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Mathematical Analysis of Within - Cell Model of Viral Hepatitis

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Abstract

We examine a single-scale model of viral hepatitis at the cellular level of biological organisation, where complete virions and incomplete particles are treated separately. This model enables the exploration of viral dynamics within infected cells, as well as the processes of subsequent cell infections and viral release from infected cells. At the within-cell scale of the infectious disease system, viral replication is the central process, and this has been thoroughly analysed both analytically and numerically in this study.

Keywords: Within-cell scale, Individual cell infectiousness, Complete virions, Incomplete particles, Life cycle.

INTRODUCTION

Mathematical models are essential tools for understanding complex biological systems and making predictions about their behaviour. A single-scale model focuses on a specific level of biological organisation, providing a framework to analyse phenomena occurring at that scale. Developing such a model involves identifying key variables and parameters that define the system's behaviour at the chosen scale. This approach allows for a detailed examination of specific processes within the broader context of biological complexity. In the context of viral hepatitis, there are five primary viruses responsible for liver infections: Hepatitis A Virus (HAV), Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), Hepatitis D Virus (HDV) and Hepatitis E Virus (HEV). These viruses vary significantly in transmission modes and disease progression: Hepatitis B, C, and D are predominantly bloodborne and have a high likelihood of leading to chronic infections while Hepatitis A and E are transmitted via the fecal-oral route and never result in chronic conditions.

The taxonomy of these viruses, often organised by their transmission pathways, clinical features, and genetic classifications, serves as a critical foundation for building such models. The table below provides a structured overview of these distinctions, highlighting differences in their biology and epidemiology, which are essential for model construction and analysis.

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Table 1. Taxonomy of Viral Hepatitis. Source: Blanco-Diaz et-al (2024).

Family	Subfamily	Species	Morphology	Envelope
DNA and RNA reverse transcribing virus				
Hepadnaviridae	Orthohepadnavirus	HBV	Icosahedral with envelope.	+
RNA virus ssRHA viruses				
Picornaviridae	Hepatovirus	HAV		
Flaviviridae	Hepacivirus	HCV		
Subviral agents	Deltavirus	HDV	Spherical	+
Hepeviridae	Hepevirus	HEV	Icosahedral	-

In this study, a within-cell single-scale model of the hepatitis B virus (HBV) was developed at the cellular level of biological organisation. HBV, classified as a partially double-stranded DNA virus under the Baltimore classification scheme VII, utilises reverse transcriptase to transition through an RNA intermediate. The covalently closed circular DNA (cccDNA) serves as the transcriptional template for all HBV transcripts. To complete its life cycle, the HBV genome must be transported to the nucleus, as highlighted in Ortega-Prieto et-al (2018). The structure of the paper is as follows. Section 2 presents the development of a mathematical model describing the life cycle of HBV infection. Section 3 provides the mathematical analysis of the proposed model. Section 4 includes the numerical simulation results. Section 5 concludes the study by summarizing the findings.

FORMULATION OF MODEL

In this study, we developed a within-cell single-scale model of hepatitis B virus (HBV) dynamics, focusing on five interacting populations: core particle in cytoplasm h_r, cccDNA inside the nucleus h_t, complete virions h_c, incomplete particles h_i and within-cell viral load V_s. We propose a simplified version of the more complex within-cell scale model described in Nakabayashi (2016). We employed a system of ordinary differential equations (ODEs) to model the transitions between different states of the hepatitis B virus (HBV) within an infected cell, utilising the law of mass action to describe these interactions.

The following assumptions were made regarding the within-cell processes:

i. no superinfection that is each cell is infected by a single virion, preventing multiple infections within the same cell,
ii. only capsids containing mature relaxed circular DNA (rcDNA) are either secreted from the cell or recycled back to the nucleus to replenish the covalently closed circular DNA (cccDNA) pool,
iii. the intracellular replication dynamics of the core particle are captured through the reverse transcription of the initial value in the cytoplasm, denoted as $h_C = h_c(0)$,
iv. the influence of the within-cell viral load on assembly and export, as well as individual cell infectiousness, represented by $V_s = V_s(t)$,
v. the within-cell processes occur on a fast time scale, denoted by s, so that $h_r = h_r(t)$, $h_t = h_t(t)$, $h_c = h_c(t)$, $h_i = h_i(t)$ and $V_s = V_s(t)$. These assumptions aim to simplify the complex intracellular dynamics of HBV replication, allowing for a more tractable mathematical analysis. Our model is given by:

$$\begin{aligned}
\frac{dh_r(t)}{dt} &= \Lambda_r - \eta_r h_r(t) V_s(t) - \delta_r h_r(t), \\
\frac{dh_t(t)}{dt} &= \eta_r h_r(t) V_s(t) - (\alpha_t + \mu_t + \delta_t) h_t(t), \\
\frac{dh_c(t)}{dt} &= \alpha_t h_t(t) - (\alpha_c + \rho_c) h_c(t) \qquad (2.1) \\
\frac{dh_i(t)}{dt} &= \mu_t h_t(t) - (\alpha_i + \delta_i) h_i(t), \\
\frac{dV_s(t)}{dt} &= N_c \alpha_c h_c(t) + N_i \alpha_i h_i(t) - (r_c + r_i) V_s(t),
\end{aligned}$$

Equation (1) is the dynamics of core particle internalisation and DNA repair. This equation models the change in the concentration of core particles in the cytoplasm h_r over time. Λ_r represents the production rate of mature rcDNA-containing core particles in the cytoplasm, η_r denotes the rate at which rcDNA-containing core particles are transported into the nucleus and converted into cccDNA and degradation rate δ_r accounts for the natural degradation of core particles in the cytoplasm.

Equation (2) is the dynamics of transcription from cccDNA. This equation describes the concentration of cccDNA in the nucleus (h_t) over time. The terms include: replenishment rate which reflects the contribution to the cccDNA pool from the nuclear import and conversion of rcDNA-containing core particles, transcription rate of pgRNA which represents the rate at which cccDNA serves as a template for the transcription of pregenomic RNA (pgRNA), transcription rate of mRNAs which accounts for the transcription of precore mRNAs and other subgenomic mRNAs from cccDNA and degradation rate of cccDNA which represents the natural degradation of cccDNA within the nucleus. These equations are adapted from the model described by Goyal et-al (2019), which provides a detailed framework for understanding the intracellular dynamics of HBV replication.

Equation (3) of model system (2.1) describes the dynamics of pregenomic RNA (pgRNA) translation leading to the formation of complete virions. The terms in this equation represent the following processes: template contribution from cccDNA (α_t) which accounts for the transcriptional activity of covalently closed circular DNA (cccDNA), which serves as the template for pgRNA synthesis. The pgRNA not only encodes the core and polymerase proteins but also acts as the precursor for new viral genomes through reverse transcription, association rate of pregenome-polymerase complex (RNP) and core protein which represents the rate at which the pgRNA-polymerase complex associates with core proteins to form nucleocapsids.

The encapsulation of pgRNA is a critical step in the viral replication cycle, leading to the formation of new virions. Translation rate of pgRNA to reverse transcription which describes the process by which pgRNA is reverse transcribed into relaxed circular DNA (rcDNA) within the nucleocapsid. These processes are integral to the HBV replication cycle, highlighting the central role of pgRNA in both protein synthesis and genome replication.

Equation (4) of model system (2.1) describes the dynamics of subgenomic RNA (mRNA) translation leading to the formation of incomplete particles, such as RNA-containing particles, empty virions, and subviral particles (SVPs). The terms in this equation represent the following processes: transcription rate of mRNAs which accounts for the rate at which covalently closed circular DNA (cccDNA) in the nucleus is transcribed into subgenomic mRNAs.

These mRNAs encode various viral proteins, including envelope proteins that are

essential for particle formation, translation rate of mRNAs: following transcription, the subgenomic mRNAs are translated in the cytoplasm to produce viral proteins. This term represents the rate of translation, leading to the synthesis of proteins that will assemble into incomplete particles and degradation rate of mRNAs which accounts for the natural degradation of subgenomic mRNAs within the cell, which reduces the availability of mRNAs for translation.

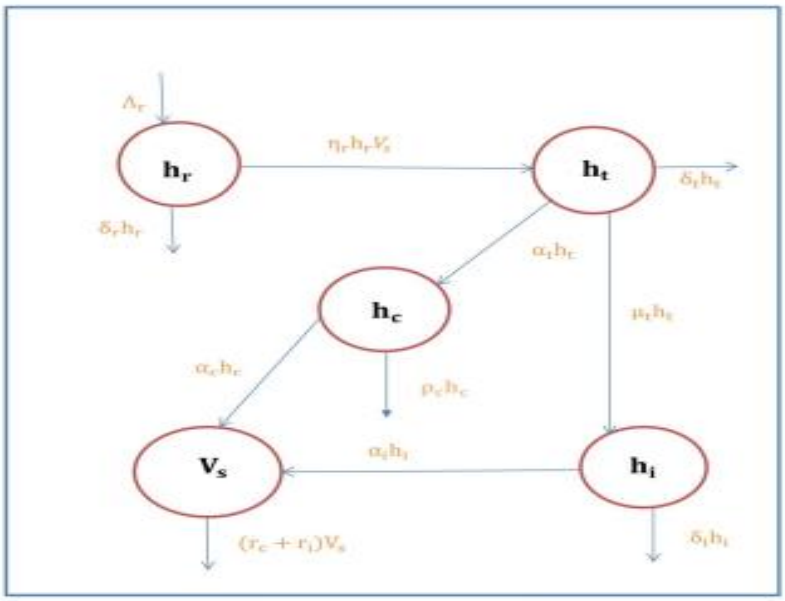

Figure 1. A conceptual diagram of the within-cell model.

Equation (5) of model system (2.1) describes the dynamics of the intracellular viral load, encompassing both complete virions and incomplete particles within the cytoplasm. The envelopment and release of these particles can occur through two primary pathways: recycling to the nucleus (mature core particles or nucleocapsids can be transported back to the nucleus to replenish the covalently closed circular DNA (cccDNA) pool, thereby sustaining viral replication) or envelopment and secretion (these particles can undergo envelopment by passing through the post endoplasmic reticulum and pre-Golgi compartments, leading to their secretion as virions into the extracellular space).These pathways are integral to the hepatitis B virus (HBV) life cycle, as detailed by Goyal et-al (2019). The Figure below shows the parameters of the model and their description.

Table 2. Parameters values of the within - cell model and their description.

Parameter	Description	Value / unit	References
Λ_r	Recruitment rate of rcDNA	100min^{-1}	Assumed
η_r	Reaction rate of DNA repair	0.1min^{-1}	Nakabayaski (2016)
δ_r	Degradation rate of rcDNA	0.001min^{-1}	Nakabayaski (2016)
δ_t	Degradation rate of cccDNA	0.001min^{-1}	Nakabayaski (2016)
ρ_c	Recycling rate of rcDNA	0.01min^{-1}	Nakabayaski (2016)
α_t	Transcription rate of DNA to RNA code	0.1molecule^{-1}min^{-1}	Nakabayaski (2016)
μ_t	Transcription rate of mRNA	0.1molecule^{-1}min^{-1}	Nakabayaski (2016)

α_c	Association rate of RNP and core protein	$0.1molecule^{-1}min^{-1}$	Nakabayaski (2016)
α_j	Translation rate of mRNAs	$0.1min^{-1}$	Nakabayaski (2016)
δ_j	Degradation rate of mRNAs	$0.001min^{-1}$	Nakabayaski (2016)
r_c	Shedding rate of complete virions	$0.1min^{-1}$	Nakabayaski (2016)
r_i	Shedding rate of incomplete particles	$0.1min^{-1}$	Nakabayaski (2016)
N_c	Viral burst size of complete virions	$10mins^{-1}$	Assumed
N_i	Viral burst size of incomplete particles	$20min^{-1}$	Assumed

MATHEMATICAL ANALYSIS

Positivity of the solutions of the Model

We now examine the positivity of the model system (2.1) by demonstrating that if the system begins with non-negative initial conditions $(h_r(0), h_t(0), (h_c(0), (h_i(0), V_s(0))$, the solution / trajectories $(h_r(t), h_t(t), (h_c(t), (h_i(t), V_s(t))$ of the model system (2.1) will remain positive for all t > 0. This ensures consistency with a fundamental aspect of biological reality. The result is summarised in the following theorem.

Theorem 3.1: Given that the initial conditions of the model (2.1) remain nonnegative (i.e $h_r(0) \geq 0,\ h_t(0) \geq 0,\ h_c(0) \geq 0,\ h_i(0) \geq 0,\ V_s(0) \geq 0$), the resulting solutions $(h_r(t), h_t(t), (h_c(t), (h_i(t), V_s(t))$ are all positive for all $t \geq 0$.

Proof: From the Equation (1) of the model system (2.1), a differential inequality which describes the dynamics of susceptible cell population in time is given by:

$$\frac{dh_r(t)}{dt} \geq \alpha_t h_t(t) - (\eta_r V_s(t) + \delta_r) h_r(t) \qquad (3.2)$$

Therefore, the expression of the differential inequality in equation (3.2) can be solved by the separation of variables as follows

$$\frac{dh_r(t)}{h_r(t)} \geq \alpha_t h_t(t) - (\eta_r V_s(t) + \delta_r) dt \qquad (3.3)$$

Now, letting

$t^* = \sup \{t > 0: h_r(0) > 0,\ h_t(0) > 0, h_c(0) > 0,\ h_i(0) > 0,\ V_s(0) > 0\} \in [0, t]$,

and integrating equation (3.3), we thus have

$$\ln(h_r(t)) \geq -(\delta_r(t) + \int_0^t V_s(t^*) dt^*) + \ln(h_r(0)) \qquad (3.4)$$

given by

Thus, the solution of the differential inequality for the susceptible cell population is

$$h_r(t) \geq h_r(0).\exp\{-(\delta_r t + \int_0^t V_s(t^*) dt^*\} > 0 \qquad (3.5)$$

This implies that.

$$\lim_{t \to \infty} \inf(h_r(t)) \geq 0$$

Applying the same principle to the Equation (2), Equation (3) and Equation (4) of the model system (2.1), it can be shown that

$$\lim_{t \to \infty} \inf(h_t(t)) \geq 0,$$

$$\lim_{t \to \infty} \inf(h_c(t)) \geq 0,$$

$\lim_{t\to\infty} \inf(h_i(t)) \geq 0.$

Now, using the Equation (5) of the model system (2.1) that describes the evolution of the viral load of the HBV, we can have the following differential inequality given as:

$$\frac{dV_s(t)}{dt} \geq -(r_c + r_i)V_s(t) \quad (3.6)$$

Therefore, by the separation of variables we obtained.

$$V_s(t) \geq V_s(0).\exp\{-(r_c + r_i)t\} \geq 0 \quad (3.7)$$

This implies.

$$\lim_{t\to\infty} \inf(V_s(t)) \geq 0.$$

Thus, when starting with nonnegative initial value conditions in the model system (2.1), the solutions of the model will remain nonnegative for all $t \geq 0$, and this complete the proof.

Feasible Region of the Equilibrium of the Model

The within-cell model system (2.1) for the HBV replication dynamics can be analyzed in the region $\Omega \in \mathbb{R}_+^5$. Since the within-cell model (2.1) monitors the dynamics of the hepatocytes of a cell, it is empirical that all the model variables always stay positives. We introduce a region of feasibility.

$$\Omega = \{h_r, h_t, h_c, h_i, V_s \in \mathbb{R}_+^5; 0 \leq h_r(t) + h_t(t) + h_c(t) + h_i(t) \leq M_1, 0 \leq V_s \leq M_2\}$$

Letting $h_N = h_r + h_t + h_c + h_i$ and adding Equation (1) to Equation (4) of the model system (2.1) gives

$$\frac{dV_s(t)}{dt} \geq \Lambda_r - \delta_r h_N(t)$$

Then we have $\lim_{t\to\infty} \inf(h_N(t)) = \frac{\Lambda_r}{\delta_r}$. Hence, all feasible solutions of the model system (2.1) enter the region Ω, where

$$\{M_1 = \frac{\Lambda_r}{\delta_r}, M_2 = \frac{\Lambda_r(N_c\alpha_c + N_i\alpha_i)}{\delta_r(r_c + r_i)}\}$$

Thus, Ω is positively invariant and attracting. It is therefore sufficient to search for the solutions of the model system (2.1) in Ω.

Reproduction Number

In the analysis of the within-cell model of hepatitis B virus (HBV) infection, setting the right-hand sides of the model system (2.1) to zero allows for the determination of equilibrium states. The model admits two primary equilibrium states (i) virus free equilibrium (VFE) state: this state represents a scenario where no infection is present within the hepatocyte. It is denoted as $E^0 = (\frac{\Lambda_r}{\delta_r}, 0, 0, 0, 0)$. $\frac{\Lambda_r}{\delta_r}$ represents the steady-state level of core particles in the cytoplasm and the zeros indicate the absence of cccDNA in the nucleus, complete virions, incomplete particles, and within-cell viral load and (ii) endemic equilibrium (EE) state which corresponds to a persistent infection within the cell and is denoted as $E^* = (h_r^*, h_t^*, h_c^*, h_i^*, V_s^*)$.

A critical parameter in understanding the dynamics of HBV infection is the basic reproduction number, $\mathfrak{R}_0$. This number indicates the average number of new infections produced by a single infected cell in a fully susceptible environment. It serves as a threshold parameter: If $\mathfrak{R}_0 < 1$, the infection is expected to die out over time, leading to the stability of the VFE state and if $\mathfrak{R}_0 > 1$, the infection can persist, making the EE state stable. The calculation of the reproduction number, $\mathfrak{R}_0$ involves analysing the rates of viral

production, infection, and clearance within the model. Accurate estimation of $\mathfrak{R}_0$ is essential for predicting the potential for viral spread and for designing effective intervention strategies. Understanding these equilibrium states and the basic reproduction number provides valuable insights into the conditions under which HBV infection can be controlled or may persist within hepatocytes. The reproduction number which is one of the necessary and important parameters in the analysis of disease outbreak is defined and calculated for the model system (2.1).

According to the author in Diekmann et-al (1990), the reproduction number is the anticipated number of secondary cases that a typical infected individual would create in a population that is fully susceptible to infection over the course of the individual's infectivity. The reproduction number is computed using the next generation approach Van den Driessche and Watmough (2002). We assume that the model system (2.1) can be written in the form:

$$\begin{cases} \frac{dX}{dt} = f(X, Y, Z), \\ \frac{dY}{dt} = g(X, Y, Z), \\ \frac{dZ}{dt} = h(X, Y, Z). \end{cases}$$

Where $X \in R^r,\ Y \in R^s, Z \in R^n; r, s, n \geq 0$. The component X, Y, Z are represented as follows:

$$\begin{cases} X = (h_r)\ \text{non} - \text{infected}, \\ Y = (h_t, h_c, h_i) - \text{infected but not infectious}, \\ Z = (V_s)\ - \text{infectious}. \end{cases}$$

Now let $U_0 = (X^*, 0, 0) \in R^{r+s+n}$ (where $r = 1, s = 3$ and $n = 1$) virus free equilibrium (VFE), that is, $U_0 = (X^*, 0, 0)$ such that $f(X^*, 0, 0) = g(X^*, 0, 0) = h(X^*, 0, 0)$. Also, we assume that the equation $g(X^*, Y, Z) = 0$ implicitly determines the function $Y = \tilde{g}(X^*, Z)$. Now expressing h_t, h_c and h_i in terms of h_r and V_s , we have:

$$\widetilde{g_1}(X^*, Z) = \frac{\eta_r \Lambda_r Z}{\delta_r(\alpha_t + \mu_t + \delta_t)},$$

$$\widetilde{g_2}(X^*, Z) = \frac{\eta_r \Lambda_r \alpha_t Z}{\delta_r(\alpha_t + \mu_t + \delta_t)(\alpha_c + \rho_c)}, \qquad (3.9)$$

$$\widetilde{g_3}(X^*, Z) = \frac{\eta_r \Lambda_r \mu_t Z}{\delta_r(\alpha_t + \mu_t + \delta_t)(\alpha_i + \delta_i)}.$$

Now we let $A = D_z h(h^*, \tilde{g}(X^*, 0), 0)$ and also assume that A can be written in the form $A = M - D$ with $M \geq 0$ and $A > 0$, a diagonal matrix. Since $\frac{dZ}{dt} = h(X, Y, Z)$, it follows that:

$$h(X, Y, Z) = N_c \alpha_c Y_2 + N_i \alpha_i Y_3 - (r_c + r_i)Z \qquad (3.10)$$

The expression (3.9) and (3.10) imply that:

$$h(X^*, \tilde{g}(X^*, Z), Z) = \frac{N_c \alpha_c \eta_r \Lambda_r \alpha_t Z}{\delta_r(\alpha_t + \mu_t + \delta_t)(\alpha_c + \rho_c)} + \frac{N_i \alpha_i \eta_r \Lambda_r \mu_t Z}{\delta_r(\alpha_t + \mu_t + \delta_t)(\alpha_i + \delta_i)} - (r_c + r_i)Z$$

Now differentiating $h(X^*, \tilde{g}(X^*, Z), Z)$ with respect to Z, we get

$$D_z h(X^*, \tilde{g}(X^*, Z), Z) = \frac{N_c \alpha_c \eta_r \Lambda_r \alpha_t (\alpha_i + \delta_i) + N_i \alpha_i \eta_r \Lambda_r \mu_t (\alpha_c + \rho_c)}{\delta_r(\alpha_t + \mu_t + \delta_t)(\alpha_c + \rho_c)(\alpha_i + \delta_i)} - (r_c + r_i)$$

At the virus free equilibrium (VFE), $(h_r^0, h_t^0, h_c^0, h_i^0, V_s^0) = (\frac{\Lambda_r}{\delta_r}, 0, 0, 0, 0)$ such that

$$D_z h(X^*, \tilde{g}(X^*, 0), 0) = \frac{N_c \alpha_c \eta_r \Lambda_r \alpha_t (\alpha_i + \delta_i) + N_i \alpha_i \eta_r \Lambda_r \mu_t (\alpha_c + \rho_c)}{\delta_r(\alpha_t + \mu_t + \delta_t)(\alpha_c + \rho_c)(\alpha_i + \delta_i)} - (r_c + r_i)$$

But $D_z h(X^*, \tilde{g}(X^*, 0), 0) = M - D$ which implies

$$\begin{cases} M = \dfrac{N_c \alpha_c \eta_r \Lambda_r \alpha_t (\alpha_i + \delta_i) + N_i \alpha_i \eta_r \Lambda_r \mu_t (\alpha_c + \rho_c)}{\delta_r(\alpha_t + \mu_t + \delta_t)(\alpha_c + \rho_c)(\alpha_i + \delta_i)}, \\ D = (r_c + r_i). \end{cases}$$

Since the basic reproduction number is given by $\rho(MD^{-1})$, we proceed to find D^{-1} which is.

$$D^{-1} = \frac{1}{(r_c + r_i)}$$

This implies that the within-cell reproduction number is given by:

$$\Re_0 = \frac{N_c \alpha_c \eta_r \Lambda_r \alpha_t (\alpha_i + \delta_i) + N_i \alpha_i \eta_r \Lambda_r \mu_t (\alpha_c + \rho_c)}{\delta_r(\alpha_t + \mu_t + \delta_t)(\alpha_c + \rho_c)(\alpha_i + \delta_i)(r_c + r_i)} \qquad (3.11)$$

The basic reproduction number is the spectral radius of the matrix $\rho(MD^{-1})$. Hence, the basic reproduction number of the model system (2.1) is expressed as shown in (3.11).

The reproduction number (3.11) represents the number of infectious hepatocytes in the cell when introduced to the completely susceptible healthy cell during the infected hepatocyte's period of infectiousness. In mathematical terms, the expression for $\Re_0$, implies that infection by HBV will only cease to infect other healthy hepatocytes if and only if $\eta_r\Lambda_r[N_c\alpha_c\eta_r\Lambda_r\alpha_t(\alpha_i+\delta_i)+N_i\alpha_i\mu_t(\alpha_c+\rho_c)]$ is less than $\delta_r[(\alpha_t+\mu_t+\delta_t)(\alpha_c+\rho_c)(\alpha_i+\delta_i)(r_c+r_i)]$.

Local Stability of the Virus Free Equilibrium

To find the local stability of the virus free equilibrium of model system (2.1), Theorem 2 of Van den Driessche and Watmough (2002) was applied the following result was established.

Theorem 3.2: The virus free equilibrium (VFE) of model system (2.1) is locally asymptotically stable whenever $\Re_0 < 1$ and unstable otherwise.

Proof: Given $E^0 = (\frac{\Lambda_r}{\delta_r}, 0, 0, 0, 0)$, the Jacobian of (2.1) is found as follows:

$$J = \begin{pmatrix} -\eta_r V_s - \delta_r & 0 & 0 & 0 & -\eta_r h_r \\ \eta_r V_s & -(\alpha_t+\mu_t+\delta_t) & 0 & 0 & \eta_r h_r \\ 0 & \alpha_t & -(\alpha_c+\rho_c) & 0 & 0 \\ 0 & \mu_t & 0 & -(\alpha_i+\delta_i) & 0 \\ 0 & 0 & N_c\alpha_c & N_i\alpha i & -(r_c+r_i) \end{pmatrix}$$

$$J(E^0) = \begin{pmatrix} -\delta_r & 0 & 0 & 0 & -\eta_r\frac{\Lambda_r}{\delta_r} \\ 0 & -a & 0 & 0 & \eta_r\frac{\Lambda_r}{\delta_r} \\ 0 & \alpha_t & -b & 0 & 0 \\ 0 & \mu_t & 0 & -c & 0 \\ 0 & 0 & N_c\alpha_c & N_i\alpha i & -d \end{pmatrix}$$

Where:

$$\begin{cases} a = (\alpha_t+\mu_t+\delta_t) \\ b = (\alpha_c+\rho_c) \\ c = (\alpha_i+\delta_i) \\ d = (r_c+r_i) \end{cases}$$

Through the characteristic polynomial solution, one can determine the eigenvalues of the Jacobian matrix: $|J(E^0)-\lambda I|=0$. After simplifying, we get the following equation:

$$(\lambda+\delta_r)[a_0\lambda^4+a_1\lambda^3+a_2\lambda^2+a_3\lambda+a_4]=0. \quad (3.12)$$

where:

$$\begin{cases} a_0 = 1, \\ a_1 = a+b+c+d, \\ a_2 = [a(b+c+d)+b(c+d)+cd], \\ a_3 = Q[1-\dfrac{\eta_r\Lambda_r(N_c\alpha_c\alpha_t+N_i\alpha_i\mu_t)}{\delta_r(ab(c+d)+cd(a+b))}], \\ a_4 = abcd[1-\Re_0]. \end{cases}$$

where

$$Q = ab(c+d)+cd(a+b)$$

It is obvious from equation (3.12) that one of the eigenvalues is equal to $-\delta_r$. We apply the Routh Hurwitz stability criterion to determine the nature of the remaining eigenvalues of the equation (3.12). The following is denoted as the determinant of the hurwitz matrices whose elements are coefficients ($a_i's$) of the characteristic's polynomial equation.

$$D_1 = a_1 = a + b + c + d > 0$$

$$D_2 = a_1 a_2 = P > 0$$

$$D_3 = a_3 D_2 - a_3^2 = PQR - (QR)^2 > 0$$

$$D_4 = a_4 D_3 - a_1 a_4^2 = abcd(1 - \Re_0)[PQR - (QR)^2] - (a + b + c + d)[abcd(1 - \Re_0)]^2 > 0$$

where

$$P = a^2(b + c + d) + b^2(a + c + d) + c^2(a + b + d) + d^2(a + b + c) + 3ab(c + d) + 3cd(a + c)$$

$$R = 1 - \frac{\eta_r \Lambda_r (N_c \alpha_c \alpha_t + N_i \alpha_i \mu_t)}{\delta_r (ab(c + d) + cd(a + b))}$$

It should be noted that all the coefficients of the polynomial in equation (3.12) are greater than zero and that all the determinants of the four matrices are positive whenever $\Re_0 < 1$. Hence, all the roots of the polynomial are either negative or have negative real parts, it is concluded that the virus free equilibrium is locally asymptotically stable otherwise unstable.

Global Stability of Virus Free Equilibrium of the Model

It is investigated that the global stability of Virus Free Equilibrium by using the Castillo Chavez's Approach, Castillo-Chavez (2002) as illustrated in the Lemma below.

Lemma 3: Consider the model system (2.1) written in the form

$$\begin{cases} \dfrac{dX}{dt} = F(X, Z), \\ \dfrac{dZ}{dt} = G(X, Z), G(X, 0) = 0 \end{cases}$$

where $X = (h_r)$ comprises of the uninfected components, $Z = (h_t, h_c, h_i, V_s)$Z comprises infected and infectious components and $(X^*, 0)$ denotes the virus free equilibrium (VFE) of the system. For the VFE to be globally asymptotically stable, the conditions of (H1) and (H2) below must hold:

(H1) : For $\dfrac{dX}{dt} = F(X, 0), X^*$ is GAS

(H2) : $G(X, Z) = AZ - \tilde{G}(X, Z), \tilde{G}(X, Z) \geq 0$, for $(X, Z) \in R$, where $A = D_Z G(X, 0)$ is an M-matrix (whose off-diagonal elements are non-negative) and Ω is the region where the model makes biological sense.

Then the VFE $(X^*, 0)$ is globally asymptotically stable provided that $\Re_0 < 1$.

Theorem 3.3: The virus free equilibrium $E^0$of model system (2.1) is globally asymptotically stable if $\Re_0 < 1$ and assumptions (H1) and (H2) holds.

Proof: The GAS of VFE of the model system (2.1) will be established using the Lemma 3 above. Then

$$F(X, Z) = (\Lambda_r - \eta_r h_r V_s - \delta_r h_r),$$

$$\hat{G}(X, Z) = \begin{pmatrix} \eta_r h_r V_s - (\alpha_t + \mu_t + \delta_t) h_t \\ \alpha_t h_t - (\alpha_c + \rho_c) h_c \\ \mu_t h_t - (\alpha_i + \delta_i) h_i \\ N_c \alpha_c h_c + N_i \alpha_i h_i - (r_c + r_i) V_s \end{pmatrix},$$

$$F(X, 0) = (\Lambda_r - \delta_r h_r),$$

and

$$A = \begin{pmatrix} -(\alpha_t + \mu_t + \delta_t) & 0 & 0 & \eta_r \frac{\Lambda_r}{\delta_r} \\ \alpha_t & -(\alpha_c + \rho_c) & 0 & 0 \\ \mu_t & 0 & -(\alpha_i + \delta_i) & 0 \\ 0 & N_c\alpha_c & N_i\alpha i & -(r_c + r_i). \end{pmatrix}$$

Using AZ and $G(X,Z)$, we deduce $\tilde{G}(X,Z)$ as follows:

$$\tilde{G}(X,Z) = \begin{pmatrix} \left(\frac{\Lambda_r}{\delta_r} - h_r\right)\eta_r V_s \\ 0 \\ 0 \\ 0 \end{pmatrix}$$

Since $\frac{\Lambda_r}{\delta_r} \geq h_r$ it is clear that $\tilde{G}(X,Z) \geq 0$ for all$(X,Z) \in \Omega$. Also A is an M-matrix, since the off-diagonal elements of A are nonnegative. Hence, the virus free equilibrium is globally asymptotically stable.

Virus Infection Equilibrium

In this section, we derive the expressions for the endemic equilibrium. At the virus infection equilibrium, the cells are infected by the virus and is denoted by $E^* = (h_r^*, h_t^*, h_c^*, h_i^*, V_s^*)$. The point can be derived by equating each of the equations of (2.1) to zero and then solve simultaneously. We have:

$$\begin{aligned} \Lambda_r - \eta_r h_r V_s - \delta_r h_r &= 0 \\ \eta_r h_r V_s - (\alpha_t + \mu_t + \delta_t)h_t &= 0 \\ \alpha_t h_t - (\alpha_c + \rho_c)h_c &= 0 \\ \mu_t h_t - (\alpha_i + \delta_i)h_i &= 0 \\ N_c\alpha_c h_c + N_i\alpha_i h_i - (r_c + r_i)V_s &= 0 \end{aligned} \qquad (3.13)$$

Solving (3.13), we obtained the following expressions for the endemic states:

$$\begin{cases} h_r^* = \dfrac{\Lambda_r}{\delta_r \Re_0}, \\ h_t^* = \dfrac{\delta_r(\alpha_c + \rho_c)(\alpha_i + \delta_i)(r_c + r_i)}{\eta_r[N_c\alpha_c\alpha_t(\alpha_i + \delta_i) + N_i\alpha_i\mu_t(\alpha_c + \rho_c)]}[\Re_0 - 1], \\ h_c^* = \dfrac{\delta_r\alpha_t(\alpha_i + \delta_i)(r_c + r_i)}{\eta_r[N_c\alpha_c\alpha_t(\alpha_i + \delta_i) + N_i\alpha_i\mu_t(\alpha_c + \rho_c)]}[\Re_0 - 1], \\ h_i^* = \dfrac{\delta_r\mu_t(\alpha_c + \rho_c)(r_c + r_i)}{\eta_r[N_c\alpha_c\alpha_t(\alpha_i + \delta_i) + N_i\alpha_i\mu_t(\alpha_c + \rho_c)]}[\Re_0 - 1], \\ V_s^* = \dfrac{\delta_r}{\eta_r}[\Re_0 - 1]. \end{cases} \qquad (3.14)$$

where $\Re_0$ is defined by equation (3.12). From result (3.14), the expressions of h_t^*, h_c^*, h_i^* and V_s^* that the endemic equilibrium for the model system (2.1) exist for $\Re_0 > 1$. The results

obtained above can be summarised by stating the following.

Theorem 3.4: The model system (2.1) has a unique positive equilibrium point whenever $\Re_0 > 1$ and does not exist when $\Re_0 \leq 1$.

Local Stability of the Virus Infection Equilibrium

To determine the local stability of virus infection equilibrium for the model system (2.1), the equation of the model system was linearised to obtain the jacobian matrix. It was then evaluated in the jacobian matrix of the model system (2.1) at the virus infection equilibrium E^*.

$$E^* = \left(\frac{\Lambda_r}{\delta_r \Re_0}, \frac{\delta_r(\alpha_c+\rho_c)(\alpha_i+\delta_i)(r_c+r_i)}{\eta_r[N_c\alpha_c\alpha_t(\alpha_i+\delta_i)+N_i\alpha_i\mu_t(\alpha_c+\rho_c)]}[\Re_0-1], \frac{\delta_r\alpha_t(\alpha_i+\delta_i)(r_c+r_i)}{\eta_r[N_c\alpha_c\alpha_t(\alpha_i+\delta_i)+N_i\alpha_i\mu_t(\alpha_c+\rho_c)]}[\Re_0-1],\right.$$
$$\left.\frac{\delta_r\mu_t(\alpha_c+\rho_c)(r_c+r_i)}{\eta_r[N_c\alpha_c\alpha_t(\alpha_i+\delta_i)+N_i\alpha_i\mu_t(\alpha_c+\rho_c)]}[\Re_0-1], \frac{\delta_r}{\eta_r}[\Re_0-1]\right).$$

We analyzed the model system (2.1) at the infection equilibrium and get its Jacobian matrix as:

$$J(E^*) = \begin{pmatrix} -\delta_r\Re_0 & 0 & 0 & 0 & -\frac{\eta_r\Lambda_r}{\delta_r\Re_0} \\ \delta_r(\Re_0-1) & -(\alpha_t+\mu_t+\delta_t) & 0 & 0 & \frac{\eta_r\Lambda_r}{\delta_r\Re_0} \\ 0 & \alpha_t & -(\alpha_c+\rho_c) & 0 & 0 \\ 0 & \mu_t & 0 & -(\alpha_i+\delta_i) & 0 \\ 0 & 0 & N_c\alpha_c & N_i\alpha i & -(r_c+r_i) \end{pmatrix}$$

We assess for stability of the infection equilibrium (E^*) by calculating the eigenvalues (λ_s) of the above Jacobian matrix. The characteristic equation for the Jacobian matrix is given by:

$$P(\lambda) = k_0\lambda^5 + k_1\lambda^4 + k_2\lambda^3 + k_3\lambda^2 + k_4\lambda + k_5 \qquad \textbf{(3.15)}$$

where

$$\begin{cases} k_0 = 1, \\ k_1 = a+b+c+d+e, \\ k_2 = a(b+c+d+e)+b(c+d+e)+c(d+e)+de, \\ k_3 = ab(c+d+e)+ac(d+e)+bc(d+e)+de(a+b+c)+\frac{\eta_r\Lambda_r}{\delta_r\Re_0}(N_c\alpha_c\alpha_t+N_i\alpha_i\mu_t), \\ k_4 = abcd+abce+abde+acde+\frac{\eta_r\Lambda_r}{\delta_r\Re_0}(N_c\alpha_c\alpha_t+N_i\alpha_i\mu_t), \\ k_5 = bcde(\delta_r\Re_0-1). \end{cases}$$

For $(a, b, c, d, e) = (\delta_r \Re_0, (\alpha_t + \mu_t + \delta_t), (\alpha_c + \rho_c), (\alpha_i + \delta_i), (r_c + r_i)$. In order to determine the stability of the infection equilibrium (E^*), we use the Routh Hurwitz stability criteria to determine the sign of the eigenvalues of the characteristic polynomial (3.16). According to Routh Hurwitz criteria, given a polynomial.

$$P(\lambda) = k_0\lambda^n + k_1\lambda^{n-1} + k_2\lambda^{n-2} + k_3\lambda^{n-3} + \ldots + k_{n-1}\lambda + k_n \qquad \textbf{(3.16)}$$

with a real constant coefficient k_i where $i = 1, 2, 3, \ldots, n$ is considered. Now from (3.16), we will form the routh array as shown below:

λ^n	k_0	k_2	k_4	k_6	k_8	–	–	–
λ^{n-1}	k_1	k_3	k_5	k_7	–	–	–	–
λ^{n-2}	a_1	a_2	a_3	a_4	–	–	–	–
λ^{n-3}	b_1	b_2	b_3	–	–	–	–	–
λ^{n-4}	c_1	c_2	–	–	–	–	–	–
λ^{n-5}	d_1							
.	.							
.	.							
λ^2	.							
λ^1	.							
λ^0	.							

k_i (For $i = 1, 2, 3, \ldots, n$) coefficients are taken the characteristic equation $P(\lambda)$ and are arranged as shown in the Routh array above. Other elements were calculated from these elements. Coefficients a_i (for $i = 1, 2, 3, \ldots, n$) are calculated as:

$$\begin{cases} a_1 = \dfrac{k_1k_2 - k_3k_0}{k_1}, \\ a_2 = \dfrac{k_1k_4 - k_5k_0}{k_1}. \end{cases}$$

Using the routh hurwitz criteria, the process is continued until we get a zero in the row with a_i coefficients. Similarly, b_i coefficients, c_i coefficients and d_i coefficients are calculated as follows:

$$\begin{cases} b_1 = \dfrac{k_3a_1 - k_1a_2}{a_1}, \\ b_2 = \dfrac{k_5a_1 - k_1a_3}{a_1}, \\ c_1 = \dfrac{a_2b_1 - a_1b_2}{b_1} \\ d_1 = \dfrac{b_2c_1 - b_1c_2}{c_1} \end{cases}$$

In our case, we define the routh array table whose elements are the coefficients ($k_i's$) of the characteristic polynomial $P(\lambda)$ in (3.15):

λ^5	k_0	k_2	k_4	k_6
λ^4	k_1	k_3	k_5	k_7
λ^3	a_1	a_2		
λ^2	b_1	b_2		
λ^1	c_1			
λ^0	d_1			

where,

$$
\begin{aligned}
a_1 &= \frac{k_1k_2 - k_3k_0}{k_1}, \\
&= \frac{a^2(b+c+d+e) + b^2(a+c+d+e) + c^2(a+b+d+e) + d^2(a+b+c+e)}{+e^2(a+b+c+d) + 2ab(c+d+e) + 2de(a+b+c) + 2ce(a+b)} > 0. \\
a_2 &= k_4, \\
&= abcd + abce + abde + acde + \frac{\eta_r \Lambda_r}{\delta_r \Re_0}(N_c\alpha_c\alpha_t + N_i\alpha_i\mu_t) > 0, \textbf{if and only if} \Re_0 > 1 \\
b_1 &= \frac{k_3a_1 - k_1a_2}{a_1}, \\
&= \frac{k_1(k_2k_3 - k_1k_4) - k_3^2}{k_1k_2 - k_3} > 0, \\
b_2 &= k_5, \\
&= bcde(\delta_r\Re_0 - 1) > 0, \textbf{if and only if} \Re_0 > 1, \\
c_1 &= \frac{a_2b_1 - a_1b_2}{a_1} > 0, \\
d_1 &= b_2 > 0.
\end{aligned}
$$

Since $k_0, k_1, a_1, b_1, c_1, d_1 > 0$, we have shown that the signs in the first column of routh array are all the same and this confirms stability of the infection equilibrium. Consequently, this completes our test for stability and subsequently conclude that the infection equilibrium for the model system (2.1) is stable.

SENSITIVITY ANALYSIS

The sensitivity analysis was conducted for the two Hepatitis B replication metrics at the within-cell scale from the model (2.1). Using parameters from existing literatures Table 2, we parameterise the model (2.1) to produce outcomes that can help with hepatitis B prevention, control, and eradication. At the within-cell scale, the two metrics are: (i) V_s^* which is the proxy for the individual cell infectiousness. This quantity requires further investigation because when HBV infection has fully established it may require completely different strategies to manage and control it effectively and efficiently. (ii) $\Re_0$ which is the within-cell basic reproduction number.

The sensitivity analysis of the two metrics ($\Re_0$, V_s^*) with respect to all the parameters will assist in informing Hepatitis B prevention and treatment policy by using high impact preventions medical interventions. Since the study considered the cell level of biological organisation, for the 2 Hepatitis B replication metrics, ($\Re_0$, V_s^*) the normalised sensitivity index with respect to a parameter P is given by:

$$S_{\Gamma_i}^P = \frac{\partial \Gamma_i}{\partial P} \times \frac{P}{\Gamma_i}, i = \Re_0, V_s^*$$

where

$$
\begin{cases}
V_s^* = \dfrac{\delta_r}{\eta_r}(\Re_0 - 1), \\
\Re_0 = \dfrac{\Lambda_r[N_c\alpha_c\eta_r\alpha_t(\alpha_i + \delta_i) + N_i\alpha_i\eta_r\mu_t(\alpha_c + \rho_c)]}{\delta r(\alpha_t + \mu_t + \delta_t)(\alpha_c + \rho_c)(\alpha_i + \delta i)(r_c + r_i)}.
\end{cases}
\tag{4.17}
$$

Table 3. The evaluation of how sensitive the two Hepatitis B replication measures, ($\mathfrak{R}_0$, V_s^*), were to the baseline parameters of the HBV within-cell model.

S/N	Parameter	Sensitivity Index of $\mathfrak{R}_0$	Sensitivity Index of V_s^*
1	Λ_r	0.999950741	0.999952125
2	η_r	0.999950741	0.000001392
3	δ_r	-0.999950741	-0.000001392
4	ρ_c	-0.028602386	-0.028602423
5	δ_t	-0.004974879	-0.004974886
6	α_t	-0.184426990	-0.184427245
7	α_c	0.028602386	0.028602426
8	μ_t	0.184426990	0.184427245
9	α_i	0.006785391	0.006785400
10	δ_i	0.006785391	0.006785400
11	r_c	-0.499975370	-0.499976063
12	r_i	-0.499975370	-0.499976063
13	N_c	0.314626246	0.314626681
14	N_i	0.685324495	0.685325444

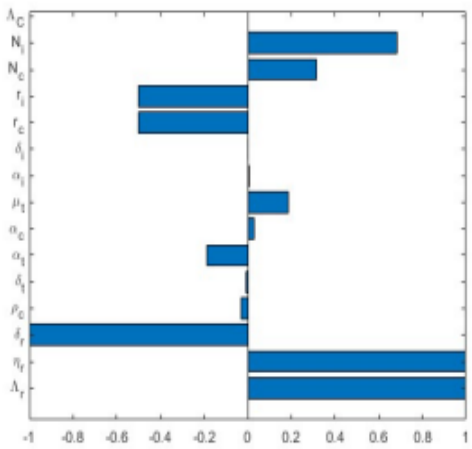

Figure 2. The normalized sensitivity indices of all the model parameters that influence the HBV replication metric of the model parameters of the within-cell HBV metric $\mathfrak{R}_0$.

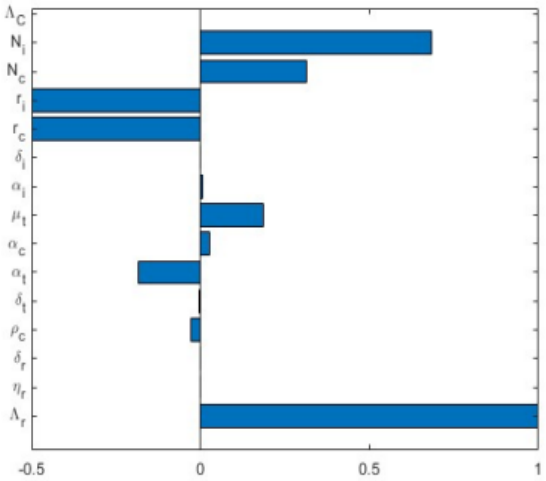

Figure 3: The normalized sensitivity indices of all the model parameters that influence the HBV replication metric of the model parameters of the within-cell HBV metric V_s^*.

Based on the sensitivity analysis results of both $\mathfrak{R}_0$ and V_s^* to all the parameters of the baseline model (2.1) as depicted in Figure 2 and Figure 3, the following conclusions were drawn:

i. Positive parameters will cause the values of $\mathfrak{R}_0$ and V_s^* to grow as they increase, whereas negative parameters will cause the values of $\mathfrak{R}_0$ and V_s^* to fall as they increase. The metric $\mathfrak{R}_0$ is extremely sensitive to the four of the

parameters (N_i , N_c, η_r, Λ_r). The sensitivity of V_s^* to the same parameters is variable, with V_s^* being least sensitive to η_r. Given that $\Re_0$ and V_s^* exhibit notable sensitivity to (N_i , N_c, Λ_r), it follows that, in order to improve the validity and reliability of the model system (2.1), attention must be made to ensure that these within-cell model parameters are accurate when collecting data.

ii. The V_s^* is less sensitive to η_r while $\Re_0$ is significantly sensitive to η_r,this implies that medical intervention such as cccDNA Inhibitors wound help to reduced replication of the virus at the start of the epidemic.

CONCLUSION

The primary contribution of this study to scientific understanding is the development of a within-cell, single-scale model of hepatitis B that distinguishes between the secretion of complete virions and incomplete particles from the cytoplasm of a cell. This model underscores the importance of considering the pathogen's life cycle to accurately capture the replication cycle. Developing microscale model only for the studying of biological system may not ideal and such system cannot be said to be a complex system due to its limitations (this scale is in isolation and lacks the ability to predict system-wide behaviour or guide interventions effectively). Multiscale modeling that involves both scales should be encouraged by modelers in the formation and development of infectious diseases as a complex system which required processes and mechanisms (this approach by contrast allows for the integration of these interconnected layers into a unified framework).

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