Stochastic dynamics of *Francisella tularensis* infection and replication

Jonty Carruthers

University of Leeds

International Centre for Theoretical Sciences, Bangalore

July 2019

Overview of the talk

1. Introduction: *Francisella tularensis* infection

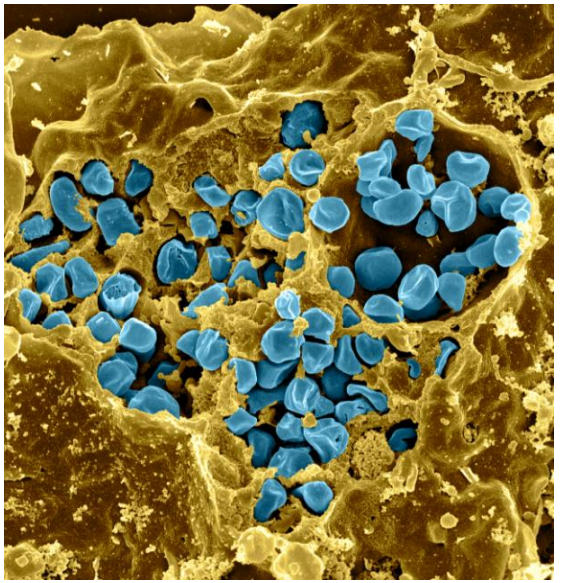
2. Modelling the intracellular life-cycle of bursting bacteria
   - Assuming a known distribution of burst times
   - Deriving the distribution of burst times

3. Agent-based modelling of early infection dynamics
   - Cohort analysis
   - Parameter inference

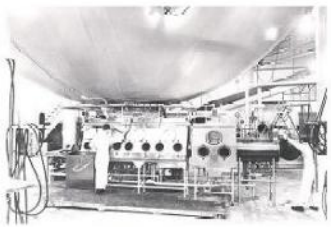
Overview of the talk

Introduction: *Francisella tularensis*

- *Francisella tularensis* is a gram-negative bacterium and the causative agent of tularemia.
- It is highly infectious and able to cause a debilitating disease in humans with as few as 10 CFUs.
- Most reported infections are acquired through the skin, but inhalation of *F. tularensis* bacteria results in the most dangerous form of tularemia
- The case fatality rate is approximately 30% when untreated.
- Infection can still be fatal even after treatment with antibiotics.
- There is currently no licensed vaccine



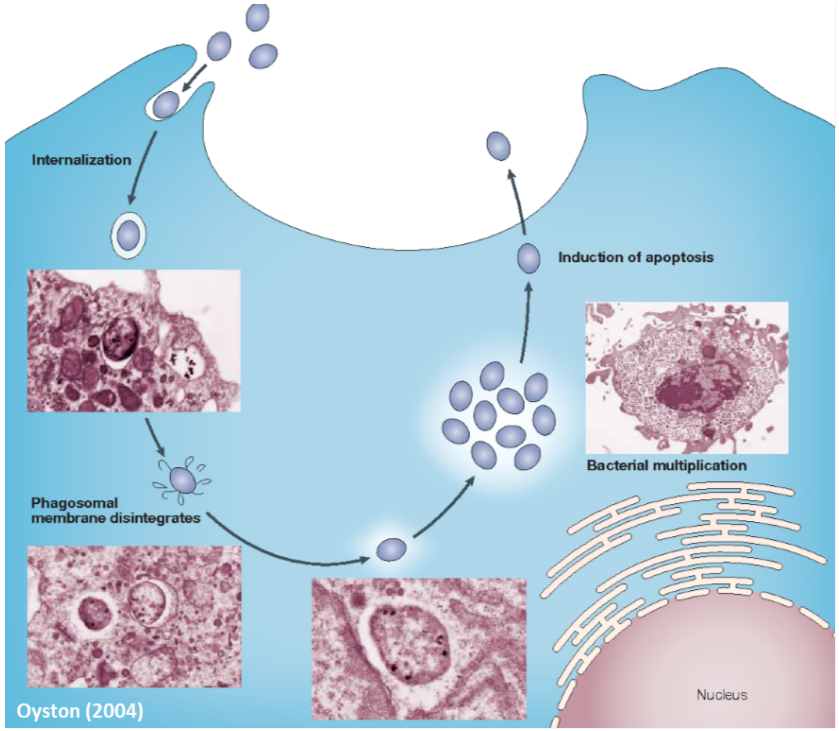
F. tularensis - a biothreat agent



- The SCHU S4 strain of *F. tularensis* has previously been developed for use as a biological weapon.
- Operation Whitecoat, USA - volunteers were infected with bacteria and later treated using antibiotics in order to study disease progression and dose dependent effects
- Similar human studies were also performed in prisons.
- *F. tularensis* is now classified as a category A bioterrorism agent by the CDC.
- We are interested in modelling the response following inhalation of bacteria.

F. tularensis - the intracellular lifecycle

- Following inhalation, *F. tularensis* bacteria primarily infect alveolar macrophages, entering without triggering the respiratory burst.
- After escaping phagosomes, bacteria replicate to high numbers in the cytosol.
- This results in the rupturing of the infected cell, releasing its bacterial contents.



- Initially undetected by the immune response, the rapid release of pro-inflammatory cytokines that follows is often "too much, too late".

Overview of the talk

Deterministic or stochastic?

Deterministic models cannot capture the discrete nature of the rupture event or the variability in size.

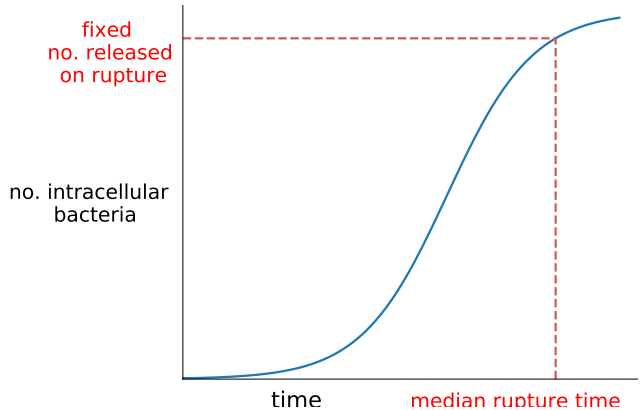
Extracellular dynamics:

$$\frac{d}{dt}T = -\beta TB$$
$$\frac{d}{dt}I = \beta TB - \frac{1}{\tau_I}I$$
$$\frac{d}{dt}B = -\beta TB + \underbrace{pI}_{\text{continuous release}} - cB$$

- As soon as cells become infected, release of bacteria occurs continuously.
- This assumption is suitable when there are plenty of cells infected at different times.

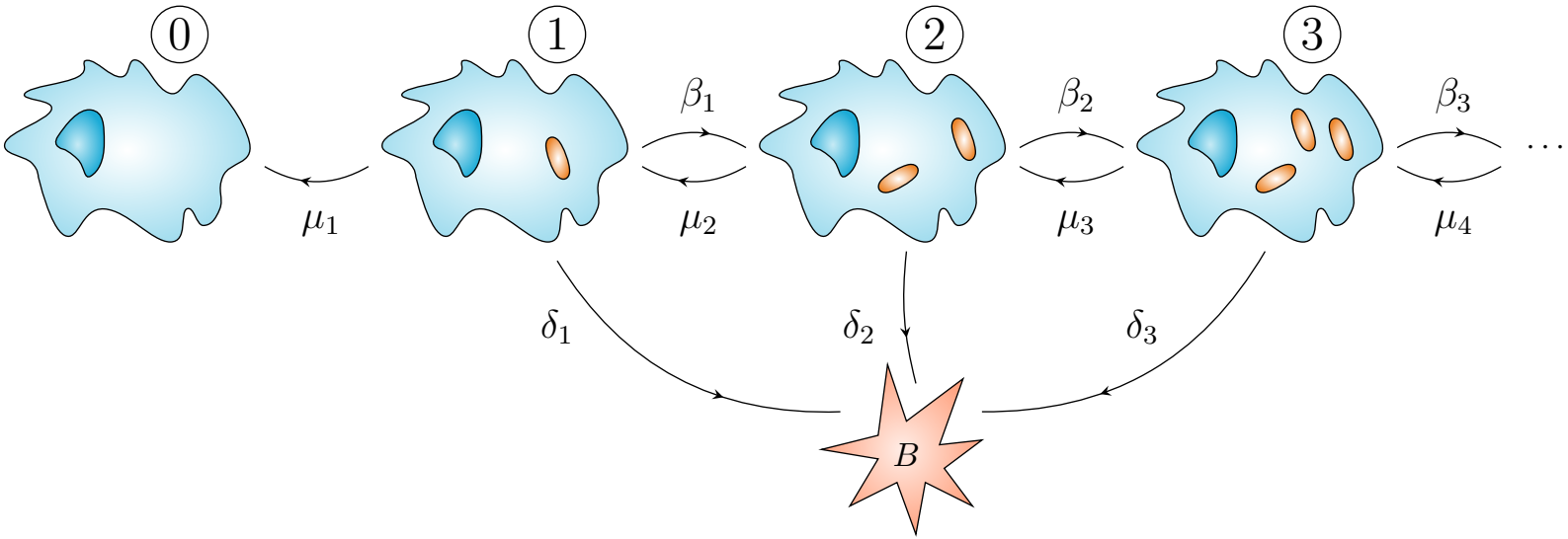
Intracellular dynamics:

$$g(t) = \begin{cases} 1, & 0 \leq t < 1 \\ \dfrac{C}{1 + (C-1)e^{-\omega(t-1)}}, & t \geq 1 \end{cases}$$



- There is no variability in the number of bacteria released when a cell ruptures.

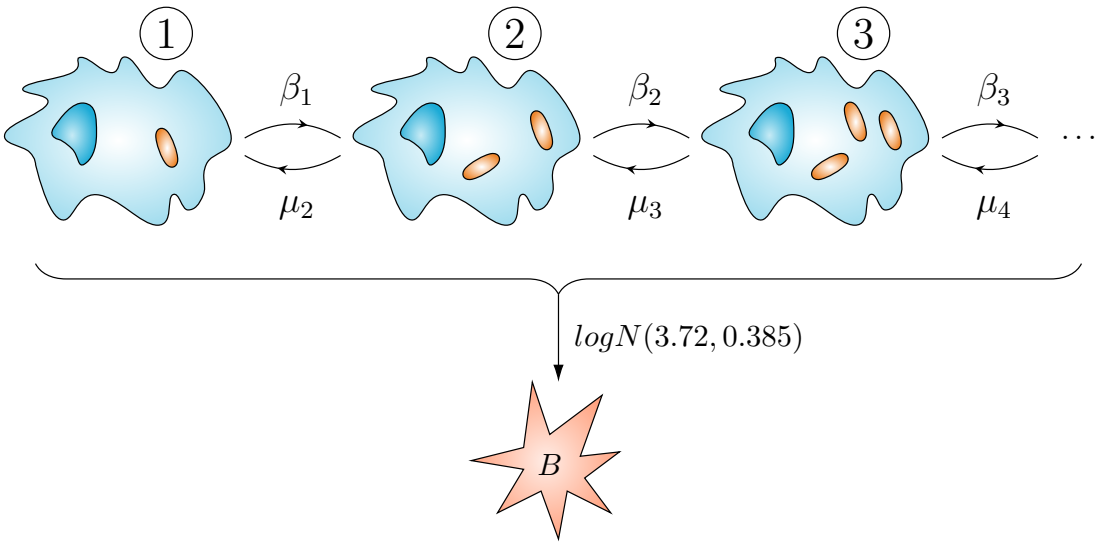
Stochastic intracellular model

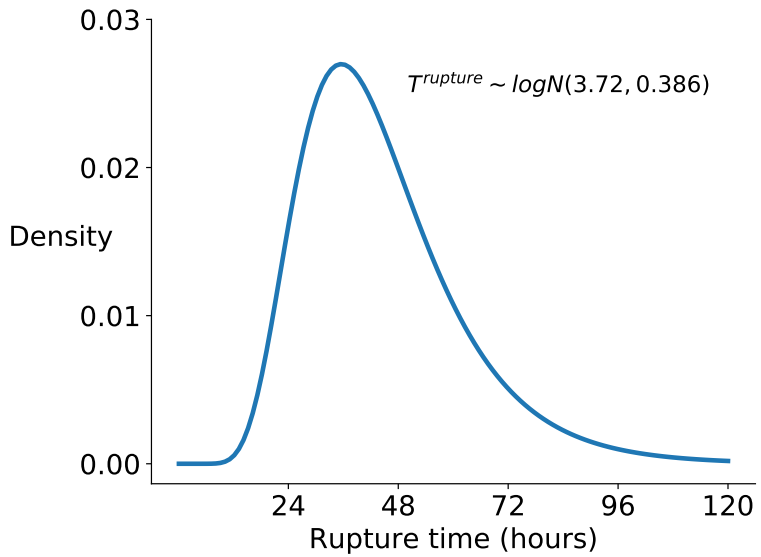


- We consider a Markov process $\mathcal{X} = \{X(t) : t \geq 0\}$, where $X(t)$ is the number of cytosolic bacteria at time $t \geq 0$.
- A single state B, represents the rupture of the macrophage - it can be entered into from any state.
- To study the process, two scenarios are considered:
  - when we **assume** the distribution of the time until rupture,
  - when we **derive** the distribution of the time until rupture.

Assuming log-normal rupture times

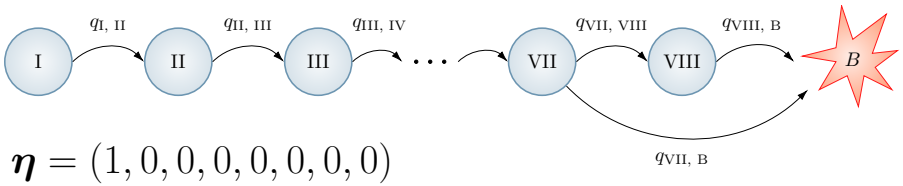
- By measuring LDH release from *F. tularensis* infected human macrophages, the distribution of rupture times is believed to follow a log-normal distribution ($T^{rupture} \sim logN(3.72, 0.385)$).
- We can think of this as an independent 'rupture clock' that starts when the cell becomes infected.
- Our stochastic process, $\mathcal{X}$, is now no longer Markovian.





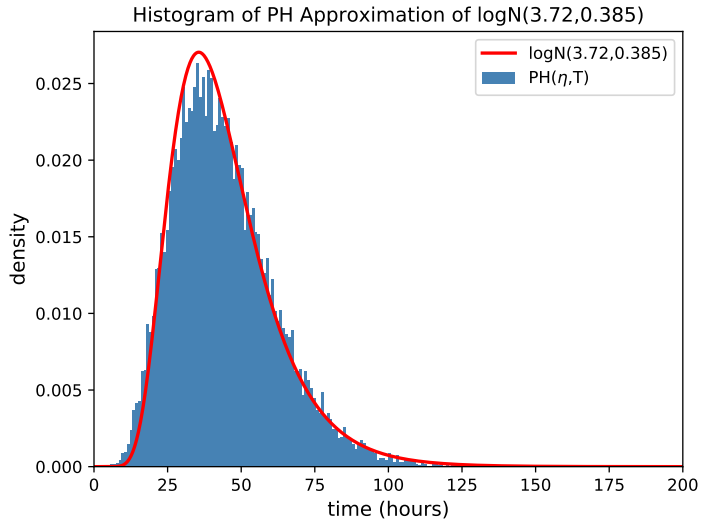
Phase-type approximation

- The time until absorption of an absorbing Markov process is a phase-type (PH) distributed random variable
- A separate Markov process can be defined, whose time until absorption follows a $logN(3.72, 0.385)$ distribution.
- A moment matching algorithm chooses:
  - the number of states in the auxiliary process,
  - matrix of transition rates, $\boldsymbol{T}$,
  - initial probability vector, $\boldsymbol{\eta}$.

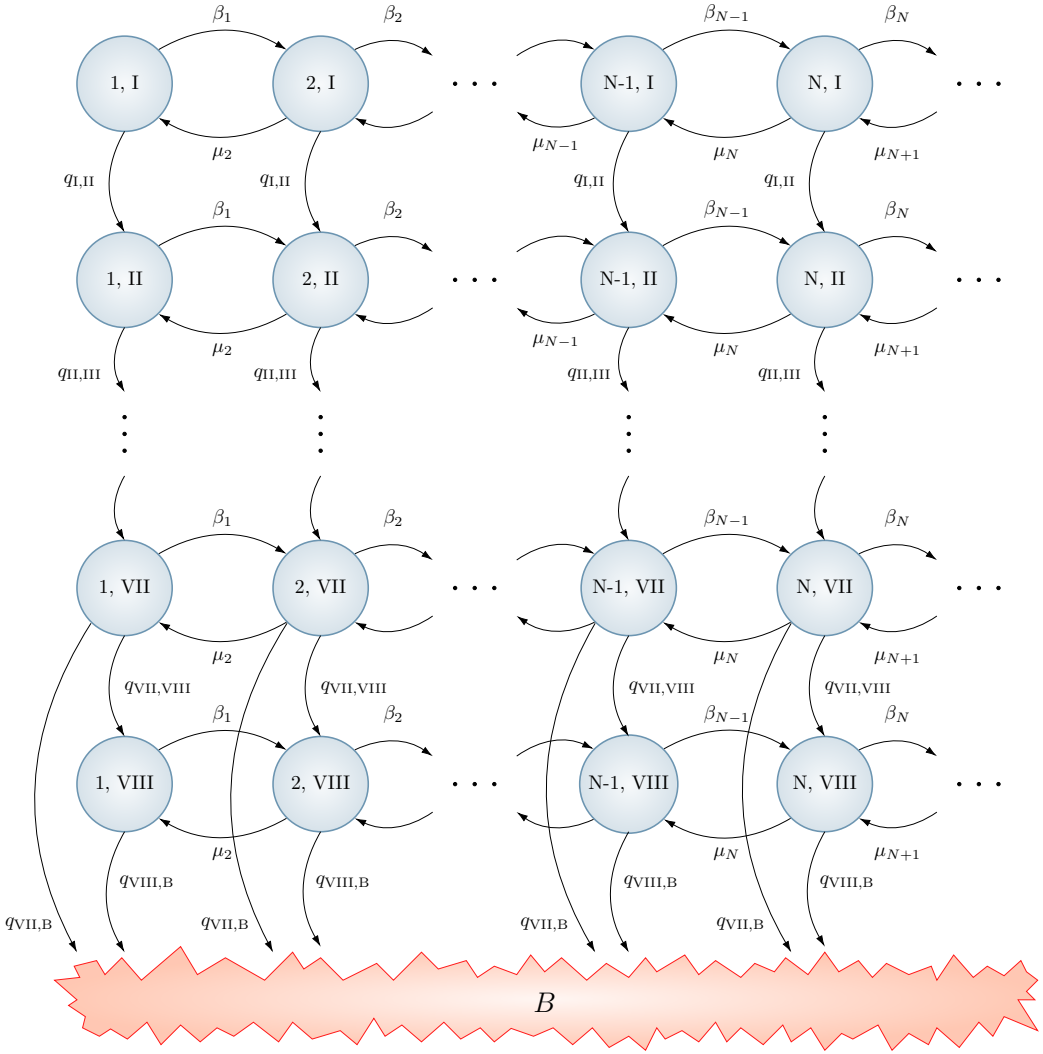


$$\boldsymbol{\eta} = (1, 0, 0, 0, 0, 0, 0, 0)$$

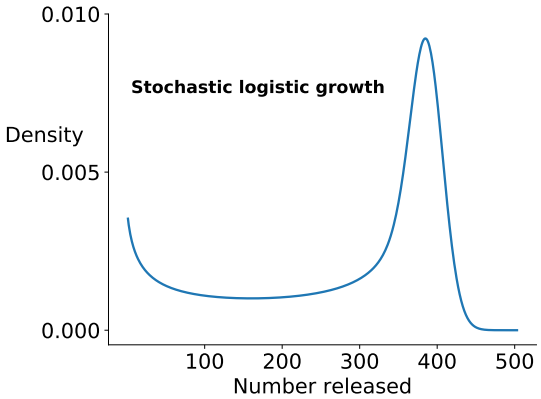
$$\boldsymbol{T} = \begin{pmatrix} -0.1447 & 0.1447 & 0 & \cdots & 0 \\ 0 & -0.1447 & 0.1447 & \cdots & 0 \\ 0 & 0 & \ddots & & \vdots \\ \vdots & & & -0.3396 & 0.0003 \\ 0 & \cdots & \cdots & 0 & -0.0127 \end{pmatrix}$$



Rupture distribution



- First step analysis can be used to find the probability that n bacteria are released upon cell rupture.



- The 'rupture clock' and growth process are not linked - a faster rate of bacterial growth does not result in shorter rupture times.
- Can we instead derive the time to rupture from β_n, μ_n and δ_n?

Linear birth-death-catastrophe process (survival function)

- Suppose that all rates are linear, that is, $\beta_n = \beta n$, $\mu_n = \mu n$ and $\delta_n = \delta n$.
- Let $S^{(k)}(t)$ be the probability that an infected macrophages survives to time t.

$$S^{(k)}(t) = \Pr(X(t) \neq B | X(0) = k) = [\Pr(X(t) \neq B | X(0) = 1)]^k = [S(t)]^k .$$

- If $X(0) = k$, then at time Δt, either:
  - $X(\Delta t) = k + 1$ with probability $\beta k \Delta t$,
  - $X(\Delta t) = k - 1$ with probability $\mu k \Delta t$,
  - $X(\Delta t) = B$ with probability $\delta k \Delta t$,
  - $X(\Delta t) = k$ with probability $1 - (\beta + \mu + \delta) k \Delta t$.

So,

$$S^{(k)}(t + \Delta t) = \beta k \Delta t S^{(k+1)}(t) + \mu k \Delta t S^{(k-1)}(t) + [1 - (\beta + \mu + \delta) k \Delta t] S^{(k)}(t),$$

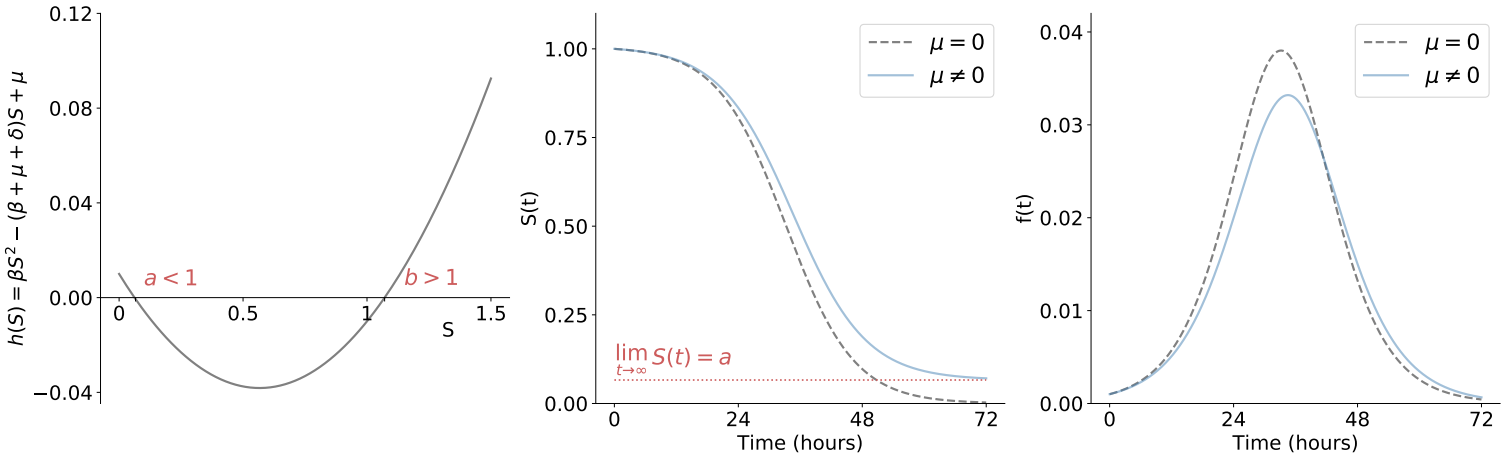
$$\boxed{\frac{d}{dt} S^{(k)}(t) = \beta k S^{(k+1)}(t) + \mu k S^{(k-1)}(t) - (\beta + \mu + \delta) k S^{(k)}(t)}$$

Time until rupture

- To get the density of the time until rupture, $f(t)$, we know that $f(t) = -\frac{d}{dt}S(t)$.
- Alternatively, consider a population of N macrophages each initially infected with 1 bacterium, X_1, X_2,...,X_N. The number of cells that die in the interval $(t, t + \Delta t)$ is:

$$N(S(t + \Delta t) - S(t)) = -\sum_{i=1}^{N} \delta X_i \Delta t,$$

$$f(t) = -\frac{d}{dt}S(t) = \delta \mathbb{E}(X(t)|X(0) = 1)$$



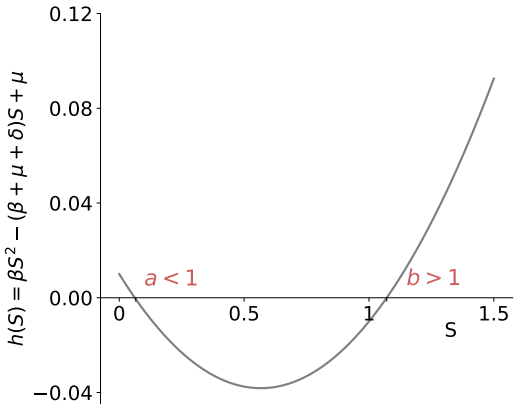
Release of bacteria

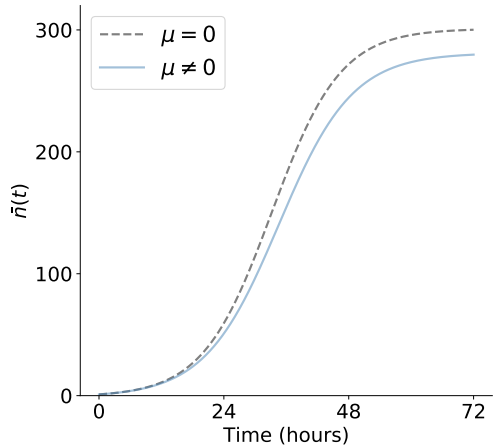
- Let $\tilde{f}(t)$ denote the mean number of bacteria released by an infected cell at time t:

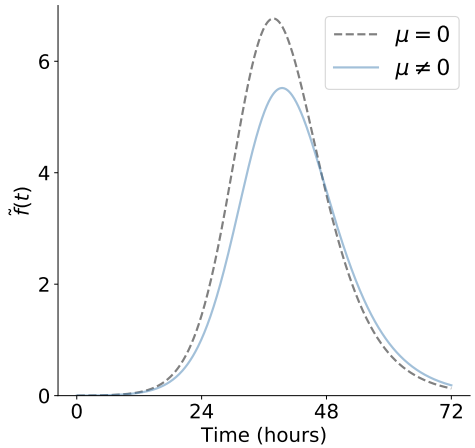
$$\tilde{f}(t) = \sum_{n=1}^{\infty} \Pr(X(t) = n \,|\, X(0) = 1)\,(\delta n)\, n$$
$$= \mathbb{E}(X^2(t) \,|\, X(0) = 1) = f(t)\bar{n}(t)$$

- The average number of bacteria released by a cell is given by

$$\int_0^{\infty} \tilde{f}(t)\, dt = (1-a)\frac{b}{b-1}.$$



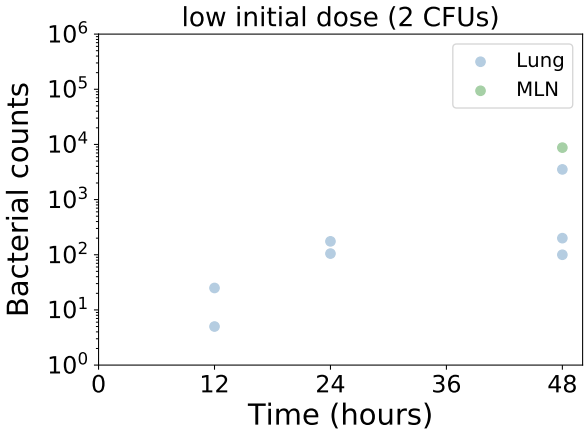


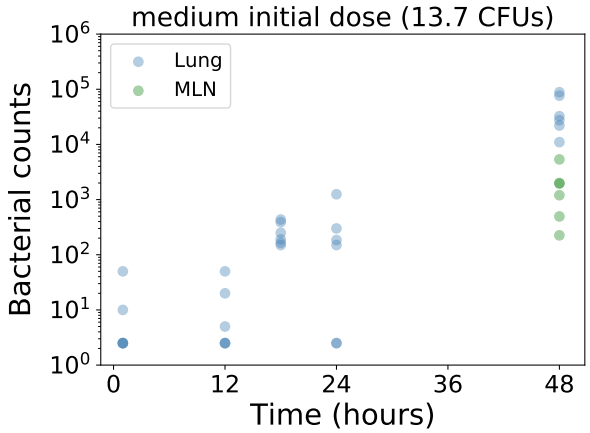


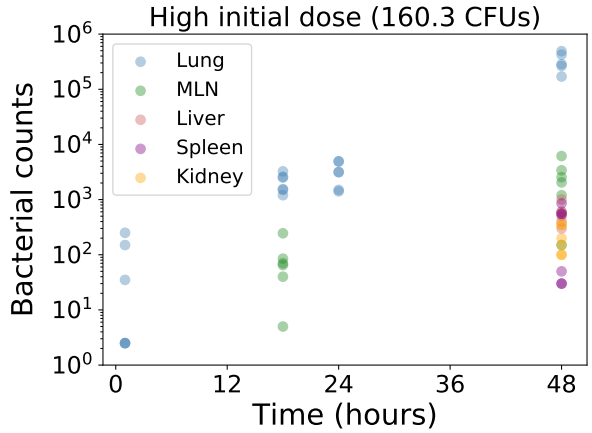
Overview of the talk

Experimental data

- Mice were challenged with *F. tularensis* SCHU S4 using a Henderson-type apparatus and Collison nebuliser
- Using the flow rate of the apparatus, the bacterial count of the sample and the breathing rate of the mice, initial doses are estimated to be **2 CFUs (low), 13.7 CFUs (medium) and 160.3 CFUs (high)**.
- At 1, 18, 24 and 48 hours post infection, mice are culled and bacterial counts are measured in the **lung, liver, kidney, spleen and mediastinal lymph node (MLN)**.





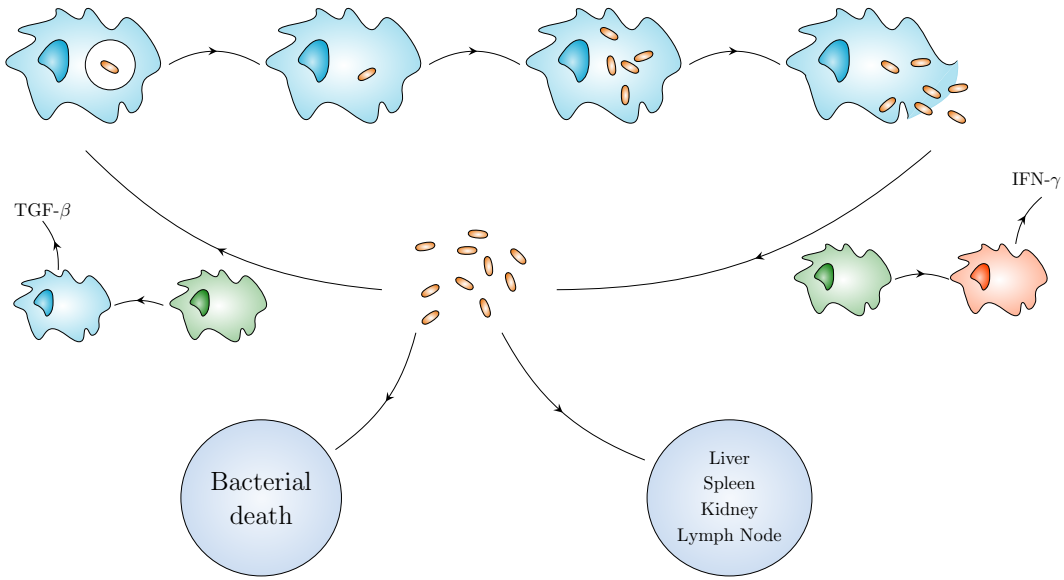


An agent based model for *F.tularensis* infection

We can now use the stochastic intracellular model to study the early stages of infection, first considering an agent based model.

Agents:

- Bacterium:
  - location,
  - intracellular compartment,
  - cohort number,
- Macrophage
  - location,
  - intracellular bacteria,
  - cohort number,
  - activation state.



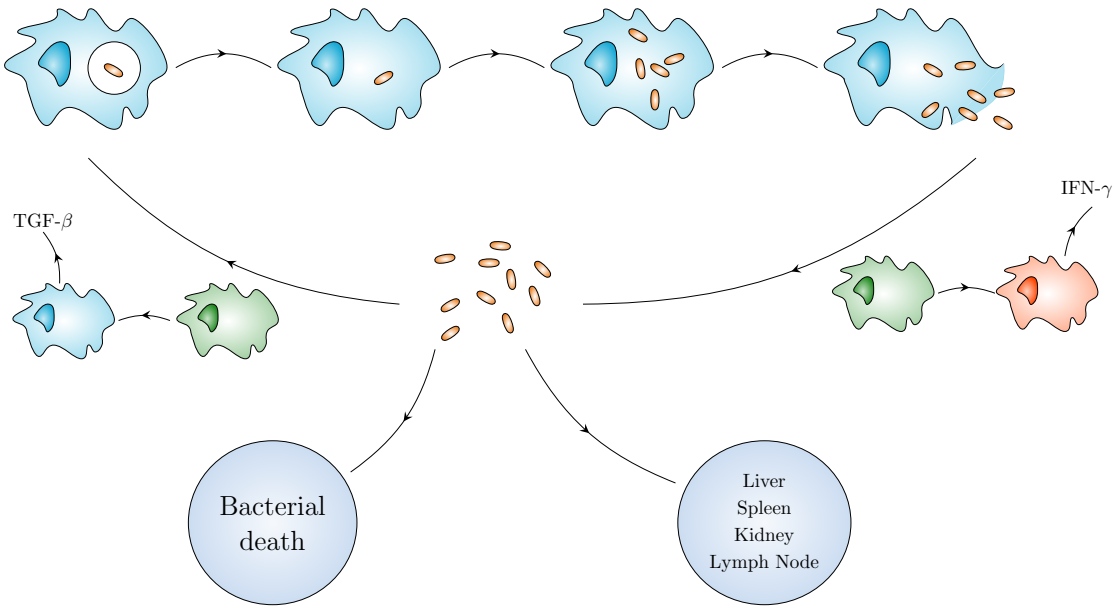
Pro-inflammatory cytokine IFN-γ and anti-inflammatory cytokine TGF-β are described using ODEs - their production is proportional to the number of activated and suppressed cells.

$$\frac{d}{dt}G = \alpha_G A(t) - \mu_G G\,, \qquad \frac{d}{dt}T = \alpha_T I(t) - \mu_T T\,.$$

An agent based model for *F.tularensis* infection

Reactions:

- Phagocytosis, ρ
- Phagosomal escape, ϕ
- Intracellular replication, β
- Infected cell rupture, δ
- Extracellular bacterial death, μ_E
- Migration, γ, with weights w_j
- Macrophage activation by IFN$-\gamma$
- Macrophage suppression by TGF$-\beta$



- Agent based simulations can be performed using two types of time-stepping, the Gillespie algorithm and tau-leaping.
- Initially there are N extracellular *F. tularensis* bacteria, and M uninfected, resting macrophages

Cohort analysis

- The cohort number of a bacterium approximately tells us how many cells it has infected - cohort numbers are inherited by progeny bacteria.
- To model the size of each cohort, first consider the lung and define:

  $P_n(t) =$ number of phagosomal bacteria with cohort number n at time $t \geq 0$

  $C_n(t) =$ number of cytosolic bacteria with cohort number n at time $t \geq 0$

Cohort 1:

- Assuming the initial N bacteria are phagocytosed quickly and escape phagosomes at rate $\phi\, h^{-1}$:

$$P_1(t) = Ne^{-\phi t}.$$

- The mean of the intracellular process can be used to describe the first cohort of bacteria in cytosols:

$$C_1(t) = \int_0^t \phi P_1(s)\mathbb{E}(X(t-s)|X(0)=1)\,ds = \frac{N\phi}{\delta}\int_0^t e^{-\phi s} f(t-s)\,ds$$

Cohort analysis

Cohort *n*: Let $r_n(t)$ be the number of bacteria released by cohort n macrophages at time $t \geq 0$.

$$r_n(t) = \int_0^t \phi P_n(s) \tilde{f}(t-s)\, ds$$

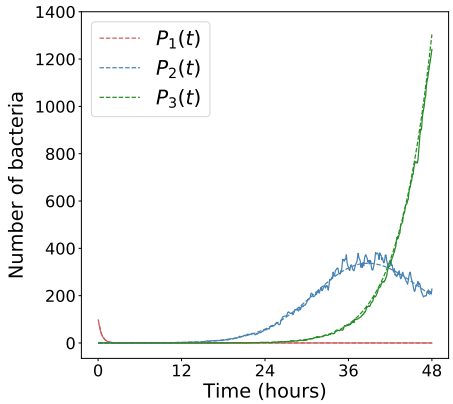
$$\frac{d}{dt} P_n(t) = -\phi P_n(t) + r_{n-1}(t)\,, \qquad P_n(0) = 0 \qquad n = 2, 3, \ldots$$

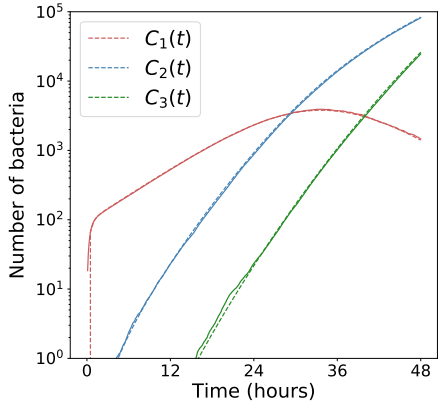
$$C_n(t) = \frac{\phi}{\delta} \int_0^t P_n(s) f(t-s)\, ds\,, \qquad C_n(0) = 0 \qquad n = 1, 2, \ldots$$

Extracellular:

$$\frac{d}{dt} E(t) = \sum_n r_n(t) - E(t)\left[M\rho + \gamma + \mu_E\right]$$

Remaining organs:
Dynamics in the lymph nodes, liver, kidney and spleen are the same as in the lung.

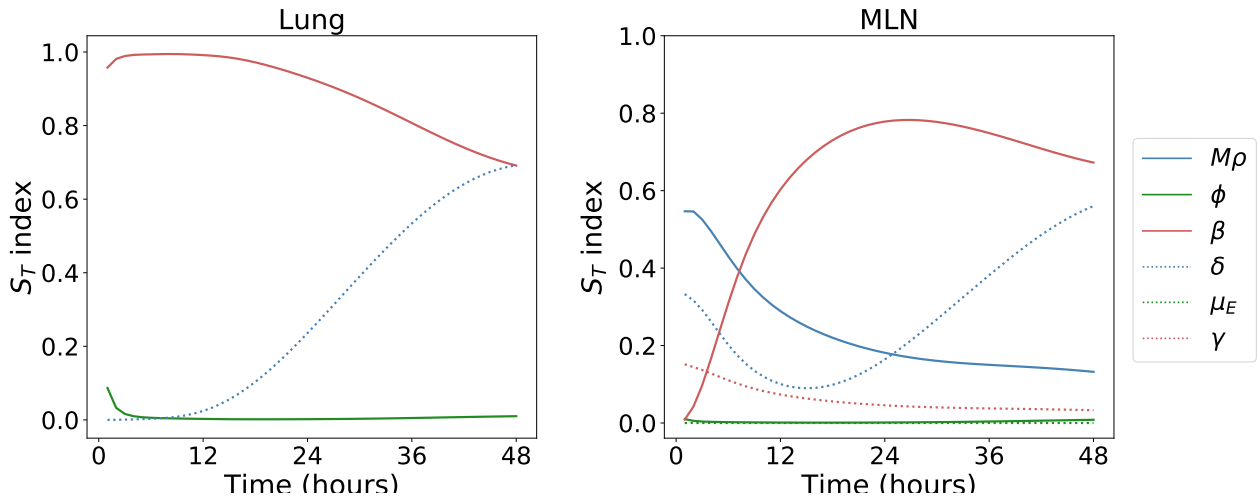




Sensitivity analysis

- Global sensitivity analysis can be used to identify which parameters have the greatest influence on bacterial counts.
- The Sobol method quantifies the reduction in variance by fixing combinations of parameters.

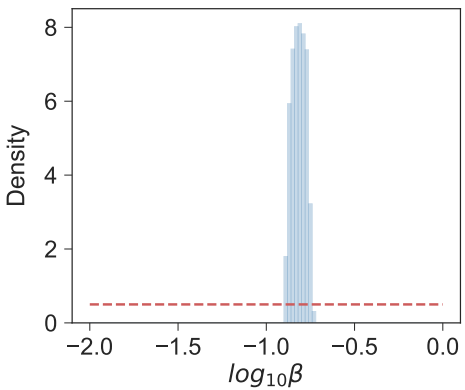
- Intracellular replication (β) and macrophage rupture (δ) are the most important parameters.
- $M\rho$ and γ have some importance - they determine whether a bacterium migrates or re-infects.

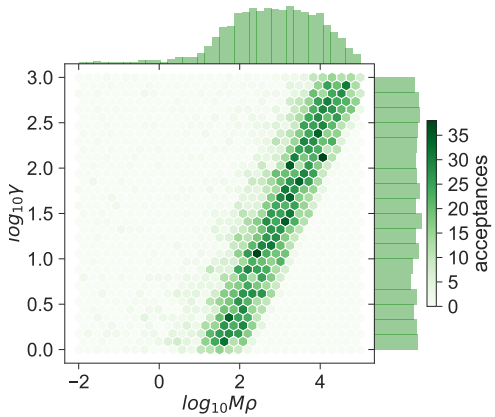


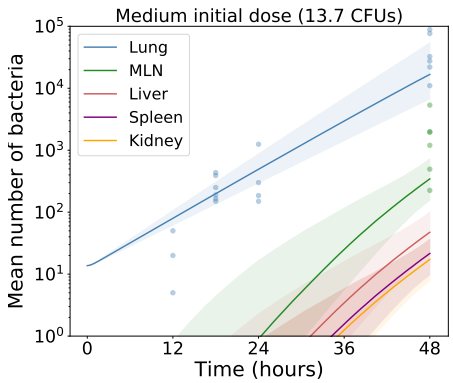
Bayesian parameter inference

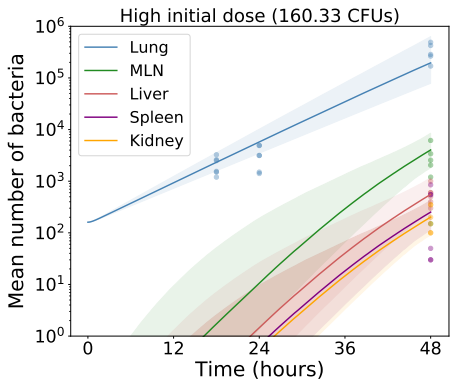
- Approximate Bayesian Computation (ABC) is used to infer the most important parameters (β, δ, γ, $M\rho$).
- Prior distributions: $\log_{10}\beta \sim U(-2,0)$, $\log_{10}\delta \sim U(-5,-1)$, $\log_{10}\gamma \sim U(0,3)$ and $\log_{10}(M\rho) \sim U(-2,5)$.
- Model predictions are compared to experimental data using the distance:

$$d^2(\text{mod},\text{exp}) = \sum_{i\in\mathcal{D}}\sum_{j\in\mathcal{S}}\sum_{t\in\mathcal{T}_{i,j}}\left[\frac{\log_{10}(B^{(\text{mod})}_{i,j}(t)) - \log_{10}(\bar{B}^{(\text{exp})}_{i,j}(t))}{\sigma_{i,j}(t)}\right]^2.$$







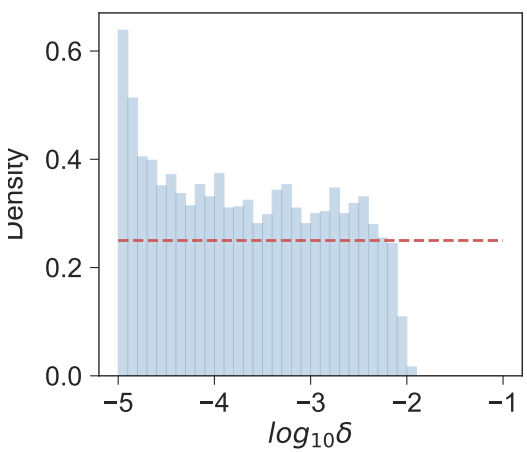


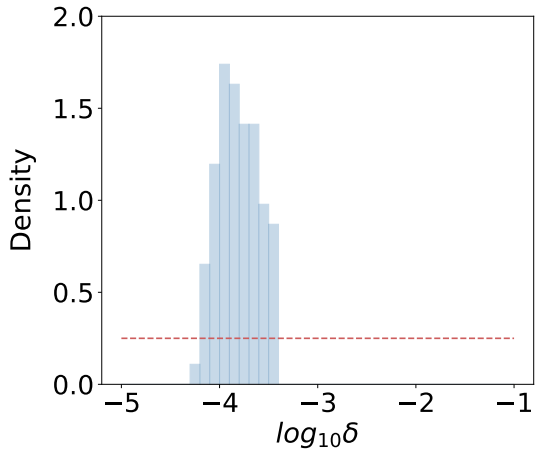
Bayesian parameter inference

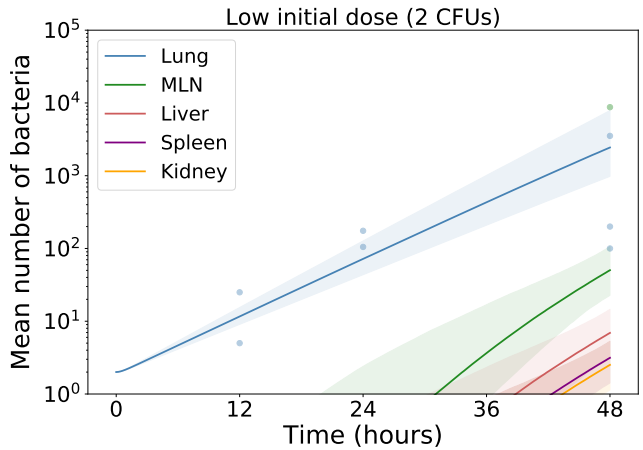
- It is not possible to learn much about δ from measurements of total bacterial counts alone.
- For a birth-catastrophe process, the mean time until rupture is a function of only β and δ:

$$\mathbb{E}(T^{rupture}|X(0)=1) = \int_0^\infty f(t)t\,dt = \frac{1}{\beta}\log\left(\frac{\beta+\delta}{\delta}\right).$$

- Posterior predictions can be produced for the growth of bacteria following infection with a low initial dose (2 CFUs).







Conclusions

- Two approaches for modelling intracellular pathgoens that burst have been presented:
  - **Using a known distribution of rupture times**:
    - Phase-type approximations can be used to incorporate the time until rupture
    - First-step analysis can be used to describe the release of bacteria
  - **Deriving the time until rupture**:
    - A birth-death-catastrophe process can be used to obtain the distribution of rupture times and the release of bacteria as a function of time
- The intracellular model can be used to describe the early stages of *F. tularensis* infection by dividing the population into cohorts.
- Using *in vivo* infection data, an estimate for the intracellular replication rate has been found - this is consistent with estimates from *in vitro* studies.

Acknowledgments

This is a joint work between the University of Leeds and the Defence Science and Technology Laboratory (DSTL, Ministry of Defence, UK).

University of Leeds

- Dr. Martin López-García
- Prof. Grant Lythe
- Prof. Carmen Molina-París

DSTL

- Dr. Joseph J. Gillard
- Dr. Thomas. R Laws
- Dr. Roman Lukaszewski

EPSRC Engineering and Physical Sciences Research Council

MRC Medical Research Council

Project MR/N014855/1

[dstl]

UNIVERSITY OF LEEDS