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LOW VIRAL PERSISTENCE OF AN IMMUNOLOGICAL MODEL

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Abstract. Hepatitis B virus can persist at very low levels in the body in the face of host immunity, and reactive during immunosuppression and sustain the immunological memory to lead to the possible state of 'infection immunity'. To analyze this phenomena quantitatively, a mathematical model which is described by DDEs with relative to cytotoxic T lymphocyte (CTL) response to Hepatitis B virus is used. Using the knowledge of DDEs and the numerical bifurcation analysis techniques, the dynamical behavior of Hopf bifurcation which may lead to the periodic oscillation of populations is analyzed. Domains of low level viral persistence which is possible, either as a stable equilibrium or a stable oscillatory pattern, are identified in parameter space. The virus replication rate appears to have influence to the amplitude of the persisting oscillatory population densities.

1. **Introduction.** Viral infections caused by hepatitis B virus (HBV) are the major global health problems due to more than 300 million people being persistently infected[1,2]. It is suggested that HBV can persist at various levels rather than being completely eliminated to a sterile state from the host[3] but are controlled by infection-immunity, which refers to a coexistence state of immunity together with low level infection. Recent experiment data shows that HBV can persist below the detection limit of conventional assays of about $10^2 - 10^3$ HBV DNA copies/ml of serum[4], which continuously restimulate a low level immune response. The qualitative parameters of low level HBV coexistence with immunity memory are difficult to assess and the mechanism of the viral persistence is not yet understood. Biologically and mathematically, the dynamics of the HBV virus infection is characterized by the relationship between interacting populations of viruses and virus-specific cytotoxic T lymphocytes[5]. Recently, a number of mathematical models have been proposed to explore the population dynamics of the virus and cells[6-7]. In general, the hepatis B virus acts as a positive regulator of the CTL population due to adaptive immunity, whilst CTL function to eliminate the virus population.

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Based on the general mechanism of within-host dynamics, the interaction relationship between virus and CTL population are described as the following:

$$\begin{aligned} \frac{dx}{dt} &= \beta x(t)(1-\frac{x(t)}{K}) - \gamma x(t)z(t), \\ \frac{dy}{dt} &= b\frac{x(t-\tau)y(t-\tau)}{\theta + x(t)} - \alpha y(t) + C, \\ \frac{dz}{dt} &= s\frac{x(t-\tau)y(t-\tau)}{\theta + x(t)} - dz(t), \end{aligned} \tag{1.1}$$

where x denotes virus number(or density), while y and z represent the resting CTL population and effector CTL population number(or density), respectively. The biological basis underlying the model (1.1) lies in that: (i) The virus population is assumed to obey the logistic growth rule; (ii) the virus population induces the clonal expansion and differentiation of the specific resting CTL population into effector CTL population; (iii) CTL population caught the virus cells and the like predator-prey relationship is reflected by Holling-II type function response.

Parameter	Biological meaning	Units	Value used	Plausible range
β	Replication rate constant of viruses	l/day	0.3	$0-0.4$
γ	Rate constant of virus clearance due to CTLs	ml/copies day	1.75×10^{-3}	$10^{-6}-10^{-3}$
K	Virus carrying capacity	copies/ml	8×10^{9}	$<10^{10}$
τ	Duration of CTL division cycle	day	0.6	0.4-1
b	Rate constant of CTL stimulation	l/day	0.07	0-4
θ	Viral load saturation in CTL expansion rate	copies/ml	1200	$<10^{10}$
α	Rate constant of CTL death	ml/day	45	$0.1-10^{3}$
C	Rate of CTL export from thymus	cell/ml/day	0.1	0-10
s	Rate of effector cells stimulation	l/day	0.05	0-4
d	Rate constant of effector cells death	ml/day	45	$0.1-10^{3}$

Table 1 The values of parameters used in system (1.1).

In the equation for virus state $x(t)$, the first term on the right-hand side describes the virus growth with an resource capacity K due to the limited amount of sensitive tissue cells to support the virus replication. The second term illustrates the elimination of anti-virus control.

In the equation for resting state $y(t)$ of CTL, the maintenance of virus-specific CTL population through exporting from thymus with the rate C and death in the periphery system are considered. The Holling-II type function reflects the virus-induced proliferation with the inhibitory effect of cumulative virus on clonal expansion, and time delay τ is the necessary time in division cycle.

The dynamics of effector cells $z(t)$ is governed by the differentiation of antigen-stimulated resting CTL population and the natural death due to its finite life span.The relative information about model parameters [5] are listed as in Table 1.

We are interested to know the necessary condition for the stability of steady states of system. The bifurcation analysis is done to understand how solutions and their stability change as the parameters in the system vary. Due to the infinite-dimensional nature of DDEs, the characteristic matrix, appearing in the stability theory for DDEs, has an infinite number of eigenvalues[8,9]. The recently developed analyzing scheme known as the Sturm sequence [10,11] is used to decide the stability and the existence of imaginary roots $i\omega$ as the characteristic polynomial equation for ω has high multiplicity.

In this paper, we outline the quantitative analysis of the stability of the positive equilibrium solution of system (1.1) in Section 2. The relevant numerical methods for bifurcation analysis with the use of software DDE-BIFTOOL obtaining the continuation of steady states and periodic solutions is done in Section 3. A brief conclusion is given finally.

2. **Model properties.** Initial data for the system has the form

$$x(s) = x_0(s) > 0, \quad y(s) = y_0(s) > 0, \quad z(s) = z_0(s) > 0$$

with $s \in [-\tau, 0]$.

Using the method of steps, it is easily seen that each component of the solution of system (1.1) remains non-negative for all $t > 0$.

By straightforward calculation, we have $E_0 = (0, \frac{C}{\alpha}, 0)$ is a disease-free state of system (1.1) which is unstable. Suppose $E^* = (x^*, y^*, z^*)$ is the positive equilibrium state, then it satisfies

$$y^* = \frac{C(\theta + x^*)}{-bx^* + \alpha\theta + \alpha x^*}, \qquad z^* = \frac{\beta(K - x^*)}{K\gamma}$$

and

$$d\beta(\alpha - b){x^*}^2 + (sC\gamma K + d\beta Kb - d\beta K\alpha + d\beta\alpha\theta)x^* - d\beta K\alpha\theta = 0 \tag{2.1}$$

If $x^* \in (0, K)$, then E^* is an interior positive equilibrium solution in $\mathbb{R}^3_+ = ((x, y, z) : x, y, z > 0)$. The solutions of Eq.(2.1) have the formula

$$x^*_\pm = \frac{-B \pm \sqrt{\Delta}}{2d\beta(\alpha - b)}$$

with

$$B = sC\gamma K + d\beta Kb - d\beta K\alpha + d\beta\alpha\theta;$$
$$\Delta = (sC\gamma K + d\beta Kb - d\beta K\alpha + d\beta\alpha\theta)^2 + 4d^2\beta^2 K\alpha\theta(\alpha - b).$$

We are interested to know the necessary conditions for the coexistence of a small scale virus population and CTL population as an equilibrium or periodic oscillating state of system. With assumption $\alpha < b$, it is easily verified $x^*_+ \in (0, K)$.Therefore, the case $x^* = x^*_+$ is considered hereafter .

In the context of dynamical system analysis, we will investigate the stability of equilibrium E^* in detail. The local asymptotic stability of a steady state can usually be determined from the roots of characteristic equation, $det(P + Qe^{-\lambda\tau} - \lambda I) = 0$. For our model,

$$P = \begin{pmatrix} -\frac{\beta x^*}{K} & 0 & -\gamma x^* \\ -\frac{bx^*y^*}{(\theta + x^*)^2} & -\alpha & 0 \\ -\frac{sx^*y^*}{(\theta + x^*)^2} & 0 & -d \end{pmatrix} \qquad Q = \begin{pmatrix} 0 & 0 & 0 \\ \frac{by^*}{\theta + x^*} & -\frac{bx^*}{\theta + x^*} & 0 \\ \frac{sy^*}{\theta + x^*} & -\frac{sx^*}{\theta + x^*} & 0 \end{pmatrix}$$

In the case of a positive delay, the characteristic equation for the linearized equation around the equilibrium point E^* is

$$P(\lambda) + Q(\lambda)e^{-\lambda\tau} = 0 \tag{2.2}$$

where

$$P(\lambda) = a_1\lambda^3 + a_2\lambda^2 + a_3\lambda + a_4,$$
$$Q(\lambda) = b_1\lambda^2 + b_2\lambda + b_3,$$

with

$$
\begin{aligned}
a_1 &= 2x^*K\theta + K\theta^2 + x^{*2}K,\\
a_2 &= K\theta^2 d + 2x^*K\alpha\theta + 2x^*K\theta d + K\alpha\theta^2 + x^{*2}K\alpha + x^{*2}Kd\\
&\quad +2x^{*2}\beta\theta + x^*\beta\theta^2 + \beta x^{*3},\\
a_3 &= K\alpha\theta^2 d - sy\gamma x^{*2}K + 2x^{*2}\beta\theta d + 2x^{*2}\beta\alpha\theta + x^{*2}K\alpha d\\
&\quad +x^*\beta\alpha\theta^2 + x^*\beta\theta^2 d + 2x^*K\alpha\theta d + x^{*3}\beta\alpha + x^{*3}\beta d,\\
a_4 &= x^*\beta\alpha\theta^2 d - x^{*2}sy^*\gamma K\alpha + x^{*3}\beta\alpha d + 2x^{*2}\beta\alpha\theta d,\\
b_1 &= x^*Kb\theta + Kbx^{*2},\\
b_2 &= Kbx^{*2}d + sy^*\gamma x^{*2}K + x^{*2}\beta b\theta + x^*Kbd\theta + sy^*\gamma x^*K\theta + \beta x^{*3}b,\\
b_3 &= sy^*\gamma x^*K\theta\alpha + x^{*2}\beta bd\theta + \beta x^{*3}bd + x^{*2}sy^*\gamma K\alpha.
\end{aligned}
$$

The equilibrium solution is stable in the absence of the delay if the roots of $P(\lambda) + Q(\lambda) = 0$ have negative real parts. Equivalently,

$$\lambda^3 + \frac{a_2 + b_1}{a_1}\lambda^2 + \frac{a_3 + b_2}{a_1}\lambda + \frac{a_4 + b_3}{a_1} = 0 \tag{2.3}$$

By Routh Hurwitz's criteria, this occurs if and only if $a_2 + b_1 > 0, a_4 + b_3 > 0$ and $(a_2 + b_1)(a_3 + b_2) - a_1(a_4 + b_3) > 0$ since $a_1 > 0$. This is satisfied with the set of parameters shown in Table1.

Now substituting $\lambda = i\omega$ in Eqs(2.2) and separating the real and the imaginary parts, we obtain the system of transcendental equations

$$
\begin{aligned}
-a_2\omega^2 + a_4 - b_1\omega^2\cos(\omega\tau) + b_2\omega\sin(\omega\tau) + b_3\cos(\omega\tau) = 0,\\
-a_1\omega^3 + a_3\omega + b_1\omega^2\sin(\omega\tau) + b_2\omega\cos(\omega\tau) - b_3\sin(\omega\tau) = 0.
\end{aligned}
$$

Therefore,

$$
\begin{aligned}
\cos(\omega\tau) &= \tfrac{b_2\omega^4 a_1 - b_2\omega^2 a_3 + b_1\omega^2 a_4 + b_3 a_2\omega^2 - b_1\omega^4 a_2 - b_3 a_4}{b_2^2\omega^2 + (b_1\omega^2 - b_3)^2},\\
\sin(\omega\tau) &= \tfrac{\omega(b_1\omega^4 a_1 - b_1\omega^2 a_3 - b_3\omega^2 a_1 + b_3 a_3 + a_2\omega^2 b_2 - a_4 b_2)}{b_2^2\omega^2 + (b_1\omega^2 - b_3)^2},
\end{aligned}
\tag{2.4}
$$

Squaring and adding both sides of Eqs.(2.4), we have

$$a_1^2\omega^6 + (-b_1^2 + a_2^2 - 2a_3a_1)\omega^4 + (-2a_4a_2 - b_2^2 + 2b_3b_1 + a_3^2)\omega^2 + a_4^2 - b_3^2 = 0 \tag{2.5}$$

By the method of the discrimination sequence of polynomials, we have seriously discussed the roots of Eqs.(2.5) in paper [12,13] , and the conclusion is cited as the following: Proposition 1: If $a_2^2 - b_1^2 - 2a_3a_1 > 0$ and $a_4^2 - b_3^2 < 0$, then Eqs.(2.5) has only one pair of roots $\pm i\omega_0$, and τ_n^* corresponds to $\pm i\omega_0$ is given by

$$\tau_n^* = \frac{1}{\omega_0}\arccos\frac{b_2\omega^4 a_1 - b_2\omega^2 a_3 + b_1\omega^2 a_4 + b_3 a_2\omega^2 - b_1\omega^4 a_2 - b_3 a_4}{b_2^2\omega^2 + (b_1\omega^2 - b_3)^2} + \frac{2n\pi}{\omega_0} \tag{2.6}$$

For $\tau = 0$, E^* is stable. Hence, E^* will remain stable for $\tau = \tau_0$ where $\tau_0 = \tau_0^*$ as $n = 0$.

For example, with the parameters given in Table 1, the corresponding discrimination sequence is $[1, -1, -1, -1, -1, 1]$ and the pair of roots is given as $\pm i\omega_0 = \pm 11.2377i$. The critical value of delay τ_0 is also calculated by Eq.(2.6) as $\tau_0 = 0.06380$.

3. **Low level viral persistence.** In Section 2, we use analytical methods to give some results about the existence of steady states and the condition for the possible Hopf bifurcation. Due to an infinite-dimensional nature of system (1.1), we will show some relevant issues to the bifurcation analysis of system (1.1) further based on the numerical methods upon the package DDE-Biftool [14,15].

It is suggested that HBV virus can persist at various levels rather than being completely eliminated from the host. The low level HBV persistence below the detection limit of conventional assays of about $10^2 - 10^3$ HBV DNA copies/ml pf serum can have negative consequences for the host. The coexistence of low level HBV virus can reactive during immunosuppression and sustain the immunological memory to form the infection immunity, that is, the likelihood that these individuals or their organs may be infectious to others. To analyze the low level HBV coexistence, we use the numerical bifurcation techniques to explore the quantitative features of the nonlinear mathematical model described by system

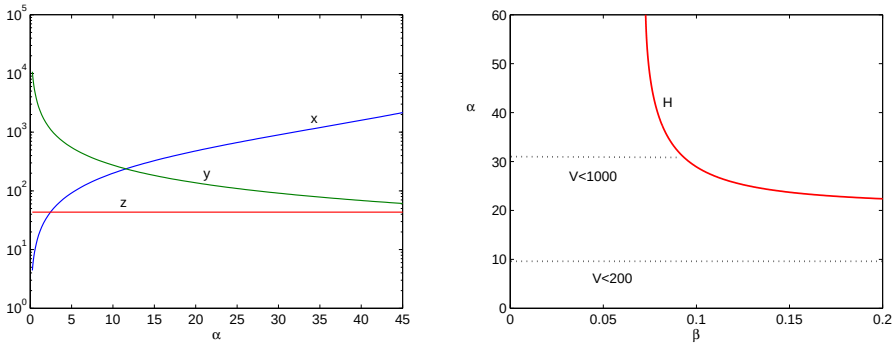

FIGURE 1. Left: Steady-state solutions x, y, z as varying CTL death rate α ($\beta = 0.0761$). Right: regions in $\beta - \alpha$ plane corresponding to solutions with $x < 10^3$ and $x < 200$ respectively. The solid line denotes the Hopf bifurcation curve.

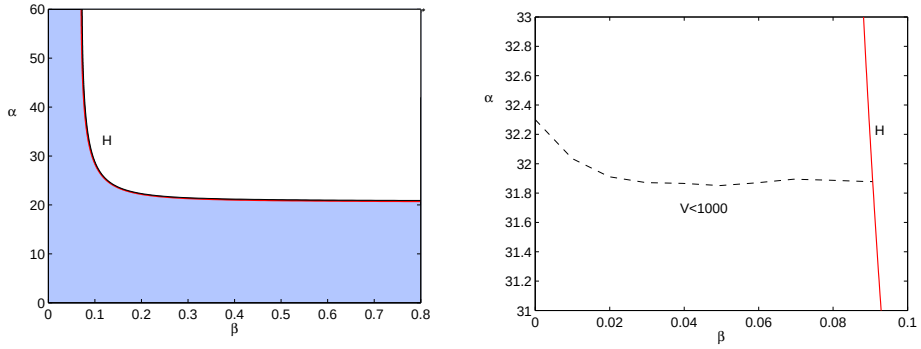

FIGURE 2. Left: the stability region (depicted in color) of the steady-state solution of system (1.1) in $(\beta - \alpha)$ plane. Right: The amplifying figure of the region corresonds to solutions with $x < 10^3$, the solid line denotes a Hopf bifurcation curve.

(1.1). The domains where low level HBV coexistence with CTL memory is possible, either as an equilibrium state or an oscillatory pattern, are identified in parameter space.

To start the analysis, we set the virus growth rate $\beta = 0.0761$ and all other parameters as in Table 1. We compute the steady state by using continuation technique to examine the dependence of the corresponding steady state on the parameter α. By varying parameter α within its ranges, as shown in Fig.1(left), the steady state density (x, y, z) of system (1.1) are calculated numerically, which indicate effect of virus and CTL death rate on the populations level and the numerical values are in agreement with the explicit analytical results derived in Section 2. One can seen that the value of x can keep at a very low level along the boundary of the stability region(the curve of Hopf points) in Fig.1(right). As it shown further, if the CTL death rate is small, change of β have little impact on the equilibrium values of x. Hence the features remain true in the stability region in the amplifying figure shown in Fig.2(right). HBV virus can persist at a low level and have little change as varying $\beta \in [0, 0.089]$ and α is less than 32.4. The branch of Hopf bifurcation

points in $(\beta - \alpha)$ plane, as shown in Fig.2(left), is drawn by the continuation calculation from the emanating Hopf point, and the adoptable value of delay is $\tau = 0.02$.

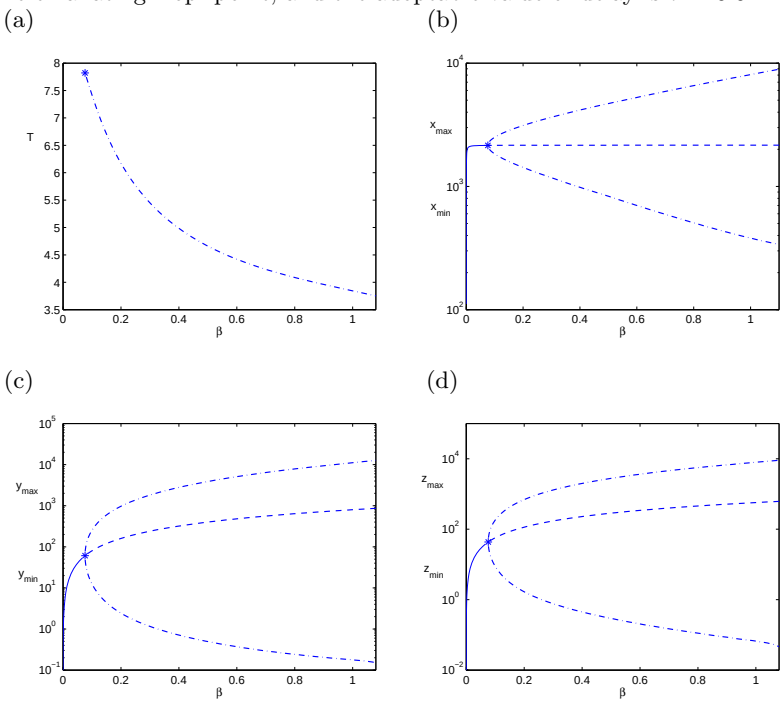

FIGURE 3. Period T, evolution of maximal and minimal values of x, y, z along a branch of bifurcating periodic solutions of system (1.1) emanating from a Hopf point $(*)$ versus parameter β for $\alpha = 45, \tau = 0.02$.Branches of stable (-) and unstable (–) steady state solutions and branch of stable periodic solutions (-.).

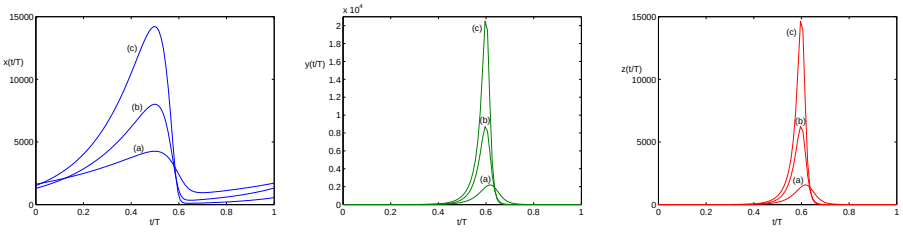

FIGURE 4. Periodic solutions with different virus growth rate β. (a)$\beta = 0.4429, T = 4.8328$;(b) $\beta = 1.0395, T = 3.7988$;(c) $\beta = 1.6344, T = 2.9941$.

We study the existence of oscillatory patterns in different level viral persistence by computing branches of periodic solutions emanating from the Hopf point. Fig.3 depicts a branch of bifurcating periodic solutions as a function of the parameter β ($\alpha = 45, \tau = 0.02$), whilst Fig.5 depicts a branch of periodic solutions as varying parameter α ($\beta = 0.6419, \tau =$

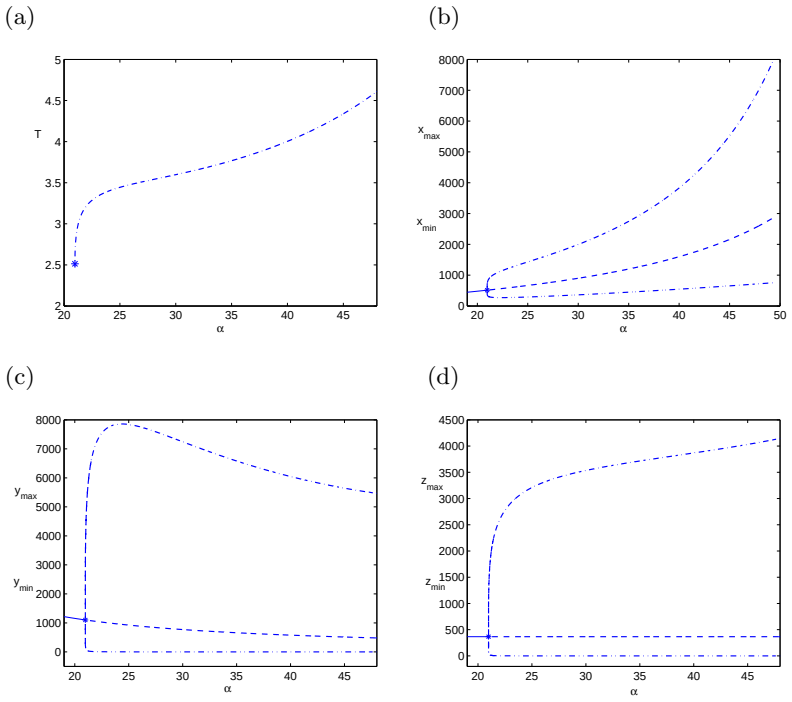

FIGURE 5. Period T, evolution of maximal and minimal values of x, y, z along a branch of bifurcating periodic solutions of system (1.1) emanating from a Hopf point $(*)$ versus parameter α for $\beta = 0.6419, \tau = 0.02$.Branches of stable (-) and unstable (–) steady state solutions and branch of stable periodic solutions (-.).

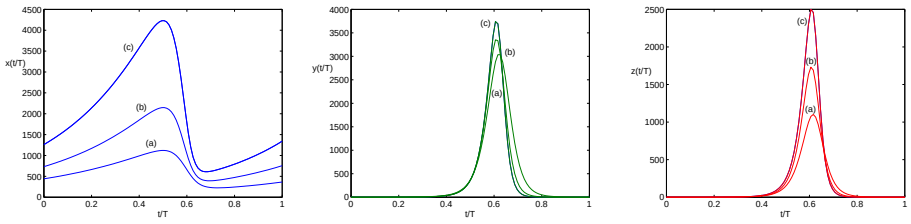

FIGURE 6. Periodic solutions with different values of parameter α. (a)$\alpha = 21.9792, T = 3.2129$; (b) $\alpha = 31.9682, T = 3.6589$; (c)$\alpha = 41.9740, T = 4.1214$.

0.02). Define $x_{max}(\beta) = max_{t\in[0,T]}x(t,\beta), x_{min}(\beta) = min_{t\in[0,T]}x(t,\beta)$,etc., then variation of solutions along the branch is characterized by their maximal and minimal values over the period for each computed point on the branch. As β grows from its Hopf point value, the amplitude of oscillations in populations densities x, y, z grows. Reversely, the oscillation period T of the periodic solutions decrease rapidly. After time is scaled by the factor T^{-1}, as shown in Fig.4 and Fig.6, respectively, we depict the periodic solutions on the time interval $[0, 1]$ with values of β chosen as $\beta = 0.4429, T = 4.8328$; $\beta = 1.0395, T = 3.7988$;

$\beta = 1.6344, T = 2.9941$ and with different values of α as $\alpha = 21.9792, T = 3.2129$; $\alpha = 31.9682, T = 3.6589$; $\alpha = 41.9740, T = 4.1214$.

The results on existence of periodic solutions in $(\beta - \alpha)$ plane is summarized in Fig.7. The region of our interest, where periodic oscillation are such that $x_{max} < 1000$ is quite small. It becomes narrow with increasing virus growth rate β. The possible high amplitude oscillation is obtained outside the coexistence region of low level virus.

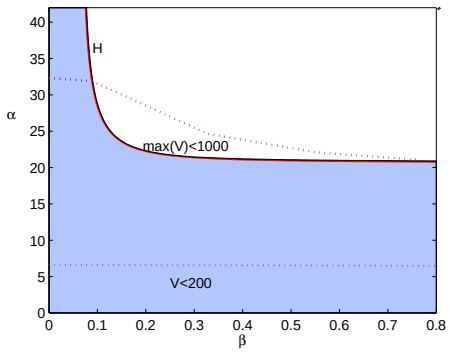

FIGURE 7. Stability regions of steady state and periodic solutions.

4. **Conclusion.** Hepatitis B virus can coexist with expanded clones of virus specific CTL in the memory phase below the detection limit. The mathematical model to predict the population dynamics of the anti-virus CTL response to Hepatitis B virus was reported. In this paper, we analyzed in quantitative terms the kinetic basis of coexistence of a small virus population with CTL and effector cells. It was seen, the combination of virus growth rate and death rate for CTL population led to a possible stable coexistence, either as an equilibrium state or an oscillatory pattern. Based on the numerical continuation techniques upon package DDE-Biftool, we also performed the bifurcation analysis of the population model, which predicted the combinations of the parameters such that viral load oscillate with different levels. Pulse form of oscillations was instructive to understand the HBV virus kinetics and the period of the periodic 'bursts' of viral replication ranged from 2 to 9 days .

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