Uncertainty and Sensitivity Analysis Applied to an In-Host Malaria Model with Multiple Vaccine Antigens

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Abstract

Results of uncertainty and sensitivity analysis have significant epidemiological importance in malaria control. In this paper, the efficient technique of latin hypercube sampling (LHS) and partial rank correlation coefficient is applied to an in-host *Plasmodium falciparum* malaria model. Sensitivity indices of the basic reproduction number are derived using the normalised forward approach. By a theoretical analysis, we show the existence and stability of the model steady states based on threshold value R_0 . Our results show that the in-host model is sensitive to variations in the efficacy of malaria vaccines. The rates of parasite to cell invasions, the density of merozoites released per bursting infected erythrocyte and the proportions of merozoites that become gametocytes are highly significant in determining the severity of malaria infection. Moreover, a highly effective vaccine combination is critical for malaria disease elimination goal. This study further shows that the long term precise predictions of the concentrations of infected cells during malaria infection would be difficult until these key parameters are correctly determined. These results are vital in the on-going malaria vaccine development.

Keywords Sensitivity analysis · Hepatocytes · Erythrocytes · *P. falciparum* · Vaccine combination

Introduction

During disease model formulation, we are bound to make simplifications and assumptions on the model itself and on the parameters that represent the different transitions and interactions in the model. Owing to uncertainty on parameter values, it is important to correctly understand the possible effects of the such parameter values to the anticipated model output [21,52]. Uncertainty in the set of parameter values generates variability in the model's predictive capabilities. The lower the number of uncertain parameters in a model, the lower the significance of variability introduced into a model. On the other hand, a higher number of uncertain parameters result into a higher significance of variability introduced [21].

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In-host malaria transmission models have become very complex. Unlike simple models, complex models may best be understood by numerical analysis [8]. Uncertainty and sensitivity analysis are instrumental in analyzing the dynamics of such structurally complex models that experiences high levels of uncertainty in the estimation of their input parameter values. The concept of uncertainty analysis enables us to evaluate the variability in the output [30]. Conversely, a sensitivity analysis helps us to discern important parameters in the dynamics of infection under study. There are three techniques of uncertainty and sensitivity analysis [30]. These include: (i) latin hypercube sampling/partial rank correlation coefficient (LHS/PRCC), (ii) response surface methodology, and (3) differential analysis. The more sophisticated and efficient approach of LHS/PRCC [9,30] that permits synchronous variation of all input variables is considered in this study.

LHS/PRCC sensitivity analysis is a synergy of Latin Hypercube Sampling [38] and the Partial Rank Correlation Coefficient [26]. It aims to identify and rank pivotal model parameters whose uncertainties contribute to prediction imprecision [21]. In this paper, uncertainty and sensitivity analysis is applied to an in-host malaria model subject to malaria vaccines. We endeavour to identify influential parameters in determining malaria disease progression and control within the human host. See [2,50] for a rigorous description of the different malaria vaccines.

Malaria is a mosquito-borne infectious disease caused by *Plasmodium* parasite. The disease is typically transmitted through the bite of an infected mosquito vector [20]. The injected sporozoites traverse host dermis and enter the liver hepatocytes for further asexual development. Hepatocyte—invading sporozoites, grow, divide mitotically and differentiate into liver merozoites [12]. The release of these merozoites into blood stream marks the beginning of the blood stage cycle. The merozoites invade susceptible erythrocytes to feed on hemoglobin. Like the liver stage, the merozoites also differentiate, causing infected erythrocytes to burst open, releasing more merozoites into blood stream. Higher densities of blood merozoites increases the severity of clinical malaria. The daughter merozoites quickly invade other red blood cells to renew the cycle or develop into male or female gametocytes [13]. This cycle is associated with malaria characteristics symptoms [17,69]. See [6,49] for a detailed discussion on stages of malaria infection. Although malaria is both preventable and curable, severe malaria may lead to seizures, coma and death [22].

Currently, there are more than 30 malaria vaccine candidates under development [68]. These vaccines are generally categorised into: (1) pre-erythrocytic vaccines (PEV), (2) blood stage vaccines (BSV) and (3) transmission blocking vaccines (TBV) [6]. RTS,S/AS01 (also known as Mosquirix) is the only malaria vaccine that has received a positive scientific opinion from the European Medicines Agency (EMA) [18]. This pre-erythrocytic vaccine has been evaluated in large phase 3 trial in sub-Saharan Africa. The vaccine showed a 45.7% (95% CI, 41.7–49.5) efficacy against all episodes of clinical *P. falciparum* malaria across all sites in the 5–17 month age category [54]. Despite positive assessment from EMA, the World Health Organization (WHO) recommends further evaluation of RTS,S/AS01 in a series of pilot implementations before it can be introduced on a wide scale [70]. Nonetheless, an efficacious malaria vaccine forms a crucial component of future malaria control and elimination tool.

Several research studies [1,13,14,23,25,27,29,39–41,55] have focused on understanding the dynamics and control of *P. falciparum* infections. Although most of these models have investigated parameter importance in driving malaria infection, this is mainly based on the basic reproduction number of the model, which often do not have all the model parameters. Sensitivity analysis for the entire parameter space provides the right rigour for parameter evaluation in driving the disease progression. Previous studies [44,71], used parameter-driven LHS/PRCC procedure to ascertain important parameters and their relative importance in

the disease model output. In [21], LHS/PRCC procedure is coupled with the optimal control numerical procedure to simultaneously examine the effects parameters on the objective functional value. In this paper, the LHS/PRCC uncertainty and sensitivity analysis applied to an in-host *P. falciparum* malaria model with vaccine controls. Moreover, multi-component and multi-stage vaccine combinations is evaluated to establish optimal vaccine combinations.

This paper is organised as follows: we provide details of the in-host malaria transmission model and its properties such as non-negativity and boundedness of solutions in “The Model” section. We carry out model analysis including model steady states and their stability conditions in “Model Analysis” section. In “Uncertainty and Sensitivity Analysis” section, we derive sensitivity indices of the basic reproduction number relative to model parameters. We also perform uncertainty and sensitivity analysis of model parameters subject to infective and infected state variables. The existence of model equilibrium points is numerically demonstrated in “Numerical Simulation” section. Numerical evaluation of multi-component and multi-stage vaccine cocktail completes “Numerical Simulation” section. A discussion on epidemiological implications of uncertainty, sensitivity analysis and vaccine combinations completes the paper in “Epidemiological Implications and Conclusion” section.

The Model

We consider an in-host *P. falciparum* malaria model. The model analyses the dynamics of interactions between the human red blood cells (erythrocytes), the liver hepatocytes, the malaria parasite (*P. falciparum*) and the cytotoxic CD8⁺ T-cells in the presence of multi-component and multi-stage vaccine antigens. The compartmental deterministic model is an extension of in-host malaria model by the authors in [49]. The model is thus composed of nine compartments: the malaria sporozoites $S(t)$, the susceptible hepatocytes $H(t)$, the infected hepatocytes $X(t)$, the susceptible erythrocytes $R(t)$, the infected red blood cells $T(t)$, the malaria gametocytes $G(t)$, the malaria merozoites $M(t)$, unactivated CD8⁺ T-cells $W_n(t)$ and activated CD8⁺ T-cells $W_a(t)$.

The activation of CD8⁺ T-cells is caused by the presence of infected hepatocytes and infected erythrocytes only. This activation is further assumed to occur at a constant rate λ_w for both X and T . Unactivated CD8⁺ T-cells are produced at a fixed rate Ω from the thymus. Unlike the model in [49], we have assumed that a proportion q of infected erythrocytes and a proportion ρ of infected hepatocytes get eradicated by the CD8⁺ T-cells before they develop to the bursting stage. The rest of infected hepatocytes develop asexually and burst open to release blood stage merozoites. We assume that some of the newly generated merozoites develop into gametocytes. This proportion is denoted by the parameter π . The commitment to gametocytogenesis is presumably made during the immediately preceding cycle of blood schizogony [13]. These blood floating gametocytes are transmitted to the mosquito vector during feeding, marking the onset of the sporogonic cycle (not considered in this study).

During blood meal, infected mosquito injects Λ sporozoites into the skin of the human host. The sporozoites traverse the skin and invade the hepatocytes H at the rate β_s to generate infected hepatocytes X at the liver stage (see [64] for detailed description of the pre-erythrocytic stage infection). We assume a natural death rate of μ_s for malaria sporozoites. Although hepatocytes are generated at a rate λ_h from the bone marrow, we assume that both the infected and uninfected hepatocytes experience natural death at the rates μ_x and μ_h , respectively.

Three malaria vaccines are considered in this model to reduce the severity of *P. falciparum* malaria infection. These are: pre-erythrocytic vaccine (PEV) (see, [6,7,46]), blood stage

Table 1 Description of the state variables

Variable	Description
$W_n(t)$	Concentration unactivated CD8 ⁺ T-cells
$W_a(t)$	Concentration activated CD8 ⁺ T-cells
$H(t)$	Concentration of susceptible (uninfected) liver hepatocytes
$X(t)$	Concentration of infected hepatocytes
$S(t)$	Population of malaria sporozoites
$R(t)$	Concentration of susceptible (healthy) erythrocytes
$T(t)$	Concentration of infected erythrocytes
$M(t)$	Population of merozoites in blood
$G(t)$	Concentration of gametocytes in blood

vaccine (BSV) (see [5,42,47]), and transmission blocking vaccines (TBV) (see [3,28]). The leading PEV, BSV and TBV considered in this study are RTS,S/AS01, merozoite surface protein 3 (MSP3), *P. falciparum* ookinete surface antigens (Pfs25), respectively. The PEV, BSV and TBV vaccines are assumed to possess efficacies denoted by ν , ϱ and χ , respectively, where $0 < \nu < 1$, $0 < \varrho < 1$ and $0 < \chi < 1$.

A summary of the descriptions of the state variables and model parameters is as shown in Tables 1 and 2, respectively. Furthermore, the compartmental flow diagram for the in-host malaria model is provided in Fig. 1. We are motivated by the structural complexity of the in-host malaria model to apply LHS/PRCC technique for uncertainty and sensitivity analysis. The structural complexity in the model could lead to high levels of uncertainty in approximating the initial values of the input parameters.

Based on the dynamics described in Fig. 1, the following deterministic system describes the dynamics of the in-host malaria infections with multi-stage malaria vaccines:

$$\left. \begin{aligned}
 \frac{dS}{dt} &= (1 - \chi)\Lambda - \mu_s S - \beta_s S H, \\
 \frac{dH}{dt} &= \lambda_h - \mu_h H - \frac{\beta_s(1 - \nu)SH}{1 + dW_a}, \\
 \frac{dX}{dt} &= \frac{\beta_s(1 - \nu)SH}{1 + dW_a} - \mu_x X - \frac{\rho X W_a}{1 + \varepsilon_0 X}, \\
 \frac{dR}{dt} &= \lambda_r - \frac{(1 - \varrho)\beta_r R M}{1 + dW_a} - \mu_r R, \\
 \frac{dT}{dt} &= \frac{(1 - \varrho)\beta_r R M}{1 + dW_a} - \mu_t T - \frac{q T W_a}{1 + \varepsilon_1 T}, \\
 \frac{dM}{dt} &= \frac{P(1 - q)(1 - \pi)(1 - a)\mu_t T}{1 + dW_a} + (1 - \rho)(1 - b)N\mu_x X - \mu_m M - \beta_r R M, \\
 \frac{dG}{dt} &= \frac{P(1 - q)(1 - a)\pi\mu_t T}{1 + dW_a} - \mu_g G, \\
 \frac{dW_n}{dt} &= \Omega - \tau\lambda_w(X + T)W_n - \mu_n W_n, \\
 \frac{dW_a}{dt} &= \tau\lambda_w(X + T)W_n - \mu_a W_a,
 \end{aligned} \right\} \quad (1)$$

Table 2 Table showing model parameters and their descriptions

Parameter symbol	Parameter description
P	Number of merozoites released per bursting erythrocyte
N	Number of merozoites released per bursting hepatocytes
Λ	Injection rate for sporozoites
μ_h	Death rate of susceptible liver hepatocytes
μ_x	Death rate of susceptible erythrocytes
μ_s, μ_m	Death rates of sporozoites and merozoites respectively
λ_h	The recruitment rate of hepatocytes
λ_r	The recruitment rate of erythrocytes from the bone marrow
π	A proportion of merozoites that develop into gametocytes per dying infected red blood cell.
ρ	Immunosensitivity of infected hepatocytes
q	Immunosensitivity of infected erythrocytes
β_s, β_r	Rate of infection of hepatocytes and erythrocytes, respectively
b	A rate accounting for PEV-induced reduction of merozoites released per bursting infected hepatocyte
μ_r	Death rate of susceptible erythrocytes
μ_t	Mortality rate of infected erythrocytes
μ_g, μ_m	Mortality rate of gametocytes and merozoites, respectively
χ, ϱ, ν	Vaccine efficacies
d	Inhibition rate of CD8 ⁺ T-cells response
Ω	Recruitment rate of unactivated CD8 ⁺ T-cells from the thymus
λ_w	Immunogenicity of IRBCs and infected hepatocytes
$1/\varepsilon_0$	A saturation (half) constant for infected hepatocytes
$1/\varepsilon_1$	A saturation (half) constant for infected red blood cells
τ	A rate accounting for vaccine-induced increased generation of activated CD8 ⁺ T-cells
μ_n, μ_a	Death rates of unactivated and activated CD8 ⁺ T-cells respectively
a	A rate accounting for BSV-induced reduction of merozoites released per bursting erythrocyte

with the general initial conditions:

$$\begin{aligned}
 S(0) \geq 0, \quad H(0) > 0, \quad X(0) \geq 0, \quad R(0) > 0, \quad T(0) \geq 0, \quad M(0) \geq 0, \\
 G(0) \geq 0, \quad W_n(0) > 0, \quad W_a(0) \geq 0.
 \end{aligned} \tag{2}$$

Fig. 1 A compartmental representation of the dynamics of in-host malaria infection with vaccine controls

Properties of the Model

Positivity of Solutions

The long term behaviour of any dynamical system is instrumental in the understanding of the system. The model system (1) monitors changes in the concentrations of blood cells, hepatocytes, malaria parasite and $CD8^+$ T-cells. To warrant biological sense, the state variables of system (1) should be non-negative. Specifically, we show that all solutions of model system (1) with non-negative initial conditions will remain non-negative for all time $t \geq 0$.

Theorem 1 *Given non-negative initial conditions in Eq. (2), the in-host model (1) has a non-negative solution $(S(t), H(t), X(t), R(t), T(t), M(t), G(t), W_n(t), W_a(t))$, for all time $t \geq 0$.*

Proof From the first equation in system (1), we can clearly see that

$$\frac{dS}{dt} \geq -(\mu_s + \beta_s H)S. \quad (3)$$

Upon integrating equation (3), we get

$$S(t) \geq S_0 \exp \left\{ - \int_0^t (\mu_s + \beta_s H(s)) ds \right\}. \quad (4)$$

Therefore, $S(t) \geq 0$ for all $t \geq 0$. $\square$

Similarly, from the last equation in system (1), we have

$$\frac{dW_a}{dt} \geq -\mu_a W_a. \quad (5)$$

Upon integrating the separable equation in (5), we obtain

$$W_a(t) = W_a(0) \exp(-\mu_a t) \geq 0.$$

Using the same argument, the rest of the state variables can similarly be shown to be non-negative for all time $t \geq 0$. That is, $H(t) > 0$, $X(t) \geq 0$, $R(t) > 0$, $T(t) \geq 0$, $M(t) \geq 0$, $G(t) \geq 0$ and $W_n(t) > 0 \forall t \geq 0$. This completes the proof.

Boundedness of Solutions

The non-negative solutions to model system (1) must be well posed in regions of biological sense. We therefore prove that the solutions are bounded.

Let N_r be the sum of the density of susceptible and infected erythrocytes in the host. We thus have $N_r(t) = R(t) + T(t)$. Therefore,

$$\frac{dN_r}{dt} = \lambda_r - \mu_r R - \mu_t T - qTW_a, \quad (6)$$

$$\leq \lambda_r - \hat{\mu} N_r \quad \text{where} \quad \hat{\mu} = \min\{\mu_r, \mu_t\}. \quad (7)$$

Using the initial condition $N_r(0) = N_{r0} > 0$, Eq. (7) is solved by integration factor method to obtain

$$N_r(t) \leq \frac{\lambda_r}{\hat{\mu}} + e^{-\hat{\mu}t} \left(N_r(0) - \frac{\lambda_r}{\hat{\mu}} \right).$$

This implies that at any time t , $N_r(t) \leq \max \left\{ N_r(0), \frac{\lambda_r}{\hat{\mu}} \right\}$.

Again, let $N_p(t) = S(t) + M(t) + G(t)$, be the sum of malaria parasites at any time t during clinical *P. falciparum* infection. So that,

$$\begin{aligned} \frac{dN_p}{dt} &= (1 - \chi)\Lambda + \frac{P(1 - q)(1 - \pi)(1 - a)\mu_t T}{1 + dW_a} + (1 - \rho)(1 - b)N\mu_x X \\ &\quad + \frac{P(1 - q)(1 - a)\pi\mu_t T}{1 + dW_a} - \mu_s S - \mu_m M - \mu_g G - \beta_s SH - \beta_r RM, \\ &\leq (1 - \chi)\Lambda + \Psi_1(t) - \bar{\mu} N_p \quad \text{where} \quad \bar{\mu} = \min\{\mu_s, \mu_m, \mu_g\}, \quad \text{and} \end{aligned} \quad (8)$$

$$\begin{aligned} \Psi_1(t) &= \frac{P(1 - q)(1 - \pi)(1 - a)\mu_t T}{1 + dW_a} + (1 - \rho)(1 - b)N\mu_x X \\ &\quad + \frac{P(1 - q)(1 - a)\pi\mu_t T}{1 + dW_a}. \end{aligned} \quad (9)$$

Upon solving (8), we obtain:

$$N_p(t) \leq \frac{(1 - \chi)\Lambda}{\bar{\mu}} + e^{-\bar{\mu}t} \int_0^t e^{\bar{\mu}t} \Psi_1(t) dt + C_1 e^{-\bar{\mu}t},$$

where the constant of integration C_1 is given by,

$$C_1 = \left(N_p(0) - \frac{(1 - \chi)\Lambda}{\bar{\mu}} \right) - \int_0^s \Psi_1(0) ds.$$

Observe that $N_p(t) \leq \max \left\{ N_p(0), \frac{(1 - \chi)\Lambda}{\bar{\mu}} \right\}$. The above procedure can similarly be applied to the total concentrations of the liver hepatocytes $N_l(t) = H(t) + X(t)$ and that of CD8⁺ T-cells $N_w(t) = W_a(t) + W_n(t)$ so that we arrive at:

$$\frac{dN_l}{dt} \leq \lambda_h - \mu^* N_l \quad \text{and} \quad \frac{dN_w}{dt} \leq \Omega - \mu_w N_w, \quad (10)$$

where $\mu^* = \min\{\mu_h, \mu_x\}$ and $\mu_w = \min\{\mu_a, \mu_n\}$.

Upon integrating and simplifying the equations in (10), we get,

$$N_l(t) \leq \max \left\{ N_l(0), \frac{\lambda_h}{\mu^*} \right\} \quad \text{and} \quad N_w(t) \leq \max \left\{ N_w(0), \frac{\Omega}{\mu_w} \right\} \quad \text{for all time } t \geq 0.$$

Therefore, all feasible solutions of in-host malaria model (1) enter the region:

$$\begin{aligned} \mathcal{D} = & \left\{ (S, H, X, R, T, C, M, G, W) \in \mathbb{R}_+^9 : N_r(t) \leq \max \left\{ N_r(0), \frac{\lambda_r}{\hat{\mu}} \right\} \right. \\ & N_p(t) \leq \max \left\{ N_p(0), \frac{(1-\chi)\Lambda}{\bar{\mu}} \right\}, \\ & \left. N_l(t) \leq \max \left\{ N_l(0), \frac{\lambda_h}{\mu^*} \right\}, N_w(t) \leq \max \left\{ N_w(0), \frac{\Omega}{\mu_w} \right\} \right\}. \end{aligned} \quad (11)$$

which is a positively invariant set of model system (1). This implies that all solutions in $\mathcal{D}$ remain in $\mathcal{D} \quad \forall t \geq 0$. It thus suffices to study the dynamics of model system (1) in $\mathcal{D}$.

Model Analysis

Existence and Stability Analysis of Disease-Free Equilibrium Point (DFE)

The disease-free equilibrium E_0 occurs when there is no *P. falciparum* infection within the human host. At E_0 , $\Lambda = 0$ and there are no activated CD8^+ -T cells; that is, $W_a = 0$. By setting the right hand-side of system (1) to zero, we obtain the following disease free equilibrium:

$$E_0 = (S^0, H^0, X^0, R^0, T^0, M^0, G^0, W_n^0, W_a^0) = \left(0, \frac{\lambda_h}{\mu_h}, 0, \frac{\lambda_r}{\mu_r}, 0, 0, 0, \frac{\Omega}{\mu_n}, 0 \right). \quad (12)$$

The disease-free steady state is very crucial in the understanding of in-host malaria infection dynamics. In order to eradicate in-host malaria, we have to identify and establish necessary conditions for E_0 to be stable. This stability could be achieved through the use of effective anti-malarial drugs such as ACT or by using highly efficacious *Plasmodium falciparum* malaria vaccines.

Next, we apply the next generation matrix (NGM) approach (see [14,63] for detailed explanation of this technique) to compute the basic reproduction number R_0 of the in-host model (1). The NGM method presumes the local stability of E_0 for $R_0 < 1$ and instability for $R_0 > 1$. R_0 is the spectral radius of the next generation matrix (FV^{-1}) of system (1); that is, $R_0 = \rho(FV^{-1})$. Adopting the matrix notations in [63], the matrices F and V are respectively given by:

$$F = \begin{pmatrix} 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & \frac{(1-\nu)\beta_s\lambda_h}{\mu_h} & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & \frac{(1-\varrho)\beta_r\lambda_r}{\mu_r} & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix} \quad (13)$$

and

$$V = \begin{pmatrix} \frac{\beta_s\lambda_h}{\mu_h} + \mu_s & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & \mu_x & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & \mu_t & 0 & 0 & 0 & 0 \\ 0 & -(1-\rho)(1-b)N\mu_x & \mathcal{A}_1 & \frac{\beta_r\lambda_r}{\mu_r} + \mu_m & 0 & 0 & 0 \\ 0 & 0 & -P\pi(1-a)(1-q)\mu_t & 0 & \mu_g & 0 & 0 \\ 0 & \frac{\tau\Omega\lambda_w}{\mu_n} & \frac{\tau\Omega\lambda_w}{\mu_n} & 0 & 0 & \mu_n & 0 \\ 0 & -\frac{\tau\Omega\lambda_w}{\mu_n} & -\frac{\tau\Omega\lambda_w}{\mu_n} & 0 & 0 & 0 & \mu_a \end{pmatrix}, \quad (14)$$

where $\mathcal{A}_1 = -P(1-\pi)(1-q)(1-a)\mu_t$.

Thus, the basic reproduction number R_0 is given by

$$R_0 = \rho(FV^{-1}) = \max\{R_{01}, R_{02}\}$$

where

$$R_{01} = \frac{(1-\nu)\beta_s\lambda_h}{\mu_h\mu_x} \quad (15)$$

and

$$R_{02} = \frac{P(1-\pi)(1-a)(1-\varrho)(1-q)\beta_r\lambda_r}{\beta_r\lambda_r + \mu_m\mu_r}. \quad (16)$$

Observe that R_{01} is the contribution of new cell invasions at the liver stage due to merozoites and R_{02} is the contributions of new cell invasions at the blood stage due to merozoite parasites. From Eqs. (15) and (16), it is evident that new cell invasions occur both at the liver stage (as depicted by R_{01}) and at the blood stage (as depicted by R_{02}). This implies that clinical control of *P. falciparum* malaria should target the parasite at both stages of its life cycle within the human host. An anti-malarial drug with the capacity to eradicate the sporozoites at the pre-erythrocytic stage and merozoites at the erythrocytic stage is most recommended. Furthermore, malaria vaccine development should equally focus on both stages of parasite development. A novel malaria vaccine should begin parasite eradication from the pre-erythrocytic stage and this should continue to the blood stage. This result is very important to the ongoing research and development of malaria vaccines.

The global stability of the disease-free equilibrium is established using a suitable Lyapunov candidate function [34,35,48]. We thus have the following theorem.

Theorem 2 *The disease-free equilibrium E_0 is globally asymptotically stable in R_+^9 if $R_0 < 1$.*

Proof Let $V(X, T, S, M, G) = \psi_1 X + \psi_2 T + \psi_3 S + \psi_4 M + \psi_5 G$ be a candidate Lyapunov function for some non-negative coefficients $\psi_1, \psi_2, \psi_3, \psi_4$ and ψ_5 . The derivative of V with respect to time is by

$$\begin{aligned}
\frac{dV}{dt} &= \psi_1 \frac{dX}{dt} + \psi_2 \frac{dT}{dt} + \psi_3 \frac{dS}{dt} + \psi_4 \frac{dM}{dt} + \psi_5 \frac{dG}{dt}, \\
&= \psi_1 \left(\frac{\beta_s(1-\nu)SH}{1+dW_a} - \mu_x X - \frac{\rho X W_a}{1+\varepsilon_0 X} \right) + \psi_2 \left(\frac{(1-\varrho)\beta_r RM}{1+dW_a} - \mu_t T - \frac{qTW_a}{1+\varepsilon_1 T} \right) \\
&\quad + \psi_3 ((1-\chi)\Lambda - \mu_s S - \beta_s SH) \\
&\quad + \psi_4 \left(\frac{P(1-q)(1-\pi)(1-a)\mu_t T}{1+dW_a} + (1-\rho)(1-b)N\mu_x X - \mu_m M - \beta_r RM \right) \\
&\quad + \psi_5 \left(\frac{P(1-q)(1-a)\pi\mu_t T}{1+dW_a} - \mu_g G \right), \\
&= (-\mu_x (N\psi_4(b(-\rho) + b + \rho - 1) + \psi_1) - \frac{\Omega}{\mu_n} (\rho\psi_1 + q\psi_2))X \\
&\quad + (-\mu_t ((a-1)P(q-1)((\pi-1)\psi_4 - \pi\psi_5) + \psi_2))T \\
&\quad + (\psi_3(-\mu_s) - \frac{\lambda_h}{\mu_h} ((\nu-1)\psi_1 + \psi_3)\beta_s)S \\
&\quad + (\psi_4(-\mu_m) - \frac{\lambda_r}{\mu_r} ((e-1)\psi_2 + \psi_4)\beta_r)M \\
&\quad + (-\mu_g\psi_5)G.
\end{aligned}$$

□

Setting the coefficients of X , T , S , M and G to zero and solving, we get

$$\begin{aligned}
\psi_1 &= \frac{N(1-b)(1-\rho)\mu_x(1-\varrho)\beta_r\lambda_r\mu_a^2 - q\Omega(\beta_r\lambda_r + \mu_m\mu_r)\mu_a}{(1-\varrho)\beta_r\lambda_r\mu_a(\rho\Omega + \mu_x\mu_a)}\psi_4, \\
\psi_3 &= \frac{\frac{\lambda_h}{\mu_h}(1-\nu)\beta_s}{\frac{\lambda_h}{\mu_h}\beta_s + \mu_s} \left[\frac{N(1-b)(1-\rho)\mu_x(1-\varrho)\beta_r\lambda_r\mu_a^2 - q\Omega(\beta_r\lambda_r + \mu_m\mu_r)\mu_a}{(1-\varrho)\beta_r\lambda_r\mu_a(\rho\Omega + \mu_x\mu_a)} \right] \psi_4, \\
\psi_2 &= \left[\frac{\frac{\lambda_r}{\mu_r}\beta_r + \mu_m}{(1-\varrho)\frac{\lambda_r}{\mu_r}\beta_r} \right] \psi_4 \quad \text{and} \quad \psi_5 = \frac{\frac{1}{1-\varrho} - P(1-a)(1-\pi)(1-q) + \frac{\mu_m\mu_r}{\beta_r\lambda_r(1-\varrho)}}{\pi P(1-a)(1-q)} \psi_4.
\end{aligned}$$

Using these coefficients, the time derivatives of the Lyapunov function is given as

$$\begin{aligned}
\frac{dV}{dt} &= - \frac{(q + (1-b)(1-\varrho)N(1-\rho)\rho)\beta_r\lambda_r + q\mu_m\mu_r)(\mu_a + \mu_n)\mu_x}{(1-\varrho)\beta_r\lambda_r\mu_n(\rho\Omega + \mu_a\mu_x)} X \\
&\quad - \frac{\mu_m\mu_r\mu_t(1 + (1-\varrho)^2\lambda_r^2)}{(1-e)\beta_r\lambda_r} T - \frac{\mu_g(1-\varrho)\lambda_r\mu_m\mu_r}{\pi\beta_r(1-a)(1-q)} G(1-R_0).
\end{aligned} \tag{17}$$

If $R_0 < 1$ in (17) then $[\mu_g(1-\varrho)\lambda_r\mu_m\mu_r]/[\pi\beta_r(1-a)(1-q)](1-R_0) > 0$. We thus obtain $\frac{dV}{dt} \leq 0$. Furthermore, $\frac{dV}{dt} = 0$ if and only if $T = X = G = 0$. Therefore, V is a Lyapunov function in $\mathcal{D}$. Given that $\mathcal{D}$ is positively invariant and attracting, it follows that the maximum invariant set in $\{(S, H, X, R, T, M, G, W_n, W_a) \in \mathcal{D} : \dot{V} = 0\}$ is the singleton E_0 . By LaSalle's Invariant principle [33], E_0 is globally asymptotically stable in $\mathcal{D}$ provided that $R_0 < 1$.

The Malaria Persistent Steady State

We establish the malaria persistent equilibrium E_p for model system (1) in the presence of $CD8^+$ T-cells by equating the right hand side of system (1) to zero. Using Mathematica software [31], model system (1), exhibits a malaria-persistent equilibrium point

$$E_p = (S^\dagger, H^*, X^*, R^*, T^*, M^*, G^*, W_n^*, W_a^*),$$

where

$$H^* = \frac{(1 + dW_a^*)\lambda_h}{(1 - \nu)\beta_s S^* + (1 + dW_a^*)\mu_h}, \quad (18)$$

$$R^* = \frac{(1 + dW_a^*)\lambda_r}{(1 - \varrho)\beta_r M^* + (1 + dW_a^*)\mu_r}, \quad (19)$$

$$W_n^* = \frac{\Omega}{\tau\lambda_w(X^* + T^*) + \mu_n}, \quad (20)$$

$$W_a^* = \frac{\tau\lambda_w(X^* + T^*)W_n^*}{\mu_a}, \quad (21)$$

$$G^* = \frac{P(1 - q)(1 - a)\pi\mu_t T^*}{\mu_g(1 + dW_a^*)}, \quad (22)$$

$$M^* = \frac{(1 + dW_a^*)[(1 - b)^2(1 - \varrho)NX^*\beta_r\mu_m\mu_x - (1 + dW_a^*)\mu_r] \pm \sqrt{\Delta}}{2(1 - \varrho)(1 + dW_a^*)\beta_r}, \quad \text{where}$$

$$\begin{aligned} \Delta_m = (1 + dW_a^*) \{ & -(e - 1)\beta_r(-4(1 - \pi)(1 - a)P(1 - q)T^*\mu_t \\ & + (1 - b)^2NX^*(1 + dW_a^*)\beta_r\mu_x(4(1 + dW_a^*)\lambda_r + (1 - b)^2(1 - e)NX^*\mu_m^2\mu_x) \\ & - 2(1 - b)^2(1 - e)NX^*(1 + dW_a^*)^2\mu_m\beta_r\mu_r\mu_x + (1 + dW_a^*)^3\mu_r^2\}, \end{aligned} \quad (23)$$

$$S^* = \frac{1}{-2(1 - \nu)\beta_s v_s} \left\{ -\Delta_s \pm \sqrt{\Delta_s^2 + 4((1 - \nu)\beta_s\mu_s)((1 - \chi)\Lambda(1 + dW_a^*)\mu_h)} \right\},$$

$$\text{where } \Delta_s = \beta_s(\Lambda(1 - \chi)(1 - \nu) - (1 + dW_a^*)\lambda_h) - (1 + dW_a^*)\mu_h\mu_s, \quad (24)$$

$$T^* = \frac{1}{-2\epsilon_1((1 - \varrho)\beta_r M^* + (1 + dW_a^*)\mu_r)\mu_t} \left\{ -\Delta_t \pm \sqrt{\Delta_t^*} \right\},$$

$$\text{where } \Delta_t = \beta_s(\Lambda(1 - \chi)(1 - \nu) - (1 + dW_a^*)\lambda_h) - (1 + dW_a^*)\mu_h\mu_s;$$

$$\Delta_t^* = \Delta_t^2 + 4\epsilon_1\mu_t((1 - \varrho)\beta_r M^* + (1 + dW_a^*)\mu_r)(\beta_r\lambda_r(1 - \varrho)M^*) \quad \text{and} \quad (25)$$

$$X^* = \frac{\Delta_x \pm \sqrt{\Delta_x^2 + 4\epsilon_0((1 - \nu)\beta_s S^* + (1 + dW_a^*)\mu_h)\mu_x}(1 - \nu)\beta_s\lambda_h S^*}{2\epsilon_0((1 - \nu)\beta_s S^* + (1 + dW_a^*)\mu_h)\mu_x}, \quad \text{where}$$

$$\begin{aligned} \Delta_x = (1 - \nu)(\epsilon_0\lambda_h - \mu_x)\beta_s S^* - d\rho W_a^{*2}\mu_h - \mu_h\mu_x \\ - W_a^*((1 - \nu)\rho\beta_s S^* + \mu_h(\rho + d\mu_x)). \end{aligned} \quad (26)$$

Based on the formulated model in Eq. (1), the activated CD8⁺ T-cells only exists due to the presence of infected erythrocytes and infected liver hepatocytes. Hence, in the absence of malaria infection ($X = T = 0$), $W_a = 0$. Moreover, non of the malaria parasites exists; that is $S^* = M^* = G^* = 0$. We further observe that when $S^* = M^* = X^* = T^* = W_a^* = 0$, the susceptible erythrocytes, susceptible hepatocytes and the unactivated CD8⁺ T-cells become $H^* = \frac{\lambda_h}{\mu_h}$, $R^* = \frac{\lambda_r}{\mu_r}$ and $W_n^* = \frac{\Omega}{\mu_n}$ respectively. We thus arrive at an equilibrium point called the disease free equilibrium point of model (1) illustrated in Eq. (12). From the above computations, it is evident that the analytical expressions of E_p is too complex. We shall therefore provide a numerical illustration of the model equilibrium points in “Numerical Simulation” section.

Theorem 3 *The malaria persistent steady state E_p is locally asymptotically stable if $R_0 > 1$ in the interior of the biologically feasible region $\mathcal{D}$.*

Proof The stability of the malaria persistent equilibrium E_p (when $R_0 > 1$ but close to 1) is proved using the center manifold theory (CMT) presented by Castillo-Chavez and Song in [11]. Adopting the notations used in [11] and without re-stating the theorem, we calculate the values of arguments **a** and **b**. The signs (+ or −) of **a** and **b** determine the local dynamics of system (1) around E_p [66].

Suppose we consider β_* to be our bifurcation parameter, then at $R_0 = 1$, we have $\beta_* = \max\{\beta_s^*, \beta_r^*\}$, where

$$\beta_r^* = \frac{\mu_m \mu_r}{[P(1-q)(1-\pi)(1-\varrho)(1-a)-1]\lambda_r} \quad \text{and} \quad \beta_s^* = \frac{\mu_h \mu_x}{(1-\nu)\lambda_h}. \quad (27)$$

□

In applying the CMT, we begin by transforming the state variables ($S, H, X, R, T, M, G, W_n, W_a$) into $(x_1, x_2, x_3, x_4, x_5, x_6, x_7, x_8, x_9)$ respectively. The model system (1), is hence represented as $\frac{dg}{dt} = g(\mathbf{x})$, where $\mathbf{x} = (x_1, x_2, x_3, x_4, x_5, x_6, x_7, x_8, x_9)$. The model system (1) thus becomes

$$\left. \begin{aligned} \dot{x}_1 &= (1-\chi)\Lambda - \mu_s x_1 - \beta_s x_1 x_2, \\ \dot{x}_2 &= \lambda_h - \mu_h x_2 - \frac{\beta_s(1-\nu)x_1 x_2}{1+dx_9}, \\ \dot{x}_3 &= \frac{\beta_s(1-\nu)x_1 x_2}{1+dx_9} - \mu_x x_3 - \frac{\rho x_3 x_9}{1+\varepsilon_0 x_3}, \\ \dot{x}_4 &= \lambda_r - \frac{(1-\varrho)\beta_r x_4 x_6}{1+dx_9} - \mu_r x_4, \\ \dot{x}_5 &= \frac{(1-\varrho)\beta_r x_4 x_6}{1+dx_9} - \mu_t x_5 - \frac{qx_5 x_9}{1+\varepsilon_1 x_5}, \\ \dot{x}_6 &= \frac{P(1-q)(1-\pi)(1-a)\mu_t x_5}{1+dx_9} + (1-\rho)(1-b)N\mu_x x_3 - \mu_m x_6 - \beta_r x_4 x_6, \\ \dot{x}_7 &= \frac{P(1-q)(1-a)\pi\mu_t x_5}{1+dx_9} - \mu_g x_7, \\ \dot{x}_8 &= \Omega - \tau\lambda_w(x_3 + x_5)x_8 - \mu_n x_8, \\ \dot{x}_9 &= \tau\lambda_w(x_3 + x_5)x_8 - \mu_a x_9, \end{aligned} \right\} \quad (28)$$

such that;

$$x_1(0) \geq 0, x_2(0) \geq 0, x_3(0) \geq 0, x_4(0) \geq 0, x_5(0) \geq 0, x_6(0) \geq 0, x_7(0) \geq 0, x_8(0) \geq 0, x_9(0) \geq 0.$$

The new model system (28) with the bifurcation point β_* has a simple zero eigenvalue. This attribute enables us to use the CMT to analyze the stability of system (28) near $\beta_* = \max\{\beta_s^*, \beta_r^*\} = \max\{\beta_s, \beta_r\}$.

Therefore, a right eigenvector $w = [w_1, w_2, w_3, w_4, w_5, w_6, w_7, w_8, w_9]^T$ associated with the simple zero eigenvalue is obtained from the linearization matrix corresponding to the zero eigenvector as follows:

$$\begin{pmatrix}
-A_2 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
-A_3 & -\mu_h & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
A_3 & 0 & -\mu_x & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & -\mu_r & 0 & -A_4 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & -\mu_t & A_4 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & A_0 & -A_5 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & A_1 & 0 & -\mu_g & 0 & 0 \\
0 & 0 & -\frac{\tau\Omega\lambda_w}{\mu_n} & 0 & -\frac{\tau\Omega\lambda_w}{\mu_n} & 0 & 0 & -\mu_n & 0 \\
0 & 0 & \frac{\tau\Omega\lambda_w}{\mu_n} & 0 & \frac{\tau\Omega\lambda_w}{\mu_n} & 0 & 0 & 0 & -\mu_a
\end{pmatrix}
\begin{pmatrix}
w_1 \\ w_2 \\ w_3 \\ w_4 \\ w_5 \\ w_6 \\ w_7 \\ w_8 \\ w_9
\end{pmatrix}
=
\begin{pmatrix}
0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0
\end{pmatrix}
\quad (29)$$

where $A_0 = (1-a)P(1-\pi)(1-q)\mu_t$, $A_1 = (1-a)P\pi(1-q)\mu_t$, $A_2 = \left(\frac{\beta_s\lambda_h}{\mu_h} + \mu_s\right)$, $A_3 = \frac{(1-v)\beta_s\lambda_h}{\mu_h}$, $A_4 = \frac{(1-q)\beta_r\lambda_r}{\mu_r}$ and $A_5 = \left(\frac{\beta_r\lambda_r}{\mu_r} + \mu_m\right)$.

Solving for the right eigenvectors, we obtain

$$\begin{aligned}
w_5 &= \frac{-\left(\frac{\beta_r\lambda_r}{\mu_r} + \mu_m\right)}{(1-a)P(1-\pi)(1-q)\mu_t}, \quad w_7 = \frac{-(1-a)P\pi(1-q)\mu_t}{\mu_g} \\
&\quad + \frac{\left(\frac{\beta_r\lambda_r}{\mu_r} + \mu_m\right)}{(1-a)P(1-\pi)(1-q)\mu_t}, \\
w_4 &= -\frac{(1-q)\beta_r\lambda_r}{\mu_r^2}, \quad w_8 = \frac{\tau\Omega\lambda_w}{(1-a)P(1-\pi)(1-q)\mu_t\mu_n^2} \left(\frac{\beta_r\lambda_r}{\mu_r} + \mu_m\right), \quad w_6 = 1, \\
w_9 &= -\frac{\tau\Omega\lambda_w}{\mu_a\mu_n} \left(\frac{\beta_r\lambda_r}{\mu_r} + \mu_m\right), \quad w_1 = w_2 = w_3 = 0.
\end{aligned}$$

Similarly, upon solving for the left eigenvector $v = [v_1, v_2, v_3, v_4, v_5, v_6, v_7, v_8, v_9]^T$ associated with the zero eigenvalue in system (28), we obtain

$$v_1 = v_2 = v_3 = v_4 = 0, \quad v_5 = P(1-a)(1-\pi)(1-q), \quad v_6 = 1, \quad v_7, v_8, v_9 = 0.$$

Next, we calculate the values of **a** and **b** as described in [11]. That is,

$$\mathbf{a} = \sum_{i,j,k=1}^9 v_k w_i w_j \frac{\partial^2 g_k(\mathbf{O}, 0)}{\partial x_i \partial x_j} \quad \text{and} \quad \mathbf{b} = \sum_{i,k=1}^9 v_k w_i \frac{\partial^2 g_k(\mathbf{O}, 0)}{\partial x_i \partial \beta_*}. \quad (30)$$

The non-zero partial derivatives of $g(x)$ in system (28) associated with the argument **a** are given as

$$\begin{aligned}
\frac{\partial^2 g_1}{\partial x_1 \partial x_2} &= -\beta_s, \quad \frac{\partial^2 g_2}{\partial x_1 \partial x_2} = -(1-v)\beta_s, \quad \frac{\partial^2 g_2}{\partial x_1 \partial x_9} = d(1-v)\frac{\lambda_h}{\mu_h}\beta_s, \\
\frac{\partial^2 g_4}{\partial x_4 \partial x_6} &= -(1-q)\beta_r, \\
\frac{\partial^2 g_4}{\partial x_6 \partial x_9} &= \frac{d(1-q)\beta_r\lambda_r}{\mu_r}, \quad \frac{\partial^2 g_5}{\partial x_5 \partial x_9} = -q, \quad \frac{\partial^2 g_5}{\partial x_4 \partial x_6} = (1-q)\beta_r, \\
\frac{\partial^2 g_5}{\partial x_6 \partial x_9} &= \frac{-d(1-q)\beta_r\lambda_r}{\mu_r}, \\
\frac{\partial^2 g_6}{\partial x_4 \partial x_6} &= -\beta_r, \quad \frac{\partial^2 g_6}{\partial x_5 \partial x_9} = P(1-a)(1-\pi)(1-q)d\mu_t,
\end{aligned}$$

$$\begin{aligned}\frac{\partial^2 g_7}{\partial x_5 \partial x_9} &= P\pi(1-a)(1-q), & \frac{\partial^2 g_8}{\partial x_3 \partial x_8} &= \frac{\partial^2 g_8}{\partial x_5 \partial x_8} = -\tau\lambda_w, \\ \frac{\partial^2 g_9}{\partial x_3 \partial x_8} &= \frac{\partial^2 g_9}{\partial x_5 \partial x_8} = \tau\lambda_w.\end{aligned}$$

Thus, the expression for **a** is:

$$\begin{aligned}\mathbf{a} &= v_5 w_5 w_9 \frac{\partial^2 g_5}{\partial x_5 \partial x_9} + v_5 w_4 w_6 \frac{\partial^2 g_5}{\partial x_4 \partial x_6} + v_5 w_6 w_9 \frac{\partial^2 g_5}{\partial x_6 \partial x_9} + v_6 w_4 w_6 \frac{\partial^2 g_6}{\partial x_4 \partial x_6} \\ &\quad + v_6 w_5 w_9 \frac{\partial^2 g_6}{\partial x_5 \partial x_9} \\ &= -\left(\frac{\beta_r \lambda_r}{\mu_r} + \mu_m\right) \left(\frac{d(1-\varrho)\beta_r \lambda_r}{\mu_r}\right) \left(\frac{P(1-a)(1-\pi)(1-q)\mu_t \tau \Omega \lambda_w}{\mu_a \mu_t \mu_n}\right) < 0. \quad (31)\end{aligned}$$

We repeat the above procedure in computing the values of **b**. The non-zero partial derivatives of $g(x)$ associated with **b** are given by

$$\begin{aligned}\frac{\partial^2 g_1}{\partial x_1 \partial \beta_*} &= -\frac{\lambda_h}{\mu_h}, & \frac{\partial^2 g_2}{\partial x_1 \partial \beta_*} &= \frac{-(1-v)\lambda_h}{\mu_h}, & \frac{\partial^2 g_3}{\partial x_1 \partial \beta_*} &= \frac{(1-v)\lambda_h}{\mu_h}, \\ \frac{\partial^2 g_4}{\partial x_6 \partial \beta_*} &= \frac{-(1-\varrho)\lambda_r}{\mu_r}, \\ \frac{\partial^2 g_5}{\partial x_6 \partial \beta_*} &= \frac{(1-\varrho)\lambda_r}{\mu_r}, & \frac{\partial^2 g_6}{\partial x_6 \partial \beta_*} &= \frac{-\lambda_r}{\mu_r}.\end{aligned}$$

Upon substituting these derivatives into Eq. (30), it follows that

$$\begin{aligned}\mathbf{b} &= v_5 w_6 \frac{\partial^2 g_5}{\partial x_6 \partial \beta_*} + v_6 w_6 \frac{\partial^2 g_6}{\partial x_6 \partial \beta_*} \\ &= \frac{\lambda_r}{\mu_r} (P(1-a)(1-\pi)(1-q)(1-\varrho) + 1) > 0. \quad (32)\end{aligned}$$

Thus, a is positive ($a > 0$) and b is negative ($b < 0$). Based on item (iv) of theorem 2 in [11], the malaria persistent equilibrium E_p is locally asymptotically stable for $R_0 > 1$, but close to 1.

Uncertainty and Sensitivity Analysis

Sensitivity Analysis of Infected and Infective States of the In-Host Malaria Model

The in-host malaria model in this paper has numerous unknown parameters, coupled with limited data. Therefore, there is a considerable uncertainty [9] in estimating the values of the 30 transmission parameters in model system (1). The uncertainty in the input values allows significant variability in the model predictions of the future malaria parasite density within the human host and the concentrations of the infected red blood cells and hence the severity of clinical *P. falciparum* malaria. Consequently, we perform the LHS/PRCC sensitivity analysis to evaluate variabilities in model predictions. With LHS/PRCC technique, we are able to explore the entire parameter space of model (1), with minimal computer simulation.

We simultaneously examine the effects of the LHS parameters on the two infected state variables: the infected liver hepatocytes $X(t)$ and the infected erythrocytes $T(t)$ and the

three infective parasite states: sporozoite $S(t)$, merozoites $M(t)$ and Gametocytes $G(t)$ in model system (1). The PRCCs are used to identify the key parameters contributing to the imprecision in predicting the future density of infected liver hepatocytes and infected red blood cells [9].

The process of performing LHS on disease models is described elsewhere [8,38] and references cited therein. Following PRCC methodology described in [52], we rank the LHS parameters in the LHS matrix together with the outcome measures: $T(t)$, $X(t)$, $M(t)$, $G(t)$ and $S(t)$. Two linear regression models are generated in response to each parameter and outcome measure. A Pearson rank correlation coefficient for the residuals from the two regression models gives the PRCC values for that specific parameter. Due to lack of data on the distribution function, we consider a uniform distribution for all model parameters in Table 3.

Results of Analyzing the LHS/PRCC for the In-Host Malaria Model

Using 1000 runs of Latin hypercube sampling, the PRCC and p value data from the 30 parameters in model system (1) are summarized in **Appendices A, B, C, D** and **E**. The PRCC and p values are computed based on the regression coefficient for outcome measures ($T(t)$, $X(t)$, $M(t)$, $S(t)$ and $G(t)$) and displayed in Tables 5, 6, 7 8 and 9, respectively. The size of PRCC shows the importance of the uncertainty in estimating the value of the specific variable in contributing to the prediction imprecision [9]. The PRCC sign (+ or −), however, shows the qualitative relationship between the input parameter and the output variables. Parameters with large PRCC values (PRCC from 0.5 to 1 or PRCC from −1 to −0.5) coupled with a corresponding small p value (p value < 0.05) are considered as the most influential [21].

A slight change in a highly influential parameter is likely to produce a significant change in the outcome variable. The symbol (*) indicates less influential parameters with p values < 0.05 and PRCC values in the range (−0.1 to −0.49 or 0.1 to 0.49). Further, the symbol (**) is used to indicate moderately critical parameters in prediction imprecision, and have p value < 0.05 and PRCC values in the range (−0.5 to −0.79 or 0.5 to 0.79). Lastly, the symbol (***) indicates the most critical and likely contributors to uncertainty, with PRCC values in the range (−0.8 to −0.99 or +0.8 to +0.99).

Results in Appendices A, B, C, D and E indicate that the rate of invasion by merozoites β_r , the rate of invasion of liver hepatocytes β_s , malaria vaccine efficacies (ϱ , ν , and χ) and the recruitment rates for the hepatocyte and erythrocytes λ_h and λ_r respectively are statistically significant in determining the variation of concentrations of erythrocytes, infected hepatocytes and the density of malaria parasites (sporozoites, merozoites and gametocytes). The infected erythrocyte burst size P and the number of merozoites released per bursting sporozoite N is shown to significantly increase the the density of the merozoites and the concentration of infected red blood cells T in blood. Clinical malaria controls should thus target these infected cells for eradication before they mature to bursting stage.

Moreover, from Appendices A and D, the proportion of merozoites that develop into gametocytes per dying infected erythrocyte π and immunosensitivity of infected erythrocytes q are shown to have significant influence in determining the concentrations of gametocytes and merozoites, respectively, during clinical *P. falciparum* infection. An efficacious blood stage vaccine coupled with a transmission blocking vaccine antigens has the potential of reducing the density of gametocytes during clinical malaria. This reduces parasite transmission to mosquito vector.

Table 3 Table showing the numerical values of model parameter values used in sensitivity analysis and numerical simulations

Symbol	Value	Min	Max	Unit	Source
Λ	30	20	35	Sporozoites/day	[56]
μ_s	1.2	0.8	1.5	/day	[56]
λ_r	3E5	3E3	3E6	cells/ml/day	[36]
λ_h	3E5	3E3	3E6	cells/ μl^{-1} /day	[61]
β_r	8E−5	8E−7	8E−3	/mm ³ /day	[15]
β_s	5E−4	5E−5	5E−3	/mm ³ /day	[56]
μ_h	0.029	0.01	0.03	/day	[49]
μ_x	0.02	0.01	0.05	/day	[56]
π	0.2	0.1	0.9	Unitless	[59]
μ_r	8.3E−5	8.3E−6	0.0001	/day	[1]
μ_m	48	46	49	/day	[36]
μ_t	0.7	0.5	0.8	/day	[37]
μ_g	0.0625	0.5	0.78	/day	[56]
χ	0.6	0	1	Unitless	Assumed
ϱ	0.45	0	1	Unitless	[6]
ν	0.7	0	1	Unitless	Assumed
d	5E−4	1E−4	0.001	Unitless	Assumed
τ	1.5	1.2	1.9	Unitless	[45]
λ_w	30	25	35	/unitless/day	[45]
b	0.17	0.2	0.5	Unitless	[45]
a	0.16	0.2	0.4	Unitless	[45]
P	16	13	20	/erythrocytes/day	[13]
N	10,000	8000	11,000	/day	[60]
ϵ_0	1E−5	1E−6	1E−4	/day	[60]
ϵ_1	1E−5	1E−6	1E−4	/day	[60]
q	0.01	0.001	0.02	Unitless	[45]
ρ	0.3	0.2	0.45	Unitless	[45]
μ_n	0.4	0.3	0.72	/day	[1]
μ_a	0.4	0.3	0.72	/day	[36]
Ω	30	24	33	Unitless	[37]

Figures 2 and 3 illustrates Monte Carlo simulations for some of the parameters with the greatest PRCC values against model R_0 .

It is evident that a highly efficacious pre-erythrocytic vaccine such as RTS,S/SA01 (with efficacy ν) and blood stage vaccine such as MSP3 (with efficacy ϵ) could effectively help in reducing parasitemia and malaria severity during clinical *P. falciparum* infections. Sporozoite invasion rate β_s and the merozoite invasion parameter β_r are also shown to increase the model R_0 and hence the severity of the disease within the human host.

Although the LHS/PRCC technique is useful in identifying significant parameters in the in-host malaria model, it fails to rank the parameters in order of their contribution to variations and disease progression. This ranking is very crucial in the design of clinical interventions

Fig. 2 The Monte Carlo simulations for some of the parameters with the greatest PRCC magnitudes, using values in Table 3 and 1000 simulations per run. The parameters are: **a** sporozoite invasion rate β_s and **b** efficacy of pre-erythrocytic vaccine ν

Fig. 3 The Monte Carlo simulations for some of the parameters with the greatest PRCC magnitudes, using values in Table 3 and 1000 simulations per run. They are: **a** proportion of parasites that become gametocytes π , **b** merozoite invasion rate β_r , **c** efficacy of blood stage vaccine e and **d** the number of merozoites produced per bursting blood schizont P

Table 4 The sensitivity results

Parameter	S.I	Parameter	S.I
P	+1	a	−0.785714
β_s	+1	π	−0.785714
λ_h	+1	β_r	+0.62402
μ_h	−1	λ_r	+0.62402
μ_x	−1	μ_m	−0.62406
ϱ	−0.785714	μ_r	−0.62406
ν	−0.785714	q	−0.428571

and in the development of malaria vaccines. In response, we consider the normalized forward sensitivity index of the model reproduction number in (15) and (16) against LHS parameters in Table 3 in the next section.

Sensitivity Analysis Based on Model R_0

We adopt the normalized forward sensitivity (NFS) index technique described in [4] to compute the sensitivity indices of the model R_0 with respect to input parameters P , β_r , β_s , λ_h , λ_r , μ_h , μ_r , μ_x , μ_m , ϱ , a , π , ν are presented in Table 4. The sensitivity indices (S.I) are evaluated in Mathematica software based on the baseline parameter values in Table 3.

From Table 4, we observe that the population of malaria merozoites that are released per bursting infected erythrocyte P , the rates of invasions of healthy erythrocytes β_r and hepatocytes β_s , the rate of recruitment of susceptible hepatocytes λ_h and erythrocytes λ_r have a direct effects on the severity of clinical malaria. A 10% increase (or decrease) in the magnitude of P , increase (or decrease) model R_0 by 10%. Clinical malaria control should aim at eradicating or reducing these parameters. On the other hand, malaria vaccines (ϱ and ν) are shown to reduce R_0 when their efficacies are improved. An increase (or decrease) in the immunosensitivity of infected red blood cells q is similarly shown to reduce (or increase) the model R_0 . By rank, the parameters P , β_s , λ_h are the most influential in model (1), followed by the vaccine efficacies ϱ and ν . Clinical considerations should thus favour these parameters in the development of malaria vaccines.

In conclusion, the LHS/PRCC technique is robust and considers the entire parameter space in determining significant parameters in a disease model. However, it fails the ranking test. On the other hand, although the NFS index technique is beneficial in discerning and ranking significant parameters in a model, it does not explain the considerable uncertainties in estimating parameter values. It therefore fails to uncover parameter effects on particular variables or populations in the model. Due to the importance attached to the significance and rank of each parameter in the model, we advise that both approaches be used in future analysis of parameter uncertainty and sensitivity analysis in disease models.

Numerical Simulation

In this section, we illustrate numerically the dynamics of the in-host model system (1). When the basic reproduction number R_0 is less than unity, clinical *P. falciparum* malaria is observed to die out (see Figs. 4, 5) and the malaria-free state $E_0 = (0, 5 \times 10^5, 0, 0, 5 \times$

Fig. 4 The dynamics of model (1) when the basic reproduction number $R_0 = 0.8881 < 1$, the malaria-free equilibrium E_0 is globally asymptotically stable

$10^6, 0, 0, 0, 0, 0, 0$) is globally asymptotically stable. Additionally, only an efficacious BSV has the chance to totally eradicate the merozoites from the blood stream of the human host. Notice that even if R_0 is less than unity, some activated CD8⁺ T-cells $W_a(t)$ still exists (see Fig. 4e). This shows that individual-level acquired immunity against malaria is maintained for some period of time even if all malaria parasites are eradicated from the host. All simulations are performed using parameter values in Table 3.

Fig. 5 Population dynamics of the liver hepatocytes and the sporozoites when the reproduction number $R_0 = 0.8881 < 1$. Parameter values are available in Table 3

Similarly, the density of sporozoites decline rapidly when $R_0 = R_{01} < 1$, leading to a corresponding decrease in the density of infected hepatocytes as shown in Fig. 5.

The persistent steady state, $E_p = (S^*, H^*, X^*, R^*, T^*, M^*, G^*, W_n^*, W_a^*) = (10^3, 3 \times 10^5, 3 \times 10^3, 5 \times 10^6, 5 \times 10^3, 10^3, 5 \times 10^2, 5 \times 10^3, 5 \times 10^3)$ is shown to be stable when R_0 is greater than unity, $R_0 = 1.3364 > 1$ (See Figs. 6, 7). We observe that the densities of sporozoites, merozoites, gametocytes, infected hepatocytes and infected erythrocytes grow and persists both at the pre-erythrocytic and blood stages of parasite development.

Multi-component and Multi-stage Vaccine Cocktail

Here, we illustrate the need to develop a multi-component and multi-stage *P. falciparum* malaria vaccines with sufficient prospect to minimize the severity of malaria infection from human host. Several strategies have been considered in the development of efficient multi-component and multi-stage *P. falciparum* vaccine formulations [16,51,53,57,58,67]. A synthetic peptide (SPf66) that combines antigens from the blood stages of malaria linked together with an antigen from the sporozoite stage reduced the number of first attacks with *P. falciparum* by 28% in South America [24]. High level of sterile efficacy and safety of combining pre-erythrocytic vaccine, RTS,S/AS01B with vectored vaccines are reported in [53].

Fig. 6 When the reproduction number $R_0 = 1.3364 > 1$, the malaria-persistent equilibrium point E_p is stable for the chosen set of parameter values. The model parameter values are available in Table 3

Plasmodium falciparum chimeric protein 2.9 which is a combination of the C-terminal regions of domain III of AMA-1 and 19-kDa of MSP1 induced both anti-MSP1–19 and anti-AMA-1(III) Abs at levels 11- and 18-fold higher, respectively, than individual components did [51]. Studies in [19] have also shown that a combination of multiple vaccine candidates in fusion proteins may lead to improved characteristics of the vaccine.

Fig. 7 When the reproduction number $R_0 = 1.3364 > 1$, the malaria-persistent equilibrium E_p is stable

Synergism from combination of transmission blocking vaccines is shown in [67] to confer protection by simultaneously inducing multiple, independent immune responses directed towards *P. falciparum* sporozoites. This ensures high levels of vaccine efficacy against the parasite. The need for multi-stage malaria vaccine is also emphasised in [16] where DNA vaccines are shown to have high capacity to induce CD8(+) cytotoxic T lymphocytes and interferon-gamma responses in humans during *P. falciparum* infection [62].

From these studies, it is evident that a multi-stage specific vaccine cocktail has the potential to prevent initial malaria infection at the pre-erythrocytic stage, reduce or eradicate clinical manifestation and prevent the transmission of gametocytes from infected human hosts to susceptible feeding female anopheles mosquito [10]. We therefore evaluate numerically the effects of malaria vaccine combinations on the severity of *P. falciparum* malaria infection. The vaccines are broadly categorized as: pre-erythrocytic vaccines, blood stage vaccines and transmission blocking vaccines. The vaccines are assumed to have an optimal efficacy of 75%. We chose this figure in consistency with the WHO strategic goal of developing a *P. falciparum* vaccine with atleast 75% efficacy [43].

Results in Fig. 8, shows the effects of all the three vaccines combined with a constant optimal efficacy of 75%. The density of infected erythrocytes T and infected hepatocytes X is shown to fall significantly. The severity of malaria infection is therefore highly reduced when the three vaccine antigens are combined.

Fig. 8 Impact of a 75% efficacious combined malaria vaccines on the dynamics of infected red blood cells T and infected hepatocytes X . The simulations are performed when $R_{01} = 2.564 > 1$, and $R_{02} = 5.564 > 1$

Fig. 9 Impact of a 75% efficacious transmission blocking vaccine on the dynamics of infected red blood cells and infected hepatocytes. The simulations are performed when $R_{01} = 2.564 > 1$, and $R_{02} = 5.564 > 1$

The use of only one vaccine is shown in Figs. 9, 10 and 11 to have minimal effects on the severity of malaria infection.

Finally, a combination of any two vaccines (with different antigens) is likely to moderately reduce the severity of malaria infection. The effects of paired—vaccine combinations are as shown in Figs. 12, 13 and 14. Unlike infected hepatocytes, transmission blocking vaccines are shown to have minimal effects on the density of infected red blood cells (see Fig. 10).

A combination of an efficacious pre-erythrocytic vaccine and an effective blood stage vaccine antigens has the potential to significantly reduce malaria severity as shown in Fig. 14. Moreover, a combination of antigens from transmission blocking vaccine and those of pre-erythrocytic vaccine at the liver stage, as shown in Fig. 12b, are very effective in suppressing the density of infected hepatocytes in the liver.

Fig. 10 Impact of a 75% efficacious pre-erythrocytic vaccine on the dynamics of infected red blood cells and infected hepatocytes. The simulations are performed when $R_{01} = 2.564 > 1$, and $R_{02} = 5.564 > 1$

Fig. 11 Impact of a 75% efficacious blood stage vaccine on the dynamics of infected red blood cells and infected hepatocytes. The simulations are performed when $R_{01} = 2.564 > 1$, and $R_{02} = 5.564 > 1$

Fig. 12 The effect of combining a PEV and TBV that are both 75% efficacious on the dynamics of infected red blood cells and infected hepatocytes. The simulations are performed when $R_{01} = 2.564 > 1$, and $R_{02} = 5.564 > 1$

Fig. 13 The effect of combining a TBV and a PEV that are both 75% efficacious on the dynamics of infected red blood cells and infected hepatocytes. The simulations are performed when $R_{01} = 2.564 > 1$, and $R_{02} = 5.564 > 1$

Fig. 14 The effect of combining a PEV and a BSV that are both 75% efficacious on the dynamics of infected red blood cells and infected hepatocytes. The simulations are performed when $R_{01} = 2.564 > 1$, and $R_{02} = 5.564 > 1$

Epidemiological Implications and Conclusion

Results of uncertainty and sensitivity analysis have significant epidemiological importance in malaria control. The presented model is shown to be sensitive to variations in vaccine efficacies χ , ϱ and v . Only efficacious malaria vaccines have a chance to eradicate *P. falciparum* malaria or to reduce its severity, significantly, during clinical infection. Sensitivity indices based on the model reproduction number indicates that the rate of invasion of susceptible erythrocytes by merozoite parasites β_r and the rate of invasion of liver hepatocytes by the sporozoites β_s are highly sensitive to malaria disease progression. Malaria vaccines must therefore target and eradicate the infective parasites and infected cells. Other parameters such as the proportions of merozoites that become gametocytes per dying blood schizont π and the average number of merozoites released per bursting blood schizonts P are also shown to have a considerable impact on the model R_0 and hence the severity of infection.

A highly efficacious blood stage vaccine is necessary to lower the rate π . A reduction in the density of blood gametocytes has the potential of lowering malaria transmission from the human host to the mosquito vector.

Given the protective capacity of liver stage-specific CD8⁺ T-cells [65], a lot more research that focuses on developing an efficacious blood stage vaccine that would prime blood trophozoite-specific CD8⁺ T-cells is called for. The parameter τ which accounts for vaccine-induced production of CD8⁺ T-cells is shown to have significant negative effects on the concentration of infected hepatocytes. The more efficient the pre-erythrocytic vaccine is, the lower the density of released merozoites into the host's blood. Moreover, a rapid eradication of parasitized liver hepatocytes is likely to result into less infected erythrocytes at the blood stage. An efficacious pre-erythrocytic vaccines has a potential to eradicate infected liver hepatocytes. Such efficacious liver stage vaccines may also reduce the overall burst size of an infected hepatocyte, leading to reduced erythrocytic schizonogy and hence the severity of clinical *P. falciparum* malaria.

Multiple combination of malaria vaccine antigen has a great potential in reducing the severity of malaria infections due to *P. falciparum* parasite. The synergy of combining pre-erythrocytic vaccines, blood stage vaccines and transmission blocking vaccines antigens induces multiple immune responses with the potential to prevent or eradicate malaria infection. As highlighted in [32], our study shows that a highly effective vaccine combination is critical for *P. falciparum* malaria disease elimination goal.

Although efficacious malaria vaccines are shown to be very effective in reducing severity of clinical malaria, our results further confirm the need to combine vaccine with existing anti-malarial therapy to achieve a complete eradication of the parasites from the host. Finally, the LHS/PRCC analysis revealed that the prediction imprecision was mainly due to certain key parameters. Long term precise predictions of the concentrations of infected liver hepatocytes and infected red blood cells would be difficult until these key parameters are correctly determined. A great understanding of parameter uncertainty and sensitivity analysis of in-host malaria in humans is necessary in order to develop effective vaccines against *P. falciparum* pathogen.

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Compliance with Ethical Standards

Conflict of interest The authors declare that there is no conflict of interest regarding the publication of this article.

Appendix A: PRCC Between Infected Red Blood Cells T and Each Parameter

See Table 5.

Table 5 PRCC between infected red blood cells T and each parameter

Parameter	PRCC	p value	Parameter	PRCC	p value
Λ	0.040124	0.69185	ν	-0.35423*	0.0001255
μ_s	0.092654	0.35921	d	-0.021968	0.82825
λ_h	0.16815	0.094459	λ_w	0.02639	0.79438
λ_r	0.99993***	1.2467E-191	τ	0.028815	0.77596
β_r	0.60394**	0.0030343	a	-0.033076	0.7439
β_s	-0.072861	0.47128	b	-0.037844	0.70854
μ_h	0.045043	0.65633	P	0.28652*	0.0038515
μ_x	0.022164	0.82674	N	0.50348**	0.0003056
π	0.24376	0.14528	ϵ_0	-0.050167	0.62012
μ_r	-0.54174**	5.8708E-9	ϵ_1	0.0092028	0.92759
μ_t	-0.043585	0.66677	ρ	0.087162	0.38852
μ_m	-0.08574	0.39634	q	-0.261905*	0.00154063
μ_g	-0.13006	0.19715	μ_n	-0.093091	0.35694
χ	-0.13607	0.17706	μ_a	-0.054864	0.58771
ϱ	-0.30701	0.0018909	Ω	0.041721	0.68024

The results are significant at the 0.05 level

Appendix B: PRCC Between Infected Hepatocytes X and Each Parameter

See Table 6.

Table 6 PRCC between infected hepatocytes X and each parameter

Parameter	PRCC	p value	Parameter	PRCC	p value
Λ	-0.034437	0.73375	ν	-0.307831*	0.00093836
μ_s	0.071002	0.48269	d	-0.1442	0.15231
λ_h	0.9749***	9.5419E-66	λ_w	-0.094174	0.35135
λ_r	0.06407	0.52654	τ	-0.20137*	0.000044531
β_r	-0.080809	0.42415	a	-0.075135	0.45751
β_s	0.53926**	0.00016701	b	0.061933	0.54045
μ_h	-0.87273***	2.7908E-23	P	0.076347	0.45026
μ_x	0.14762	0.14273	N	0.10516	0.29776
π	-0.045846	0.6506	ϵ_0	0.21125	0.034877
μ_r	0.094998	0.34713	ϵ_1	-0.16583	0.09918
μ_t	0.12588	0.21205	ρ	0.033235	0.74272
μ_m	0.13493	0.18073	q	0.095106	0.34658
μ_g	0.033064	0.74399	μ_n	0.039223	0.69843
χ	-0.28902	0.0035417	μ_a	-0.052237	0.60575
ϱ	-0.061742	0.5417	Ω	0.10411	0.30264

The results are significant at the 0.05 level

Appendix C: PRCC Between Merozoites M and Each Parameter

See Table 7.

Table 7 PRCC between merozoites M and each parameter

Parameter	PRCC	p value	Parameter	PRCC	p value
Λ	0.013086	0.89718	ν	-0.61866**	6.9194E-12
μ_s	0.12012	0.23389	d	0.12239	0.22509
λ_{fh}	-0.005884	0.95367	λ_w	-0.30208	0.0022545
λ_r	-0.78852**	2.0731E-22	τ	-0.11155	0.26918
β_r	-0.77199**	5.3565E-21	a	0.14416	0.15243
β_s	0.10834	0.28328	b	-0.18907	0.059575
μ_h	0.080773	0.42436	P	0.28652*	0.0038515
μ_x	0.46617*	1.0183E-06	N	-0.50348**	0.0003056
π	-0.29849	0.0025577	ϵ_0	-0.23892	0.01667
μ_r	0.060227	0.55168	ϵ_1	-0.057548	0.56955
μ_t	-0.05975	0.55485	ρ	-0.25708	0.0098207
μ_m	-0.090522	0.37042	q	-0.047855	0.63635
μ_g	0.18256	0.069072	μ_n	-0.0015737	0.9876
χ	-0.67251**	1.8298E-14	μ_a	0.1363	0.17632
ϱ	-0.024582	0.80818	Ω	-0.12172	0.22769

The results are significant at the 0.05 level

Appendix D: PRCC Between Gametocytes G and Each Parameter

See Table 8.

Table 8 PRCC between gametocytes G and each parameter

Parameter	PRCC	p value	Parameter	PRCC	p value
Λ	0.09861	0.32902	ν	-0.54919**	3.2858E-09
μ_s	-0.18356	0.067537	d	-0.33627	0.00062476
λ_h	0.16929	0.092216	λ_w	-0.14215	0.15831
λ_r	-0.79454***	5.8878E-23	τ	-0.080527	0.42578
β_r	-0.76958**	8.4228E-21	a	-0.16499	0.10092
β_s	-0.021251	0.83378	b	-0.31287	0.0015279
μ_h	0.060337	0.55095	P	0.059063	0.55941
μ_x	0.40198*	3.3899E-05	N	0.24601	0.013619
π	0.698883**	0.0032768	ϵ_0	-0.28727	0.0037563
μ_r	-0.022582	0.82353	ϵ_1	0.075806	0.45349
μ_t	0.075502	0.45531	ρ	-0.047433	0.63933
μ_m	0.067991	0.50149	q	0.12264	0.22416
μ_g	-0.084857	0.40124	μ_n	-0.12368	0.22021
χ	-0.55186**	2.6601E-09	μ_a	0.17443	0.082611
ϱ	-0.55799**	1.6273E-09	Ω	0.15261	0.12956

The results are significant at the 0.05 level

Appendix E: PRCC Between Sporozoites S and Each Parameter

See Table 9.

Table 9 PRCC between sporozoites S and each parameter

Parameter	PRCC	p value	Parameter	PRCC	p value
Λ	0.31314*	0.00097533	ν	0.0067811	0.94661
μ_s	-0.68707**	0.0094591	d	0.010905	0.91425
λ_h	-0.012635	0.90071	λ_w	0.0043436	0.96579
λ_r	0.03593	0.72266	τ	0.023508	0.81642
β_r	0.025432	0.80168	a	0.0065303	0.94859
β_s	-0.0091069	0.92835	b	-0.017604	0.86199
μ_h	0.022838	0.82156	P	-0.019561	0.84683
μ_x	0.0056574	0.95545	N	-0.015923	0.87505
π	-0.0047473	0.96261	ϵ_0	-0.00027845	0.99781
μ_r	0.00025243	0.99801	ϵ_1	-0.0003386	0.99733
μ_t	-0.011016	0.91338	ρ	-0.031317	0.75709
μ_m	0.0096995	0.9237	q	-0.0037326	0.9706
μ_g	-0.023075	0.81974	μ_n	0.030253	0.7651
χ	-0.699368*	0.0001184	μ_a	-0.0042258	0.96672
ϱ	0.01863	0.85403	Ω	-0.0052564	0.95861

The results are significant at the 0.05 level

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