Mathematical analysis of the effect of a pulse vaccination to an HBV mutation model

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ABSTRACT. It has been proven that vaccine can play an important role for eradication of hepatitis B infection. When the mutant strain of virus appears, it changes all treatments strategies. The current problem is to find the critical vaccine threshold which can stimulate the immune system for eradicate the virus, or to find conditions at which mutant strain of the virus can persist in the presence of a CTL vaccine. In this paper, the dynamical behavior of a new hepatitis B virus model with two strains of virus and CTL immune responses is studied. We compute the basic reproductive ratio of the model and show that the dynamic depend of this threshold. After that, we extend the model incorporating pulse vaccination and we find conditions for eradication of the disease. Our result indicates that if the vaccine is sufficiently strong, both strains are driven to extinction, assuming perfect adherence.

RÉSUMÉ. Il a été démontré que le vaccin peut jouer un rôle très important dans le processus d'éradication de l'hépatite B. Lorsque la souche mutante apparait, elle modifie les stratégies de luttes. Le problème courant est celui de déterminer le seuil vaccinal capable de stimuler le système immunitaire pour éliminer le virus, ou de trouver les conditions pour lesquelles la souche mutante persistera en présence du vaccin. Dans ce travail, nous considérons un nouveau modèle d'hépatite B dans lequel nous prenons en compte une souche de virus sauvage et une souche mutante et leur interaction avec les cellules du système immunitaire. Nous calculons le taux de reproduction de base et montrons que la dynamique dépend de ce seuil. Une extension de ce modèle est faite afin d'inclure un schéma impulsionnel de vaccination et de déterminer les conditions pour éliminer la maladie. Nos résultats montrent que si le vaccin est suffisamment puissant, les deux souches pourront être éliminées.

KEYWORDS : Mutant strains, pulse vaccination, Hepatitis B.

MOTS-CLÉS : Souche mutante, vaccination par impulsion, hépatite B.

1. Introduction

Hepatitis B virus (HBV) infection is a disease of global health. According to the data of World Health Organization (WHO), approximately 30% of world's population, i.e. about 2 billion people have been infected with HBV at some time in their lives. Of these, about 350 million remain infected chronically and become carriers of the virus. Every year, there are over 4 million acute clinical cases of HBV, and about 25% of carriers. Hepatitis B causes about 1 million people to die from chronic active hepatitis, cirrhosis, or primary liver cancer annually [13].

The rate of chronicity of viral infection is dramatically higher in neonates born from infected mothers, suggesting that mature immunity is important to clear infection. Patients with chronic hepatitis B (CHB) are at increased risk of developing severe liver disease, including cirrhosis and hepatocellular carcinoma (Figure 1) [7, 8]. As HBV is currently viewed as a non-cytopathic virus, HBV-associated liver damage is thought to be the consequence of a long lasting cytolytic immune response against infected hepatocytes [10, 11].
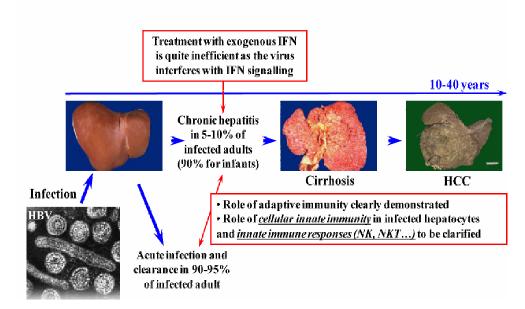

Figure 1: Natural history of HBV infections. HCC, hepatocellular carcinoma; NK, natural killer cells; NKT, natural killer T cells.

Both innate and adaptive arms of the immune system are generally involved in responding to viral infection, with innate responses being important for control of viral replication and dissemination very early after infection, as well as for timely orchestration of virus-specific adaptive responses [12]. In the case of HBV, it has been clearly shown that the adaptive response is needed for efficient and persistent control of infection [10, 11]. However, the role of innate immunity has been more difficult to analyze, as HBV infection is usually diagnosed several weeks after the onset of infection when

viremia is already high; thus the role of innate immunity in defense against HBV remains controversial.

HBV mutants have recently been identified in patients with acute, fulminant, or chronic infections. Sequence analysis of virus isolates from many individual patients has revealed the occurrence of certain mutational hot spots in the genome, some of which appear to correlate with the patient's immunological and/or disease status; however, cause and effect are not always easily discernible [5]. This holds particularly for the issue of whether virus variants exist that have an increased pathogenic potential; due to the scarcity of appropriate in vivo experiment models, such hypotheses are difficult to prove. Similarly, because of the compact organization of the HBV genome, almost every single mutation may have pleiotropic phenotypic effects.

Naturally occurring mutations have been identified in all viral genes and regulatory elements, most notably in the genes coding for the structural envelope and nucleocapsid proteins [6]. Mutations in the gene coding for the HBsAg may result in infection or viral persistence despite the presence of antibodies against HBsAg (anti-HBs). Mutations in the gene encoding the pre-core/core protein (pre-core stop codon mutant) result in a loss of HBeAg (HBeAg minus mutant) and seroconversion to antibodies to HBeAg (anti-HBe) with persistence of HBV replication [5]. Mutations in the core gene may lead among others to an "immune escape" due to a T cell receptor antagonism [5]. Mutations in the gene coding for the polymerase/reverse transcriptase can be associated with viral persistence or resistance to nucleoside analogues [5]. Thus, HBV mutations may affect the natural course of infection, viral clearance and response to antiviral therapy. The exact contribution of specific mutations to diagnosis and therapy of HBV infection as well as patient management in clinical practice remain to be established. In addition, despite the availability of an effective prophylactic vaccine, further extensive efforts are required to monitor the emergence of vaccination and therapy-resistant HBV variants and to prevent their spread in the general population.

Current treatment options for chronic hepatitis B depend on interferon (IFN) α and direct antivirals, i.e. nucleoside or nucleotide analogues. Although there now are seven approved therapies for HBV infection (two IFN formulations and five nucleoside analogues) [18, 19]. HBV cannot be cleared by currently available antiviral therapy and therefore requires long-term antiviral treatment, which is costly, often selects for drug resistant viral variants and may have long-term side effects [18]. So there is a need for alternative treatment approaches. For HBV highly effective and safe prophylactic vaccines are available. These, however, showed no effect in the setting of chronic infection [20, 21, 22, 23], indicating the need for a specific therapeutic vaccine design. We here focus on the options to design and develop a therapeutic hepatitis B vaccine.

Since its widespread introduction in 1983, the hepatitis B vaccine has become an essential part of infant immunization programmes globally, and is the key component of the global hepatitis B control programme for the World Health Organization (WHO)[1]. Infection with hepatitis B virus (HBV) can cause acute liver disease, as well as chronic infection that may lead to liver failure or hepatocellular carcinoma. The vaccine has been particularly important for countries where the incidence of HBV-related hepatocellular carcinoma is high. In effect, the hepatitis B vaccine was the world's first anticancer vaccine.

Therapeutic vaccines are currently developed for chronic viral infections, such as human papillomavirus (HPV), human immunodeficiency virus (HIV), herpes virus and hepatitis B (HBV) and C (HCV) virus infections. As an alternative to antiviral treatment or to support only partially effective therapy a therapeutic vaccine shall activate the patient's

immune system to fight and finally control or ideally even eliminate the virus. HBV can be eliminated by the immune system after acute infection or sometimes even when the immune balance in chronic infection tips. Since shifting this balance towards immune control is the aim of therapeutic vaccination, those viruses are primary targets for a proof-of-concept of therapeutic vaccination.

Within-host models are used for different purposes : explanation of observations, to predict impact of interventions (antimalarial drugs), estimate hidden states or parameters. Experimental results show that one of the main reasons for CTL response failure is viral escape from CTLs [2]. Moreover, if escape mutation to the vaccine occurs, then either the wild type or the mutant can outcompete the other strain [14]. In this paper, we investigate the effect of viral mutation on the ability of CTLs to control the viral infection when a post-infection vaccine is administered at regular intervals. The novelty in our model is that its not only takes into account two strains of virus, but also assumes that a regulatory negative feedback force, operates to suppress immune population growth at a net rate proportional to the square of its density. This implies regulation of the response at hight antigen concentration.

This work is organized as follows. In the next Section, we propose our model. We analyze the model in Section 3. We extend this model in Section 4 to incorporate pulse vaccination and to find conditions for eradication of the viruses. We give some numerical simulations in Sect. 5 to explain our mathematical results. We end this paper with a brief discussion and conclusion.

2. The model formulation

We begin by introducing the model constructed by Nowak and Bangham [9] when there is no mutation. This model is given by

$$\begin{cases} \dot{x} &= \lambda - d_x x - \beta x v, \\ \dot{y}_1 &= \beta x v - d_y y - \rho y I, \\ \dot{v}_1 &= k y - d_v v, \\ \dot{I} &= \alpha y I - \gamma I. \end{cases} \tag{1}$$

where x is the number of susceptible host cells, y_1 is the number of infected cells, v_1 is the number of free virus, and I is the number of CTL cells. All the parameters λ, d_x, β, d_y, ρ_y, k_y, d_v, α and γ are positive. d_x, d_y, d_v, and γ are the death rates of uninfected cells, infected cells, free virus, and CTL cells, respectively. λ represents a constant production of the target cells. β is the contact rate between uninfected cells and free virus. Infected cells are removed at rate ρI by CTL immune responses. The virus-specific CTL cells proliferate at rate αy by contact with infected cells. Free virus is produced from infected cells at rate $k y$.

Now we include mutant virus and we use a special function to describe immune responses. This function assume a regulatory negative feedback force, which operates to suppress immune population growth at a net rate proportional to the square of its density.

This implies regulation of the response at hight antigen concentration. So, the model we consider in this paper is given as follows:

$$
\begin{cases}
\dot{x} &=& \lambda - d_x x - \beta_1 v_1 x - \beta_2 v_2 x, \\
\dot{v}_1 &=& k_1 y_1 - d_v v_1, \\
\dot{v}_2 &=& k_2 y_2 - d_v v_2, \\
\dot{y}_1 &=& (1-\varepsilon)\beta_1 v_1 x - \rho_1 y_1 I - d_y y_1, \\
\dot{y}_2 &=& \varepsilon\beta_1 v_1 x + \beta_2 v_2 x - \rho_2 y_2 I - d_y y_2, \\
\dot{I} &=& \alpha(y_1 + y_2)I + pI - qI^2.
\end{cases}
\tag{2}
$$

where x is the number of uninfected liver cells, v_1 is the number of wild-type virus, v_2 is the number of mutant virus, y_1 is the number of liver cells infected with wild-type, y_2 is the number of liver cells infected with mutant strains, and I is the number of CTL cells. All the parameters $\lambda, d_x, \beta_1, \beta_2, k_1, d_v, \varepsilon, k_2, d_y, \alpha, p$, and q are positive. Uninfected liver cells are produced with constant rate λ and die with rate d_x. They are infected with wild-type and mutant strains respectively at rate β_1 and β_2. The chance of the novo mutation is ε. Free virus particles and infected liver cells die at rate d_v and d_y respectively. Infected liver cells are also cleared by the body's defensive CTLs; this happens respectively at rate ρ_1 and ρ_2. CTLs reproduced in the presence of infected liver cells at rate α. The parameter p denotes the proliferation rate of immune cells and q the density-dependent rate of immune cells suppression. New virus particles are produced at rate k_1 by wild-type virus and k_2 by mutant virus respectively.

3. Mathematical analysis of the model

Herein, we present some basic results, such as the positive invariance of model system (2), the boundedness of solutions, the existence of equilibria and its stability analysis.

3.1. Positivity and boundedness of solutions

The following result guarantees that model system (2) is biologically well behaved and its dynamics is concentrated on a bounded region of $\mathbb{R}_+^6$. More precisely, the following result holds.

Theorem 3.1. *Let $R_+^6 = \{(x, v_1, v_2, y_1, y_2, I) \in \mathbb{R}^6 : x \geq 0, v_1 \geq 0, v_2 \geq 0, y_1 \geq 0, y_2 \geq 0, I \geq 0\}$. Then, $\mathbb{R}_+^6$ is positively invariant under the flow induced by model system (2). Moreover, the region*

$$
\Delta = \left\{ (x, v_1, v_2, y_1, y_2, I) \in \mathbb{R}^6 : x + y_1 + y_2 \leq \frac{\lambda}{\mu}, \; v_1 + v_2 \leq \frac{(k_1 + k_2)\lambda}{\mu d_v}, \right.
$$
$$
\left. \frac{p}{q} \leq I \leq \frac{p\mu + \alpha\lambda}{\mu q} \right\}.
$$

is positively invariant and absorbing with respect to model system (2), where $\mu = \min\{d_x, d_y\}$.

Proof. Let $\mu = \min\{d_x, d_y\}$. No solution of model system (2) with initial conditions $(x(0), v_1(0), v_2(0), y_1(0), y_2(0), I(0)) \in \mathbb{R}_+^6$ is negative. In fact, for $(x(t), v_1(t), v_2(t), y_1(t), y_2(t), I(t)) \in \mathbb{R}_+^6$, we have $\dot{x}\mid_{x=0} = \lambda > 0, \dot{v}_1\mid_{v_1=0} = k_1 y_1 \geq 0, \dot{v}_2\mid_{v_2=0} = k_2 y_2 \geq 0, \dot{y}_1\mid_{y_1=0} = (1-\varepsilon)\beta_1 v_1 x \geq 0, \dot{y}_2\mid_{y_2=0} = \varepsilon\beta_1 v_1 x + \beta_2 v_2 x \geq 0,$

$\dot{I}\mid_{I=0} = 0 \geq 0$, this immediately implies that all solutions of model system (2) with initial condition $(x(0), v_1(0), v_2(0), y_1(0), y_2(0), I(0)) \in \mathbb{R}_+^6$ stay in the first quadrant.

For the invariance property of Δ, it suffices to show that the vector field, on the boundary, does not point to the exterior. Adding the first, fourth and fifth and second equations of model system(2) yields on the boundary of Δ:

$$\frac{d(x + y_1 + y_2)}{dt}\bigg|_{x+y_1+y_2 = \frac{\lambda}{\mu}} = \lambda - d_x x - d_y y_1 - d_y y_2 - (\rho_1 y_1 + \rho_2 y_2)I \mid_{x+y_1+y_2 = \frac{\lambda}{\mu}}$$

$$\leq (\lambda - \mu(x + y_1 + y_2))|_{x+y_1+y_2 = \frac{\lambda}{\mu}} = 0$$

Similarly, we get

$$\frac{d(v_1 + v_2)}{dt}\bigg|_{v_1+v_2 = \frac{(k_1+k_2)\lambda}{\mu d_v}} \leq \frac{(k_1+k_2)\lambda}{\mu} - d_v(v_1 + v_2)\bigg|_{v_1+v_2 = \frac{(k_1+k_2)\lambda}{\mu d_v}} = 0,$$

$$\frac{dI}{dt}\bigg|_{I=\frac{p}{q}} \geq (p - qI)\,I|_{I=\frac{p}{q}} = 0 \quad i.e\ I(t) \geq \frac{p}{q}, \ \forall t \geq 0,$$

and

$$\frac{dI}{dt}\bigg|_{I=\frac{p\mu+\alpha\lambda}{\mu q}} \leq \left(\frac{\alpha\lambda}{\mu} + p - qI\right)I\bigg|_{I=\frac{p\mu+\alpha\lambda}{\mu q}} = 0$$

Therefore, solutions starting in Δ will remain there for $t \geq 0$.

Now, we prove the attractiveness of the trajectories of model system (2). To do so, from model system (2), one has $\frac{d(x + y_1 + y_2)}{dt} \leq \lambda - \mu(x + y_1 + y_2)$. Therefore, $\limsup\limits_{t\to\infty}(x + y_1 + y_2)(t) \leq \frac{\lambda}{\mu}$. Similarly, since $\frac{d(v_1 + v_2)}{dt} \leq \frac{(k_1 + k_2)\lambda}{\mu} - d_v(v_1 + v_2)$, one has $\limsup\limits_{t\to\infty}(v_1(t) + v_2(t)) \leq \frac{(k_1 + k_2)\lambda}{\mu d_v}$. Concerning the variable I, we have $\frac{dI}{dt} \leq \left(\frac{\alpha\lambda}{\mu} + p\right)I - qI^2$ which implies that $I(t) \leq \dfrac{1}{\frac{\mu q}{p\mu+\alpha\lambda} + \left(\frac{1}{I(0)} - \frac{\mu q}{p\mu+\alpha\lambda}\right)e^{-\left(p+\frac{\alpha\lambda}{\mu}\right)t}}$.

So, I is bounded and hence, Δ is attracting, that is, all solutions of model system (2) eventually enters Δ. This concludes the proof. $\qquad\square$

3.2. Basic reproduction number and equilibria

The basic reproduction number is defined as the average number of secondary infections produced by one infected cell during the period of infection when all cells are uninfected. This threshold is obtained at the virus free equilibrium. The virus free equilibrium is obtained by setting $v_1 = 0$ in all equations of model system (2) at the equilibrium. We obtain that the virus free equilibrium is $P_1(x^*, 0, 0, 0, 0, 0, I^*)$, where $x^* = \frac{\lambda}{d_x}$ and $I^* = \frac{p}{q}$. We use the method of van den Driessche[3] to compute the basic reproduction number. Using the notations of van den Driessche and Watmough[3], for model system (2), we have

$$F = \begin{pmatrix} 0 & 0 & k_1 & 0 \\ 0 & 0 & 0 & k_2 \\ (1-\varepsilon)\beta_1 x^* & 0 & 0 & 0 \\ \varepsilon\beta_1 x^* & \beta_2 x^* & 0 & 0 \end{pmatrix} \qquad \text{and}$$

$$V = \begin{pmatrix} d_v & 0 & 0 & 0 \\ 0 & d_v & 0 & 0 \\ 0 & 0 & \rho_1 I^* + d_y & 0 \\ 0 & 0 & 0 & \rho_2 I^* + d_y \end{pmatrix}.$$

Thus, the basic reproduction number is given by:

$$\mathcal{R}_0 = \max \{ \mathcal{R}_{01} \, ; \, \mathcal{R}_{02} \}. \tag{3}$$

where $\mathcal{R}_{01} = \dfrac{k_1 (1-\varepsilon)\beta_1 x^*}{d_v(d_y + \rho_1 I^*)}$ and $\mathcal{R}_{02} = \dfrac{k_2 \beta_2 x^*}{d_v(d_y + \rho_2 I^*)}$. From theorem 2 of Van Den Driessche[3], we have the following result.

Lemma 3.1. *The virus-free equilibrium P_1 of the model system (2) is locally asymptotically stable (LAS) if $\mathcal{R}_0 < 1$, and unstable if $\mathcal{R}_0 > 1$.*

We now study the existence of equilibria of model system (2). Setting the right-hand sides of model system (2) equals to zero gives

$$\lambda - d_x x - \beta_1 v_1 x - \beta_2 v_2 x = 0, \tag{4}$$

$$k_1 y_1 - d_v v_1 = 0, \tag{5}$$

$$k_2 y_2 - d_v v_2 = 0, \tag{6}$$

$$(1-\varepsilon)\beta_1 v_1 x - \rho_1 y_1 I - d_y y_1 = 0. \tag{7}$$

$$\varepsilon \beta_1 v_1 x + \beta_2 v_2 x - \rho_2 y_2 I - d_y y_2 = 0, \tag{8}$$

$$\alpha(y_1 + y_2) I + pI - qI^2 = 0. \tag{9}$$

Model system (2) has always equilibrium $P_1 = (x^*, 0, 0, 0, 0, I^*)$ which is the virus free equilibrium and represents the state when the viruses are absent. If $\mathcal{R}_{02} > 1$, then the model has one mutant-only equilibrium $\tilde{P}_2(\tilde{x}, 0, \tilde{v}_2, 0, \tilde{y}_2, \tilde{I})$, where $\tilde{x} = \frac{\lambda d_v}{d_x d_v + \beta_2 k_2 \tilde{y}_2}$, $\tilde{v}_2 = \frac{k_2}{d_v} \tilde{y}_2$, $\tilde{I} = \frac{p}{q} + \frac{\alpha}{q} \tilde{y}_2$, and $\tilde{y}_2$ is the unique positive solution of the following equation:

$$\frac{\rho_2 \alpha \beta_2 k_2}{q} \tilde{y}_2^2 + \left(d_y \beta_2 k_2 + \frac{\rho_2 \alpha d_x d_v + \rho_2 p \beta_2 k_2}{q} \right) \tilde{y}_2 + (1 - \mathcal{R}_{02}) d_x d_v (d_y + \rho_2 I^*) = 0 \tag{10}$$

In what follows, we study the existence of the third equilibrium $\bar{P} = (\bar{x}, \bar{v}_1, \bar{v}_2, \bar{y}_1, \bar{y}_2, \bar{I})$, which is obtained when the two virus strains coexist. From equations (4), (5), (6) and (9), we have

$$\bar{x} = \frac{\lambda d_v}{d_x d_v + \beta_1 k_1 \bar{y}_1 + \beta_2 k_2 \bar{y}_2}, \quad \bar{v}_1 = \frac{k_1}{d_v} \bar{y}_1, \quad \bar{v}_2 = \frac{k_2}{d_v} \bar{y}_2, \quad and \quad \bar{I} = \frac{p}{q} + \frac{\alpha}{q} (y_1 + y_2).$$

Substituting the above relations in Eqs. (7)−(8), we obtain the following system:

$$\begin{cases} \bar{y}_1 = \dfrac{d_x(1-\varepsilon)\beta_1 k_1 x^* \bar{y}_1}{(d_x d_v + \beta_1 k_1 \bar{y}_1 + \beta_2 k_2 \bar{y}_2) \left[d_y + \rho_1 \left(\frac{p}{q} + \frac{\alpha}{q} (\bar{y}_1 + \bar{y}_2) \right) \right]} \\ \bar{y}_2 = \dfrac{\varepsilon d_x \beta_1 k_1 x^* \bar{y}_1 + \beta_2 k_2 \bar{y}_2 x^* d_x}{(d_x d_v + \beta_1 k_1 \bar{y}_1 + \beta_2 k_2 \bar{y}_2) \left[d_y + \bar{y}_2 \rho_2 \left(\frac{p}{q} + \frac{\alpha}{q} (\bar{y}_1 + \bar{y}_2) \right) \right]} \end{cases} \tag{11}$$

Solving the above fixed point problem, we obtain the following result:

Proposition 3.1. *If $\mathcal{R}_0 > 1$, then the model has a unique endemic equilibrium $\bar{P} = (\bar{x}, \bar{v}_1, \bar{v}_2, \bar{y}_1, \bar{y}_2, \bar{I})$.*

Remark 3.1.   *1) The proof of Proposition 3.1 is given in Appendix C.*

2) Due to mutation, there is no wild-type-only equilibrium. In fact, when $v_2 = 0$ in (4)-(9), then $v_1 = 0$.

3.3. Stability of equilibria

Here, we analyze the stability of equilibria obtained in the previous section. We have the following result.

Lemma 3.2. *If $\mathcal{R}_{01} < 1$, then $\dfrac{k_1(1-\varepsilon)\beta_1\tilde{x}}{d_v(\rho_1\tilde{I} + d_y)} < 1$.*

Proof :   If $\mathcal{R}_{01} < 1$, then $k_1(1-\varepsilon)\beta_1 x^* < d_v(\rho_1 I^* + d_y)$. since $x^* > \tilde{x}$ and $I^* < \tilde{I}$, we have the result. $\square$

Theorem 3.2. *Let us consider the model system (2). If $\mathcal{R}_{01} < 1$ and $\mathcal{R}_{02} > 1$, then the mutant-only equilibrium $\tilde{P}_2$ exists and is locally asymptotically stable in Δ.*

Proof :   The characteristic equation of the jacobian matrix evaluated at $\tilde{P}$ is given by

$$P(\xi) = \left[\xi^2 + \xi(\rho_1\tilde{I} + d_y + d_v) + d_v(\rho_1\tilde{I} + d_y)\left(1 - \frac{k_1(1-\varepsilon)\beta_1\tilde{x}}{d_v(\rho_1\tilde{I} + d_y)}\right) \right]\left(\sum_{i=0}^{4} a_i\xi^{4-i}\right)$$

(12)

where the coefficients a_0, a_1, a_2, a_3 and a_4 are positive and are given in the appendix A. The Maple program shows that $a_1 a_2 a_3 > a_3^2 + a_1^2 a_4$. It follows that the stability of $\tilde{P}_2$ is determined by the solution of equation:

$$\xi^2 + \xi(\rho_1\tilde{I} + d_y + d_v) + d_v(\rho_1\tilde{I} + d_y)\left(1 - \frac{k_1(1-\varepsilon)\beta_1\tilde{x}}{d_v(\rho_1\tilde{I} + d_y)}\right) = 0.$$ (13)

Since the discriminant is $delta = (d_v - \rho_1\tilde{I} - d_y)^2 + 4k_1(1-\varepsilon)\beta_1\tilde{x} > 0$, if $\mathcal{R}_{01} < 1$ then from lemma 3.2, equation (13) has two negative solutions. So, it follows from the Routh-Hurwitz criterion that if $\mathcal{R}_{01} < 1$ then $\tilde{P}_2$ is locally asymptotically stable. $\square$

Using the Routh-Hurwitz criterion, about the stability of equilibrium $\bar{P}$ when the two virus co-exist, we have the following result.

Theorem 3.3. *If $\mathcal{R}_0 > 1$, there exists one endemic equilibrium $\bar{P} = (\bar{x}, \bar{v}_1, \bar{v}_2, \bar{y}_1, \bar{y}_2, \bar{I})$ for the model system (2) which is locally asymptotically stable in Δ if the following conditions holds:*

1) $u_i > 0$, $i = 2, ..., 6$ since $u_1 > 0$,

2) $u_1 u_2 u_3 > u_3^2 + u_1^2 u_4$,

3) $(u_1 u_4 - u_5)(u_1 u_2 u_3 - u_3^2 - u_1^2 u_4) > u_5(u_1 u_2 - u_3)^2 + u_1 u_5^2$,

4) $u_5(u_1 u_2 u_3 u_4 + 2u_1^2 u_2 u_6 + u_1 u_4 u_5 + u_1 u_5 u_4 + u_2 u_3 u_5) + u_3 u_6(u_1^2 u_4 + u_3) > u_5(3u_1 u_3 u_6 + u_1^2 u_4^2 + u_1 u_2^2 u_5 + u_3^2 u_4 + u_5^2) + u_1 u_6(u_2 u_3^2 + u_1^2 u_6)$.
where u_i, $i = 1, ..., 6$ are given in Appendix D.

4. The model with pulse vaccination

In this section, we consider the previous model and we extend that model by incorporating pulse vaccination. We suppose that at fixed vaccination times t_k, $k = 1, 2, ...$ vaccination increases CTL cells by a fixed amount $\hat{I}$ which is proportional to the total number of CTLs the vaccine stimulates. This hypothesis is make to determine the critical vaccine threshold for eradication of the virus or to find conditions at which mutant strain of the virus can persist in the presence of a CTL vaccine. For $t \neq t_k$, the model is

$$\begin{cases} \dot{x} &= \lambda - d_x x - \beta_1 v_1 x - \beta_2 v_2 x, \\ \dot{v}_1 &= k_1 y_1 - d_v v_1, \\ \dot{v}_2 &= k_2 y_2 - d_v v_2, \\ \dot{y}_1 &= (1 - \varepsilon)\beta_1 v_1 x - \rho_1 y_1 I - d_y y_1, \\ \dot{y}_2 &= \varepsilon \beta_1 v_1 x + \beta_2 v_2 x - \rho_2 y_2 I - d_y y_2, \\ \dot{I} &= \alpha(y_1 + y_2)I + pI - qI^2, \end{cases} \tag{14}$$

and for $t = t_k$,

$$\Delta I \equiv I(t_k^+) - I(t_k^-) = \hat{I} \tag{15}$$

where $I(t_k^-)$ is the CTL concentration immediately before the impulse and $I(t_k^+)$ is the CTL concentration immediately after the impulse.

4.1. Impulsive orbit and local stability

Because of the impulsive effect in I, there are no classical equilibria for System (14). In this case, we have the impulse orbit which are obtained when $\dot{x} = \dot{y}_1 = \dot{y}_2 = \dot{v}_1 = \dot{v}_2 = 0$ and $I \neq 0$. Let $r_1 = \rho_1 I + d_y$ and $r_2 = \rho_2 I + d_y$. We obtain the disease-free orbit $\hat{P}_0 = \left(\frac{\lambda}{d_x}, 0, 0, 0, 0\right)$, the mutant-only orbit $\hat{P}_1 = (\hat{x}, 0, \hat{v}_2, 0, \hat{y}_2)$, where $\hat{x} = \frac{r_2 d_v}{\beta_2 k_2}$, $\hat{v}_2 = \frac{\lambda \beta_2 k_2 - d_x r_2 d_v}{\beta_2 r_2 d_v}$, $\hat{y}_2 = \frac{\lambda \beta_2 k_2 - d_x r_2 d_v}{\beta_2 r_2 k_2}$. Due to mutation, there is no wild-type only orbit. For both strains of the virus to be eradicated, the disease-free orbit must be locally stable. If both the disease-free and mutant-only orbits are locally unstable, then the two virus strains will coexist in the presence of the vaccine.

Definition 4.1. *Let I_1^* be the value of I such that $d_x d_v r_1 = (1 - \varepsilon)\beta_1 k_1 \lambda$. That is $I_1^* = \frac{(1-\varepsilon)\beta_1 k_1 \lambda - d_x d_v d_y}{d_x d_v \rho_1}$. Let I_2^* be the value of I such that $d_x d_v r_2 = \beta_2 k_2 \lambda$. That is $I_2^* = \frac{\beta_2 k_2 \lambda - d_x d_v d_y}{d_x d_v \rho_2}$. Finally, let I_3^* be the value of I such that $(1 - \varepsilon)\beta_1 k_1 r_2 = \beta_2 k_2 r_1$. That is $I_3^* = \frac{(1-\varepsilon)\beta_1 k_1 d_y - \beta_2 k_2 d_y}{\beta_2 k_2 \rho_1 - (1-\varepsilon)\beta_1 k_1 \rho_2}$. The value I_1^* determines the long-term behavior of the wild-type strain, while I_2^* determines the long-term behavior of the mutant strain. The parameter I_3^* can take any sign and is critical in the analysis of the competition between the two virus strains.*

Lemma 4.1. *Either $I_1^* = I_2^* = I_3^*$ or $I_3^* > I_1^* > I_2^*$ or $I_2^* > I_1^* > I_3^*$.*

Remark 4.1. *The proof of the previous lemma is given in the appendix B.*

About the local stability of the disease free orbit and mutant-only orbit, we have the following result:

Theorem 4.2. *The disease-free orbit is locally stable if and only if $I > I_1^*$ and $I > I_2^*$. The mutant-only orbit is locally stable if and only if $I_3^* < I < I_2^*$.*

Proof :   The characteristic polynomial of the jacobian matrix at the disease-free $\hat{P}_0$ orbit is given by

$$
\begin{aligned}
P(\zeta) &= (-d_x - \zeta)\left(\zeta^2 + (d_v + r_1)\zeta + d_v r_1 - r_1(1-\varepsilon)\beta_1 \frac{\lambda}{d_x}\right) \times \\
&\quad \left(\zeta^2 + (d_v + r_2)\zeta + d_v r_2 - k_2\beta_2 \frac{\lambda}{d_x}\right).
\end{aligned} \tag{16}
$$

At the disease free orbit, $-d_x$ is an eigenvalue which is negative. From equation (16), the equilibrium $\hat{P}$ is locally asymptotically stable if $d_v r_1 - r_1(1-\varepsilon)\beta_1 \frac{\lambda}{d_x} > 0$ and $d_v r_2 - k_2\beta_2 \frac{\lambda}{d_x} > 0$; i.e $I > I_1^*$ and $I > I_2^*$.

The characteristic polynomial of the jacobian matrix at the mutant-only orbit $\hat{P}_1$ is given by:

$$
Q(\zeta) = -\left(\zeta^2 + (d_v + r_1)\zeta + d_v r_1 - k_1(1-\varepsilon)\beta_1 \frac{r_2 d_v}{\beta_2 k_2}\right)\left(\zeta^3 + b_1\zeta^2 + b_2\zeta + b_3\right) \tag{17}
$$

where $b_1 = r_2 + d_v + \frac{\lambda\beta_2 k_2}{r_2 d_v}$, $b_2 = (r_2 + d_v)\frac{\lambda\beta_2 k_2}{r_2 d_v}$ and $b_3 = \lambda\beta_2 k_2 - d_x r_2 d_v$. Since $b_1 > 0$ and $b_1 b_2 > b_3$, from the Routh-Hurwitz criterion, the equilibrium $\hat{P}_1$ is locally asymptotically stable if only if $d_v r_1 - k_1(1-\varepsilon)\beta_1 \frac{r_2 d_v}{\beta_2 k_2} > 0$ and $b_3 > 0$. These lead to $I > I_3^*$ and $I < I_2^*$; that is $I_3^* < I < I_2^*$. $\square$

Remark 4.2.   *1) By Lemma 4.1, for stability of mutant only orbit, either $I_3^* < I_1^* < I < I_2^*$ or $I_3^* < I < I_1^* < I_2^*$.*

2) The condition for eradication of the both strains of the virus is $I > I_1^*$ and $I > I_2^*$. So, it is important for the vaccine to be strong enough to guarantee $I > I_1^*$ and $I > I_2^*$. Moreover, if $I_2^* > I_1^*$ and if the vaccine is such that $I_3^* < I < I_2^*$, then the mutant strain may become dominant. The both strains coexist when $0 \le I < I_3^* < I_1^* < I_2^*$. The coexistence both strains can also exists when $0 < I < I_3^* < I_1^* < I_2^*$ or $I_2 < I < I_1 < I_3^*$.

This remark is summarized in Figure 2.

4.2. Perfect adherence of vaccine

We have established in the previous section, the critical level of CTLs necessary to control the virus. Here, we determine the maximal time between the vaccinations, depending on the vaccine strength, to ensure that the CTL amount always exceeds the desired level. Establishing this maximal time frame allows us to recommend a CTL vaccine treatment.

The vaccination is operate at time t_k and $I(t_k^+)$ (is the CTL concentration immediately after the impulse. From the impulsive differential equation for I, we have: $\dot{I} = \alpha(y_1 + y_2)I + pI - qI^2 > pI - qI^2$ as y_1 and y_2 are positive. So, we obtain

$$
I(t) > \frac{1}{\frac{p}{q} + \left(\frac{1}{I(t_k^+)} - \frac{p}{q}\right)e^{-p(t-t_k)}} \quad \text{for } t_k < t < t_{k+1}.
$$

Let us suppose that vaccination is successful (y_1 and y_2 are very small) and let us consider the following approximaltion of $I(t)$:

$$
I(t) = \frac{1}{\frac{p}{q} + \left(\frac{1}{I(t_k^+)} - \frac{p}{q}\right)e^{-p(t-t_k)}} \quad \text{for } t_k < t < t_{k+1}.
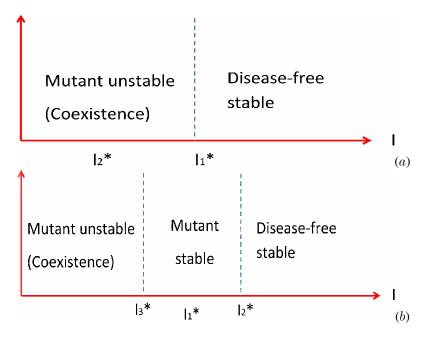$$

Figure 2: Regions of stability of impulsive orbits. When $I_2^* < I_1^*$, if $I > I_1^*$, the disease-free orbit becomes stable and the mutant cannot survive on its own (see Fig. 2 (a)). Conversely, if $I < I_1^*$, both strains coexist. If $I_2^* > I_1^*$ The disease-free orbit is stable if $I > I_2^*$ and unstable otherwise. When $I_3^* < I < I_2^*$, the mutant survives on its own, while if $0 \leq I < I_3^*$, both strains coexist.

Let us suppose that $I(0)) = 0$ and the vaccine is taken at regular intervals with length τ. Then, we have
$I(t_1^+) = \hat{I}, \quad I(t_2^-) = \frac{p\hat{I}}{q\hat{I}+(p-q\hat{I})e^{-p\tau}}, \quad I(t_2^+) = \frac{(p+q)\hat{I}+\hat{I}(p-q\hat{I})e^{-p\tau}}{q\hat{I}+(p-q\hat{I})e^{-p\tau}}, \dots$ We will show
numerically that trajectories converge to an impulsive periodic orbit.

5. Numerical simulation

In this section, we use numerical simulations to illustrate the results. The parameter values are taken as: $\lambda = 252666.6667$, $d_x = 0.0039$, $\beta_1 = 7 \times 10^{-5}$, $\beta_2 = 7 \times 10^{-5}$; $k_1 = 100$; $d_v = 0.021$; $k_2 = 100$, $\varepsilon = 3 \times 10^{-5}$, $d_y = 0.0693$, $\rho_1 = 0.03$, $\rho_2 = 0.042$, $\alpha = 0.03$, $p = 0.5$, $q = 0.03$ and $\hat{I} = 50$ (these data based on [4, 15, 16], others values are assumed) in the following simulations except as noted in the figures.

Firstly, Fig. 3(a) illustrates that under regular vaccinations, the CTL count oscillates in an impulsive periodic orbit. If the vaccine is sufficiently strong, both strains are driven to extinction, assuming perfect adherence (see Fig. 3.(b)). In this figure we have also increase the value of d_v. So, if the vaccine can reduce the life span of viruses, the two strains can be eradicated.

Fig. 4(a) show that the mutant and wild type can coexist if vaccination is low, but nonzero, and if we increase the time life of virus. Both values approach a stable orbit. When $I_3^* < I_2^*$, the mutant persists at high levels, while the wild type is driven to extinc-
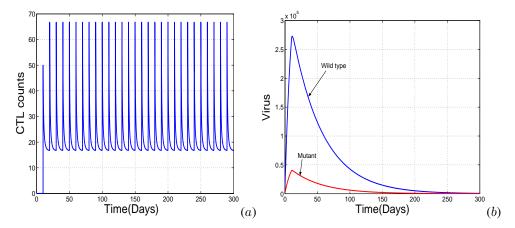
Figure 3: When the vaccine is taken every ten days, stimulating 50 cells/1L, CTL cells converge to an impulse periodic orbit (Fig 3.(a)); if the vaccination frequency is increased, then we have eradication of both strains (Fig 3.(b)).

tion if vaccination is low or zero. The mutant go to an impulsive orbit (see Fig. 4 b). In this case, the disease-free orbit is also unstable.

From Figure 5. (a), we observe that when the immune system is against only infected cells y_1, it has an important effect on wild strain virus v_1. In this case, the behavior of v_1 is the same as if the immune system is against the two strains. The result is similar when immune system reacts against y_2 because its has an important effect on mutant virus v_2 (see Fig. 5. (b)). So, if the immune system reacts only against y_1, he does not affect v_2; and if the immune system is only against y_2, he does not affect v_1. From these observations, we can conclude that it is important that the immune system reacts against the both two population.

6. Conclusion

In this paper we analyse the pulse vaccination strategy in a new HBV within-host model with with cell-mediated immunity and two strains virus. We first examined the model when there is no pulse vaccine. We have analyzed the existence and the local stability of equilibria. Our analysis shows that the basic reproductive ratio satisfies a threshold property with threshold value 1. After introduction of pulse vaccine in our model, we find that a CTL vaccine can theoretically eradicate both the wild-type and resistant strains of the virus, if taken with sufficient frequency, at regular intervals and if the vaccine can reduce the life span of the virus.

We have proved that there exist three critical values of I which are I_1^*, I_2^* and I_3^*. When $I_2^* < I_1^*$, if $I > I_1^*$, the disease-free orbit becomes stable and the mutant cannot survive on its own. Conversely, if $I < I_1^*$, both strains coexist. If $I_2^* > I_1^*$ The disease-free orbit is stable if $I > I_2^*$ and unstable otherwise. So, If the vaccine is sufficiently strong, both strains are driven to extinction, assuming perfect adherence. Imperfect adherence may allow the mutant to persist at low, but nonnegligible levels. When $I_3^* < I < I_2^*$, the mutant survives on its own, while if $0 \leq I < I_3^*$, both strains coexist.

In a future project, we shall combine the model (2) with clinical data of drug therapy to study the dynamical behavior of hepatitis B virus with the two strains virus and we
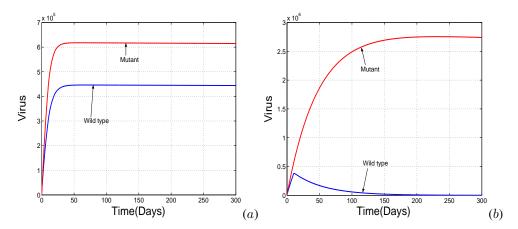
Figure 4: $0 \leq I < I_3^*$, $d_v = 0.00021$ and the two populations coexist (Fig.4 (a)). The mutant exists at high levels (approximately 2.7×10^6 virions/1L), but the wild type is eradicated. This one is obtained when $I_3^* < I_2^*$ (Fig.4.(b)).

shall also consider the role of time delay of CTL cells responses in the model. This work will be helpful to study the treatment protocols.

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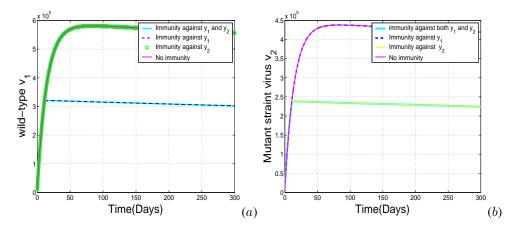
Figure 5: $0 \leq I < I_3^*$, $d_v = 0.00021$ and the two populations coexist (Fig.5 (a)) and Fig.5. (b). The two strains population exist. The target of immune response strongly influences the outcome of infection in HBV model with two strains.

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Appendix A : Coefficients of the characteristic polynomial at $\tilde{P}$

$$
\begin{aligned}
a_4 &= (d_x + \beta_2 v_2)[d_v q\tilde{I}d_y + q\tilde{I}k_2\beta_2\tilde{x} + \rho_2 d_v p\tilde{I}] + \beta_2^2\tilde{v}_2\tilde{x}k_2 q\tilde{I} \\
a_3 &= (d_x + \beta_2 v_2)[(\rho_2\tilde{I} + d_y + q\tilde{I})d_v + q\tilde{I}d_y + k_2\beta_2\tilde{x} + \rho_2 p\tilde{I}] \\
&+ d_v q\tilde{I}d_y + q\tilde{I}k_2\beta_2\tilde{x} + \rho_2 d_v p\tilde{I} + \beta_2^2\tilde{v}_2\tilde{x}k_2 \\
a_2 &= (d_x + \beta_2 v_2)(d_v + \rho_2\tilde{I} + d_y + q\tilde{I}) + d_v(\rho_2\tilde{I} + d_y + q\tilde{I}) + q\tilde{I}d_y + k_2\beta_2\tilde{x} \\
&+ \rho_2 p\tilde{I} \\
a_1 &= d_x + d_v + d_y + \beta_2 v_2 + (\rho_2 + q)\tilde{I} \\
a_0 &= 1
\end{aligned}
$$

Appendix B : Proof of lemma 4.1.

Proof :   Case 1: Suppose that $I_1^* = I_2^* = I$. Since $\frac{\lambda}{d_x d_v} = \frac{r_1}{(1-\varepsilon)\beta_1 k_1}$ and $\frac{\lambda}{d_x d_v} = \frac{r_2}{k_2\beta_2}$, we obtain $\frac{r_1}{(1-\varepsilon)\beta_1 k_1} = \frac{r_2}{k_2\beta_2} \Rightarrow r_2(1-\varepsilon)\beta_1 k_1 = r_1\beta_2 k_2 \Rightarrow I = I_3^*$.

Case 2: Let us suppose that $I_1^* > I_2^*$. Set $I = I_2^*$ then $I < I_1^* \Leftrightarrow r_1 < \frac{(1-\varepsilon)\beta_1 k_1\lambda}{d_x d_v} \Leftrightarrow I < I_3^*$. Hence $I_3^* > I_2*$. By the same method, we establish that $I_3^* > I_2^*$. The case 3 is obtained by the same method as in case 2. $\square$

Appendix C : Proof of Proposition 3.1.

We use a theorem for the existence and uniqueness of a positive fixed point of a multi-variable function. We labeled this theorem as follows.

Theorem 7.1. *(Thieme [17], theorem 2.1) Let $F(x)$ be a continuous, monotone non-decreasing, strictly sub linear, bounded function which maps the non-negative orthant $\mathbb{R}_+^n = [0, +\infty)$ into itself. Let $F(0) = 0$ and $F'(0)$ exists and be irreducible. Then $F(x)$ does not have a non-trivial fixed point on the boundary of $\mathbb{R}_+^n$. Moreover, $F(x)$ has a positive fixed point iff $\rho(F'(0)) > 1$. If there is a positive fixed point, then it is unique.*

Let us show that the system (11) has a positive solution. (11) can be written as: $Y = F(Y)$ where $Y = (\bar{y}_1, \bar{y}_2)^T$ and

$$
F = \left(\begin{array}{c} \dfrac{d_x(1-\varepsilon)\beta_1 k_1 x^*}{(d_x d_v + \beta_1 k_1\bar{y}_1 + \beta_2 k_2\bar{y}_2)\left[d_y + \rho_1\left(\frac{p}{q} + \frac{\alpha}{q}(\bar{y}_1 + \bar{y}_2)\right)\right]} \\ \dfrac{\varepsilon d_x\beta_1 k_1 x^*\bar{y}_1 + \beta_2 k_2\bar{y}_2 x^* d_x}{(d_x d_v + \beta_1 k_1\bar{y}_1 + \beta_2 k_2\bar{y}_2)\left[d_y + \bar{y}_2\rho_2\left(\frac{p}{q} + \frac{\alpha}{q}(\bar{y}_1 + \bar{y}_2)\right)\right]} \end{array} \right) \triangleq \left(\begin{array}{c} F_1(Y) \\ F_2(Y) \end{array} \right)
$$

We have $F_1(Y) \leq \dfrac{d_x(1-\varepsilon)\beta_1 k_1 x^*\bar{y}_1}{(d_x d_v + \beta_1 k_1\bar{y}_1)\left(d_y + \rho_1\frac{p}{q}\right)} \leq M_1$ and

$F_2(Y) \leq \dfrac{\varepsilon d_x\beta_1 k_1 x^*\bar{y}_1}{(d_x d_v + \beta_1 k_1\bar{y}_1)\left(d_y + \rho_2\frac{p}{q}\right)} + \dfrac{\beta_2 k_2\bar{y}_2 x^* d_x}{(d_x d_v + \beta_2 k_2\bar{y}_2)\left(d_y + \rho_2\frac{p}{q}\right)} \leq M_2.$

In this case, $F(Y)$ is continuous, bounded function which maps

$$
\Gamma = \{(y_1, y_2) : 0 < y_1 < M_1, \quad 0 < y_2 < M_2\}
$$

It is easy to find that $F(Y)$ is infinitely differentiable and is a monotone nondecreasing function. Moreover, $F(0,0) = (0,0)$ and

$$F'(0,0) = \begin{pmatrix} \mathcal{R}_{01} & 0 \\ \dfrac{\varepsilon\beta_1 k_1 x^*}{d_v(d_y + \rho_2 I^*)} & \mathcal{R}_{02} \end{pmatrix}$$

Hence $\rho(F'(0,0)) = \max\{\mathcal{R}_{01}, \mathcal{R}_{02}\} = \mathcal{R}_0 > 1$. Thanks to the graph theory, we claim that $F'(0,0)$ is irreducible because the associated graph of the matrix is strongly connected.

Let us now prove that F is strictly sub linear in Γ, i.e., $F(rY) > rF(Y)$, for any $Y \in \Gamma$. with $Y > 0$, and $r \in (0;1)$. Some calculations give

$$\frac{r_1 F_1(Y)}{F_1(r_1 Y)} = \frac{(d_x d_v + r_1\beta_1 k_1\bar{y}_1 + r_1\beta_2 k_2\bar{y}_2)\left[d_y + \rho_1\left(\frac{p}{q} + r_1\frac{\alpha}{q}(\bar{y}_1 + \bar{y}_2)\right)\right]}{(d_x d_v + \beta_1 k_1\bar{y}_1 + \beta_2 k_2\bar{y}_2)\left[d_y + \rho_1\left(\frac{p}{q} + \frac{\alpha}{q}(\bar{y}_1 + \bar{y}_2)\right)\right]} < 1,$$

$$\frac{r_2 F_2(Y)}{F_2(r_2 Y)} = \frac{(d_x d_v + r_2\beta_1 k_1\bar{y}_1 + r_2\beta_2 k_2\bar{y}_2)\left[d_y + \rho_2\left(\frac{p}{q} + r_2\frac{\alpha}{q}(\bar{y}_1 + \bar{y}_2)\right)\right]}{(d_x d_v + \beta_1 k_1\bar{y}_1 + \beta_2 k_2\bar{y}_2)\left[d_y + \rho_2\left(\frac{p}{q} + \frac{\alpha}{q}(\bar{y}_1 + \bar{y}_2)\right)\right]} < 1$$

So the function $F(Y)$ is strictly sub linear with $r = \min(r_1; r_2)$. This ends the proof of Proposition 3.1.

Appendix D : Coefficients u_i ($i = 1, ..., 6$) of the characteristic polynomial at $\bar{P}$

The characteristic polynomial at $\bar{P}$ is given by

$$Q(\lambda) = \lambda^6 + u_1\lambda^5 + u_2\lambda^4 + u_3\lambda^3 + u_4\lambda^2 + u_5\lambda + u_6$$

where:

$$
\begin{aligned}
u_1 &= q\bar{I} + \rho_1\bar{I} + \rho_2\bar{I} + 2d_y + d_x + \beta_1\bar{v}_1 + \beta_2\bar{v}_2 + 2d_v, \\
u_2 &= -k_2\beta_2\bar{x} + q\bar{I}(\rho_1\bar{I} + \rho_1\bar{I} + 2d_y) + (\rho_1\bar{I} + d_y)(\rho_2\bar{I} + d_y) + \alpha\bar{I}(\rho_1\bar{y}_1 + \rho_2\bar{y}_2) \\
&\quad + (d_x + \beta_1\bar{v}_1 + \beta_2\bar{v}_2 + 2d_v)(q\bar{I} + \rho_1\bar{I} + \rho_2\bar{I} + 2d_y) + 2d_v(d_x + \beta_1\bar{v}_1 + \beta_2\bar{v}_2) \\
&\quad + d_v^2, \\
u_3 &= k_2\beta_2\bar{x}(\varepsilon\beta_1\bar{v}_1 - \rho_1\bar{I} - d_y - d_x - \beta_1\bar{v}_1 - q\bar{I} - d_v) + \beta_1 k_1(1-\varepsilon)\bar{x}(\bar{v}_1\beta_1 + 1) \\
&\quad - q\bar{I}(\rho_1\bar{I} + d_y)(\rho_2\bar{I} + d_y) + \alpha\rho_1\bar{y}_1\bar{I}(\rho_2\bar{I} + d_y) \\
&\quad + (d_x + \beta_1\bar{v}_1 + \beta_2\bar{v}_2 + 2d_v)\big[q\bar{I}(\rho_1\bar{I} + \rho_2\bar{I} + 2d_y) + (\rho_1\bar{I} + d_y)(\rho_2\bar{I} + d_y) \\
&\quad + \alpha\bar{I}\rho_2\bar{y}_2(\rho_1\bar{I} + d_y) + \alpha\bar{I}(\rho_1\bar{y}_1 + \rho_2\bar{y}_2)\big] + d_v^2(d_x + \beta_1\bar{v}_1 + \beta_2\bar{v}_2) \\
&\quad + d_v(q\bar{I} + \rho_1\bar{I} + \rho_2\bar{I} + 2d_y)\big[d_v + 2(d_x + \beta_1\bar{v}_1 + \beta_2\bar{v}_2)\big] \\
u_4 &= k_2\beta_2\bar{x}(\varepsilon\beta_1\bar{v}_1 + \beta_2\bar{v}_2)(\rho_1\bar{I} + d_y + d_v + q\bar{I}) \\
&\quad - k_2\beta_2\bar{x}\big[\alpha\bar{I}\rho_1\bar{y}_1 + q\bar{I}(\rho_1\bar{I} + d_y) + (d_x + \beta_1\bar{v}_1 + \beta_2\bar{v}_2 + d_v)(\rho_1\bar{I} + d_y + q\bar{I}) \\
&\quad + ad_v\big] - (d_x + \beta_1\bar{v}_1 + \beta_2\bar{v}_2 + 2d_v)\big[q\bar{I}(\rho_1\bar{I} + d_y)(\rho_2\bar{I} + d_y) - \alpha\bar{I}\rho_1\bar{y}_1(\rho_2\bar{I} + d_y) \\
&\quad - \alpha\bar{I}\rho_2\bar{y}_2(\rho_1\bar{I} + d_y)\big] + \beta_1^2(1-\varepsilon)\bar{v}_1 k_1\bar{x}(d_v + \rho_2\bar{I} + d_y + q\bar{I}) \\
&\quad + d_v(2d_x + 2\beta_1\bar{v}_1 + 2\beta_2\bar{v}_2 + d_v)\big[q\bar{I}(\rho_1\bar{I} + \rho_2\bar{I} + 2d_y) + (\rho_1\bar{I} + d_y)(\rho_2\bar{I} + d_y) \\
&\quad + \alpha\bar{I}(\rho_1\bar{y}_1 + \rho_2\bar{y}_2)\big] + d_v^2(d_x + \beta_1\bar{v}_1 + \beta_2\bar{v}_2)(\rho_1\bar{I} + \rho_1\bar{I} + 2d_y + q\bar{I}) \\
&\quad + k_1(1-\varepsilon)\beta_1\bar{x}(d_v + \rho_2\bar{I} + d_y + q\bar{I}) \\
u_5 &= -\beta_1 k_1\bar{x}(\varepsilon\beta_1 v_1 + \beta_2 v_2)\big[\rho_1 y_1\alpha\bar{I} + k_2(1-\varepsilon)\bar{x}\beta_2\big] \\
&\quad + k_2\beta_2\bar{x}(\varepsilon\beta_1 v_1 + \beta_2 v_2)\big[\alpha\bar{I}\rho_1\bar{y}_1 + q\bar{I}(\rho_1\bar{I} + d_y) + d_v(q\bar{I} + \rho_1\bar{I} + d_y) \\
&\quad + \varepsilon\beta_1 k_1\rho_1\alpha\bar{x}\bar{y}_1\big] + \beta_1 k_1\bar{x}(1-\varepsilon)(\beta_1 v_1 + 1)[d_v(\rho_2\bar{I} + d_y) + q\bar{I}(d_v + \rho_2\bar{I} + d_y) \\
&\quad - k_2\beta_2\bar{x} + \rho_2\alpha\bar{I}\bar{y}_2] - k_2\beta_2\bar{x}[(d_v + d_x + \beta_1\bar{v}_1 + \beta_2\bar{v}_2)(\rho_1\bar{I}q\bar{I} + d_y q\bar{I} + \alpha I\rho_1 y_1) \\
&\quad + d_v(d_x + \beta_1\bar{v}_1 + \beta_2\bar{v}_2)(\rho_1\bar{I} + d_y + q\bar{I})] \\
&\quad + d_v(d_x + \beta_1\bar{v}_1 + \beta_2\bar{v}_2)[q\bar{I}(\rho_1\bar{I} + \rho_2\bar{I} + 2d_y) + (\rho_1\bar{I} + d_y)(\rho_2\bar{I} + d_y) \\
&\quad + \alpha\bar{I}(\rho_1\bar{y}_1 + \rho_2\bar{y}_2)] - d_v(d_v + 2d_x + 2\beta_1\bar{v}_1 + 2\beta_2\bar{v}_2)[q\bar{I}(\rho_1\bar{I} + d_y)(\rho_2\bar{I} + d_y) \\
&\quad - \alpha\bar{I}\rho_1\bar{y}_1(\rho_2\bar{I} + d_y) - \alpha\bar{I}\rho_2\bar{y}_2(\rho_1\bar{I} + d_y)] + k_2(1-\varepsilon)\beta_1\beta_2\bar{v}_1\bar{x}(k_1\varepsilon\beta_1\bar{x} - \rho_2\alpha\bar{I}\bar{y}_2) \\[4pt]
u_6 &= \beta_1\bar{x}\bar{I}k_1(\varepsilon\beta_1\bar{v}_1 + \beta_2\bar{v}_2)\big[qk_2\bar{x}(1-\varepsilon)\beta_2 + \bar{y}_1\rho_1\alpha d_v\big] \\
&\quad + k_2(1-\varepsilon)\beta_1\bar{v}_1\beta_2\bar{x}(k_1\varepsilon\beta_1\bar{x}q\bar{I} - \rho_2 y_2\alpha\bar{I}d_v) + \varepsilon\beta_1 k_1 d_v\rho_1 y_1\alpha\bar{I}\bar{x} \\
&\quad - \beta_1^2(1-\varepsilon)\bar{v}_1\bar{x}k_1\big[-q\bar{I}d_v(\rho_2\bar{I} + d_y) + k_2\beta_2\bar{x}q\bar{I} - \rho_2\bar{y}_2\alpha\bar{I}d_v\big] \\
&\quad - k_1(1-\varepsilon)\beta_1\bar{x}\big[-q\bar{I}d_v(\rho_2\bar{I} + d_y) + k_2\beta_2\bar{x}q\bar{I} - \alpha\rho_2\bar{y}_2\bar{I}d_v\big] \\
&\quad - k_2\beta_2\bar{x}d_v(d_x + \beta_1\bar{v}_1 + \beta_2\bar{v}_2)\big[(\rho_1\bar{I} + d_y)q\bar{I} + \alpha\bar{I}\rho_1\bar{y}_1\big] \\
&\quad - d_v^2(d_x + \beta_1\bar{v}_1 + \beta_2\bar{v}_2)\big[q\bar{I}(\rho_1\bar{I} + d_y)(\rho_2\bar{I} + d_y) - \alpha\bar{I}\rho_1\bar{y}_1(\rho_2\bar{I} + d_y) \\
&\quad - \alpha\bar{I}\rho_2 y_2(\rho_1\bar{I} + d_y)\big] + k_2\beta_2\bar{x}v_v(\varepsilon\beta_1\bar{v}_1 + \beta_2\bar{v}_2)\big[q\bar{I}(\rho_1\bar{I} + d_y) + \alpha\bar{I}\rho_1\bar{y}_1\big]
\end{aligned}
$$