$$x(t) := \Big(X_U(t), X_I(t), B(t), I_R(t), A_R(t)\Big) \in \mathbb{R}^5 \tag{2}$$

The structure of the system (1) is unchanged with respect to the baseline model presented in [7] except for an additional term to the bacteria equation. We now include a new term into the equation determining the rate of change of bacteria. This term accounts for direct killing of bacteria due to adaptive immunity (bold in $B(t)$ equation). This occurs naturally through, for example, T-cell-mediated cytotoxicity and activated macrophage engulfment, as well as antibody mediated killing (see [26] for a review). We capture this via a simple mass action term (i.e. $\alpha_4 B(t)A_R(t)$). The baseline model studied in [7] is a special case of (1) (i.e. $\alpha_4 = 0$).

Uninfected target cells (X_U) have a natural turnover (s_U) and half-life (μ_{X_U}) and can become infected (mass-action term $\alpha_1 X_U(t)B(t)$). Infected cells (X_I) can be cleared by the adaptive response (mass-action term $\alpha_2 X_I(t)A_R(t)$), or they die (half-life term $\mu_{X_I}X_I(t)$). The bacterial population (B) has a net proliferation term, represented by a logistic function $\alpha_{20}B(t)\left(1 - \frac{B(t)}{\sigma}\right)$ and is also cleared by innate immunity (mass-action term $\alpha_3 B(t)I_R(t)$) and adaptive immunity (mass-action term $\alpha_4 B(t)A_R(t)$). Both innate (I_R) and adaptive (A_R) responses have a source term and a half-life term. Two delays are included in the model. The delay

for innate immunity, τ_1, occurs on the order of minutes to hours, and τ_2 is the delay for adaptive immunity on the order of days to weeks.

Innate immunity is the first line of defense against microbial infections, is non-specific (it targets any particle that is not recognized as belonging to the human genetic repertoire) and is always present (the source term s_{I_R} in the I_R equation is always positive). Adaptive responses take place only after innate response occurs, and they are specific and effective. Background levels of A_R are zero unless the host has already been exposed to the same pathogen (either through vaccination or clearance of a previous infection). Therefore the source term s_{A_R} in the A_R equation is set to zero in the case of a first infection and positive otherwise.

We have previously considered two cases of delay for each immune type [7]. Here we consider the bacterial load as the most informative marker of infection, and therefore we assume that both responses depend solely on the bacterial load $B(s)$ in the previous τ_i time units $(i = 1, 2)$; that is,

$$f_1\Big(x(t+s)\Big) = B(t+s), s \in [-\tau_1, 0]$$
$$\text{and } f_2\Big(x(t+s)\Big) = B(t+s), s \in [-\tau_2, 0].$$

We hypothesize a relationship between the amplitude of the delay and the functional form of the kernels $(w_i(\theta)(i = 1, 2)$; to wit, the larger the time delay, the more relevant the recent history of the infection. This leads us to use two different delay kernels: a uniform kernel for innate immunity and an exponential growth kernel for adaptive immunity. In the case of a uniform kernel

$$w_1(\theta) = A > 0, \theta \in [-\tau_1, 0],$$

we assume that the innate response is uniformly dependent on the level of the bacterial load in the previous τ_1 time units of infection.

In the case of an exponential growth kernel

$$w_2(\theta) = Ae^{k\theta}, \theta \in [-\tau_2, 0], A, k \in \mathbb{R}_+,$$

we assume that the adaptive response places significant emphasis on the most recent level of infection.

To complete the development of the mathematical model, we must define values for the parameters and initial conditions, as well as measure units. In many cases, previously published data in the literature suggest large ranges in parameter choices. The values of initial conditions and parameter value ranges are given in tables 1 and 2. Below we consider a wide range of values for parameters and test the effects of this variability by our uncertainty and sensitivity analysis (see next section). To properly define the integrals in (1), we need the following initial condition:

$$B(t) \equiv B(0) \text{ for t} \in [-\tau_2, 0].$$

As an example, we will focus our studies on the intracellular human pathogen, *Mycobacterium tuberculosis*. This respiratory pathogen is the number-one cause of death from infectious diseases in the world today with an estimated 1/3 of the world's population infected and 10 million deaths per year [1]. This pathogen is an intracellular pathogen that has existed for thousands of years with records of Egyptian mummies whose lungs show signs of infection. Data from host-pathogen interaction studies with this pathogen will serve to guide the parameter choices and infection outcomes that are typically observed in tuberculosis infection (TB). A person can have either inactive tuberculosis (more frequently called latent TB) or active TB. Latent TB is the more frequent outcome of infection (approximately

90 percent of the infected). In latent TB the bacteria are alive but not active: the patient has no symptoms and is not infectious, and the bacteria coexist peacefully within the host. In active TB the bacteria multiply, causing permanent damage and even death if the patient is not treated. From every 100 people with latent TB, from 5 to 10 will develop active TB in their lifetimes (reactivation).

We use a volumetric measure unit (i.e., number of cells per cm^3 of tissue) to allow for comparison of our results with available experimental data in the respiratory tract and lung ([32, 31, 36, 37, 43, 62, 69]). Data in table 2 are mostly used from *M. tuberculosis* literature. By applying our uncertainty analysis later, we will address the effects of parameter variation, allowing us to comment more generally regarding host-pathogen interactions.

Previously, we studied local stability of the baseline model (case 1 in [7]). Here we present local stability results for the modified model and simulate different scenarios to test and validate the analytical results.

TABLE 1. Initial conditions (cells or bacteria per cm^3 of tissue)

Name	*Value*	*Range*
$X_U(0) = X_U^{baseline}$	$1e^4$	$[1e^3 - 1e^5]$
$X_I(0) = X_I^{baseline}$	0	
$B(0) = B_0$	20	$[1 - 1e^3]$
$I_R(0) = I_R^{baseline}$	$1e^3$	$[5e^2 - 1e^4]$
$A_R(0) = A_R^{baseline}$	0 (first infection)	$[5e^1 - 2e^2]$

2.1. **Uncertainty and sensitivity analysis.** Variances in the values for most parameters are due to two key factors: extensive variability in experimental systems leading to variations in data, and lack of experimental protocols to measure kinetics that define model rates and rate constants. We have adapted a method known as Latin hypercube sampling to allow us to stratify parameter space over large ranges and distributions [38]. Sampling occurs simultaneously, stratified, random, and evenly distributed for each parameter within a defined range. This technique systematically evaluates how different combinations of parameter values significantly affect infection outcome. Since we base our conclusions regarding qualitative properties of (1) on the choice of parameter values, the most relevant parameters are

TABLE 2. Parameter values for the model 1

Name	*Definition*	*Value*	*Range*	*Units*	*Reference*
μ_{X_U}	Half-life of X_U	0.011	0.011	$1/day$	[68]
α_1	Rate X_U infection	$1e^{-3}$	$[1e^{-5}, 1]$	$B(t)^{-1}/day$	Estimated
α_2	Kill rate of X_I (A_R)	$1e^{-3}$	$[1e^{-5}, 1]$	$A_R(t)^{-1}/day$	[19, 35, 58, 66, 67]
μ_{X_I}	Half-life of X_I	0.011	0.011	$1/day$	[68]
α_{20}	Growth rate of B	.5	$[1e^{-5}, 1]$	$1/day$	[48, 58, 59]
σ	Max # of bacteria	1e5	$[1e^4, 1e^6]$	$B(t)$	Estimated
α_3	Kill rate of $B(I_R)$	$1e^{-4}$	$[1e^{-5}, 1]$	$I_R(t)^{-1}/day$	[19]
α_4	Kill rate of $B(A_R)$	$1e^{-4}$	$[1e^{-5}, 1]$	$A_R(t)^{-1}/day$	estimated
μ_{I_R}	Half-life of I_R	0.11	0.11	$1/day$	[60]
μ_{A_R}	Half-life of A_R	0.33	0.33	$1/day$	[60]
τ_1	Delay innate immun.	.1	[.1 , 10]	day	Estimated
τ_2	Delay adaptive immun.	20	[5 , 40]	day	Estimated

evaluated in the modified model using this uncertainty analysis. In conjunction with the uncertainty analysis, we need to employ a measure that allows for analysis of the variation induced by variability in parameter values on model outcome values. Thus, we perform a sensitivity analysis on model (1) by investigating what parameter(s) contribute most to variation of bacterial load (defined as our outcome value).

To this end, two sensitivity indexes are calculated: partial rank correlation coefficient (PRCC) and extended Fourier amplitude sensitivity test (EFAST) [55]. PRCC deals with monotonic nonlinear associations between parameters and outcome, while EFAST is more accurate for non-monotonic nonlinear relationships. LHS and PRCC method has been originally applied in the ODE model setting ([9]. Our group has also applied it in the setting of agent based models (ABMs) ([57]) and partial differential equations (PDEs) ([20]).

We sampled eight parameters simultaneously (τ_1,τ_2, α_1, α_2, α_3, α_4, α_{20}, σ), defining uniform probability density functions for their distributions. The remainder of the parameters are held constant at their default value (see tables 1 and 2).

LHS is used to sample the parameter space for the PRCC calculation. EFAST uses its own sampling implementation based on Fourier transformation in the frequency domain (see [52, 53] for details). Tables 1 and 2 are used to initialize the sampling procedure and to define intervals (Range column).

2.1.1. *Latin Hypercube Sampling (LHS) and Partial Rank Correlation Coefficient (PRCC).* Uncertainty analysis is performed by creating an N-dimensional hypercube based on both ranges and distributions for all parameters in the model. The method is known as Latin hypercube sampling [38] and allows for simultaneous, stratified, random, evenly distributed sampling of each parameter within a defined range (basically a stratified sampling without replacement). This sampling is guided by the specification of a probability density function for each parameter, depending on *a priori* information (i.e., normal, uniform, lognormal,...). The larger N is, the more accurate is the sampling of the parameter space and more reliable conclusions can be drawn.

A matrix (which we call an LHS matrix) consisting of K columns corresponding to the number of varied parameters and N rows for the number of simulations is generated; N solutions are then created that reveal all variability in model outcome due to uncertainty in the parameters. The widest, biologically relevant range possible is used for all parameters. When applied correctly, this method identifies all relevant and distinct stable numerical solutions for the range of parameters given. Once variations in outcome variable(s) are obtained (for example bacterial load), a partial rank correlation (PRC) ([30]) is performed to identify which of the K parameters are most correlated with outcome values. Each PRC value generates a p-value that determines the significance; thus, even small correlations may be significant. Correlation coefficients vary between -1 and +1. By combining the uncertainty analyses with PRC, we can reasonably assess the sensitivity of our outcome variable to parameter variation. This allows us to identify and quantify critical parameters (i.e., interactions) that, when varied, dramatically affect the outcome and stability switches.

2.1.2. *Extended Fourier Amplitude Sensitivity Test - EFAST.* EFAST is based on the Fourier amplitude sensitivity test (FAST) and uses the behavior of the model variance to evaluate the variance contribution of the input parameters to the model output. It is a computationally efficient method that implements a small random sample to investigate the entire distribution of the input parameters. EFAST allows the computation of the main effect and total effect contribution of each input factor to the output's variance. The term "total" here means that the factor's main effect (single contribution or first-order effect), as well as all the interaction terms involving that factor, are included. In EFAST, Fourier coefficients are used to compute the proportional variance contribution (partial variance) of each input parameter. Cukier et al. [15], Schaibly et al. [56], and Collins and Avissar [13] provided details of method development and equations for sampling and computing Fourier coefficients and partial variance. Partitioning model uncertainty using EFAST involves three procedures: generating random sample, obtaining the original model predictions, and computing of Fourier coefficients and partial variances of the input parameters. The main advantages of the extended FAST are its robustness, especially at low sample size, and its computational efficiency. The computational aspects of the extended FAST include the definition of new sets of parametric equations for the search-curve exploring the input space (composition of sin functions), the selection of frequencies for the parametric equations, and the procedure adopted to estimate the total contributions. For details on how the procedure is implemented see [52, 53, 54, 55]. We follow [53].

3. **Analytical results.** The equations of the modified model are

$$\begin{cases} \frac{d}{dt}X_U(t) = s_U - \alpha_1 X_U(t)B(t) - \mu_{X_U}X_U(t) \\ \frac{d}{dt}X_I(t) = \alpha_1 X_U(t)B(t) - \alpha_2 X_I(t)A_R(t) - \mu_{X_I}X_I(t) \\ \frac{d}{dt}B(t) = \alpha_{20}B(t)\Big(1 - \frac{B(t)}{\sigma}\Big) - \alpha_3 B(t)I_R(t) - \alpha_4 B(t)A_R(t) \\ \frac{d}{dt}I_R(t) = s_{I_R} + \int_{-\tau_1}^{0}[w_1(\theta)B(t+\theta)]d\theta - \mu_{I_R}I_R(t) \\ \frac{d}{dt}A_R(t) = s_{A_R} + \int_{-\tau_2}^{0}[w_2(\theta)B(t+\theta)]d\theta - \mu_{A_R}A_R(t) \end{cases} \tag{3}$$

.

In the following we define $\Delta(\tau_i)$ as

$$\Delta(\tau_i) = \int_{-\tau_i}^{0} w_i(\theta)d\theta, i = 1, 2. \tag{4}$$

We now discuss the main mathematical properties of system (3).
Let $h = \max\{\tau_1, \tau_2\} = \tau_2$, $X(t)$ as in (2) and $X_t(\theta) = X(t+\theta), \theta \in [-h, 0]$ for all $t \geq 0$. Then (3) can be rewritten as

$$x'(t) = F(x_t) \tag{5}$$

with initial conditions at $t = 0$ given by

$$\Phi \in C([-h, 0], \mathbb{R}^5)$$

where $C([-h,0],\mathbb{R}^5)$ is the Banach space of continuous functions mapping the interval $[-h,0]$ into $\mathbb{R}^5$ equipped with the (supremum) norm

$$\| \Phi \| = \sup_{\theta\in[-h,0]} |\Phi(\theta)|$$

where $|\cdot|$ is any norm in $\mathbb{R}^5$.
For the biological relevance, according to (2) and Table 1, we define nonnegative initial conditions

$$\Phi(\theta) \geq 0, \theta \in [-h,0]$$

with

$$\Phi_i(0) > 0, i = 1,3,4,5 \quad and \quad \Phi_2(0) = X_I(0) = 0$$

to equations (3).

LEMMA 3.1. *Any solution $x(t) = x(\Phi,t)$ of (3) with $\Phi(\theta) \geq 0, \theta \in [-h,0], \Phi(0) > 0$ (except for $\Phi_2(0) = 0$) remains positive whenever it exists; i.e., $x(t) \in \mathbb{R}^5_+$ where*

$$\mathbb{R}^5_+ = \{x = (x_1,x_2,x_3,x_4,x_5) \in \mathbb{R}^5 | x_i > 0, i = 1,2,3,4,5\}$$

Proof. Consider the third equation in (3):

$$\frac{dB}{dt} = B(t)\left[\alpha_{20}\left(1 - \frac{B(t)}{\sigma}\right) - \alpha_3 I_R(t) - \alpha_4 A_R(t)\right]$$

with $B(0) = \Phi_3(0) > 0$. Then

$$B(t) = B(0)\exp\{\int_0^t [\alpha_{20}(1 - \frac{B(s)}{\sigma}) - \alpha_3 I_R(s) - \alpha_3 A_R(s)]ds\} > 0, \quad t \geq 0. \quad (6)$$

The first equation in (3) gives

$$\frac{dX_U}{dt} > -X_U(t)(\alpha_1 B(t) + \mu_{X_U}), \qquad X_U(0) = \Phi_1(0) > 0;$$

i.e.,

$$X_U(t) > X_U(0)\exp\left\{-\int_0^t [\alpha_1 B(s) + \mu_{X_U}]ds\right\} > 0 \quad t \geq 0. \quad (7)$$

Since $X_U(t) > 0, B(t) > 0$ for $t \geq 0$, the second equation in (3) gives

$$\frac{dX_I}{dt} > -X_I(t)\left(\alpha_2 A_R(t) + \mu_{X_I}\right), \qquad X_I(0) = \Phi_2(0) = 0;$$

i.e.,

$$X_I(t) > 0 \quad t \geq 0 \quad (8)$$

Consider the last two equations in (3). Since $B(\theta) = \Phi_3(\theta) \geq 0$ in $[-h,0]$ and $B(t) > 0$ for $t \geq 0$, we have

$$\frac{dI_R(t)}{dt} \geq s_{I_R} - \mu_{I_R} I_R(t), \quad I_R(0) = \Phi_4(0) > 0;$$

i.e.,

$$I_R(t) \geq I_R(0)e^{-\mu_{I_R}t} + \frac{s_{I_R}}{\mu_{I_R}}(1 - e^{-\mu_{I_R}t}) > 0, \ t \geq 0. \quad (9)$$

Similarly,

$$\frac{dA_R(t)}{dt} \geq s_{A_R} - \mu_{A_R} A_R(t), \quad A_R(0) = \Phi_5(0) > 0;$$

i.e.,

$$A_R(t) \geq A_R(0)e^{-\mu_{A_R}t} + \frac{s_{A_R}}{\mu_{A_R}}(1 - e^{-\mu_{A_R}t}) > 0,\ t \geq 0. \tag{10}$$

This completes the proof of positivity. □

Let us consider the boundedness of solutions.

LEMMA 3.2. *Any solution $x(t) = x(\Phi, t)$ of (3) is bounded.*

Proof. Because of positivity of solutions, the first two equations in (3) give

$$\frac{d}{dt}(X_U(t) + X_I(t)) < s_U - \mu\,(X_U(t) + X_I(t))\,,$$

where

$$\mu = \min\{\mu_{X_U}, \mu_{X_I}\}.$$

Hence

$$\limsup_{t\to\infty}(X_U(t) + X_I(t)) \leq \frac{s_U}{\mu}. \tag{11}$$

Positivity of solutions still implies

$$\frac{dB(t)}{dt} \leq \alpha_{20}B(t)\left(1 - \frac{B(t)}{\sigma}\right),$$

and therefore

$$\limsup_{t\to\infty} B(t) \leq \sigma. \tag{12}$$

Accordingly, there exists a $T_\epsilon > 0$ such that for all $t > T_\epsilon + h$ ($h = \max\{\tau_1, \tau_2\}$) and for sufficiently small $\epsilon > 0$, $B(t) < \sigma + \epsilon$. Hence, the last two equations in (3) give

$$\begin{array}{rcl} \dfrac{dI_R(t)}{dt} & < & s_{I_R} + (\sigma+\epsilon)\Delta(\tau_1) - \mu_{I_R}I_R(t) \\ \dfrac{dA_R(t)}{dt} & < & s_{A_R} + (\sigma+\epsilon)\Delta(\tau_2) - \mu_{A_R}A_R(t) \end{array},$$

thus implying (by letting $\epsilon \to 0$),

$$\limsup_{t\to\infty} I_R(t) \leq \frac{s_{I_R} + \sigma\Delta(\tau_1)}{\mu_{I_R}} \tag{13}$$

$$\limsup_{t\to\infty} A_R(t) \leq \frac{s_{A_R} + \sigma\Delta(\tau_2)}{\mu_{A_R}}. \tag{14}$$

This proves boundedness. □

DEFINITION 3.1. *(Permanence of (3))*
System (3) is permanent (or uniformly persistent) if there exist positive constants $m, M, m < M$, independent of initial conditions and such that for solutions of (3), we have:

$$\begin{array}{l} \max\{\limsup_{t\to\infty} X_U(t), \limsup_{t\to\infty} X_I(t), \limsup_{t\to\infty} B(t), \limsup_{t\to\infty} I_R(t), \\ \limsup_{t\to\infty} A_R(t)\} \leq M \\ \\ \min\{\liminf_{t\to\infty} X_U(t), \liminf_{t\to\infty} X_I(t), \liminf_{t\to\infty} B(t), \liminf_{t\to\infty} I_R(t), \\ \liminf_{t\to\infty} A_R(t)\} \geq m. \end{array} \tag{15}$$

LEMMA 3.3. *Provided that*

$$\alpha_{20} > \alpha_3\frac{s_{I_R} + \sigma\Delta(\tau_1)}{\mu_{I_R}} + \alpha_4\frac{s_{A_R} + \sigma\Delta(\tau_2)}{\mu_{A_R}}, \tag{16}$$

system (3) is permanent.

Proof. Let us consider the lim sup; i.e., the first of (15).
From the first equation of (3) and lemma 1 (positivity), we have:

$$\frac{dX_U}{dt} \leq s_U - \mu_{X_U} X_U(t),$$

which implies that

$$\limsup_{t\to\infty} X_U(t) \leq \frac{s_U}{\mu_{X_U}} := \bar{X}_U. \tag{17}$$

From (12), (17) and the second of equations (3), for sufficiently large $t > 0$ and small $\epsilon > 0$, we have

$$\frac{dX_I(t)}{dt} < \alpha_1 \left(\frac{s_U}{\mu_{X_U}} + \epsilon\right)(\sigma + \epsilon) - \mu_{X_I} X_I(t),$$

which gives

$$\limsup_{t\to\infty} X_I(t) \leq \frac{\alpha_1 \left(\frac{s_U}{\mu_{X_U}}\right)\sigma}{\mu_{X_I}} := \bar{X}_I. \tag{18}$$

Hence, from (12)-(14), (17), (18) we have

$$\begin{aligned}\left(\limsup_{t\to\infty} X_U(t), \limsup_{t\to\infty} X_I(t), \limsup_{t\to\infty} B(t), \limsup_{t\to\infty} I_R(t), \limsup_{t\to\infty} A_R(t)\right) \\ \leq (\bar{X}_U, \bar{X}_I, \bar{B}, \bar{I}_R, \bar{A}_R)\end{aligned} \tag{19}$$

where

$$\bar{B} = \sigma, \quad \bar{I}_R = \frac{s_{I_R} + \sigma\Delta(\tau_1)}{\mu_{I_R}}, \quad \bar{A}_R = \frac{s_{A_R} + \sigma\Delta(\tau_2)}{\mu_{A_R}}.$$

If we choose

$$M = \max(\bar{X}_U, \bar{X}_I, \bar{B}, \bar{I}_R, \bar{A}_R),$$

then there exists $M > 0$ such that the first inequality in (15) holds true.

Consider now the lim inf; i.e., the second in (15).

(i) Consider A_R, I_R. From (9) and (10) we have

$$\liminf_{t\to\infty} I_R(t) \geq \frac{s_{I_R}}{\mu_{I_R}} := \underline{I}_R, \ \liminf_{t\to\infty} A_R(t) \geq \frac{s_{A_R}}{\mu_{A_R}} := \underline{A}_R \tag{20}$$

(ii) Consider B. From (19), for sufficiently large $t > 0$ and small $\epsilon > 0$ we have

$$\begin{aligned}\frac{dB(t)}{dt} \geq \alpha_{20} B(t)\left(1 - \frac{B(t)}{\sigma}\right) - (\alpha_3 \bar{I}_R(t) + \epsilon)B(t) - (\alpha_4 \bar{A}_R + \epsilon)B(t) = \\ = \alpha_{20} B(t)\left(1 - \frac{\alpha_3 \bar{I}_R + \epsilon}{\alpha_{20}} - \frac{\alpha_4 \bar{A}_R + \epsilon}{\alpha_{20}} - \frac{B(t)}{\sigma}\right).\end{aligned} \tag{21}$$

Hence, letting $\epsilon \to 0$

$$\liminf_{t\to\infty} B(t) \geq \left(\frac{\alpha_{20} - \alpha_3 \bar{I}_R - \alpha_4 \bar{A}_R}{\alpha_{20}}\right)\sigma := \underline{B} \tag{22}$$

where $\underline{B} > 0$ provided that

$$\alpha_{20} > \alpha_3 \frac{s_{I_R} + \sigma\Delta(\tau_1)}{\mu_{I_R}} + \alpha_4 \frac{s_{A_R} + \sigma\Delta(\tau_2)}{\mu_{A_R}}.$$

(iii) Consider X_U. For large $t > 0$, small $\epsilon > 0$ we have

$$\frac{dX_U(t)}{dt} \geq s_U - (\alpha_1(\bar{B} + \epsilon) + \mu_{X_U})X_U,$$

from which, letting $\epsilon \to 0$

$$\liminf_{t\to\infty} X_U(t) \geq \frac{s_U}{\alpha_1\sigma + \mu_{X_U}} := \underline{X}_U. \tag{23}$$

(iv) Consider X_I. For large $t > 0$, small $\epsilon > 0$ we have

$$\frac{dX_I(t)}{dt} \geq \alpha_1(\underline{X}_U - \epsilon)(\underline{B} - \epsilon) - (\alpha_2(\bar{A}_R + \epsilon) + \mu_{X_I})X_I(t)$$

from which, letting $\epsilon \to 0$, we obtain

$$\liminf_{t\to\infty} X_I(t) \geq \frac{\alpha_1\underline{X}_U\underline{B}}{\alpha_2\bar{A}_R + \mu_{X_I}} := \underline{X}_I; \tag{24}$$

hence, provided that (16) holds true, (20)-(24) imply that

$$\begin{aligned}&(\liminf_{t\to\infty} X_U(t), \liminf_{t\to\infty} X_I(t), \liminf_{t\to\infty} B(t), \lim_{t\to\infty} \inf I_R(t),\\ &\liminf_{t\to\infty} A_R(t)) \geq (\underline{X}_U, \underline{X}_I, \underline{B}, \underline{I}_R, \underline{A}_R),\end{aligned} \tag{25}$$

where the constants on the right side of (25) are positive.

Thus, if we choose

$$m = \min(\underline{X}_U, \underline{X}_I, \underline{B}, \underline{I}_R, \underline{A}_R), \quad m > 0,$$

considering that $\liminf x(t) \leq \limsup x(t)$, we have found two positive constants $m, M, m \leq M$ such that (15) hold true. □

REMARK 1. *As we will see in theorem 3.1, if* $\Delta(\tau_1) = 0$ *and* $\Delta(\tau_2) = 0$ *the permanence condition (16) becomes the existence condition of the positive equilibrium* E_P. *Furthermore, if* $\Delta(\tau_1) = 0$ *and* $\Delta(\tau_2) = 0$, *then* $\underline{B} = B^*$.

Concerning the equilibria of (3), we can give the following result (we omit the computations which can be easily checked):

THEOREM 3.1. *The system (3) gives two nonnegative equilibria:*

1. *for all parameter values the boundary equilibrium exists*

$$E_B = \left(X_U^* = \frac{s_U}{\mu_{X_U}}\,,\ X_I^* = 0\,,\ B^* = 0\,,\ I_R^* = \frac{s_{I_R}}{\mu_{I_R}}\,,\ A_R^* = \frac{s_{A_R}}{\mu_{A_R}}\right) \tag{26}$$

on the boundary of the positive cone in R^5*; and*

2. *for* $\alpha_{20} - \alpha_3\,(s_{I_R}/\mu_{I_R}) - \alpha_4\,(s_{A_R}/\mu_{A_R}) > 0$ *the positive equilibrium exists*

$$E_P = (X_U^*\,,\ X_I^*\,,\ B^*\,,\ I_R^*\,,\ A_R^*)$$

with the following values for each component,

$$E_P = \begin{pmatrix} X_U^* = \dfrac{s_U}{\alpha_1 B^* + \mu_{X_U}}, & X_I^* = \dfrac{\alpha_1 B^* X_U^*}{\alpha_2 A_R^* + \mu_{X_I}} \\ B^* = \dfrac{\alpha_{20} - \alpha_3 \dfrac{s_{I_R}}{\mu_{I_R}} - \alpha_4 \dfrac{s_{A_R}}{\mu_{A_R}}}{\dfrac{\alpha_{20}}{\sigma} + \alpha_3 \dfrac{\Delta(\tau_1)}{\mu_{I_R}} + \alpha_4 \dfrac{\Delta(\tau_2)}{\mu_{A_R}}} & \\ I_R^* = \dfrac{s_{I_R} + \Delta(\tau_1) B^*}{\mu_{I_R}}, & A_R^* = \dfrac{s_{A_R} + \Delta(\tau_2) B^*}{\mu_{A_R}} \end{pmatrix}, \quad (27)$$

which is interior to the positive cone in R^5.

We observe that the positive equilibrium E_P exists whenever the parameter

$$R_0 := \alpha_{20} - \alpha_3 \frac{s_{I_R}}{\mu_{I_R}} - \alpha_4 \frac{s_{A_R}}{\mu_{A_R}} \quad (28)$$

is positive and E_P coincides with the boundary equilibrium E_B as $R_0 = 0$. When $R_0 < 0$, we have only the boundary equilibrium E_B.

4. **Characteristic equation and local stability.** System (3) linearized around either equilibria yields

$$\frac{dx(t)}{dt} = Lx(t) + \int_{-h}^{0} K(\theta) x(t+\theta) d\theta. \quad (29)$$

If we define by $x(t) = col\,(X_U(t), X_I(t), B(t), I_R(t), A_R(t))$, then by inspection of equations (3) we get that $L \in \mathrm{R}^{5\times 5}$ is the matrix

$$\mathbf{L} = \begin{pmatrix} -\alpha_1 B^* - \mu_{X_U} & 0 & -\alpha_1 X_U^* & 0 & 0 \\ \alpha_1 B^* & -\alpha_2 A_R^* - \mu_{X_I} & \alpha_1 X_U^* & 0 & -\alpha_2 X_I^* \\ 0 & 0 & V & -\alpha_3 B^* & -\alpha_4 B^* \\ 0 & 0 & 0 & -\mu_{I_R} & 0 \\ 0 & 0 & 0 & 0 & -\mu_{A_R} \end{pmatrix} \quad (30)$$

where $V = (\alpha_{20} - \alpha_3 I_R^* - \frac{2\alpha_{20}}{\sigma} B^* - \alpha_4 A_R^*)$ and $K\,(\theta) : [-h, 0] \to \mathrm{R}^{5\times 5}$ is the matrix function

$$\mathbf{K} = \begin{pmatrix} 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & \tilde{w}_1(\theta) & 0 & 0 \\ 0 & 0 & w_2(\theta) & 0 & 0 \end{pmatrix} \quad (31)$$

where $\tilde{w}_1(\theta) = \begin{cases} w_1(\theta) & \text{in } [-\tau_1\,,\, 0] \\ 0 & \text{in } [-\tau_2\,,\, -\tau_1] \end{cases}$. The associated characteristic equation is

$$\det\left(\lambda \mathbf{I} - \mathbf{L} - \int_{-h}^{0} \mathbf{K}\,(\theta)\, e^{\lambda\theta} d\theta\right) = \quad 0 \quad (32)$$

where $\mathbf{I} \in \mathrm{R}^{5\times 5}$ is the identity matrix and λ are the characteristic roots. If we define by

$$F_i\,(\lambda) := \int_{-\tau_i}^{0} w_i(\theta) e^{\lambda\theta} d\theta,\ i = 1, 2 \quad (33)$$

then we get the following explicit structure for the characteristic equation:

$$\begin{vmatrix} \lambda+Z & 0 & \alpha_1 X_U^* & 0 & 0 \\ -\alpha_1 B^* & \lambda+(\alpha_2 A_R^*+\mu_{X_I}) & -\alpha_1 X_U^* & 0 & \alpha_2 X_I^* \\ 0 & 0 & \lambda-V & \alpha_3 B^* & \alpha_4 B^* \\ 0 & 0 & -F_1(\lambda) & \lambda+\mu_{I_R} & 0 \\ 0 & 0 & -F_2(\lambda) & 0 & \lambda+\mu_{A_R} \end{vmatrix} = 0, \tag{34}$$

where $Z = (\alpha_1 B^* + \mu_{X_U})$. It is easy to check that (34) can be written as

$$[\lambda + (\alpha_1 B^* + \mu_{X_U})]\,[\lambda + (\alpha_2 A_R^* + \mu_{X_I})] \cdot$$

$$\cdot \det \begin{pmatrix} \lambda - \left(\alpha_{20} - \alpha_3 I_R^* - \frac{2\alpha_{20}}{\sigma} B^* - \alpha_4 A_R^*\right) & \alpha_3 B^* & \alpha_4 B^* \\ -F_1(\lambda) & \lambda+\mu_{I_R} & 0 \\ -F_2(\lambda) & 0 & \lambda+\mu_{A_R} \end{pmatrix} = 0;$$

i.e., we have two negative characteristic roots

$$\lambda_1 = -(\alpha_1 B^* + \mu_{X_U}) \qquad \lambda_2 = -(\alpha_2 A_R^* + \mu_{X_I}) \tag{35}$$

and the other characteristic roots are solution of

$$\det \begin{pmatrix} \lambda - \left(\alpha_{20} - \alpha_3 I_R^* - \frac{2\alpha_{20}}{\sigma} B^* - \alpha_4 A_R^*\right) & \alpha_3 B^* & \alpha_4 B^* \\ -F_1(\lambda) & \lambda+\mu_{I_R} & 0 \\ -F_2(\lambda) & 0 & \lambda+\mu_{A_R} \end{pmatrix} = 0. \tag{36}$$

Thus the study of the characteristic equation (34) is reduced to the study of the equation (36), the remaining characteristic roots being negative. We remark that unlike in the baseline model of [7], $F_2(\lambda)$ appears in (36), and also the characteristic roots in (36) are dependent on both the innate delay τ_1 and the adaptive delay τ_2. This implies that both delays determine infection outcome. Regarding local stability of the boundary equilibrium, we can prove the following:

THEOREM 4.1. *The boundary equilibrium E_B is:*

1. *asymptotically stable if*

$$\alpha_{20} - \alpha_3 (s_{I_R}/\mu_{I_R}) - \alpha_4 (s_{A_R}/\mu_{A_R}) < 0;$$

2. *linearly neutrally stable if*

$$\alpha_{20} - \alpha_3 (s_{I_R}/\mu_{I_R}) - \alpha_4 (s_{A_R}/\mu_{A_R}) = 0;$$

   with one real vanishing characteristic root, while others characteristic roots are negative;

3. *unstable (with one positive real root) if*

$$\alpha_{20} - \alpha_3 (s_{I_R}/\mu_{I_R}) - \alpha_4 (s_{A_R}/\mu_{A_R}) > 0.$$

Proof. It follows from (36), since at the boundary equilibrium E_B, $B^* = 0$, $I_R^* = (s_{I_R}/\mu_{I_R})$ and $A_R^* = (s_{A_R}/\mu_{A_R})$, which gives three characteristic roots: two negative $\lambda_1 = -\mu_{I_R}$, $\lambda_2 = -\mu_{A_R}$, and the other equal to the threshold parameter R_0 for the existence of interior equilibrium E_P:

$$\lambda = \alpha_{20} - \alpha_3 (s_{I_R}/\mu_{I_R}) - \alpha_4 (s_{A_R}/\mu_{A_R}).$$

□

We now study the local stability of the positive equilibrium E_P. Assume $R_0 > 0$. At E_P, B^* satisfies

$$\alpha_{20} - \alpha_3 I_R^* - \frac{\alpha_{20}}{\sigma} B^* - \alpha_4 A_R^* = 0,$$

and therefore (36) reduces to

$$\det \begin{pmatrix} \lambda + \frac{\alpha_{20}}{\sigma} B^* & \alpha_3 B^* & \alpha_4 B^* \\ -F_1(\lambda) & \lambda + \mu_{I_R} & 0 \\ -F_2(\lambda) & 0 & \lambda + \mu_{A_R} \end{pmatrix} = 0. \tag{37}$$

Therefore the local stability of E_P leads to the equation

$$\begin{aligned} &\lambda^3 + \lambda^2 \left(\mu_{A_R} + \mu_{I_R} + \tfrac{\alpha_{20}}{\sigma} B^*\right) + \\ &+\lambda \left(\mu_{I_R}\mu_{A_R} + \alpha_3 B^* F_1(\lambda) + \alpha_4 B^* F_2(\lambda) + \tfrac{\alpha_{20}\mu_{A_R}}{\sigma} B^* + \tfrac{\alpha_{20}\mu_{I_R}}{\sigma} B^*\right) + \\ &+B^* \left(\mu_{I_R}\mu_{A_R}\tfrac{\alpha_{20}}{\sigma} + \alpha_3 \mu_{A_R} F_1(\lambda) + \alpha_4 \mu_{I_R} F_2(\lambda)\right) = 0. \end{aligned} \tag{38}$$

The information of the delays τ_1 and τ_2 is carried by $F_1(\lambda) := \int_{-\tau_1}^{0} w_1(\theta) e^{\lambda\theta} d\theta$ and $F_2(\lambda) := \int_{-\tau_2}^{0} w_2(\theta) e^{\lambda\theta} d\theta$ and is therefore dependent on the choice of both delay kernels $w_1(\theta)$ and $w_2(\theta)$.

4.1. **Delay kernels.** Since $F_1(\lambda)$ refers to the delay in the innate response, we assume that the delay kernel w_1 is uniform, i.e.

$$w_1(\theta) = A, \quad \theta \in [-\tau_1, 0]. \tag{39}$$

Then

$$F_1(\lambda) = \frac{A}{\lambda}(1 - e^{-\lambda\tau_1}), \tag{40}$$

which is defined, since $\lambda = 0$ is not a root of equation (38). In fact, if $\lambda = 0$, then $F_1(0) = \Delta(\tau_1)$, $F_2(0) = \Delta(\tau_2)$ and equation (38) becomes

$$B^* \left(\mu_{I_R}\mu_{A_R}\frac{\alpha_{20}}{\sigma} + \alpha_3 \mu_{A_R}\Delta(\tau_1) + \alpha_4 \mu_{I_R}\Delta(\tau_2)\right) \neq 0, \quad \forall \tau_1, \tau_2 \geq 0, \tag{41}$$

where by $B^*(\tau_1, \tau_2)$ we emphasize the dependence on delays τ_1 and τ_2, as it is evident from the equilibrium components (27). $F_2(\lambda)$ accounts for the delay in the adaptive response. We assume that the delay kernel w_2 is exponential; i.e.,

$$w_2(\theta) = Ae^{k\theta}, \qquad \theta \in [-\tau_2, 0], \quad A, k \in \mathbb{R}_+. \tag{42}$$

then

$$F_2(\lambda) = \frac{A}{\lambda + K_2}\left[1 - e^{-(\lambda+k)\tau_2}\right]. \tag{43}$$

If $\tau_1 = \tau_2 = 0$, then $F_1(\lambda) = F_2(\lambda) = 0$ and equation (38) becomes

$$\begin{aligned} &\lambda^3 + \lambda^2 \left(\mu_{A_R} + \mu_{I_R} + \tfrac{\alpha_{20}}{\sigma} B^*\right) + \\ &+\lambda \left(\mu_{I_R}\mu_{A_R} + \tfrac{\alpha_{20}\mu_{A_R}}{\sigma} B^* + \tfrac{\alpha_{20}\mu_{I_R}}{\sigma} B^*\right) + B^* \left(\mu_{I_R}\mu_{A_R}\tfrac{\alpha_{20}}{\sigma}\right) = 0 \end{aligned} \tag{44}$$

which has three negative roots (easy to check from (37)); i.e., E_P is asymptotically stable at $\tau_1 = \tau_2 = 0$. We have thus the general problem to find the delay values τ_1

and τ_2, if they exist, at which for increasing τ_1 and τ_2 the stability of E_P changes or, in other words, at which E_P undergoes a stability switch. Substituting (40) and (43) in (38), the characteristic polynomial (38) takes the form

$$P(\lambda,\tau_1,\tau_2)+Q_1(\lambda,\tau_1,\tau_2)e^{-\lambda\tau_1}+Q_2(\lambda,\tau_1,\tau_2)e^{-\lambda\tau_2}=0 \tag{45}$$

where P is a fifth-order degree polynomial

$$P=p_5(\tau_1,\tau_2)\lambda^5+p_4(\tau_1,\tau_2)\lambda^4+p_3(\tau_1,\tau_2)\lambda^3+p_2(\tau_1,\tau_2)\lambda^2+p_1(\tau_1,\tau_2)\lambda+p_0(\tau_1,\tau_2) \tag{46}$$

with delay-dependent coefficients

$$\begin{cases} p_5(\tau_1,\tau_2)=\sigma, \\ p_4(\tau_1,\tau_2)=\alpha_{20}B^*+\sigma\mu_{I_R}+\sigma\mu_{A_R}+\sigma K_2, \\ p_3(\tau_1,\tau_2)=\sigma\mu_{A_R}K_2+B^*\alpha_{20}\mu_{A_R}+B^*\alpha_{20}K_2+ \\ +\sigma\mu_{I_R}\mu_{A_R}+\sigma\mu_{I_R}K_2+B^*\alpha_{20}\mu_{I_R}, \\ p_2(\tau_1,\tau_2)=\sigma\mu_{I_R}\mu_{A_R}K_2+A_2\alpha_4B^*\sigma+A_1\alpha_3B^*\sigma \\ +B^*\alpha_{20}\mu_{I_R}\mu_{A_R}+B^*\alpha_{20}\mu_{I_R}K_2++B^*\alpha_{20}\mu_{A_R}K_2, \\ p_1(\tau_1,\tau_2)=A_1\alpha_3B^*\sigma\mu_{A_R}+A_1\alpha_3B^*\sigma K_2+ \\ +B^*\alpha_{20}\mu_{I_R}\mu_{A_R}K_2+A_2\alpha_4B^*\sigma\mu_{I_R}, \\ p_0(\tau_1,\tau_2)=A_1\alpha_3B^*\sigma\mu_{A_R}K_2. \end{cases} \tag{47}$$

Q_1 is a second order polynomial

$$Q(\lambda,\tau_1,\tau_2)=q_1(\tau_1,\tau_2)\lambda^2+q_1(\tau_1,\tau_2)\lambda+q_0(\tau_1,\tau_2) \tag{48}$$

with delay dependent coefficients

$$\begin{cases} q_2(\tau_1,\tau_2)=-A_1\alpha_3B^*\sigma, \\ q_1(\tau_1,\tau_2)=-A_1\alpha_3B^*\sigma K_2-A_1\alpha_3B^*\sigma\mu_{A_R}, \\ q_0(\tau_1,\tau_2)=-A_1\alpha_3B^*\sigma\mu_{A_R}K_2. \end{cases} \tag{49}$$

Q_2 is a second order polynomial as well (same as (48)) with delay dependent coefficients

$$\begin{cases} \tilde{q_2}(\tau_1,\tau_2)=2A_2\alpha_4B^*\sigma, \\ \tilde{q_1}(\tau_1,\tau_2)=2A_2\alpha_3B^*\sigma\mu_{I_R}-A_1\alpha_3B^*\sigma\mu_{A_R}, \\ \tilde{q_0}(\tau_1,\tau_2)=0. \end{cases} \tag{50}$$

The occurrence of stability switches for characteristic equations with one delay and with delay dependent coefficients has been studied by Beretta and Kuang [6], who have proposed a geometric stability switch criterion. However, the extension of the criterion to the case of two distinct delays is still far from being realized. Here we only perform a qualitative study of stability switches in E_P by coupling LHS to a heuristic classification algorithm.

4.2. **Numerical simulations.** We simulated the system by numerically solving the differential equations using suitable numerical methods. Considering the general case for delay equations (3); i.e., of exponential delay kernels for innate and adaptive immune responses,

$$\begin{array}{lcl} w_i(\theta) & = & A_ie^{K_i\theta}, \quad \theta\in[-\tau_1,0],\ i=1,2 \\ A_i,K_i & \in & \mathbb{R}_+ \end{array}$$

in system (3) we define the new variables

$$\begin{array}{lcl} u_I(t) & := & \int_{-\tau_1}^0 w_1(\theta)B(t+\theta)d\theta \\ u_A(t) & := & \int_{-\tau_2}^0 w_2(\theta)B(t+\theta)d\theta \end{array}. \tag{51}$$

By the transformation $s = t + \theta$ equations (51) give

$$\begin{array}{rcl} u_I(t) & := & \int_{t-\tau_1}^{t} w_1(s-t)B(s)ds \\ u_A(t) & := & \int_{t-\tau_2}^{t} w_2(s-t)B(s)ds \end{array}, \tag{52}$$

which is straightforward checking that they satisfy the equations

$$\begin{array}{rcl} \dfrac{du_I}{dt} & = & A_1B(t) - A_1e^{-K_1\tau_1}B(t-\tau_1) - K_1u_I(t) \\ \dfrac{du_A}{dt} & = & A_2B(t) - A_2e^{-K_2\tau_2}B(t-\tau_2) - K_2u_A(t) \end{array};$$

hence, system (3) is transformed into

$$\begin{array}{rcl} \dfrac{dX_U(t)}{dt} & = & s_U - \alpha_1X_U(t)B(t) - \mu_{X_U}X_U(t) \\ \dfrac{dX_I(t)}{dt} & = & \alpha_1X_U(t)B(t) - \alpha_2X_I(t)A_R(t) - \mu_{X_I}X_I(t) \\ \dfrac{dB(t)}{dt} & = & \alpha_{20}B(t)\left(1 - \dfrac{B(t)}{\sigma}\right) - \alpha_3B(t)I_R(t) - \alpha_4B(t)A_R(t) \\ \dfrac{dI_R(t)}{dt} & = & s_{I_R} + u_I(t) - \mu_{I_R}I_R(t) \\ \dfrac{dA_R(t)}{dt} & = & s_{A_R} + u_A(t) - \mu_{A_R}A_R(t) \\ \dfrac{du_I(t)}{dt} & = & A_1B(t) - A_1e^{-K_1\tau_1}B(t-\tau_1) - K_1u_I(t) \\ \dfrac{du_A(t)}{dt} & = & A_2B(t) - A_2e^{-K_2\tau_2}B(t-\tau_2) - K_2u_A(t) \end{array} \tag{53}$$

with initial conditions given by (2) and table 2.1, and particularly B takes initial values on the interval $[-\tau_2, 0]$; i.e.,

$$B(s) = \Phi_3(s), \qquad s \in [-\tau_2, 0], \tag{54}$$

thus defining at $t = 0$ the initial conditions for u_I and u_A in (53) by

$$\begin{array}{rcl} u_I(0) & = & \int_{-\tau_1}^{0} w_1(s)\Phi_3(s)ds \\ u_A(0) & = & \int_{-\tau_2}^{0} w_2(s)\Phi_3(s)ds. \end{array} \tag{55}$$

Hence, system (3) is transformed into an equivalent system of delay differential equations (53) with fixed delay τ_1, τ_2, where *equivalent* means that such a new system has the same characteristic equation and same equilibria (regarding the original variables (X_U, X_I, B, I_R, A_R)) as the original system (3) (we leave this the reader to check). Note that the case of uniform delay kernel for innate response I_R is simply obtained by setting $K_1 = 0$ in (53). Such a new system (53) may be solved by any delay differential equations solver. We used the Matlab *dde23* by Shampine and Thompson [51].

5. **Results.** System (1) is comprised of 20 parameters (17 independent parameters), 7 of which are directly involved in existence condition for the boundary and interior equilibrium solution (see Theorem 3.1). The study of the zeros of (45) and consequently the stability of E_P are affected by changes in these parameter values. Here we perform two types of analysis: two different sensitivity analyses and a qualitative study of stability switches. Sensitivity analysis results are valid only in the biological ranges we investigated.

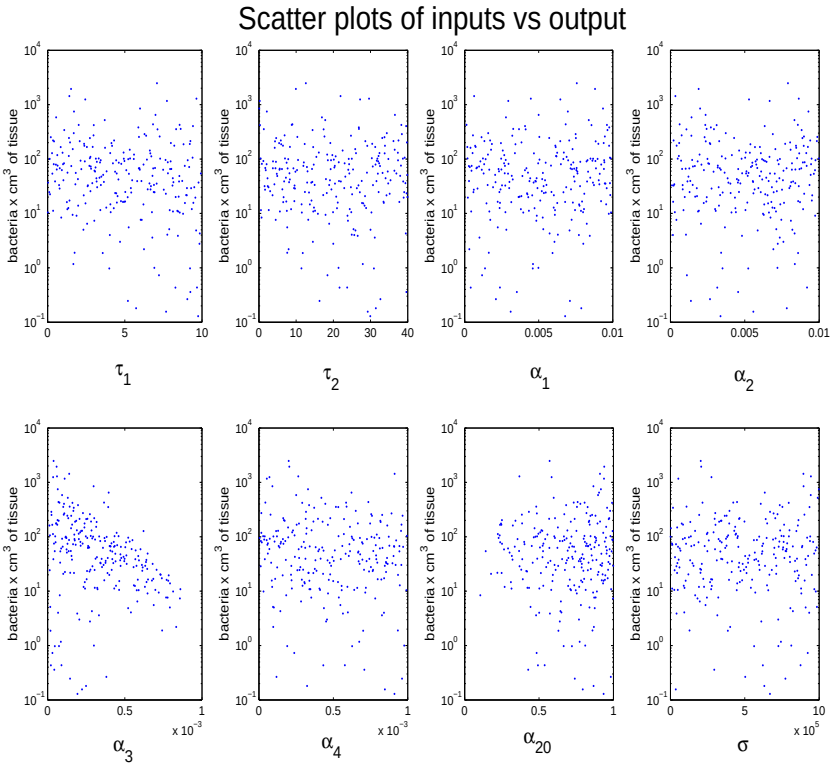

FIGURE 1. Scatter plots of bacterial load (y-axis) vs parameters (x-axis) varied in LHS. Shown on the y-axis is number of bacteria per cm^3 of tissue.

5.1. **Sensitivity analysis.** We perform a sensitivity analysis on model (1) by investigating the parameter(s) that contribute most to variations in bacterial load (our outcome variable). Two indexes are calculated as described earlier: PRCC and EFAST. PRCC deals with monotonic nonlinear associations between parameters and outcome, while EFAST is more accurate for nonmonotonic nonlinear relationships.

To compare PRCC with EFAST, we set the dimension of the sample in LHS to the minimum number of runs defined in the EFAST sampling procedure (see [53]), namely 520 (65 multiplied by the number of parameters that are varied, 8 in our case). Some parameter combinations do not satisfy the existence condition for E_P (i.e., $R_0 > 0$; see equation (28)); thus, we calculate PRCCs both on the entire LHS dataset and on the subset of samples fulfilling $R_0 > 0$. The same procedure cannot be applied to EFAST. Because of its peculiar sampling technique, EFAST must process the whole sampled dataset at once, and we can only compare the sensitivity indexes calculated on the whole parameter space.

Figure 1 shows scatter plots of the parameters that are varied in LHS versus bacterial load, our marker of disease progression. There is no clear monotonic association between parameter variation and outcome; thus, sensitivity analysis

should be performed using a variance-based method (EFAST) rather than a standard sample-based method such as PRCC (as our results clearly show). Table 3 shows PRCC values in Panel A and B. Panel A shows only the subset of parameter combinations satisfying $R_0 > 0$, while Panel B gives values from the analysis of the entire LHS matrix. EFAST sensitivity indexes are shown in Panel C, D and E. Each row of the panels corresponds to a specific time point during infection. Parameters α_3, α_4, τ_1 and τ_2 have a significant negative PRCC with bacterial load, while α_{20} is the only parameter that has a significant positive PRCC (see Panel A). The strength of these associations becomes less important over time.

All the coefficients in Panel B (the whole LHS matrix) are very low and not significant. Panels C, D and E values of EFAST analysis should be read as amounts (C and D) and percentages (E) of variance explained by varying the single parameter with (Panel D, total-order sensitivity index) and without (Panel C, first-order sensitivity index) considering interactions with the rest of the parameters. Panel E normalizes the indexes by recasting Panel D as ratios between each cell with the marginal total by time (row). Since first-order and total-order EFAST values are not equal (Panel C and D), interactions between parameters matter (the model is not additive) and only Panel D or E should be used to compare our sensitivity results.

As suggested by Figure 1, because of nonmonotonic nonlinear relationships between parameters and outcome, PRCC fails to give any significant answer in term of sensitivity (see Panel B) if the entire parameter space is sampled (regardless of whether condition $R_0 > 0$ is satisfied). EFAST performs much better, defining rankings of the relative importance of each parameter on the model outcome. From Panel E (last four columns) α_3, α_4, α_{20} and σ explain together up to 80 % of the total variability of the bacterial load. If we consider the results of Panel A, PRCCs and EFAST give similar conclusions except for the carrying capacity parameter σ (i.e., the maximum sustainable bacteria population). EFAST suggests that σ ranks first in percentage of explained variance, while PRCC considers this parameter not significant.

5.2. **Stability switch.** We use LHS technique to qualitatively investigate the existence of a stability switches for E_P. We sample only τ_1 and τ_2 from uniform probability density functions defined in very large intervals, namely τ_1 in [0.01,10] and τ_2 in [1,90] (days). The rest of the parameter values are given in tables 1 and 2. These reference values fulfill the condition for the existence of E_P (see theorem 3.1 and equation (28)). We run a total of 27,000 simulations to properly classify the outcomes based on the type of equilibrium achieved (damped or sustained oscillations). We follow a heuristic argument to classify the type of oscillatory behavior of E_P, namely either as sustained or damped. This argument comprises three different filtering steps. The first step calculates the maximum difference between the mean and the current value of a specific outcome in the first and last 50% of the simulation, then it takes the ratio. If the ratio is approximately one, the oscillation must be sustained. This first screening classifies as sustained any simulation with a ratio smaller than 1.2. The second step calculates the variance of the last 50% of the simulation and if it is larger than 1e4, E_P is classified as sustained. We got similar results for a variance-threshold of 1e3. The third step processes the last 25% of the simulation, and check for significant deviations from its mean value. This last filtering step classifies as sustained all the solutions that deviate more than 20% (plus and minus) from the mean. The above steps are evaluated simultaneously on

TABLE 3. Panel A: PRC coefficients and their respective significance calculated at different time points from the subset of the LHS matrix satisfying $R_0 > 0$ (* indicates not significant, $p > 0.05$), Panel B: PRC coefficients and their respective significance calculated at different time points from the entire LHS matrix (all the values are not significant; i.e., $p > 0.05$), Panel C: First-order EFAST sensitivity indexes at different time points, Panel D: Total-order EFAST sensitivity indexes at different time points; Panel E: Total-order EFAST sensitivity indexes (percentages) at different time points

time	τ_1	τ_2	α_1	α_2	α_3	α_4	α_{20}	σ
10	-0.335	-0.224	0.018^*	0.033^*	-0.932	-0.836	0.627	0.102^*
30	-0.464	-0.497	0.055^*	0.048^*	-0.744	-0.521	0.320	0.099^*
50	-0.217	-0.327	-0.011^*	0.046^*	-0.746	-0.465	0.533	0.142
100	-0.211	-0.181	0.025^*	0.061^*	-0.546	-0.299	0.295	0.064^*
200	-0.189	-0.148	0.057^*	0.003^*	-0.342	-0.259	0.173	0.048^*

PANEL A

time	τ_1	τ_2	α_1	α_2	α_3	α_4	α_{20}	σ
10	0.019	-0.050	0.004	-0.025	0.038	0.065	-0.011	-0.016
30	0.012	-0.076	-0.004	-0.026	0.014	0.050	0.001	-0.014
50	0.009	-0.061	-0.009	-0.033	0.023	0.066	-0.009	-0.023
100	0.001	-0.075	-0.002	-0.045	0.018	0.073	-0.001	-0.019
200	0.020	-0.078	0.016	-0.051	0.012	0.055	0.010	0.004

PANEL B

time	τ_1	τ_2	α_1	α_2	α_3	α_4	α_{20}	σ
10	0.049	0.055	0.012	0.027	0.319	0.095	0.109	0.043
30	0.004	0.127	0.024	0.024	0.510	0.158	0.185	0.007
50	0.024	0.141	0.014	0.024	0.363	0.086	0.108	0.0601
100	0.034	0.151	0.009	0.007	0.473	0.090	0.107	0.099
200	0.027	0.019	0.010	0.009	0.494	0.063	0.128	0.160

PANEL C

time	τ_1	τ_2	α_1	α_2	α_3	α_4	α_{20}	σ
10	0.384	0.391	0.162	0.249	0.872	0.552	0.720	0.368
30	0.105	0.848	0.266	0.122	0.925	0.659	0.730	0.1464
50	0.339	0.666	0.201	0.136	0.870	0.594	0.763	0.603
100	0.415	0.536	0.163	0.154	0.903	0.595	0.824	0.753
200	0.259	0.216	0.107	0.093	0.855	0.280	0.869	0.892

PANEL D

time	τ_1	τ_2	α_1	α_2	α_3	α_4	α_{20}	σ
10	0.103	0.105	0.043	0.067	0.235	0.149	0.194	0.099
30	0.027	0.223	0.070	0.032	0.243	0.173	0.192	0.038
50	0.081	0.159	0.048	0.032	0.208	0.142	0.182	0.144
100	0.095	0.123	0.037	0.035	0.207	0.137	0.189	0.173
200	0.072	0.060	0.030	0.026	0.239	0.078	0.243	0.249

PANEL E

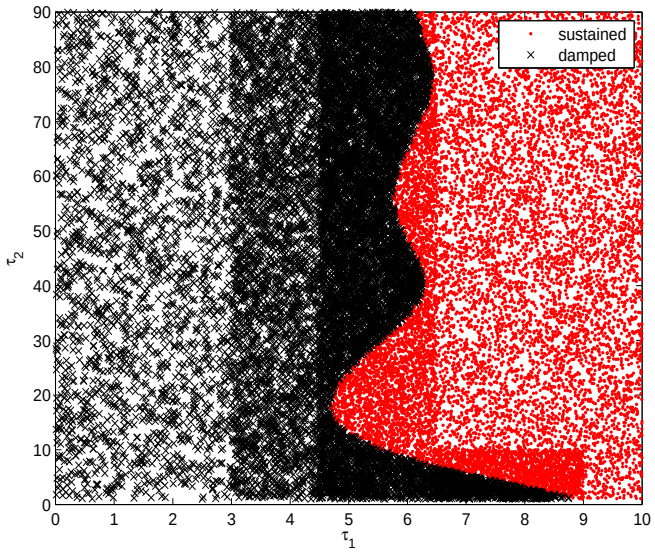

FIGURE 2. Phase diagram in τ_1 , τ_2. 27,000 runs of the system 1 are classified either as damped or sustained oscillations. Keeping the rest of the parameters constant, τ_1 is varied in the interval $[0.01, 10]$ and τ_2 is varied in the interval $[1, 90]$, .

the matrix of 27,000 solutions (only for $B(t)$). The first set of runs (8,000) explored the whole range for both parameters. Then we run 10,000 simulations restricting τ_1 in the interval [3 , 10]. The last two sets zoomed into the boundaries of the emerging stability switch manifold: we run 7,000 simulations in the interval [4.5 , 6.5] for τ_1 (τ_2 is in [1 , 90]) and 2,000 simulations in the range [6.5 , 10] for τ_1 and [1,10] for τ_2. Figure 3 shows our simulations results. There is a clear separation between the two classes and that depends on both parameters. E_P always results in damped oscillations with a delay of innate immunity response lower than 4.65 days, regardless adaptive immunity response. For $\tau_1 > 4.65$, adaptive immunity can play a major role in defining the type of E_P oscillatory behavior. For example, at τ_1=6.24, we get sustained oscillations for τ_2 equal to 20, 60 or 91 days (see Figure 4, Panel A) and damped oscillations for τ_2 equal to 7, 40 and 80 days (see Figure 4, Panel B). We run the model for 3000 days to see graphically if the oscillations are sustained. Adaptive immunity can compensate for an inefficient innate immunity (longer than a week) by a very fast response (within a week or less) that is inversely proportional to τ_1.

6. **Discussion.** We extended an existing delay differential equation model by Beretta et al. [7] by including a term representing direct bacterial killing by adaptive immunity. The baseline model described in [7] accounts for different killing capabilities of the immune system and incorporates two delays representing the two types of immune responses, namely innate and adaptive immunity. The basic structure of the model is maintained with a new term added to reflect active killing of a class of very important cells of the immune system, such as macrophages and cytotoxic

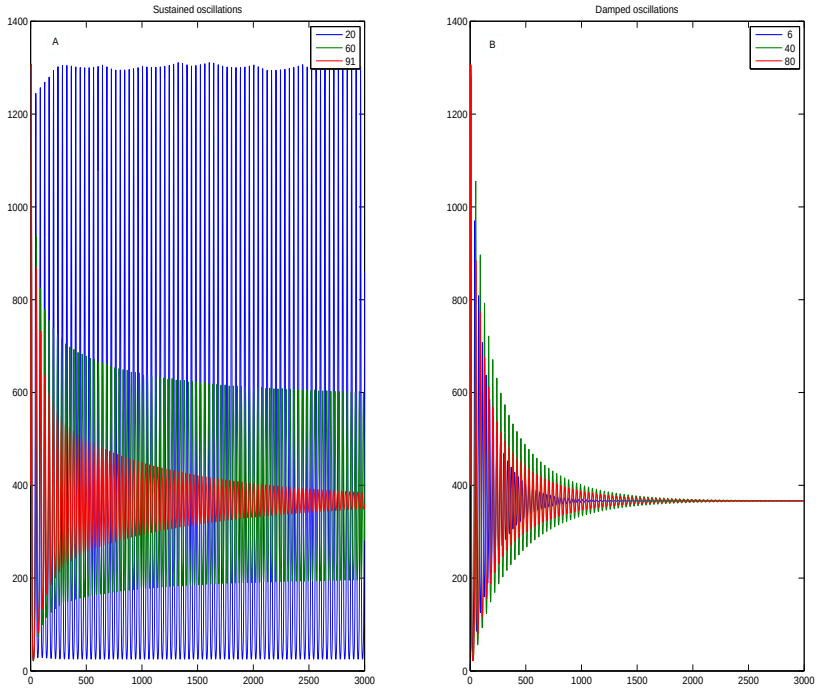

FIGURE 3. Plots of bacterial dynamics over time. Here, τ_1 is fixed at 6.24. Panel A: three values of τ_2 that give sustained oscillations for E_P (20, 60 and > 90). Panel B: three values of τ_2 that give damped oscillations for E_P (6, 40 and 80).

lymphocytes. We simplified the form of the delay functions by considering only bacterial load as the relevant information scanned by the immune system to determine the strength of the response (both innate and adaptive). We also retain the structure of delay kernels, namely uniform for innate response and positive exponential for adaptive response. As in [7], the new model admits a boundary equilibrium E_B or uninfected steady state, and only when the threshold parameter R_0 in (28) becomes positive, one positive equilibrium E_P bifurcates from E_B (transcritical bifurcation), corresponding to the infected steady state. The local stability of E_B is independent of the delays in the innate (τ_1) and adaptive (τ_2) immune responses. E_B is asymptotically stable whenever the positive equilibrium E_P is not feasible and unstable if E_P exists. The positive equilibrium E_P has components dependent on τ_1 and τ_2, and its local stability is dependent on both (see (37)). The study of the characteristic equation leads to equation (38), where the term $F_1(\lambda)$ takes information of the delay kernel $w_1(\theta)$, $\theta \in [-\tau_1, 0]$ in the innate immune response and $F_2(\lambda)$ takes information of the delay kernel $w_2(\theta)$, $\theta \in [-\tau_2, 0]$ in the adaptive immune response. Solving for the delay kernel structures, equation (38) takes the form of the polynomial exponential transcendental equation (45), with delay

dependent coefficients given by (46)-(50). The positive equilibrium E_P is asymptotically stable at $\tau_1 = 0$, whereas it undergoes a Hopf bifurcation toward sustained oscillations by increasing the values of τ_1 and τ_2. The structure of this dependence is studied qualitatively by a heuristic algorithm and, although not proven analytically, for values of $\tau_1 > 4.5$, the delay τ_2 plays a major role in defining the type of oscillatory behavior of E_P.

6.1. **Biological discussion.** We focus our studies on the intracellular bacterium *Mycobacterium tuberculosis*, a slow-growing pathogen that preferentially infects the lung. Most infected people progress to latent TB, an asymptomatic state where the pathogen and the host coexist without infecting other hosts. During their lifetimes, latently infected hosts can experience reactivation. Reactivation can be described as a switch in the host-pathogen coexistence, where bacteria can no longer be controlled by the host response anymore, causing outgrowth of the pathogen, dissemination and uncontrolled spread of infection. So, the study of factors and mechanisms involved in controlling and mantaining a latent state within the host represent a critical goal in developing TB treatments and cures.

The baseline model studied in [7] suggested a key role for innate immunity in establishing a protective response against intracellular bacterial infections. The new model presented here allowed us to determine the relevance of delays for adaptive response in addition to those present for the innate response. Taken together, our analyses suggest that a strong and rapid innate response (high innate immunity cell turnover that is a function of baseline levels and their half-life, and high bacterial killing by innate response) is always the best strategy either for clearing or for controlling bacterial growth and its damage to the host. The parameters of the bacteria equation are the most important in determining infection outcome, at least within the large biological ranges we investigated.

The role of adaptive immunity seems to be two-fold: filling the gap by eliciting a fast cell-mediated or humoral immunity (within few days) when innate response fails (or is very delayed, more than a week) or to contain undesirable bacterial outgrowth by adjusting its response to potential reactivation scenarios. The first goal is probably achievable only through targeted immunization, given that the physiological and biological constraints on the timing of adaptive response usually result in delays within the order of one to three weeks (without vaccination). As for the second goal, the stability switch study suggests that a fine-tuning of adaptive response is desirable when innate immunity is not as rapid as it should be (between 4 and 7 days). Depending on the natural memory of our adaptive response (likely variable between different hosts), either delaying or expediting adaptive responses could be advantageous.

It is not clear why and how the optimal strategy emerges. A potent and rapid adaptive immune response can be very effective in killing pathogens but will likely cause a lot of inflammation and tissue damage, sometimes unnecessarily. On the other hand, bacteria, if not controlled in their growth, can spread and disseminate causing necrosis, cavity formation and also widespread inflammation and tissue damage. It is a dynamic balance between effector and target cells, where the bacterial load is likely the driving force. In this framework, we retain the phenomenological classification drawn in [7] regarding the biological difference between damped and sustained oscillation of E_P. We still observe damped oscillations as the latent infection scenario in which a clinically asymptomatic coexistence between the host

and the microbe is established and maintained. Sustained oscillations might represent chronic infection scenarios in which bacteria undergo temporary uncontrolled growth and coexistence is achieved only by recurrent cyclic outbreaks with evident symptoms experienced by the host. These scenarios could be easily driven out of control (reactivation) either by host or pathogen factors, as well as environmental pressure. An alternative example is given by herpes symplex virus (HSV), which is associated with recurrent pathology, usually less severe than the primary pathology as a consequence of the immune response.

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