Stochastic models of *F. tularensis* infection

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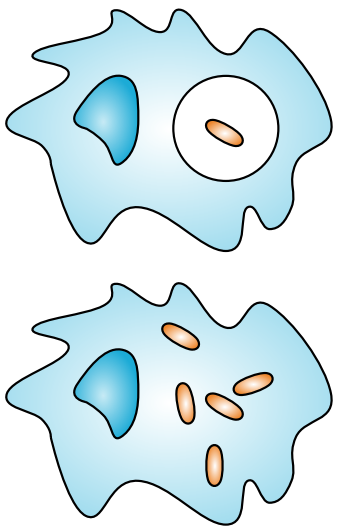
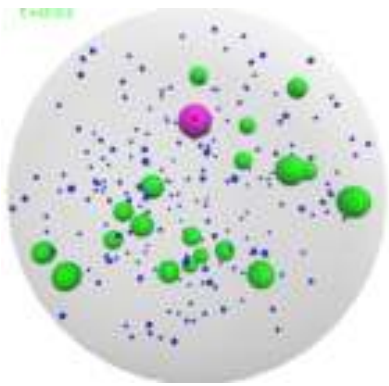
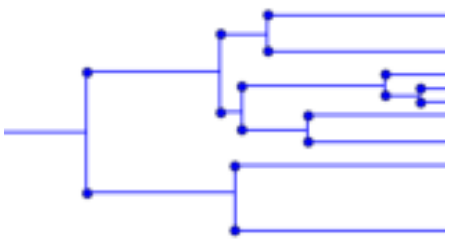
with Joe Gillard, Tom Laws and Roman Lukaszewski (Dstl)
Martín López-García, Carmen Molina-París and Jonty Carruthers (Leeds)







Gillard, Laws, Lythe, Molina-París. Frontiers in Cellular and Infection Microbiology (2014)

Carruthers, López-García, Gillard, Laws, Lythe, and Molina-París. Frontiers in Microbiology (2018)

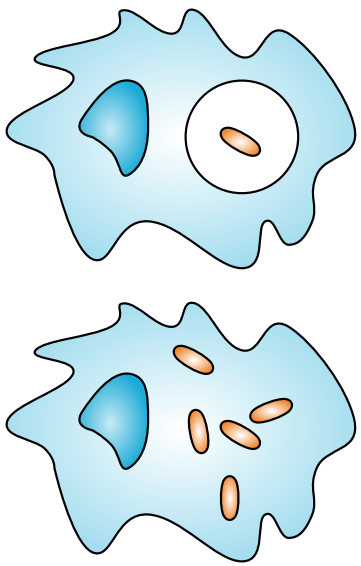
Carruthers, Lythe, López-García, Gillard, Laws, Lukaszewski, Molina-París. PLoS computational biology (2020)

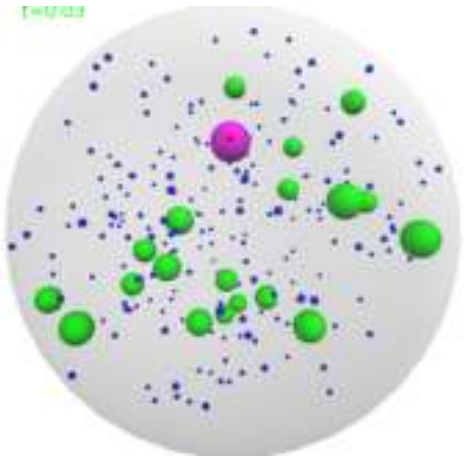
Outline

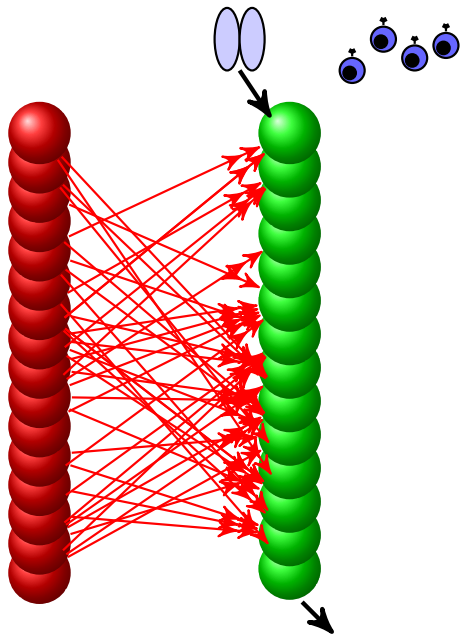
Francisella tularensis pathogenesis

- Computational model
- Birth and death process with catastrophe
- Data

Modelling Ebola virus in vitro infection







Francisella tularensis

- Gram negative, intracellular
- Extremely low infectious dose
- Untreated 40% fatality rate, no licensed vaccine

F. tularensis bacteria may be inhaled in an aerosol, with initial doses as low as 10 CFU resulting in respiratory or pneumonic tularemia. The bacteria enter alveolar macrophages, evading initial immune recognition and inflammatory response because of their atypical lipopolysaccharide.

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Carruthers, López-García, Gillard, Laws, Lythe, and Molina-París. Frontiers in Microbiology (2018)

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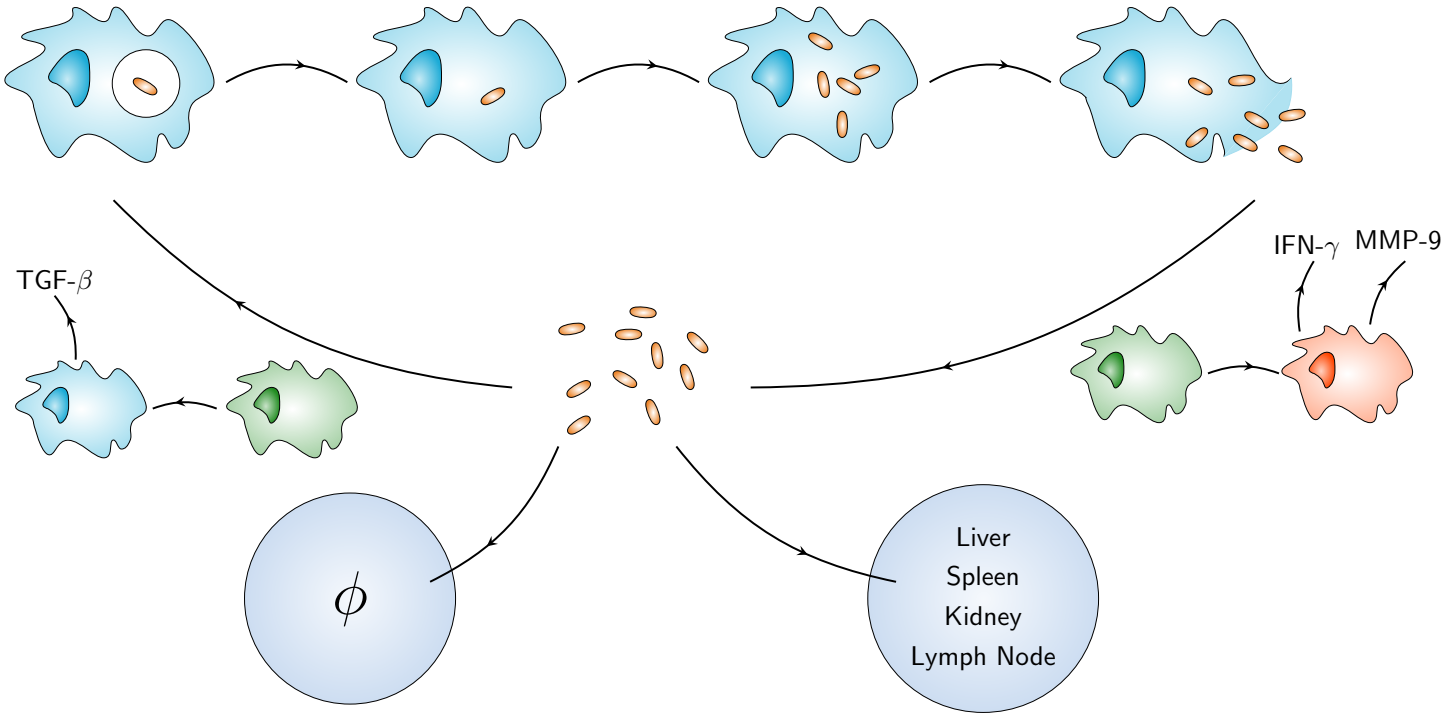
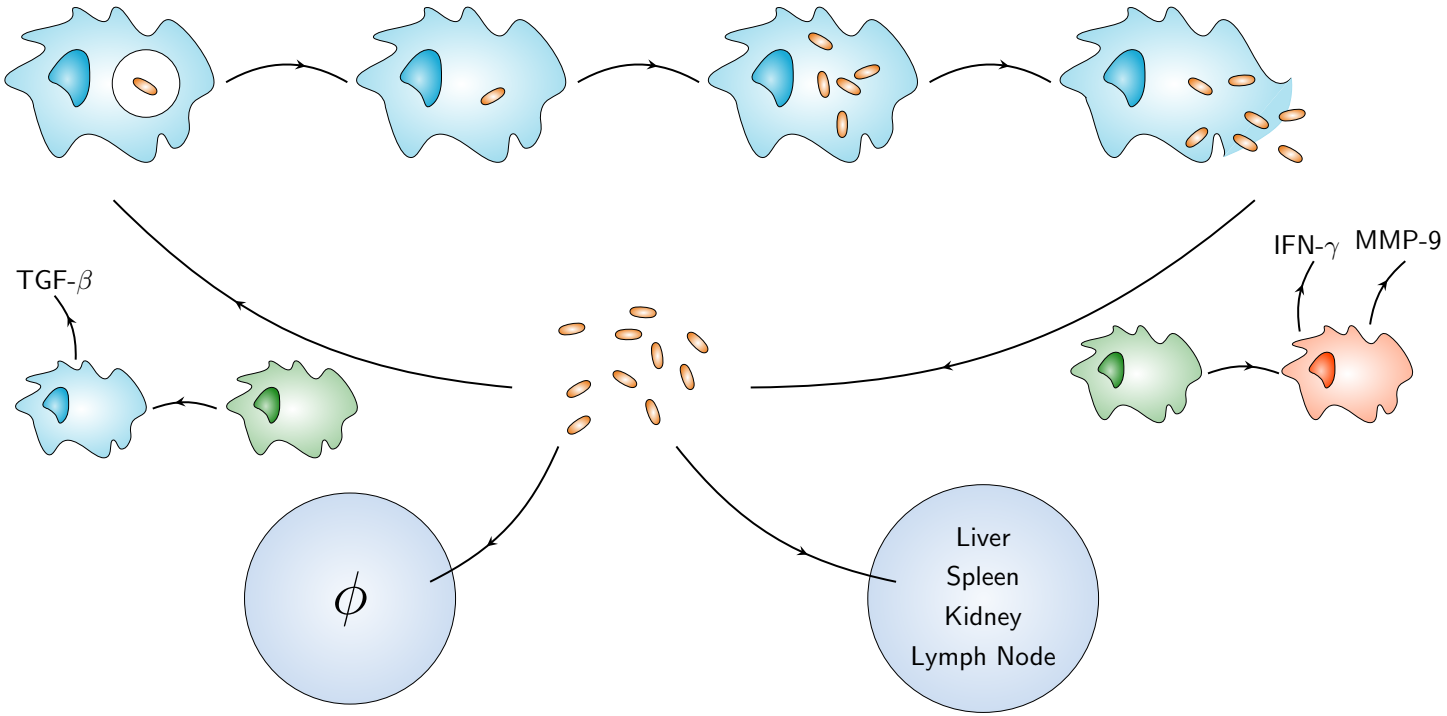
F. tularensis pathogenesis



- Bacteria escape from phagosomes in less than an hour, and begin multiple rounds of replication in the cytosol.

F. tularensis pathogenesis



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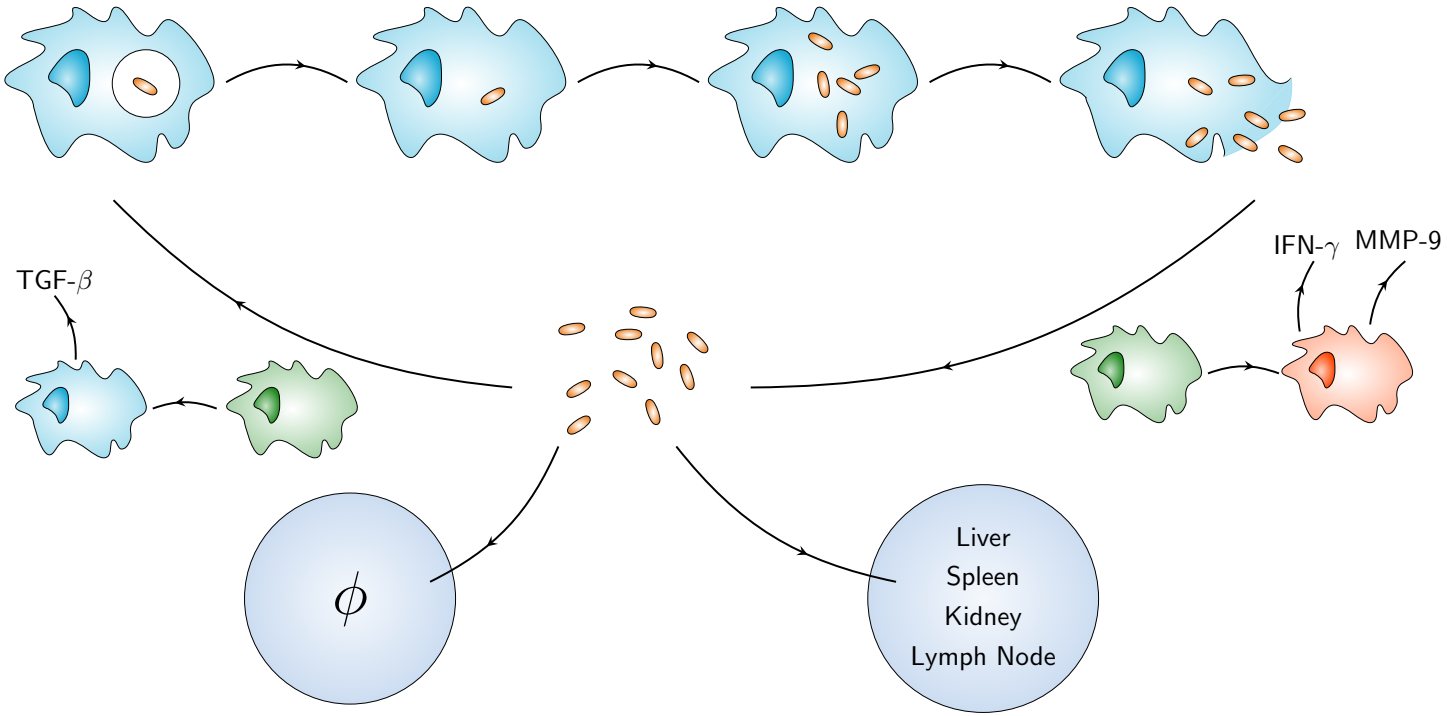

- Bacteria escape from phagosomes in less than an hour, and begin multiple rounds of replication in the cytosol.
- Instead of producing inflammatory cytokines, the first infected macrophages produce anti-inflammatory TGF-β.
- Macrophage rupture releases bacteria that can migrate to another organ, or again infect macrophages in the lung.

You might be expecting

free infectious agents: $\frac{\mathrm{d}}{\mathrm{d}t}V =$

target cells: $\frac{\mathrm{d}}{\mathrm{d}t}T =$

infected cells: $\frac{\mathrm{d}}{\mathrm{d}t}I =$

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p, c and δ are rates: measured in units of inverse time. But ...

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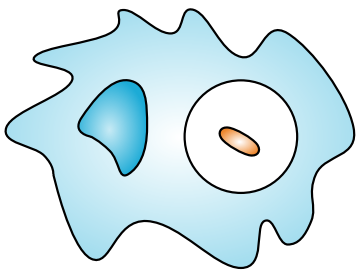
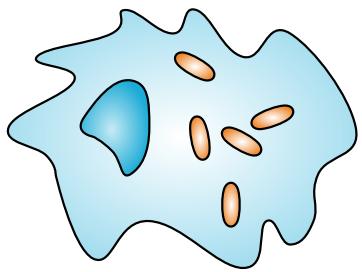
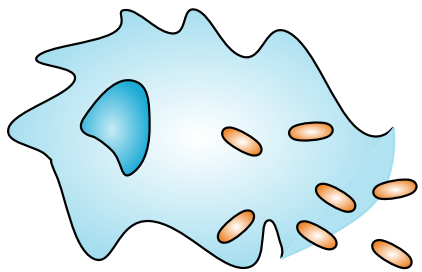
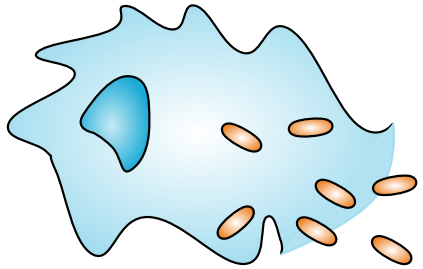




Rupture of an infected macrophage and the release of its bacterial contents



A consequence of the extreme virulence of *F. tularensis* is that initial doses of bacteria used in experiments are small enough that it is reasonable to assume that host macrophages are infected by one bacterium each. Thus the ensemble of realisations of the stochastic process describing the **dynamics of a population of bacteria inside one cell** can equally be thought of as describing the dynamics inside a set of host cells, that behave independently until they rupture.

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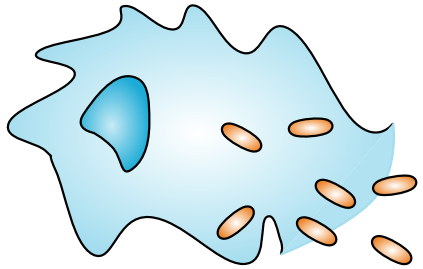

It is possible to assume that the **rupture time and number of bacteria released** per rupture event are fixed parameters that can be fitted from experimental data (Wood et al PHE), or to assume a distribution of rupture times that is independent of the intracellular dynamics. Here, we adopt a mathematical description of the rupture of an infected macrophage and the release of its bacterial contents as a catastrophe in a birth-and-death process.

Agent-based computational model

Each macrophage and each bacterium has a unique identity and set of mutable attributes.
The attributes of a macrophage are: state of activation, cohort counter, number of phagosomal bacteria, list of cytosolic bacteria and spatial location (lung, liver, spleen, MLN or kidney).
At any time, each macrophage is in one of three states: resting, suppressed (anti-inflammatory) or activated (pro-inflammatory).

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Every one of the initial macrophages begins in the resting state.
On phagocytosis, resting macrophages enter a suppressed state in which they are unable to kill bacteria and secrete the anti-inflammatory cytokine TGF-β that contributes to the suppression of other macrophages.
Resting macrophages can become activated through the detection of host damage caused by rupturing macrophages.
Activated macrophages kill the bacteria they phagocytose; they also secrete IFN-γ that provokes activation of neighbouring macrophages.

Agent-based model parameters

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$$\delta \times \text{ bacterial load.}$$

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- Bacteria released either reinfect macrophages in the same organ with rate ρ, are killed, or migrate to a different organ.
- Cytokine-mediated activation and suppression of macrophages are included by means of two dimensionless functions of time, $G(t)$ and $T(t)$, in each organ. The first represents the levels of inflammatory cytokines, such as IFNγ; the second represents the levels of anti-inflammatory cytokines, such as TGF-β.

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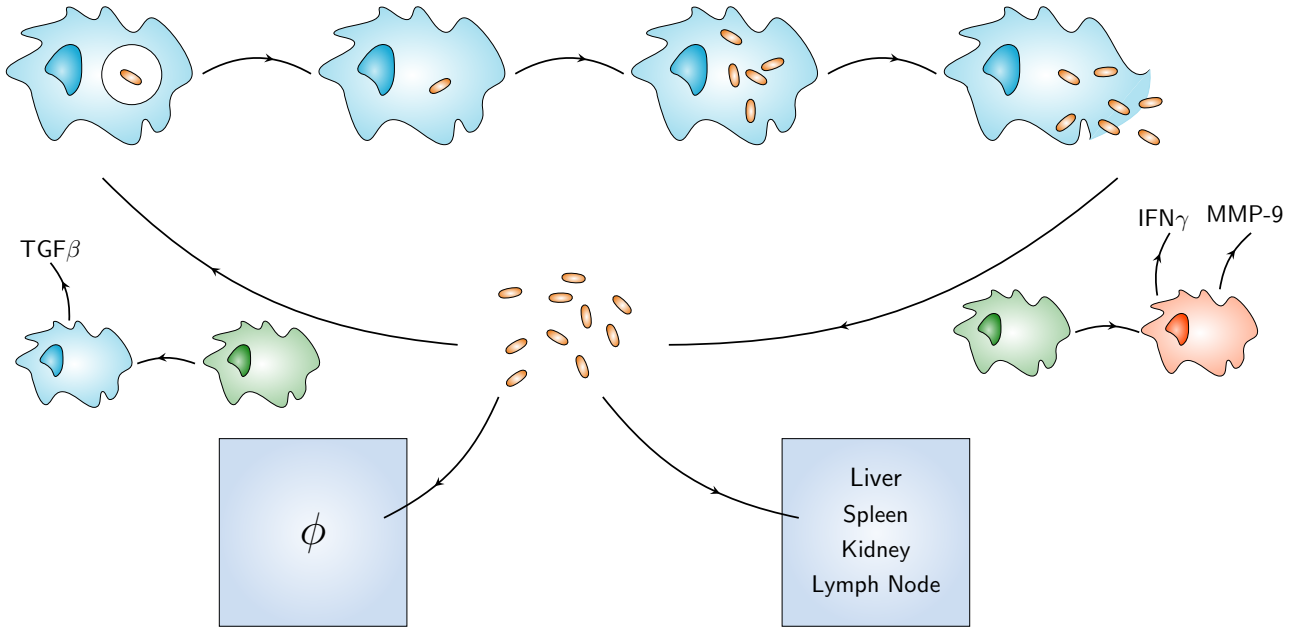
- Initial conditions: N bacteria and M macrophages. $N \ll M$.

Stochastic simulation: eight types of events



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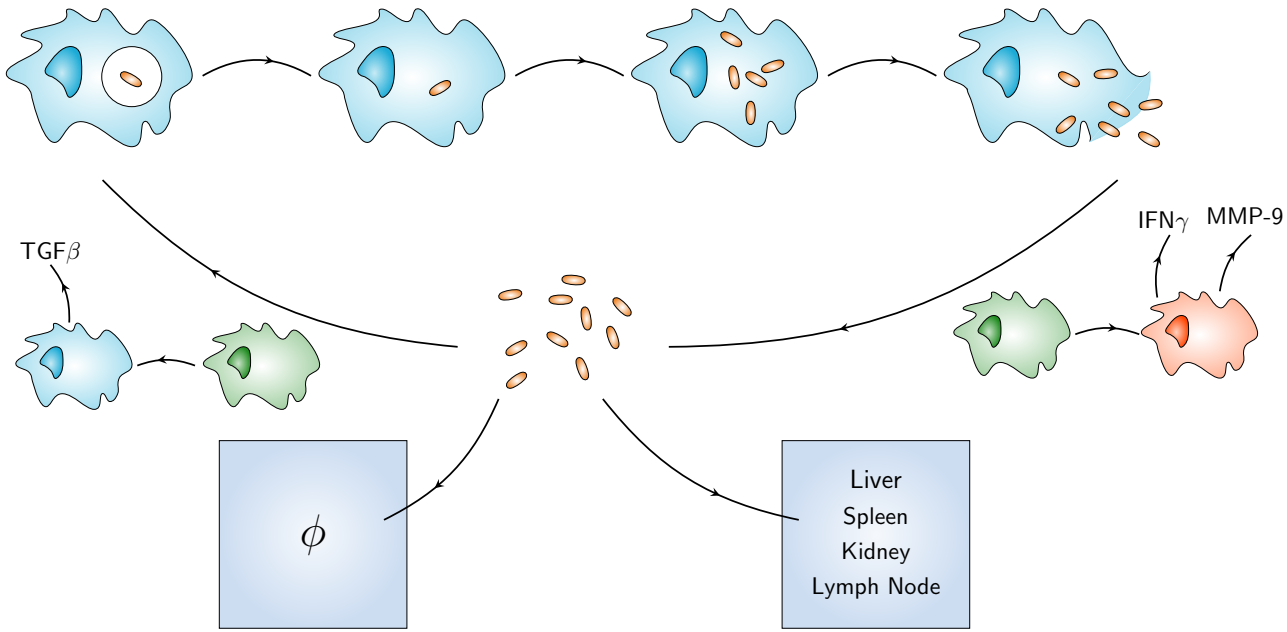
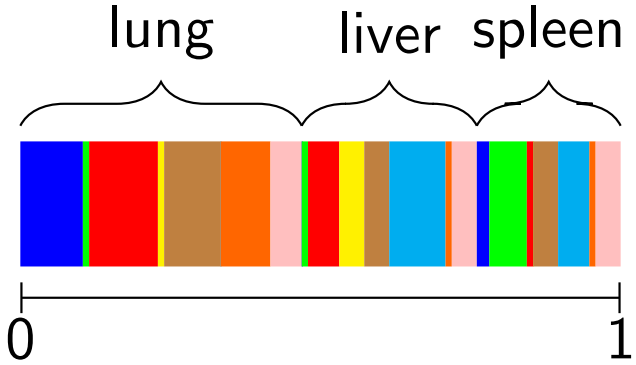
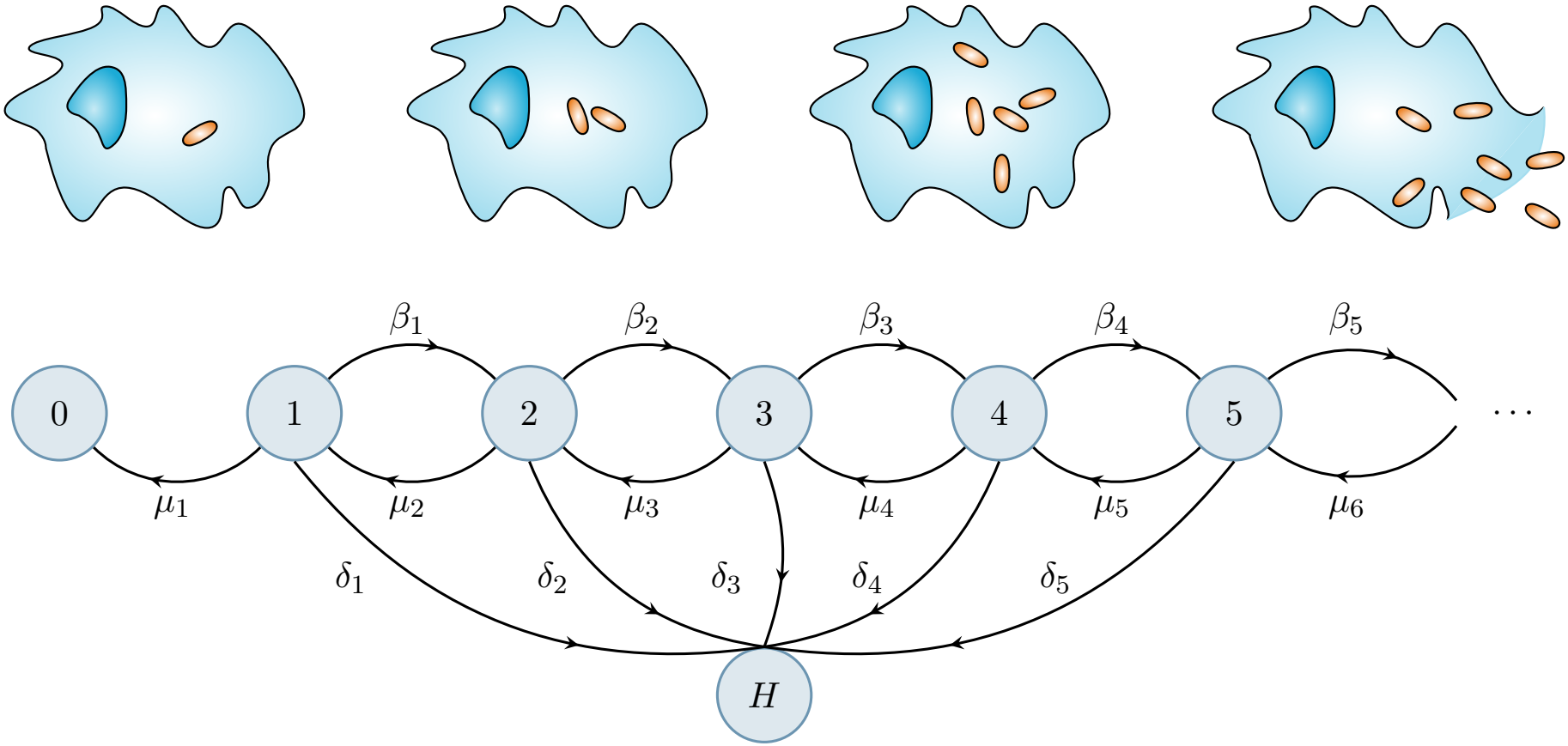




phagocytosis
escape
replication
rupture
death
migration
suppression
activation

At each step, one type of event is chosen, with probabilities weighted by the corresponding rates.

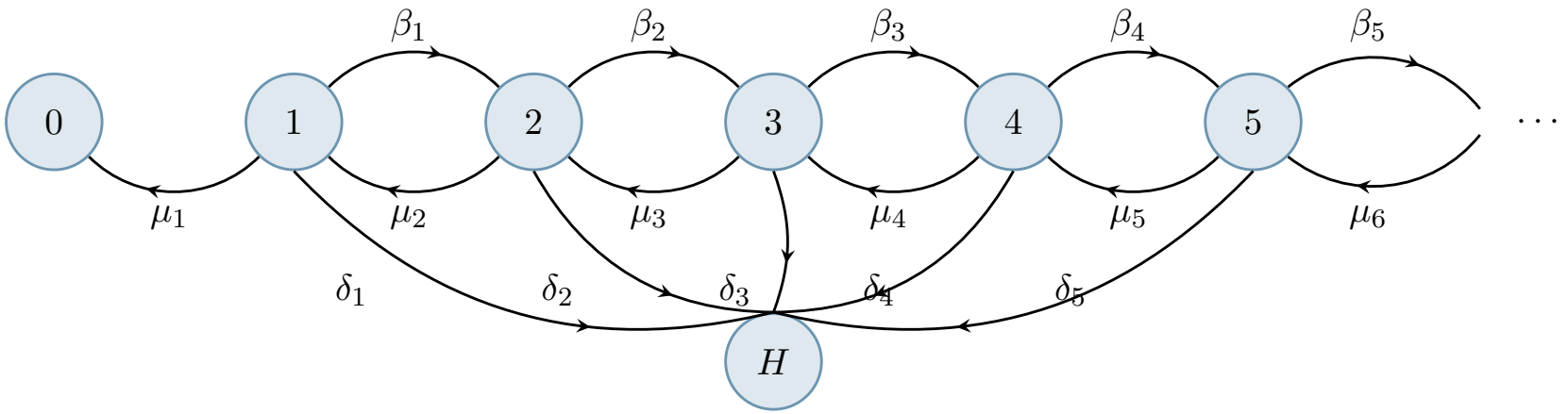
Inside one infected cell: birth and death process with catastrophe



Analysis: birth and death process with catastrophe

If $\mathbf{X}_t$ is the bacterial load **in the cytosol of one macrophage** at time t then, $\mathbf{X}_{t+\Delta t}$ is either

$$\begin{aligned}
&\mathbf{X}_t && \text{with probability } 1-(\mu+\beta+\delta)\mathbf{X}_t\Delta t\\
&\mathbf{X}_t+1 && \text{with probability } \beta\mathbf{X}_t\Delta t\\
&\mathbf{X}_t-1 && \text{with probability } \mu\mathbf{X}_t\Delta t\\
&H && \text{with probability } \delta\mathbf{X}_t\Delta t.
\end{aligned}$$



The absorbing state of zero bacteria can be reached by: (i) elimination of bacteria from the macrophage, or (ii) rupture of the macrophage and release of its contents (H).

Survival function

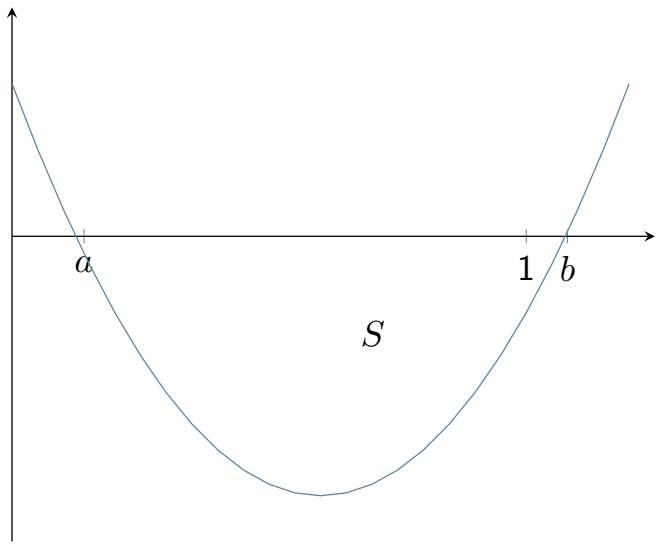
$$S(t) = \Pr[\text{macrophage survives to time } t | \mathbf{X}_0 = 1].$$

If a macrophage is carrying $\mathbf{X}_t$ bacteria in its cytosol at time t, its probability of rupture between t and $t + \Delta t$ is is equal to $\delta \mathbf{X}_t \Delta t$. The function $S(t)$ is the the average over possible values of $\mathbf{X}_t$. Thus $S(t + \Delta t) - S(t) = -\delta \mathbb{E}(\mathbf{X}_t)\Delta t$. In other words,

$$\frac{\mathrm{d}}{\mathrm{d}t} S = -\delta \mathbb{E}(\mathbf{X}_t).$$

In fact

$$\frac{\mathrm{d}}{\mathrm{d}t} S = \mu - (\beta + \mu + \delta)S + \beta S^2.$$



Mean number of bacteria released at time t

- If $\bar{n}(t)\Delta t$ is the mean number of bacteria released by a macrophage that was infected by one bacterium at $t = 0$ and ruptures between t and $t + \Delta t$, then $\bar{n}(t) = \delta\, \mathbb{E}(\mathbf{X}_t{}^2 \,|\, \mathbf{X}_0 = 1)$.
- The most elegant way to evaluate the moments of $\mathbf{X}_t$ is by making use of the probability generating function.

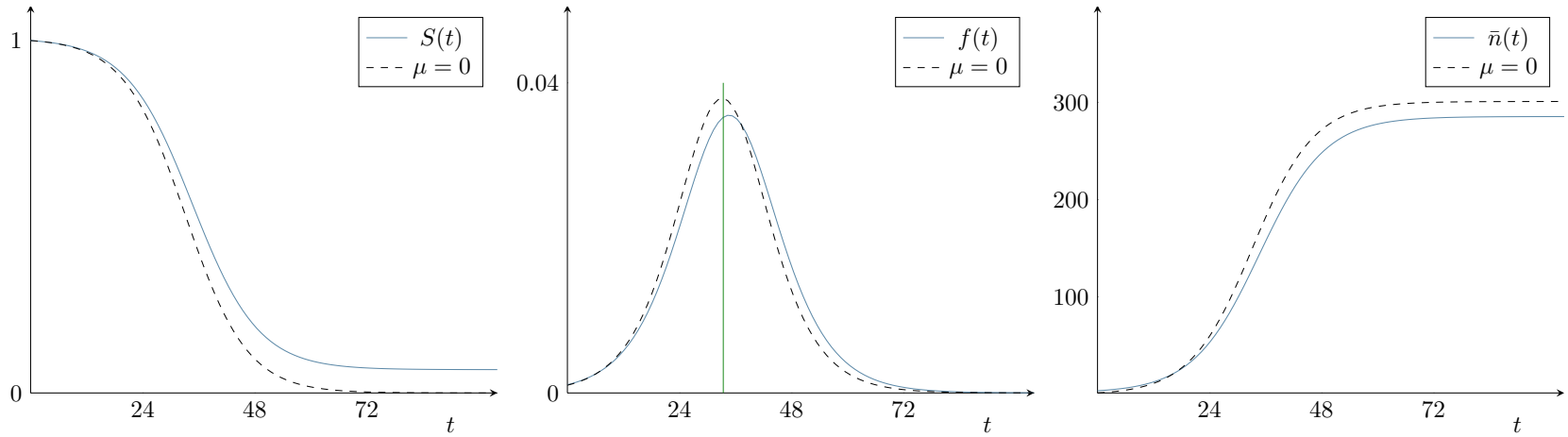
$$p_n(t) = \Pr[\mathbf{X}_t = n | \mathbf{X}_0 = 1] \qquad \text{and} \quad G(z,t) \equiv \sum_{n=0}^{+\infty} p_n(t) z^n.$$

It can be shown that

$$G(z,t) = \frac{ab(1 - \mathrm{e}^{-\beta(b-a)t}) + z(b\mathrm{e}^{-\beta(b-a)t} - a)}{b - a\mathrm{e}^{-\beta(b-a)t} - z(1 - \mathrm{e}^{-\beta(b-a)t})}.$$

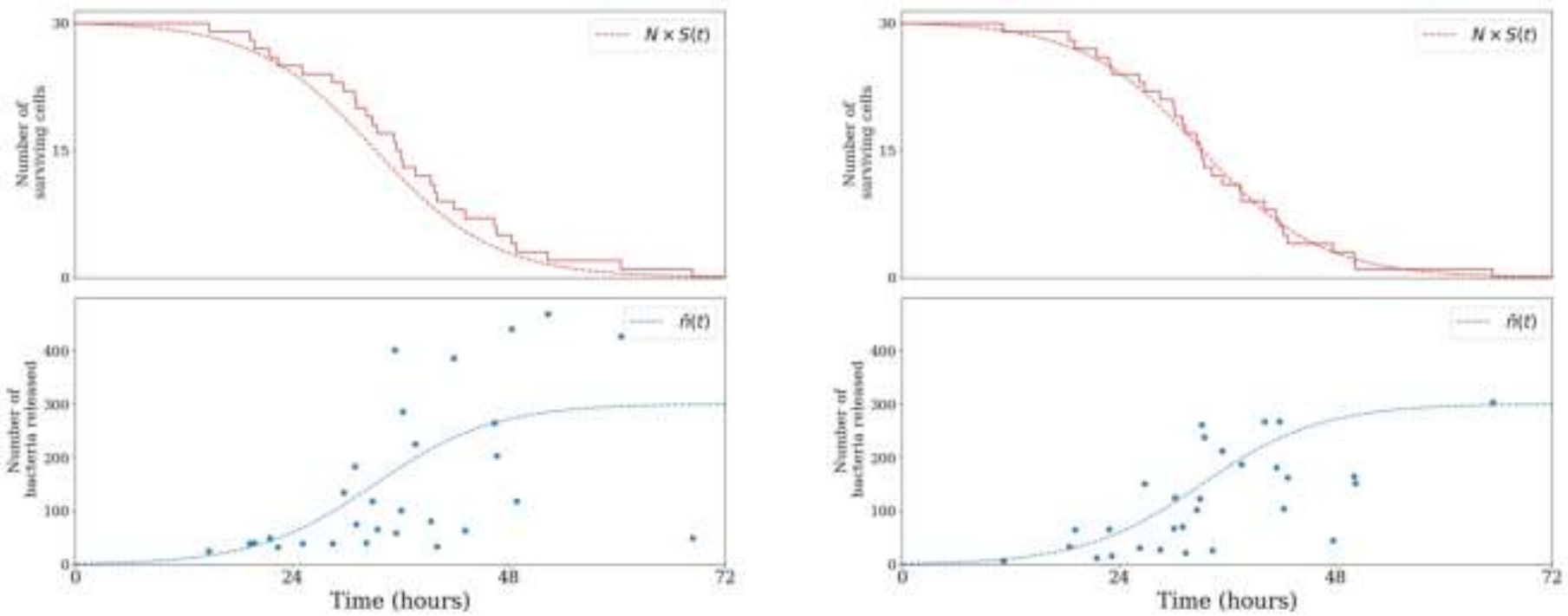
- We conclude that $\bar{n}(t) = \frac{b+1-(1+a)e^{-(\beta+\delta)t}}{b-1+(1-a)e^{-(\beta+\delta)t}}$.

The survival function and bacterial release on rupture



Left: The macrophage survival probability $S(t)$.
Centre: The mean bacterial load is proportional to $f(t) = -\frac{\mathrm{d}}{\mathrm{d}t}S(t)$.
Right: the function $\bar{n}(t)$ that gives the mean number of bacteria released per macrophage.
The parameter values are $\beta = 0.15\ h^{-1}$, $\mu = 0.01\ h^{-1}$ and $\delta = 0.001\ h^{-1}$. (Dashed lines: $\mu = 0$.)

Agent-based realisation compared to predicted means

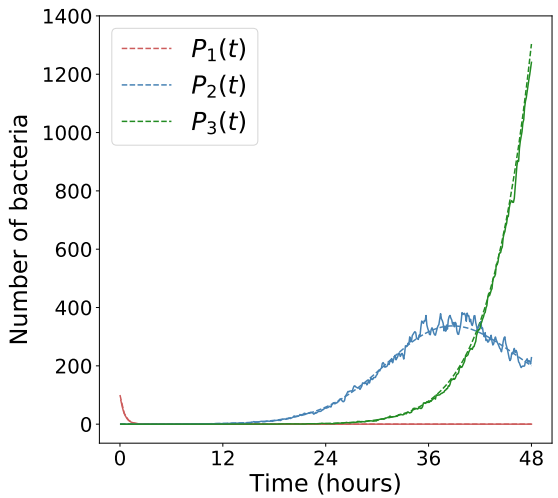


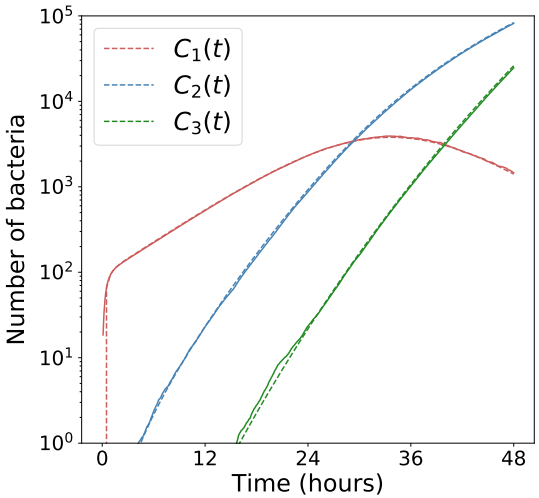
- In each numerical realisation, $N = 30$ macrophages are infected, by one bacterium each, at $t = 0$. Red line: number of those macrophages surviving up until time t. Dotted red curve: $NS(t)$.
- Each blue dot coincides with a downward step in the red line, corresponding to a macrophage rupture. Dotted blue curve: $\bar{n}(t)$.
- Parameter values: $\beta = 0.15\text{h}^{-1}$, $\mu = 0$ and $\delta = 0.001\text{h}^{-1}$. Numerical runs: $M = 10^4$, $\rho = 0.01\text{h}^{-1}$, $\phi = 2\text{h}^{-1}$, $\gamma = 1\text{h}^{-1}$.

Cohort analysis

Bacterial loads can be measured in different organs of an infected mouse (but only at one timepoint).
In an agent-based simulation, on the other hand, the entire history of every macrophage and bacterium is available. We explicitly track cohorts of bacteria by assigning a 'cohort number' attribute to each.
We define the quantities

- $P_i(t)$, the mean number of cohort i bacteria in macrophage phagosomes at time $t \geq 0$,
- $C_i(t)$, the mean number of cohort i bacteria in macrophage cytosols at time $t \geq 0$.



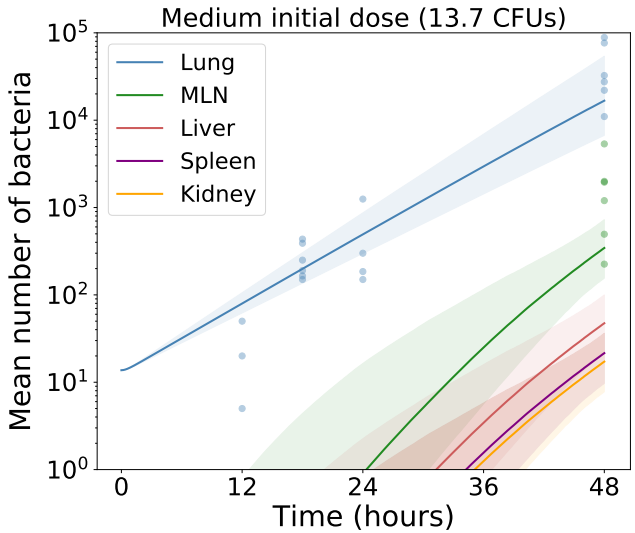


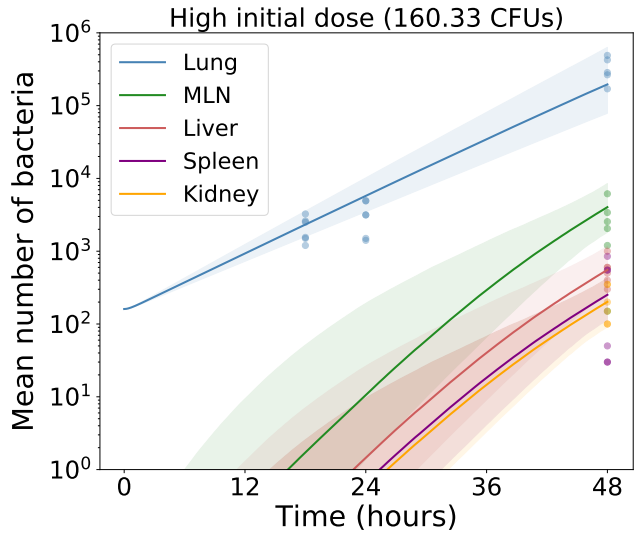
Data (Roman Lukaszewski, Dstl)

Six-to-eight week old female BALB/c mice were challenged with *F. tularensis* SCHU S4. In these experiments, mice were infected with either 160 (high), 14 (medium) or 2 (low) colony forming units.

High infectious dose (160.33 CFU)

time ($hours$)	organ	mouse 1	2	3	4	5	6	mean	SD
1	lung	0	0	2.50×10^2	1.50×10^2	0	3.50×10^1	1.05×10^1	1.42×10^1
18	lung	2.60×10^3	3.25×10^3	1.50×10^3	1.55×10^3	1.20×10^3	2.50×10^3	1.97×10^3	1.48
	MLN	4.00×10^1	8.50×10^1	5.00×10^0	7.00×10^1	2.45×10^2	6.50×10^1	5.16×10^1	3.64
24	lung	4.95×10^3	3.15×10^3	3.15×10^3	4.90×10^3	1.41×10^3	1.50×10^3	2.83×10^3	1.74
48	lung	2.65×10^5	2.85×10^5	1.30×10^6	1.70×10^5	4.90×10^5	4.25×10^5	3.89×10^5	2.01
	MLN	2.05×10^3	6.15×10^3	3.40×10^3	1.50×10^2	2.55×10^3	1.20×10^3	1.64×10^3	3.64
	liver	5.00×10^2	6.00×10^2	4.00×10^2	3.00×10^2	1.00×10^3	6.00×10^2	5.28×10^2	1.51
	kidney	3.50×10^2	2.00×10^2	3.50×10^2	1.00×10^2	1.00×10^2	1.50×10^2	1.82×10^2	1.77
	spleen	3.00×10^1	5.00×10^1	8.50×10^2	3.00×10^1	5.50×10^2	5.50×10^2	1.50×10^2	4.94





Model calibration from Roman's data

Event	Symbol	Units	Range for sensitivity analysis	Value
intracellular bacterial replication	β	h^{-1}	[0,0.5]	1.51×10^{-1}
macrophage rupture	δ	h^{-1}	[0,0.01]	1.00×10^{-3}
phagocytosis	$M\rho$		$\log_{10} M\rho \in [-2, 5]$	2.93×10^{3}
extracellular bacterial death	μ	h^{-1}	[0,0.1]	1.00×10^{-2}
phagosomal escape	ϕ	h^{-1}	[0.5,5]	2.00×10^{0}
total migration away from the lung	γ	h^{-1}	$\log_{10} \gamma \in [0, 3]$	2.35×10^{1}

Bayesian computation:

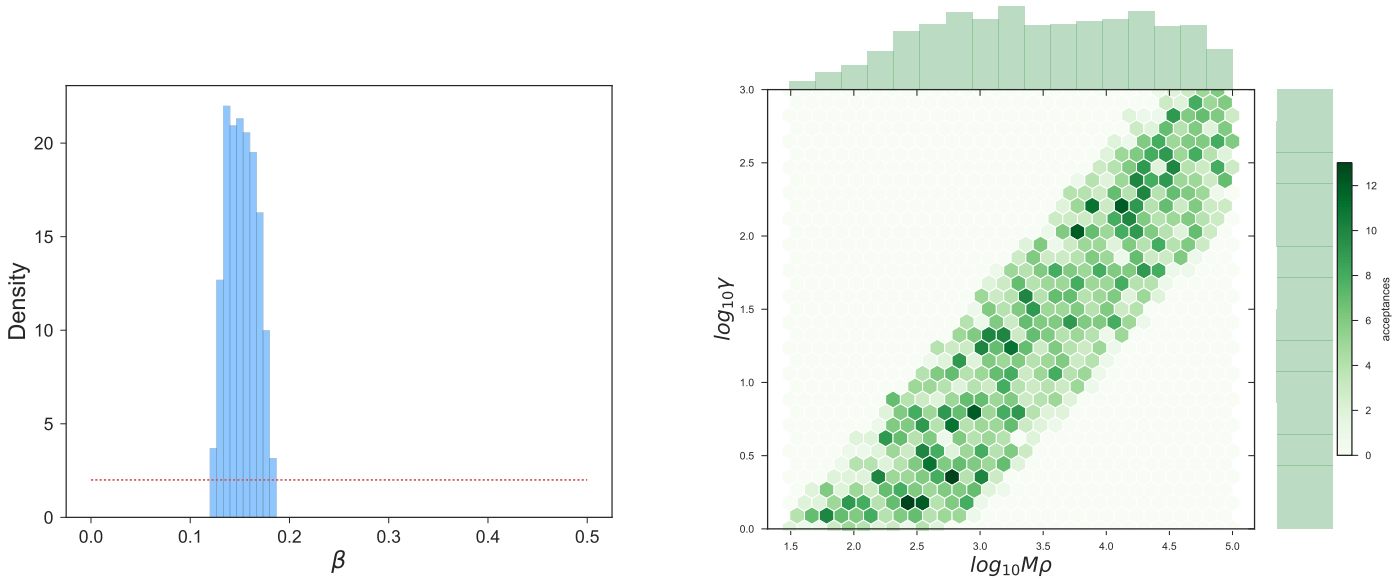


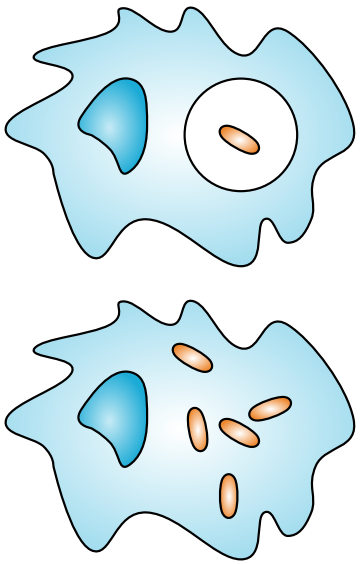
Figure: Left: Posterior histogram for β with the prior distribution in red. Right: The relationship between $\log_{10} \gamma$ and $\log_{10} M\rho$.

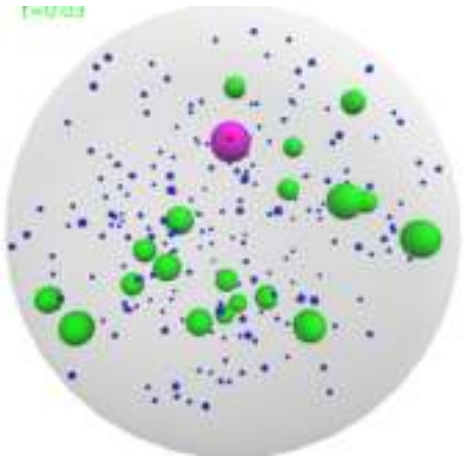
F. tularensis: summary

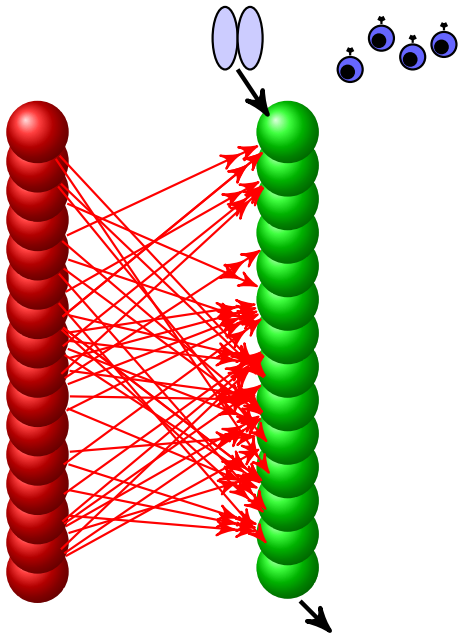
- With a mouse infection model, agent-based computation and mathematical analysis, we study *F. tularensis* infection.
- Bacteria enter host cells and proliferate inside, eventually destroying the cell and releasing copies that infect other cells.
- Analysis based on a stochastic model of a population of infectious agents inside one host cell, extending the birth-and-death process by the occurrence of catastrophes: cell rupture events that affect all bacteria in a cell simultaneously.
- Compare analysis with agent-based computation and, via Approximate Bayesian Computation, with experimental measurements carried out after murine aerosol infection.

Outline

Modelling Ebola virus in vitro infection

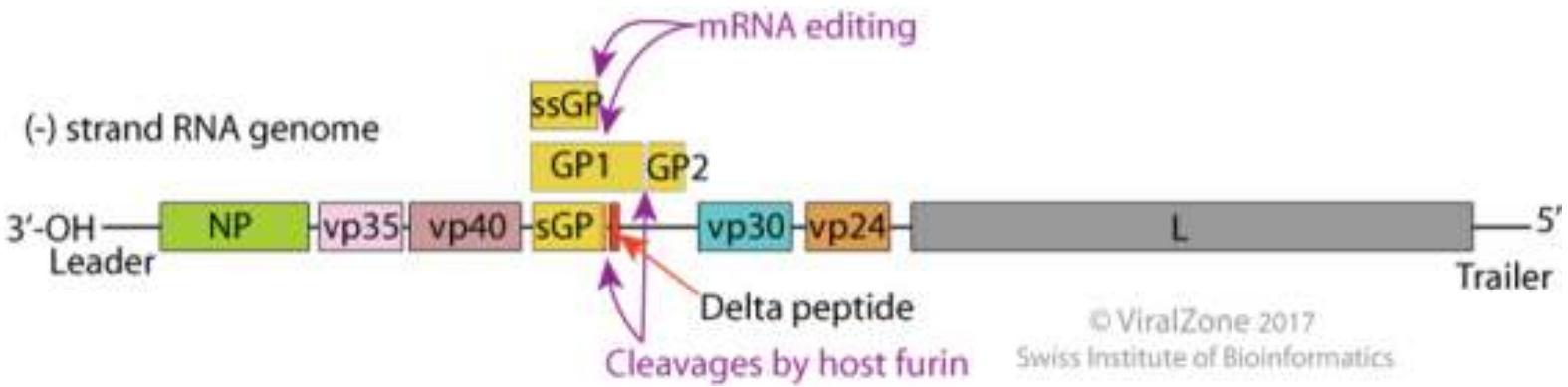




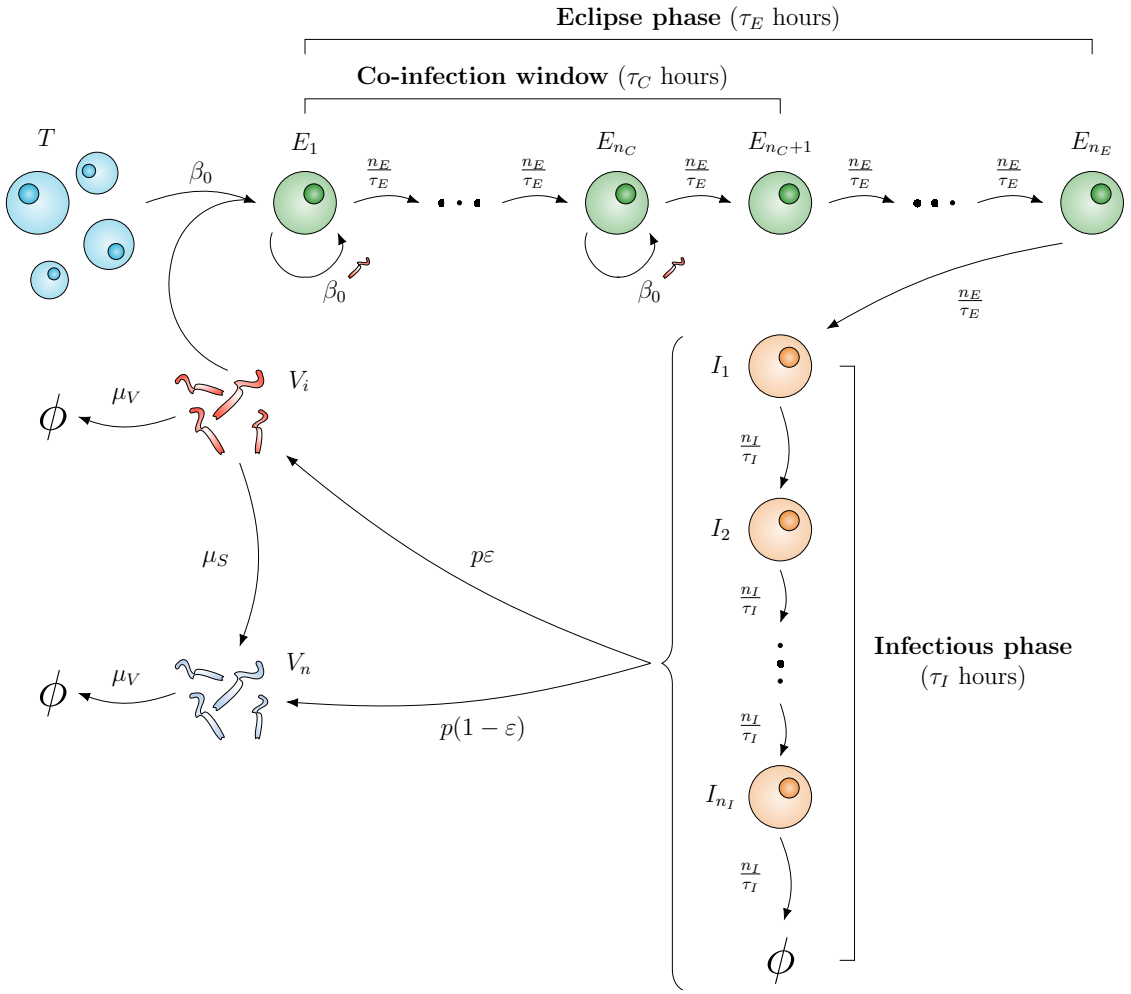


Ebola virus

- Ebola virus is a highly pathogenic filovirus, with a negative-sense single-stranded non-segmented RNA genome.
- Filoviruses replicate in many different cell types: macrophages, dendritic cells, endothelial cells, ...
- Viral release occurs by budding.
- Work with infectious filoviruses must be conducted under containment level 4.



Within-host model: co-infection window and eclipse phase



Following the eclipse phase, infected cells enter a phase where budding of infectious virus and non-infectious virus occurs.

Intra-cellular stochastic model

Dstl measured intra-cellular viral load for Ebola WT. *In vitro* infection assays (single-cycle, multiple-cycle, and viral infectivity decay assays) using EBOV "E718" and Vero cells, obtained from Culture Collection, Public Health England.

- **Co-infection window**: in this phase, virus can enter already infected cells.
- **Eclipse phase**: viral replication takes place.
- **Infectious phase**: viral replication, assembly and budding.

Posterior distributions of parameters were estimated using a Markov chain Monte Carlo (MCMC) approach.

Ebola summary

- We estimate that one EBOV-infected cell spends 30h in an eclipse phase before it releases infectious virions at a rate of 13h^{-1}, over its infectious lifetime of 83h. The number of infectious virions produced over an infected cell's lifetime is about 1000, with an estimated basic reproductive number of 600.
- Once in the culture medium, virus loses infectivity at rate 0.06h^{-1}.
- EBOV was titrated in 96-well plates using the endpoint fifty percent tissue culture infectious dose (TCID50) assay.
- Characterise WT and DI competition and/or interference.

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`http://www1.maths.leeds.ac.uk/applied/~grant`

`http://www1.maths.leeds.ac.uk/applied/BSI/`

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