# Pathogenesis throughout the systems: a reaction networks model of the infection process of *Salmonella enterica*

# Patogénesis a través de los sistemas: un modelo de redes de reacción del proceso de infección de *Salmonella enterica*

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## ABSTRACT

*Salmonella enterica* is a gut-associated bacterial pathogen that can invade host cells and disseminate through the body using complex molecular machinery. The interplay between the host immune response and the bacteria is filled with many interactions at different complexity levels and molecular scales. This host-pathogen interaction can be modelled through reaction networks (RNs). RNs are mathematical models that represent interactions and dynamics of its components to provide a quantitative framework for studying complex biological processes. Even though RNs has been used to model biological processes, the multilevel dynamics of host-pathogen interaction is hard to model with current modelling approaches that limit the insights of the system. Here we show that the infection process of *Salmonella enterica* and its interplay with the host immune system can be modelled through RNs to form a host-pathogen model and gain insight into key processes of infection.

**Keywords:**

Mathematical Modelling, Pathogenesis, Host-Pathogen Interaction, Salmonella enterica

## RESUMEN

*Salmonella enterica* es un patógeno bacteriano asociado al intestino que puede invadir las células del huésped y diseminarse a través del cuerpo usando maquinaria molecular compleja. La interacción entre la respuesta inmune del huésped y la bacteria está llena de muchas interacciones en diferentes niveles de complejidad y escalas moleculares. Esta interacción huésped-patógeno se puede modelar a través de redes de reacción. Las redes de reacción son modelos matemáticos que representan interacciones y dinámicas de sus componentes para proporcionar un marco cuantitativo para el estudio de procesos biológicos complejos. Aunque las redes de reacción se han utilizado para modelar procesos biológicos, la dinámica multinivel de la interacción huésped-patógeno es difícil de modelar con los enfoques de modelado actuales que limitan el entendimiento del sistema. Aquí mostramos que el proceso de infección de *Salmonella enterica* y su interacción con el sistema inmunologico del huésped se pueden modelar a través de redes de reacción para formar un modelo de patógeno-hospedero y obtener información sobre los procesos clave de infección.

**Palabras Claves:**

Modelamiento Matemático, Patogénesis, Interacción Patógeno-Hospedero, Salmonella enterica

# 1  INTRODUCTION

**P**athogenesis refers to the process by which a pathogen causes disease within a host organism. It involves a series of interactions between the pathogen and the host, leading to the development and progression of the disease. Understanding the pathogenic processes is crucial since it provides insights into how pathogens invade the host, evade immune responses, and cause tissue damage. By unraveling the mechanisms underlying pathogenesis, researchers can identify potential targets for intervention, develop effective treatments, and design preventive strategies such as vaccines (Karkey *et al.*, 2018).

Moreover, studying the pathogenesis of specific pathogens, such as *Salmonella enterica*, allows us to gain a deeper understanding of the factors that contribute to their virulence, transmission, and persistence within the host. Through this knowledge, we can develop more targeted approaches to combat and control infectious diseases.

There is a plethora of modelling technologies one can use, each one with their pros and cons (Veloz, 2019), but Reaction Networks (RNs) stand out for the modelling of complex host-pathogen interactions (Loskot *et al.*, 2019). In this article we rely on them since they shine where different components interact with each other using reactions. These reactions are specific to each process (e.g. cell growth, infection, cell death, etc.) and they are independent of each other in time scales (Loskot *et al.*, 2019; Lambusch *et al.*, 2018). The emergence of dangerous pathogens presents the need to research their infection process to develop better treatment strategies. The need to obtain information at different levels of complexity is more and more a necessity in biological research. Here we propose a RNs model to show that complex host-pathogen interactions can be modelled by using *S. enterica* as an example.

# 2  PATHOGENESIS OF *Salmonella enterica*

*Salmonella enterica* (*S. enterica*) is a pathogenic bacterium primarily associated with gastrointestinal infections. It is widely studied as a model organism for understanding the mechanisms of disease. Within the species, various serovars exist, with *S. enterica* sv. Typhimurium and *S. enterica* sv. Typhi being the most relevant (Jajere, 2019).

*S.* Typhimurium is known to infect a wide range of hosts, including humans and animals, while *S.* Typhi specifically targets humans. Even though there are notable differences in the severity and progression of the diseases caused by these two serovars, they share many common characteristics that contribute to their ability to infect their respective hosts (Jajere, 2019).

Both *S.* Typhimurium and *S.* Typhi possess a set of virulence factors and mechanisms that enable them to colonize and invade the host's intestinal tract. These factors include adhesins that facilitate the attachment of the bacteria to the intestinal epithelium, invasion proteins that promote the entry of *S. enterica* into host cells, and effector proteins
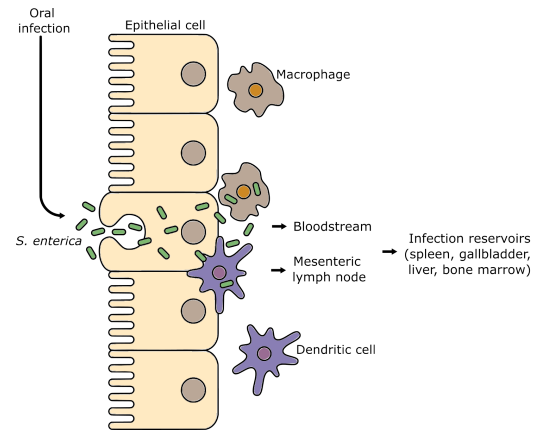


**Figure 1:** Process of *S. enterica* infection from oral infection, to invasion and persistence in organ reservoirs.

delivered via the type III secretion system (T3SS) that manipulate host cell processes and suppress the immune response (Hume *et al.*, 2017).

*S. enterica* is equipped with various survival mechanisms to evade the immune system and establish a persistent infection. These include the ability to survive and replicate within macrophages and other phagocytes, where it can evade immune clearance and disseminate to other tissues. Additionally, *S. enterica* can invade dendritic cells, which are crucial for initiating immune responses, allowing the bacterium to influence the host's immune defenses (Mastroeni *et al.*, 2009; Li, 2022; Kurtz *et al.*, 2017).

While *S.* Typhimurium causes a self-limiting gastroenteritis in humans, *S.* Typhi causes a more severe systemic infection known as typhoid fever. The latter is characterized by prolonged fever, gastrointestinal symptoms, and potential complications in various organs. However, despite the differences in disease severity, the underlying mechanisms of infection and pathogenesis are shared to a large extent between these two serovars (Jajere, 2019; Runkel *et al.*, 2013).

### INTESTINAL INFECTION

First and foremost, *S. enterica* enters the host by ingestion of contaminated food or water (Figure 1). Various sources can contribute to the contamination, including raw or undercooked poultry, eggs, unpasteurized dairy products, and raw fruits and vegetables that have been exposed to fecal matter containing *S. enterica*. Upon ingestion, *S. enterica* passes through the harsh acidic environment of the stomach and eventually reaches the small intestine, which is its primary target for colonization (Hume *et al.*, 2017; Li, 2022; Runkel *et al.*, 2013).

Within the intestinal tract, *S. enterica* encounters a dynamic microenvironment characterized by changes in pH, temperature, and nutrient availability. These environmental cues act as signals that trigger specific adaptive responses in *S. enterica*, allowing the bacterium to adapt, survive, and establish

infection within the intestinal environment (Runkel *et al.*, 2013; Spector and Kenyon, 2012; Mastroeni *et al.*, 2009; Li, 2022).

*S. enterica* exploits specialized epithelial cells called M cells, which are primarily located in the gut-associated lymphoid tissue (GALT), including the Peyer's patches (lymphoid follicles found in the small intestine) (Mastroeni *et al.*, 2009; Li, 2022; Jajere, 2019). M cells lack the protective mucus layer present in other intestinal epithelial cells, making them particularly vulnerable to bacterial invasion. *S. enterica* utilizes the molecular machinery of the T3SS to inject virulence factors directly into the M cells (Mastroeni *et al.*, 2009; Li, 2022). These injected virulence factors facilitate the breach of the epithelial barrier, enabling *S. enterica* to gain entry into the underlying tissues (Mastroeni *et al.*, 2009).

Once *S. enterica* has successfully invaded M cells, it subsequently gains access to the underlying epithelial cells. Within these cells, *S. enterica* triggers its own uptake through a process called "triggered phagocytosis". This process involves the manipulation of host cell signaling pathways by *S. enterica*, leading to the engulfment of the bacterium by the epithelial cells. Once inside, *S. enterica* harnesses the host cell's machinery to replicate, creating localized infection foci called "Salmonella-containing vacuoles" (SCVs). *S. enterica* manipulates the host cell's cytoskeleton and molecular processes to establish a protected replication niche favorable for its survival and proliferation (Mastroeni *et al.*, 2009; Li, 2022; Dandekar *et al.*, 2015; Ilyas *et al.*, 2017; Fang and Méresse, 2022).

Once epithelial cells from the intestinal tract have been infected, they act as a reservoir for further intestinal infections. *S. enterica* is able to induce cell death in these cells, making them burst open and releasing the bacteria (Mastroeni *et al.*, 2009).

## SYSTEMIC INFECTION

*S. enterica* has the ability to invade various immune system cells, macrophages being a prominent target (Gogoi *et al.*, 2018) along with dendritic cells (Mastroeni *et al.*, 2009; Li, 2022).

Once inside the SCV, *S. enterica* actively manipulates host cell's machinery to create a replication niche. The bacterium secretes effector proteins through its T3SS into the cytoplasm of the host cell. These effectors modulate various cellular processes, including cytoskeletal rearrangements, vesicular trafficking, and signaling pathways, to promote SCV integrity and nutrient acquisition (Li, 2022; Mastroeni *et al.*, 2009).

During the course of infection, macrophages are recruited to the site of *S. enterica* invasion in an attempt to suppress the pathogen. However, *S. enterica* has developed several evasion strategies to survive and replicate within macrophages. These mechanisms include the production of efflux pumps to expel antimicrobial peptides, modification of lipopolysaccharide structure to avoid immune recognition, modification of macrophage polarization from a proinflammatory (M1 type) to an antiinflamatory (M2 type), and inhibition of phagosome-lysosome fusion to prevent bacterial degradation (by modifying the lipid composition of the SCV membrane and interfering with the recruitment of lysosomal components) (Li, 2022).

By surviving within macrophages, *S. enterica* can evade immune responses and establish a persistent infection (Mastroeni *et al.*, 2009; Li, 2022). The ability of *S. enterica* to persist within macrophages contributes to its evasion of the immune system and establishment of a systemic infection.

Another set of immune cells that *S. enterica* can invade are dendritic cells. These cells are found in the Peyer's patches. Along with macrophages, Salmonella can make its way inside dendritic cells by signalling phagocytosis using pathogen-associated molecular patterns (PAMPs) that are recognized by immune system cells. After invasion, Salmonella can use dendritic cells as vehicles to reach the mesentheric lymph node, facilitating migration and dissemination through the system (Li, 2022). Dendritic cells, when detecting a bacterial pathogen, can activate killer T cells to target and remove the pathogen from the infection site (Wick, 2007; Tam *et al.*, 2008).

Infection and inflammatory responses activate the recruitment of monocytes to the affected area. Monocytes are a special type of mononuclear phagocytes that leave the bone marrow to the site of infection, releasing antimicrobial components to control the spread of *S. enterica*. Monocytes once in the site of infection will differentiate into macrophages or dendritic cells, which can be infected by *S. enterica* (Li, 2022; Tam *et al.*, 2008). Along with monocytes, neutrophils also react to inflammatory responses in the site of infection. This type of immune cell is short-lived and similarly to monocytes, they will be the first line of immune defense against *S. enterica* infection (Cheminay *et al.*, 2004). They are considered a very effective control of intracellular pathogens. Their high numbers present in the body at all times make them a constant monitoring agent against infections, although they seem to have a lower effect against non-typhoidal *S. enterica* (Castanheira and García-del Portillo, 2017).

As the infection progresses, *S. enterica* can breach the intestinal epithelial barrier and disseminate to other tissues and organs. The bacteria can access the bloodstream by directly penetrating the intestinal epithelium or by crossing the gut-associated lymphoid tissue. Once in the bloodstream, *S. enterica* can travel to various organs throughout the body, including the liver, spleen, kidneys, and bone marrow. This systemic dissemination leads to the establishment of infection in these organs and contributes to the severity of the disease. The ability of *S. enterica* to survive and multiply within host cells, including epithelial cells and immune system cells, facilitates its dissemination and persistence within different body compartments (Runkel *et al.*, 2013; Jajere, 2019; Hume *et al.*, 2017).

The process of pathogenesis of *S. enterica* although well understood still impose challenges to the treatment of the bacteria, specially with the appearance of multidrug-

resistant strains and the increase of infection rates of dangerous serovars (e.g. *S.* Typhi) (Karkey *et al.*, 2018). This is why we developed a RNs model of the infection process of *S. enterica* to determine the main processes and stages that are required for the bacteria to develop the intestinal and systemic progression of disease.

## 3   REACTION NETWORKS MODELING OF HOST-PATHOGEN INTERACTIONS

RNs are mathematical models that represent the interactions and dynamics of biochemical reactions within a biological system (Dittrich and di Fenizio, 2007). They provide a quantitative framework for studying complex biological processes and understanding the behavior of biochemical networks. RNs capture the connectivity between molecular species, the rates of biochemical reactions, and the dependencies between different reactions. By characterizing the network of interactions, RNs enable the simulation and prediction of how changes in molecular concentrations and reaction rates influence the overall behavior of the system (August and Papachristodoulou, 2009; Centler *et al.*, 2008; Styles *et al.*, 2021).

The usefulness of RNs in modeling biological processes lies in their ability to capture the intricate details of biochemical reactions and their dependencies. They provide a systematic approach to study the dynamics of cellular processes, signaling pathways, and metabolic networks. RNs allow researchers to test hypotheses, simulate different scenarios, and gain insights into the underlying mechanisms of complex biological phenomena. Moreover, they can be used to integrate experimental data, validate theoretical models, and make predictions about the behavior of the system under different conditions (Loskot *et al.*, 2019; Lambusch *et al.*, 2018; Zhang and Zhou, 2019; Duso and Zechner, 2020).

Since complex biological and chemical processes are hard to understand on their own, modelling techniques provide a great alternative to gain insight on complex systems (Wen *et al.*, 2023). RNs have been widely used for modelling complex networks and processes where computational approaches are needed. Anything from chemical processes and gene regulatory networks to population dynamics and symbiosis interaction can be benefited by them. From molecular biology to biotechnological applications, RNs are used to gain insight of processes with non-linear dynamics (Loskot *et al.*, 2019).

One of such applications is to understand the interaction of two organisms in the context of symbiosis, which in general terms could be mutualistic or parasitic. These interactions are highly regulated by genes, nutrient acquisition, immune suppression and molecular communication. Such complex systems can gain a lot of insight from RNs, recognizing that components are not limited to a on/off state or the same time scale (Centler *et al.*, 2008; August and Papachristodoulou, 2009).

In the context of host-pathogen interactions, RNs are valuable tools for modeling and understanding the dynamic interplay between the host and the pathogen. They can capture the molecular interactions involved in infection, immune responses, and pathogen evasion strategies. By constructing RNs that represent the interactions between host cells and pathogens, researchers can simulate the progression of infection of disease, investigate the effects of host immune responses, and identify potential targets for therapeutic intervention (Vlazaki *et al.*, 2019; Styles *et al.*, 2021). These models can provide valuable insights into the pathogenesis of specific pathogens, such as *S. enterica*, shedding light on the underlying molecular mechanisms driving the infection process.

It is of special interest the use of RNs to model the process of infection. Since *S. enterica* is a well known model of gastrointestinal infection, it comes as a great candidate to evaluate the systemic pathogenesis model. In this case we evaluate the process of pathogenesis from the intestinal survival and invasion, the internalization of *S. enterica* into epithelial cells and macrophages, and finally the systemic dissemination and infection.

## 4   SYSTEMIC PATHOGENESIS MODEL

The model uses *S. enterica* as its model organism. The components of the RNs model are shown in Table 1 and the network graph is shown in Figure 2. For the given components, 27 reactions ($r_1 - r_{27}$) are extracted from literature based on the main processes from *S. enterica* infection:

$r_1 : S_{en} \xrightarrow{\text{Invasion}} SM_c$ : *S. enterica* senses the intestinal environment and nutrients that activate the molecular machinery of the T3SS, thus granting it the ability to invade specialized epithelial cells. These are called M cells and are a common way of infection of *S. enterica* since they lack a key protective mucus layer.

$r_2 : S_{en} + D_c \xrightarrow{\text{Invasion}} SD_c$ : From the intestinal environment, *S. enterica* can also invade dendritic cells that are between the gap junctions of epithelial cells in the intestine, making

Table 1: Model components and their respective annotations

| | |
|---|---|
| $S_{en}$ | *S. enterica* in intestinal lumen |
| $SM_c$ | *S. enterica* in M cells |
| $M_p$ | Macrophages |
| $SM_p$ | *S. enterica* in Macrophages |
| $D_c$ | Dendritic cells |
| $SD_c$ | *S. enterica* in Dendritic cells |
| $SP_p$ | *S. enterica* in Peyer's Patches |
| $M_{ln}$ | *S. enterica* in Mesenteric Lymph Node |
| $SS_y$ | Systemic infection (bacteremia) |
| $R_s$ | *S. enterica* in Organ Reservoirs |
| $M_y$ | Monocytes |
| $N_p$ | Neutrophils |
| $\varnothing$ | Cell death |

it a good intermediary for later spreading systemically.

$r_3 : SM_c + M_p \xrightarrow{I_{nvasion}} SM_p$ : *S. enterica* can also invade macrophages that are found roaming around the epithelial barrier. It survives inside them and can travel throughout the body via blood or the mesenteric lymph node. This becomes a hostile environment for *S. enterica*, where it needs to activate and deactivate host processes to avoid the normal immune response of the cell.

$r_4 : SM_c \xrightarrow{I_{nvasion}} SP_p + N_p + M_y$ : From M cells, *S. enterica* can pierce through the epithelial barrier and reach a lymphoid tissue called Peyer's patches. This process of infection releases inflammatory responses that signal neutrophils and monocytes to fight bacteria at the site of infection.

$r_5 : SP_p + D_c \xrightarrow{I_{nvasion}} SD_c$ : From the Peyer's patches, *S. enterica* can invade dendritic cells that are recruited to the site of infection.

$r_6 : SP_p + M_p \xrightarrow{I_{nvasion}} SM_p$ : From the Peyer's patches, *S. enterica* can invade macrophages that are recruited to the site of infection.

$r_7 : M_{ln} + D_c \xrightarrow{I_{nvasion}} SD_c$ : From the mesenteric lymph node, *S. enterica* can invade dendritic cells that are recruited to the site of infection.

$r_8 : M_{ln} + M_p \xrightarrow{I_{nvasion}} SM_p$ : From the mesenteric lymph node, *S. enterica* can invade macrophages that are recruited to the site of infection.

$r_9 : R_s + M_p \xrightarrow{I_{nvasion}} SM_p$ : From the organ reservoirs, *S. enterica* can invade macrophages that are recruited to the site of infection.

$r_{10} : R_s + D_c \xrightarrow{I_{nvasion}} SD_c$ : From the organ reservoirs, *S. enterica* can invade dendritic cells that are recruited to the site of infection.

$r_{11} : SD_c \xrightarrow{D_{issemination}} SP_p + M_{ln} + SS_y + R_s + N_p + M_y$ : After invading dendritic cells, *S. enterica* can travel to different sites to spread infection, such as the Peyer's patches, mesenteric lymph node, the bloodstream and organ reservoirs. This process of infection releases inflammatory responses that signal neutrophils and monocytes to fight bacteria at the site of infection.

$r_{12} : SP_p \xrightarrow{D_{issemination}} M_{ln} + N_p + M_y$ : From the Peyer's patches, *S. enterica* can gain access to the mesenteric lymph node, and thus, disseminating from it to other sites. This process of infection releases inflammatory responses that signal neutrophils and monocytes to fight bacteria at the site of infection.

$r_{13} : M_{ln} \xrightarrow{D_{issemination}} SS_y$ : From the mesenteric lymph node, *S. enterica* can disseminate through the blood stream.

$r_{14} : SM_p \xrightarrow{D_{issemination}} SS_y + R_s + N_p + M_y$ : Infected macrophages can spread *S. enterica* through the bloodstream and organ reservoirs. This process of infection releases inflammatory responses that signal neutrophils and monocytes to fight bacteria at the site of infection.

$r_{15} : SS_y \xrightarrow{D_{issemination}} R_s + N_p + M_y$ : From the bloodstream, *S. enterica* can reach organ reservoirs, promoting a persistent infection in them.

$r_{16} : R_s \xrightarrow{D_{issemination}} SS_y$ : From the organ reservoirs, *S. enterica* can disseminate through the bloodstream, reaching different sites to infect and making it a persistent infection.

$r_{17} : N_p + SP_p \xrightarrow{I_{mmunity}} \varnothing$ : Neutrophils recruited to the site of infection in the Peyer's patches, fight *S. enterica* by engulfing and later degrading it. Since neutrophils are short-lived, the result of this process becomes cell death for both parties.

$r_{18} : N_p + M_{ln} \xrightarrow{I_{mmunity}} \varnothing$ : Neutrophils recruited to the site of infection in the mesenteric lymph node, fight *S. enterica* by engulfing and later degrading it. Since neutrophils are short-lived, the result of this process becomes cell death for both parties.

$r_{19} : N_p + R_s \xrightarrow{I_{mmunity}} \varnothing$ : Neutrophils recruited to the site of infection in the organ reservoirs, fight *S. enterica* by engulfing and later degrading it. Since neutrophils are short-lived, the result of this process becomes cell death for both parties.

$r_{20} : M_y + SP_p \xrightarrow{I_{mmunity}} D_c$ : Monocytes recruited to the site of infection in the Peyer's patches, differentiate into dendritic cells, which signal other immune cells to fight the infection of *S. enterica*.

$r_{21} : M_y + M_{ln} \xrightarrow{I_{mmunity}} D_c$ : Monocytes recruited to the site of infection in the mesenteric lymph node, differentiate into dendritic cells, which signal other immune cells to fight the infection of *S. enterica*.

$r_{22} : M_y + SS_y \xrightarrow{I_{mmunity}} D_c$ : Monocytes recruited to the site of infection in the bloodstream, differentiate into dendritic cells, which signal other immune cells to fight the infection of *S. enterica*.

$r_{23} : M_y + R_s \xrightarrow{I_{mmunity}} D_c$ : Monocytes recruited to the site of infection in the organ reservoirs, differentiate into dendritic cells, which signal other immune cells to fight the infection of *S. enterica*.

$r_{24} : M_y + SP_p \xrightarrow{I_{mmunity}} M_p$ : Monocytes recruited to the site of infection in the Peyer's patches, differentiate into macrophages, which fight the infection of *S. enterica* by engulfing and degrading it.

$r_{25} : M_y + M_{ln} \xrightarrow{I_{mmunity}} M_p$ : Monocytes recruited to the site of infection in the mesenteric lymph node, differentiate into macrophages, which fight the infection of *S. enterica* by engulfing and degrading it.

$r_{26} : M_y + SS_y \xrightarrow{I_{mmunity}} M_p$ : Monocytes recruited to the site of infection in the bloodstream, differentiate into macrophages, which fight the infection of *S. enterica* by engulfing and degrading it.

$r_{27} : M_y + R_s \xrightarrow{I_{mmunity}} M_p$ : Monocytes recruited to the site of infection in the organ reservoirs, differentiate into macrophages, which fight the infection of *S. enterica* by engulfing and degrading it.

All these reactions are a simplification of the complex process of infection, immune response and host-pathogen interaction. With these key processes it's important to notice that there are main host cells that play a role as a target of
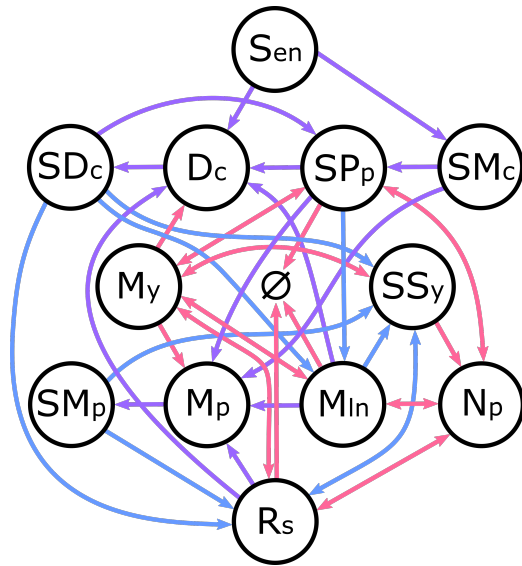
**Figure 2:** Network graph of the infection process of *Salmonella enterica*. Purple edges represent *S. enterica* invasion to host cells, blue edges represent dissemination of *S. enterica* (lymphoid tissue, bloodstream and organ reservoirs), red edges represent immune response to fight *S. enterica*.

*S. enterica* like M cells, macrophages and dendritic cells, or as specialized cells to fight the infection such as neutrophils and monocytes.

It is also important to mention that throughout the infection process of *S. enterica*, several environments are found and will affect bacteria in different ways. The high nutrient content of the intestine grants *S. enterica* the ability to grow and activate the molecular machinery to begin the infection process. In a different way, the nutrient starved and harsh environment found inside macrophages will delay bacterial growth and activate survival instead. These differences are key for further inspection of the model since they will affect the kinetics of the reactions and thus, will affect how fast certain processes will occur.

In a similar way, *S. enterica* must avoid being detected by the immune system and has to rely on specific genes to suppress the immune response of the host (such as PhoP/Q two-component system). The dynamics of these processes add another layer of complexity to the system since they will affect the kinetics of the interactions negatively. Finally, the fact that *S. enterica* uses reservoirs to maintain the infection and produce a subsequent reinfection is a key process to understanding the infection process of *S. enterica*. It not only affects the survival aspects of the bacteria in the whole process, but also how the system regulates itself.

For the identification of key processes, simulations are required to check if the model is robust, as well as to compare different scenarios of infection (e.g. typhoidal infection vs non-typhoidal infection, immunized host vs non-immunized host, etc.)

## 5   SIMULATION OF THE MODEL

Here are shown the results of the simulations of the systemic pathogenesis model. In this case, the only initial conditions were in the $S_{en}$, $M_y$, $N_p$, $M_p$ and $D_c$ components, as they are both the infection of *S. enterica* and the immune cells present in the body at all times. From literature, the kinetic constants were determined regarding invasion Tahoun *et al.* (2012), infection (Tam *et al.*, 2008; Monack *et al.*, 1996; Bueno *et al.*, 2008) and immune response (Cheminay *et al.*, 2004; Castanheira and García-del Portillo, 2017; Tam *et al.*, 2008; Hurley *et al.*, 2014).

In Figure 3 it is possible to see that the infection of typhoidal *S. enterica* is quick and proceeds to generate infection sites throughout the body. In this case, *S. enterica* survives within the host due to the generation of reservoirs which both increases bacterial density and decreases immune response. This goes in hand with literature, where common ways to test for *S. enterica* infection is to check samples of bone marrow, blood and stool (Wain *et al.*, 2001; Tennant *et al.*, 2015). Regarding the immune response, it is evident that neutrophils are the most abundant ones and the first one to fight infection, although since they have a short lifespan their response is hindered in the long run. In contrast, monocytes are seen to have a more steady production over time, which may be due to the differentiation into macrophages and dendritic cells instead of fighting the infection directly by themselves. Interestingly, Peyer's patches, the mesenteric lymph node and organ reservoirs have a small peak shortly after infection, which is rapidly reduced just to grow exponentially afterwards. This effect could be due to the rapid bacterial growth in infection sites that act as reservoirs for *S. enterica*, suggesting that the process of initial infection and shortly after, the dissemination of those bacteria to other tissues, is a key process for persistent systemic infection.

In the modern world, efforts for the development of antimicrobial treatments as well as immunization alternatives have reduced the threat of *S. enterica*. Given that non-typhoidal infection of *S. enterica* is much less lethal than typhoidal infections (30% mortality without antibiotic treatment), the progression of the disease in a common individual may look different. In Figure 4 it is possible to see that the clearance of infection is fast due to the greater immune response in these simulations. Even though *S. enterica* still achieved a systemic infection, the concentration in contrast with Figure 3 is minimal.

From this model we can extract the following points. 1) The infection process of *S. enterica* is a complex process that is filled with many host-pathogen interactions. 2) These interactions are affected by the dynamics of the immune response and the possibility of reinfections due to reservoirs. 3) Even though the presented model is a simplification of what is happening in a real situation, it can help us notice key processes of infection, such as the invasion of Peyer's patches and the dissemination through the mesenteric lymph node and organ reservoirs.

This model goes to show that RNs are a helpful way to

visualize and understand how multilevel processes like this happen.

# 6 DISCUSSION

We found that the systemic pathogenesis model brings up information that other modelling approaches fail to do. This is due to the nature of RNs where components are only a part of the model and for it to be fully functional, interactions or reactions are needed to control and understand how the dynamics of the system change at different parts of the infection process.

With the above results, it is possible to see some key processes of the host-pathogen interaction between *S. enterica* and the host. One of those key processes is the spread of *S. enterica* through the Peyer's patches and the mesenteric lymph node, mostly due to the amount of intermediaries that can allow bacteria to travel from the site of infection to organ reservoirs. The dynamics of these interactions suggest that it is at the very least, a very common path of *S. enterica* to disseminate to reservoirs.

These interactions have been modeled through biological phenomena that has been researched throughout the years. *S. enterica* is a well known pathogen and model of study for host-pathogen interaction and what we show is a simplification of complex interactions. These interactions show that *S. enterica* is a specialized bacterium that can suppress the immune response of the host cells and that it correlates with the available data.

The data available usually corresponds to genomic analysis, systems biology techniques and reaction rate models (Styles *et al.*, 2021; Stelling, 2004). These models usually are specific to gene regulatory networks of a determined species or interaction processes in a controlled environment. These models usually lack the different layers of complexity found in biological systems.

Specifically to *S. enterica*, systems of ordinary differential equations, metabolic network analysis and others has been done (Bumann, 2009; Lo, 2007; Sweilam *et al.*, 2022). These models are usually used in the area of epidemiology for disease spread in a specific situation. That is the case of typhoid fever, where a few studies have reported the emergence, spread and control of the disease by using mathematical models (Gauld *et al.*, 2018; Pitzer *et al.*, 2015).

These reports show that the insights gained from modelling dynamics allow for unexpected results. That is the case of Pitzer *et al.* (2015) where population density and cross-immunity was not enough to explain typhoid emergence in Blantyre, but increase in the duration of infectiousness and transmission rate did. This goes to show that data on its own is not enough to get the full picture of the processes and dynamics of a system, and that computational efforts are needed to uncover them.

In a similar manner, Gauld *et al.* (2018) developed a mathematical model for typhoid transmission in Santiago, Chile.

Their model showed that vaccination and reduced exposure to long-cycle transmission were important factors for the decline of incidence. Although the approach of these two cases are on a disease level and not an infection level. These studies reflect the need of better alternatives to what has been done on mathematical modelling efforts to complement the reports available, specially within the infection process bacteria.

In regards to this problematic, a study built a so called "within-host" mathematical model to understand the pathophysiology of *S. Typhi* from ingestion to the full progression of disease. What they found was that the migration of bacteria to the caecal lymph node was a key step for the dissemination of *S. Typhi* and the progression of disease. Even though the analysis is very robust, it lacks major components and interactions that are crucial in the pathogenesis of *S. enterica*, making it a powerful but simple model. This makes RNs a useful alternative to these types of models.

Even though network analysis is somewhat common in biological sciences, the use of RNs is very limited, even more in host-pathogen interactions. One such case of a network analysis is that of Zhang *et al.* (2022), where they evaluated the network model of a inter-host disease spreading with intra-host evolutionary dynamics. This study is a great example of modelling biological systems at different layers of complexity (inter-host and intra-host) since the components work independently from one another and that can have great impact on the results and comparisons of the model with what is actually happening.

Another example is that of the complex interactions between bacteriophages, bacteria and eukaryotic hosts with the goal of understanding these interactions to develop phage therapies as an alternative to antibiotics. This is another example of host-pathogen interaction at different layers of complexity, where RNs have been used in flux-balance analysis (Styles *et al.*, 2021).

Recent studies regarding endosymbiosis (a type of symbiosis where one species inhabits inside another species) proposed reaction network model to show the interactions between organisms (Veloz and Flores, 2021b,a). This study showed the endosymbiotic relationship between a coral host and its symbiont (*Symbiodinium* sp.). This is another example of host-pathogen interaction but in this case both species cooperate for survival (Veloz and Flores, 2021a). This cooperation is tightly regulated by evolution and biochemical pathways that helps both species live in harmony.

The above mentioned examples suggest that there is an unexplored niche of research to develop new models that can fill the gaps that other studies failed to complete. As such, RNs models, specially those related to host-pathogen interactions are not only an emerging technology for the modelling of host-pathogen interactions, but also a way to model them at the different levels of complexity that they appear in nature.

Some considerations that need to be made in this model are that: 1) it is necessary to refine the organismic and biochemical reactions described, and 2) they can be applied

to other pathogens with similar pathogen-host interactions, or even that these reactions serve as support to describe different cases. Nonetheless, this model shows that the intricate interactions of *S. enterica* can be simplified without losing significant information of the system, all while benefiting from the framework of RNs.

## 7   CONCLUSION

The complexity of biological processes can be simplified to accommodate a mathematical model using RNs as a modelling framework. This model sets a basis for the future development of modelling technologies for the research of host-pathogen interactions with the goal to understand the key steps in infection and develop strategies to fight pathogens.
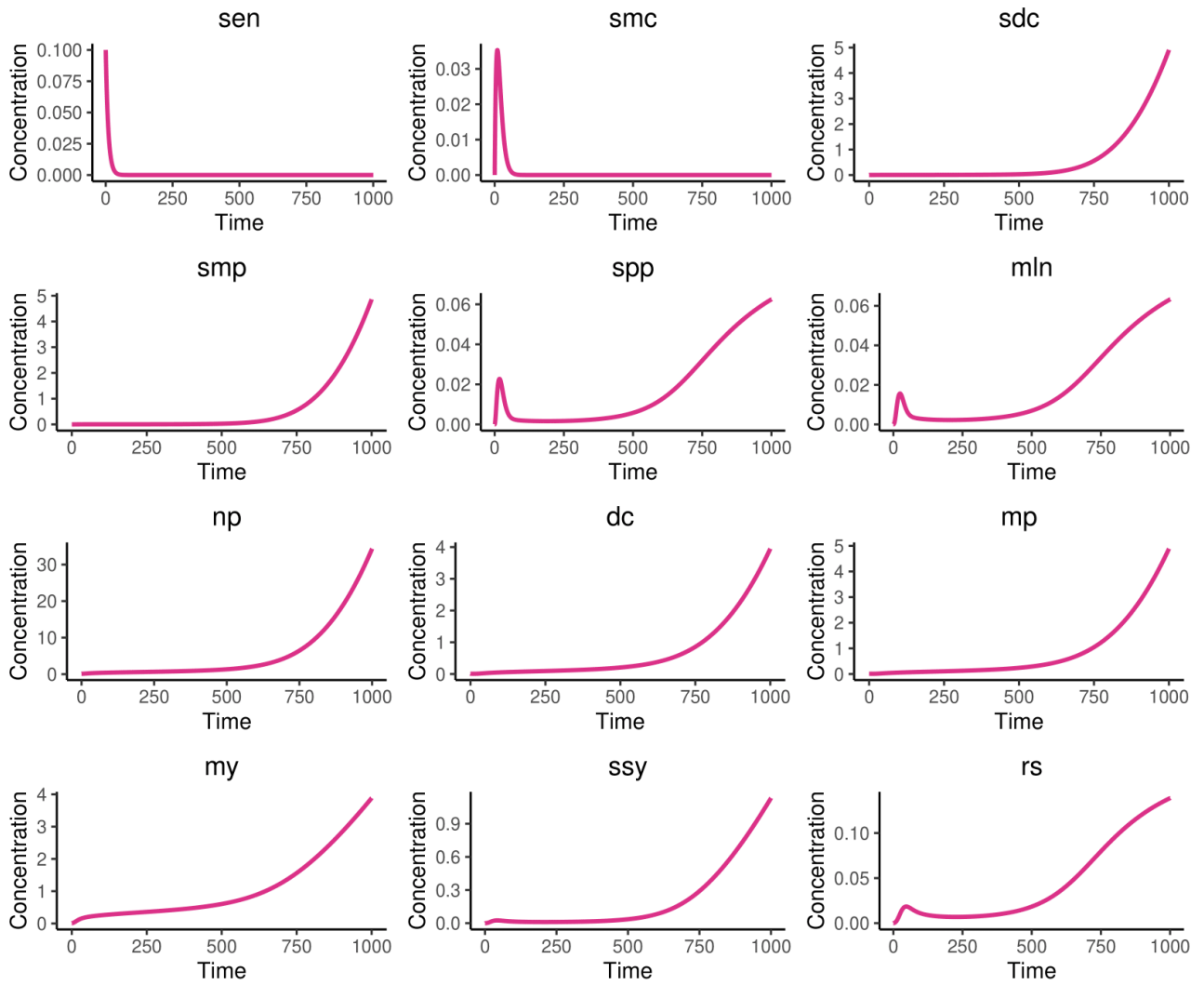
**Figure 3:** Simulation of the infection process of *S. enterica*. The evolution of the 12 components of the model are shown as the concentration change over time.
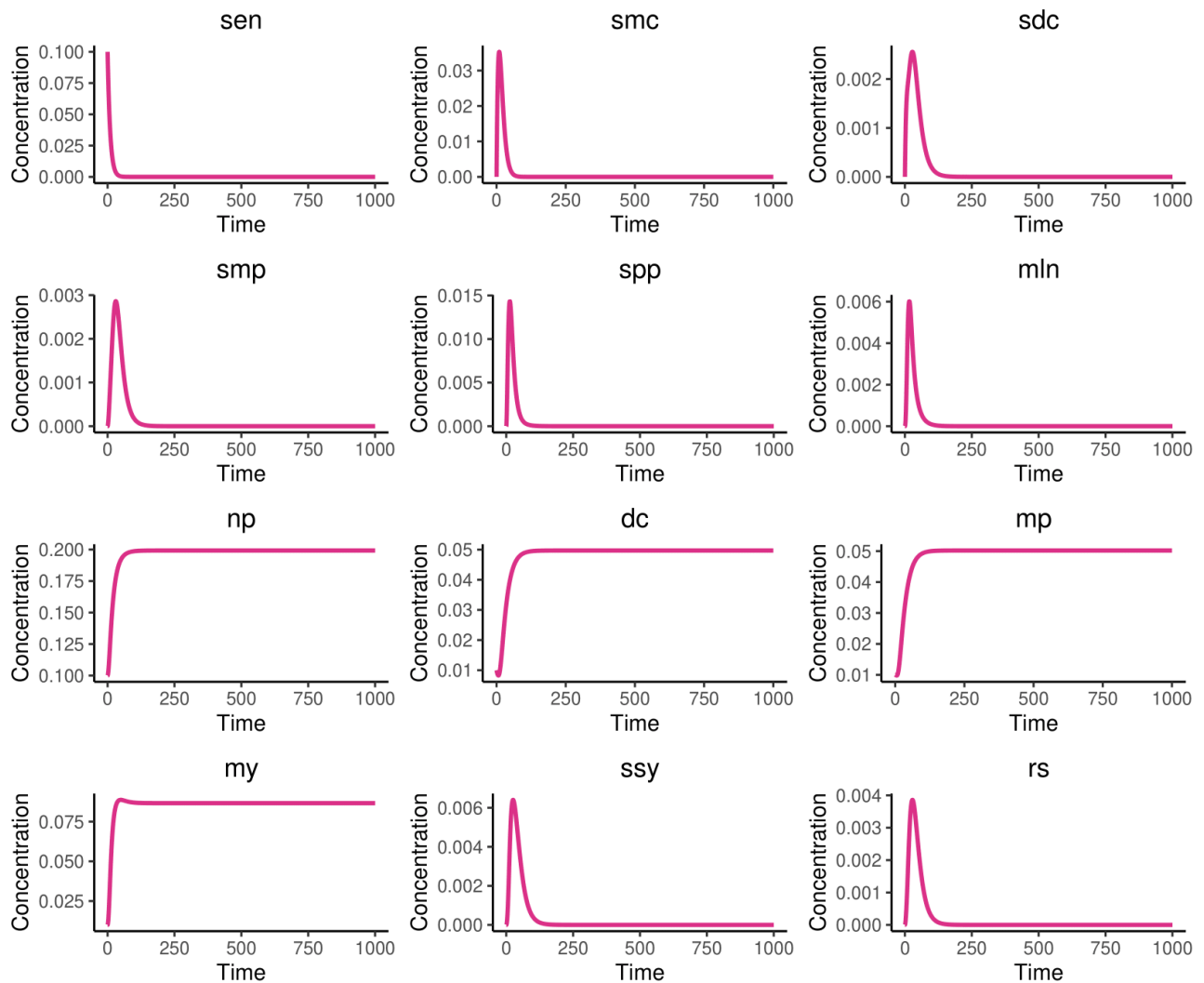
**Figure 4:** Simulation of the infection process of *S. enterica* in a immunized host. The evolution of the 12 components of the model are shown as the concentration change over time. The parameters of the immune response were increased to simulate a vaccinated host.

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