Mathematical Modelling of the Interaction of *Chlamydia Trachomatis* with the Immune System

Masoumeh Bagher Oskouei*†, Dann G. Mallet*‡, Ashkan Amirshahi‡, Graeme J. Pettet*‡,

Abstract—**In this paper, we present a two dimensional mathematical model capable of describing the process of the ascension of infection to the upper genital tract in females and investigating the number of free chlamydial particles and infected cells. This model includes diffusion and chemotaxis terms corresponding to the motion of free chlamydial particles and the migration of immune cells toward the infection site in response to diffusible chemical cues such as $IFN-\gamma$, respectively. The qualitative results of the model reflect experimentally observed phenomena.**

Keywords: mathematical model,* Chlamydia trachomatis*, partial differential equation, immune

1 Introduction

Chlamydia trachomatis is an obligate intracellular bacterial pathogen that infects the genital and ocular mucosa of humans causing sexually transmitted disease and trachoma. It is estimated that 70-75% of endocervical infections in women caused by *C. trachomatis* are asymptomatic and may persist for months to years [4]. The sequelae of *C. trachomatis* genital tract infections in women, namely chronic pain, pelvic inflammatory disease (PID), infertility and ectopic pregnancy are the most costly outcomes of any sexually transmitted infection except human immunodeficiency virus (HIV/AIDS), resulting in an estimated $US4 billion in health care costs per annum only United States alone [5].

C. trachomatis is an intracellular, complex and multifunctional process pathogen, unique among prokaryotes because of a biphasic developmental cycle of replication in which the organism exists in two distinctive forms; the Elementary Body (EB), which is the infectious form, and the Reticulate Body (RB), which is the replicating structure. In this developmental cycle (see Fig. 1), the infectious but metabolically inactive elementary body, 200-300 nm in diameter, is endocytosed by eukaryotic cells and resides within a cytoplasmic inclusion. Within the inclusion, the EBs transform into the non-infectious but metabolically active reticulate body which is larger at 1000-1500 nm in diameter. The RBs divide within the cell by binary fission and transform back to the infectious form before being released to the cell exterior [1, 3].

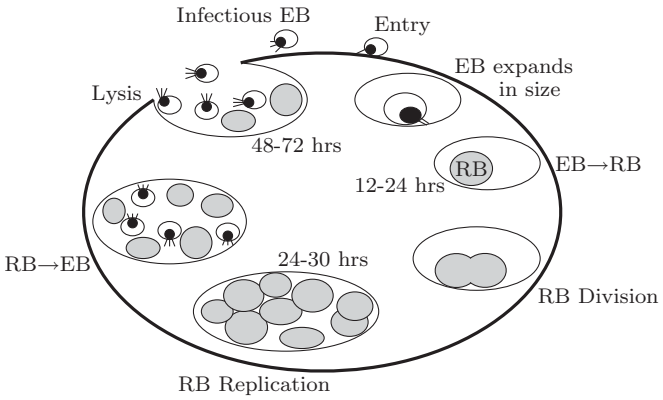

Figure 1: Schematic of the *Chlamydia trachomatis* Serovar D Developmental Cycle.

Wilson [16] indicates that 200-500 new EBs are released from each infected cell following the replication process. This is an initial indicator of how the *C. trachomatis* infection can progress and subsequently reach the upper genital tract. It is also important though to note that the host immune system has a significant role in the clearance of *Chlamydia* throughout the infection period. *C. trachomatis* is subjected to both innate and adaptive immune responses of the host. Wilson for example, has shown that CD4+ T cells play a significant role in adaptive immunity to *Chlamydia trachomatis* infection of the genital tract [14]. Moreover the recent studies on human clinical samples and also animal models clearly show that it normally takes 4 to 7 days for the immune response to be induced. The next section will focus more closely on how chlamydial particles interact with immune cells.

In addition to the effect of immune system, the female menstrual cycle influences *Chlamydia* growth. The cycle has three phases: follicular, ovulatory, and luteal (see Fig.2).The menstrual cycle is regulated by luterinizing

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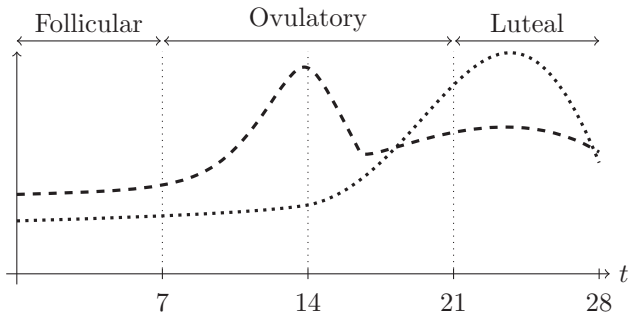

Figure 2: Schematic of hormone levels during the normal menstrual cycle (progesterone – dotted line, estrogen – dashed line).

hormone (LH), follicle-stimulating hormone (FSH), estrogen, and progesterone. A sharp drop in the levels of estrogen and progesterone results in a shedding of the endometrial lining of the uterus (see Fig.3). Estrogen and progesterone are produced in the ovaries and are regulated by other hormones produced in the brain. Of importance to this study is the fact that a simple vaginal infection, such as a chlamydial infection, will not affect the production or regulation of these reproductive hormones. However, the hormones have a significant effect on *Chlamydia* growth. Recent studies [7, 8] show that susceptibility and inflammation are induced in high progesterone environments and also estrogen decreases inflammation and protection from infection. The rate of infection increases when the level of progesterone is higher [17].

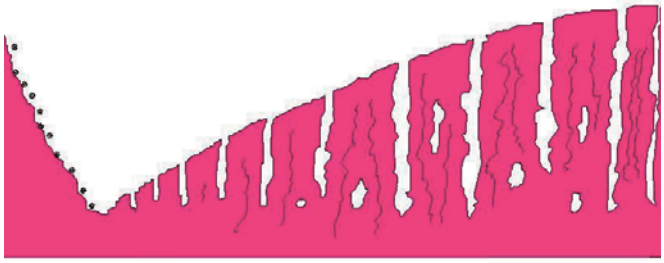

Figure 3: Endometrial changes during the normal menstrual cycle.

In vitro, providing all the factors mentioned above is not straightforward. Moreover, there are the several differences in the *Chlamydia* infection of laboratory animals such as mice, compared with the infection in humans such as length of infection and the immune-evasion mechanism. Therefore a mathematical model describing the interaction between *C. trachomatis* and the immune system is an extremely useful way to obtain further insights into the dynamics of the infection process, and the subsequent effects and possible effective control strategies.

In this paper we develop a mathematical model to investigate the interaction between *C. trachomatis* and the host immune system. We present preliminary results regarding infection dynamics and progression of infection in the genital tract and to track the motion of free chlamydial particles toward the upper genital level.

The remainder of this paper is organised as follows. A brief description of *C. trachomatis* and immune system interactions is provided in Section 2. Section 3 is a detailed description of our partial differential equation based mathematical model of the immune response related to *Chlamydia trachomatis*. Then we present preliminary numerical results from simulations of the mathematical model. Finally, a discussion and conclusions regarding the model are made in Section 5.

2 The host immune system and *C. trachomatis*

The innate and adaptive immunity are two essential barriers against *Chlamydia*. The innate immune system is a non-specific system that provides immediate defence against infection. Macrophages, neutrophils and dendritic cells are the key elements of the innate immune system. Pattern recognition receptors (PRRs) are proteins on the surface of both epithelial cells and circulating cells of the innate immune system. The toll-like receptors (TLRs) and NOD-like receptors (NLRs) are the two most important families of PRRs and are able to recognise and bind pathogen-associated molecular patterns (PAMPs). The adaptive immune system, on the other hand, is a specific system which develops after first contact with a pathogen. Humoral immunity (B lymphocytes) and cell mediated immunity (T lymphocytes) are two arms of the adaptive immune response (see Fig. 4).The B lymphocytes are triggered by antigen-presenting cells (APCs), cells of the innate response or T cells. APCs stimulate B cells to produce antibodies which bind to antigens on the surface of the invading pathogen to flag them for neutralisation. Similarly, T cells are activated by APCs, cells of the innate response or B lymphocytes. T lymphocytes consist of T helper cells (Th cells) and cytotoxic T cells (CD8+ cells or killer cells). Th cells produce proinflammatory cytokines such as IL-4, IL-5, IL-6, and IFN-γ which both support the the humoral system and inhibit pathogen growth. Cytotoxic T cells directly attack and eliminate a pathogen. Although humoral immunity is the principal immunological response effective against extracellular bacteria, the major defensive and protective response against intracellular bacteria is performed by T cells [11, 10]. Because *Chlamydia* is present both extra- and intracellularly, both of these elements of the immune system are important when modelling chlamydial infections.

In terms of *C. trachomatis* infection, when a chlamydial

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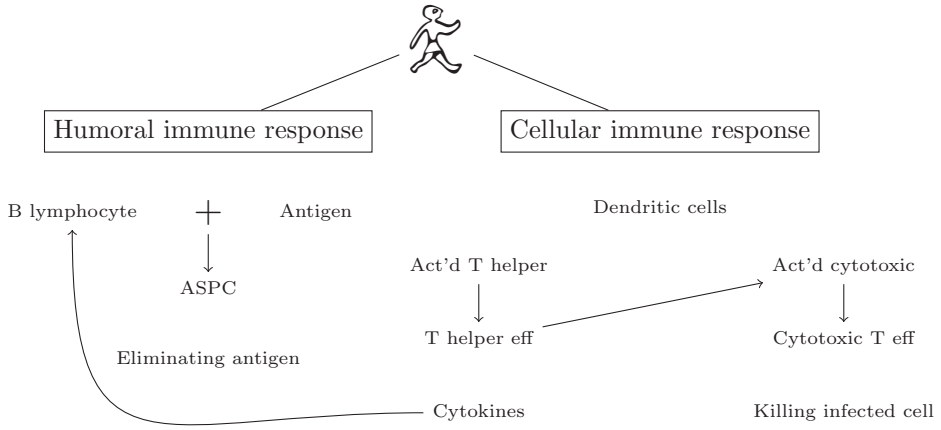

Figure 4: Schematic showing components of the immune system.

particle enters the body, epithelial cells are the first line of defence against the pathogen. PRRs on the surface of the epithelial cells bind to the antigens expressed on the surface of *C. trachomatis*. This connection stimulates the epithelial cells to secrete chemokines and other pro-inflammatory cytokines which initiate circulating cells of the innate immune response. Once the innate immune cells such as macrophages, dendritic cells and neutrophils reach the infection site, they are activated and their PRRs bind to chlamydial antigens leading to pathogen destruction. Activated macrophages and dendritic cells are able to express pathogen antigens bound to major histocompatibility complex (MHC) proteins on the surface and to serve as APCs which can activate the adaptive immune cells. Under stimulation of activated dendritic cells, T cells move toward the site of infection and differentiate into either cytotoxic T cells (CD8+ T cells or T_c cells) or helper T cells (CD4+ T cells). Cytotoxic T cells (CD8+) are activated when their receptors strongly interact with the MHC class I molecule presented at the site of infection and perform their function by releasing lymphotoxins that form pores in the target cell's plasma membrane and cause it to burst or lyse. On the other hand, T helper cells (CD4+) interact with MHC class II molecules and produce pro-inflammatory cytokines such as interferon-gamma (IFN-γ) that inhibit chlamydial growth, and activate macrophages and humoral cells in order to accelerate clearance of infection [12, 13, 9].

3 Mathematical model

To model chlamydial infection in the genital tract, we use a rectangular domain $\Omega = [0, L_x] \times [0, L_y]$, where the side boundaries are subject to a wrapping condition. This results in a domain shaped like the exterior surface of a cylinder – an oversimplified picture of the surface of the genital tract (see Fig. 5).

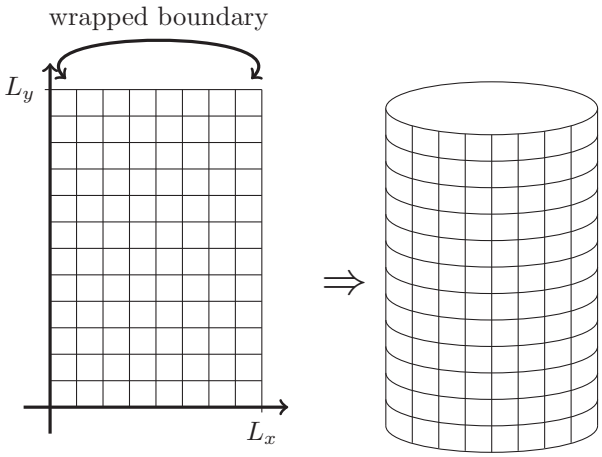

Figure 5: Computationally, the genital tract is modelled using a rectangular grid that is then subjected to wrapped boundary conditions for $x = 0$ and $x = L_x$.

In order to simulate the infection process and the effectiveness of immune responses during three different phases of the female menstrual cycle, we will solve the model equations over a time range of 28 days, the average length of the female menstrual cycle.

As we discussed in the previous section, free chlamydial particles and intracellular *Chlamydia* are subjected to the effects of several different elements of the immune system including circulating cells of the innate immune system and the adaptive immune system. Moreover, cytokines and chemokines are produced in response to the infection caused by *C. trachomatis*. In this paper, K represents the concentration of cytokines and chemokines. In order to simplify the complexity induced by considering a large number of immune cells involved in the clearance of *C. trachomatis*, here we denote by H those immune cells that clear free extracellular chlamydial particles and

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by M the components of the immune system that attack the intracellular *Chlamydia*. The free chlamydial particles, uninfected epithelial cells, and infected cells are represented by C, E and I respectively.

To model the events described in the previous section, we assume that initially there are some free chlamydial particles at the lower region of the genital tract and represent this using the initial condition

$$C(x, y, 0) = 10\mathrm{e}^{-(y-1)^2}.$$

Initially there are no infected cells (all chlamydial particles are initially outside of the host cells) and epithelial cells are at their normal concentration levels throughout the domain. Further, there is initially an absence of chemokines/cytokines and of components of the immune system that attack *Chlamydia* when it is inside host cells. Immune cells that clear free *Chlamydia* particles are initially circulating in the system at some 'normal' level given. Hence, initially we have

$$E(x, y, 0) = E_{\max},$$

$$I(x, y, 0) = K(x, y, 0) = M(x, y, 0) = 0,$$

$$H(x, y, 0) = H_0.$$

We make the assumption that each of the species of interest in our model is subject to a no-flux condition at the upper and lower ends of the domain; in the other words, they do not leave or enter the system via those end regions. Therefore, we have boundary conditions

$$\frac{\partial C}{\partial y} = \frac{\partial E}{\partial y} = \frac{\partial I}{\partial y} = \frac{\partial H}{\partial y} = \frac{\partial M}{\partial y} = \frac{\partial K}{\partial y} = 0 \qquad (1)$$

at the boundaries $y = 0$ and $y = L_y$.

For free chlamydial particles, we assume that the particles diffuse in the Fickian sense, with diffusion coefficient D_C. When infected cells burst at rate κ, P new EBs are released from each infected cell. The internalisation of EBs into the epithelial cells during the infection process occurs at rate $g(t)$ and varies depending on the current phase of the female menstrual cycle. Chlamydial particles are cleared due to the humoral immune response, however as previously mentioned, the immune response to *Chlamydia* is delayed and this is modelled using the expression $h(t)$. Combining these elements, we obtain the free particle conservation equation

$$\frac{\partial C}{\partial t} = D_C \nabla^2 C + P\kappa I - g(t)CE - h(t)CH. \qquad (2)$$

By day 5 to 7 of the menstrual cycle, shedding of the endometrial lining of the uterus results in a drop in the rate of EB internalisation. Vaginal epithelial thickness is minimal during the first phase, and increases in thickness throughout the reproductive phase [18]. Therefore, the rate of EB internalisation gradually increases right after follicular phase until reaching a steady state level in the high progestrone environment. Thus we model the rate of EB internalisation using

$$g(t) = \begin{cases} 2.7 \times 10^{-6}(\exp(t) - 1) + 0.001 & 0 \le t \le 7 \\ 1.1 \times 10^{-4} t^2 - 0.0013 & 7 < t \le 14 \\ 0.02 & \text{otherwise.} \end{cases}$$

The delay of the innate and humoral immune response is imposed using the function

$$h(t) = \begin{cases} 7.3 \times 10^{-6}(\exp(t) - 1) + 0.001 & 0 \le t \le 7 \\ 0.1 & \text{otherwise.} \end{cases}$$

The population of uninfected epithelial cells is also allowed to undergo Fickian diffusion, with coefficient D_E. Epithelial cells are also assumed to be produced according to a logistic growth law to the normal/maximum cell level of $E_{\max}$ and to die naturally at rate μ_1. The cells are also lost to the infected population when they come in contact with free Chlamydia particles at rate $g(t)$. Combining these we obtain the conservation equation

$$\frac{\partial E}{\partial t} = D_E \nabla^2 E + E(E_{max} - E) - g(t)CE - \mu_1 E. \quad (3)$$

Infected cells also diffuse randomly with coefficient D_I. The internalisation of EBs into the epithelial cells causes an increase in the number of infected cells at rate $g(t)$. The infected cell population decreases due to the lysis of cells when the intracellular developmental cycle is complete, with a lysis rate of κ. Finally, the cell mediated immune system, with a delayed response modelled by $s(t)$, decreases the number of infected cells. This gives

$$\frac{\partial I}{\partial t} = D_I \nabla^2 I + g(t)CE - \kappa I - s(t)IM \qquad (4)$$

for the population of infected cells.

Studies [6] show that CD4+ and CD8+ cells have a significant role in the clearance of *Chlamydia* compared to the innate immune response and that the rate of clearance by cell mediated immunity is higher than humoral clearance rate. Similarly, it takes 4 to 7 days for cell mediated response to be induced. The delay of the cell mediated immune response is thus modelled by the function

$$s(t) = \begin{cases} 7.3 \times 10^{-6}(\exp(t) - 1) + 0.001 & 0 \le t \le 7 \\ 0.3 & \text{otherwise} \end{cases}$$

The effect of humoral and cell mediated immunity are described in equations (5) and (6). The first term in each of these equations represents the random movement of humoral and cell mediated immune cells with constant

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coefficients D_H and D_M respectively. The second term in equations (5) and (6) represents the activation of humoral immunity in response to free chlamydial particles and cell mediated immunity in response to infected cells respectively. Natural death for elements of the humoral and cell mediated immune response has been characterised by the third terms in the corresponding equations, at rates of μ_2 and μ_3 respectively. The final terms in equations (5) and (6), model chemotactic responses. We propose that humoral and cell mediated immune cells direct their movement toward higher concentrations of chemokines and cytokines produced in response to either free chlamydial particles outside the host cell or infected cells, with chemotactic coefficients of χ_H and χ_M respectively. Together, these components give the conservation equations

$$\frac{\partial H}{\partial t} = D_H \nabla^2 H + \gamma_1 CH - \mu_2 H - \nabla(\chi_H (H \nabla K)), \quad (5)$$

$$\frac{\partial M}{\partial t} = D_M \nabla^2 M + \gamma_2 IM - \mu_3 M - \nabla(\chi_M (M \nabla K)). \quad (6)$$

Cytokines and chemokines are also assumed to undergo random Fickian diffusion with coefficient D_K. Chemokines produced by epithelial cells and some cytokines such as IFN-γ secreted by macrophages stimulate humoral immune cells to defend against free extracellular chlamydial particles at rate β_1. Additionally, chemokines secreted by infected cells at the RB stage of chlamydial developmental cycle, trigger T cells to produce cytokines in order to inhibit chlamydial growth or clear/control infection. In short, once chlamydial particles are inside the host cells, cell mediated immunity may prevent infection. These components combine to give the conservation equation

$$\frac{\partial K}{\partial t} = D_K \nabla^2 K + \beta_1 CH + \beta_2 IM. \quad (7)$$

4 Numerical results

In this preliminary study, we simulate chlamydial particles growing on a square domain $\Omega = [0, 5] \times [0, 5]$. An explicit finite difference method was used to solve the model equations (2–7) subject to the initial and boundary conditions given in Section 3. In this paper, we assume the first day of *Chlamydia* infection corresponds to the first day of menstruation and then continue to simulate the following 28 days of infection.

Wilson [15] provides a number of estimates for rate constants and other parameters, including $P = 350$, $\kappa = 0.45$, $\mu_1 = 0.3$. We estimate that $\mu_2 = \mu_3 = 0.01$, $\beta_1 = 0.4$ and $\beta_2 = 0.1$. Without access to further experimentally-informed parameters, we assume that the diffusion coefficients for cell species are an order of magnitude smaller than the coefficients for particles (simply due to relative sizes). Hence, we set $D_C = 10^{-4}$ and $D_E = D_I = D_H = D_M = D_K = 10^{-5}$. Similarly, we set the chemotactic coefficients to $\chi_H = \chi_M = 10^{-5}$.

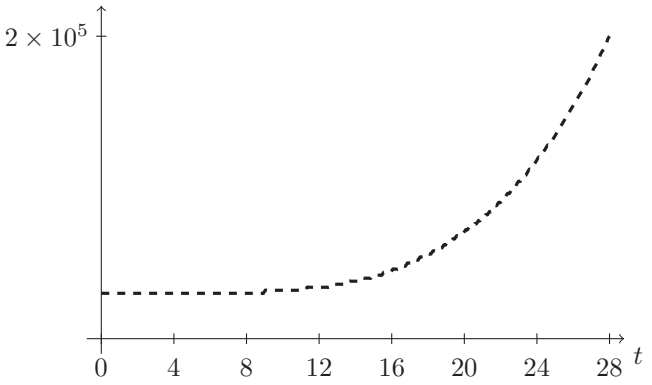

Figure 6: Number of free particles C over time (days).

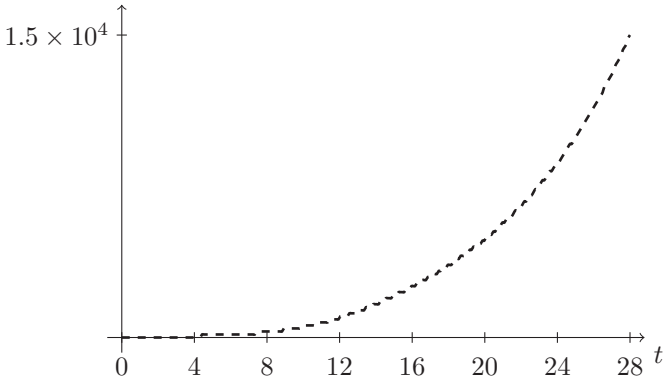

Figure 7: Number of infected cells I over time (days).

Fig. 6 shows an initially stable population of infectious particles (0-8 days) due to the low rate of cell infection caused by shedding of the uterus lining and the delayed immune clearance. Then after about 7-8 days, an increase in the amount of progesterone leads to the high rate of cell infection causing a rise in the population of chlamydial particles. Ashkan [2] state experimentally that there is a direct connection between the number of chlamydial particles and non-infected cells and as the number of infectious particles increase the number of non-infected cells decrease. This experimental result is observed with this model, but not shown here due to space restrictions. The number of infected cells (see Fig. 7) increases as the population of EBs increases. The level of humoral and cell mediated immunity also rises as the population of chlamydial particles and infected cells increase respectively (see for example, Fig. 8). Finally the model also predicts an increase in the level of secreted cytokines and chemokines in response to the free chlamydial particles and infected cells respectively (not shown). These results also confirmed by experimental results of Ashkan [2] that the level of humoral, cell mediated immunity and cytokine is at the lowest level in the first 8 days and increases dramatically afterwards.

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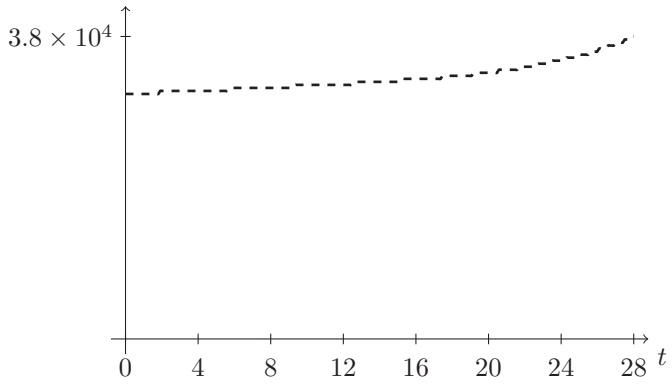

Figure 8: Humoral immunity measure H over time (days).

5 Conclusion

In this research we have extended the ordinary differential equation mathematical model of Wilson [15] to a partial differential equation model that allows for the consideration of spatio-temporal variation in the relevant biological species. This includes the incorporation of random motion of particles and cells, as well as the chemotactic response of components of the immune system. We have also introduced more explicit tracking of factors such hormones and the delayed immune response that have a significant role in the growth of Chlamydia. The model presented here has successfully reproduced experimental results observed in the laboratory by Amirshahi and others and as such this new model provides a framework for further investigation of Chlamydial infection through extension of the mathematical description. Such extensions are a topic of current research of the authors.

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