Modelling Host-Pathogen Dynamics of *Salmonella*

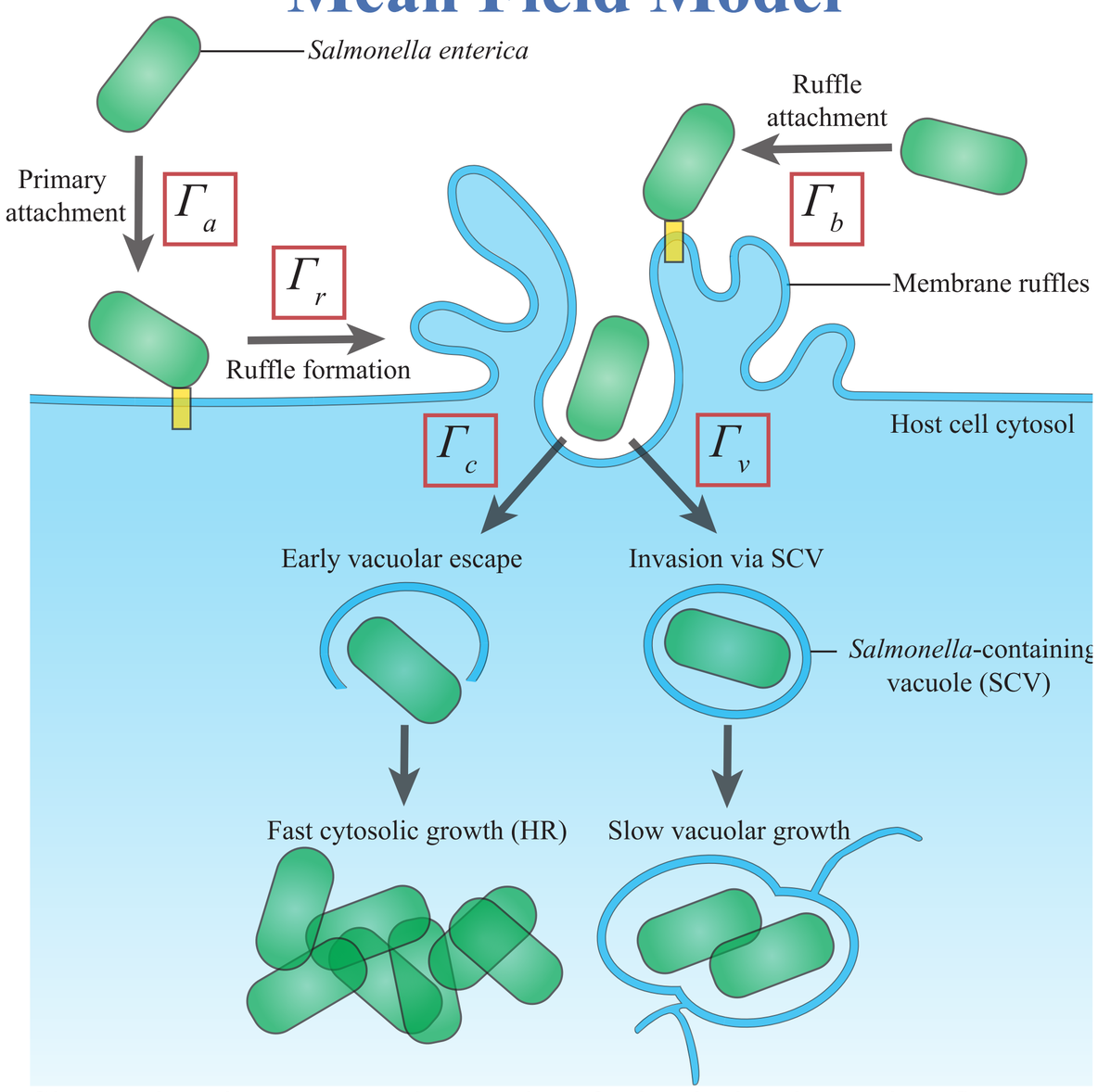
Taylor Dunn (taylor.dunn@dal.ca) and Andrew Rutenberg (adr@dal.ca)
Department of Physics & Atmospheric Science, Dalhousie University, Canada

Motivation

The dynamics of invasion by pathogenic bacteria is a stochastic process at every stage, including attachment, entry by endocytosis, vacuolar escape, replication and transmission to other cells [1]. To date, there has been little quantitative modelling of this system.

The bacterium *Salmonella enterica* has been shown to hyper-replicate (HR) within the cytoplasm of some epithelial cells, while remaining slow-growing within the vacuole of others [2]. Our key question is how the HR fraction (the fraction of infected cells which contain HR bacteria) varies with the multiplicity of infection (MOI, ratio of bacteria to host cells). To explore this, we model the invasion process using simple rates, tuned to experimental data.

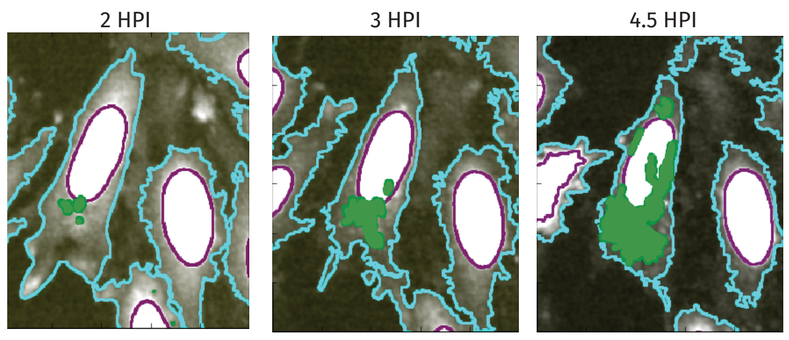
Mean Field Model



Given an invasion system with a certain MOI, incubation time and confluency, these rates ($\Gamma_a, \Gamma_b, \Gamma_r, \Gamma_v, \Gamma_c$) can be used to numerically integrate sets of differential equations (see box below) describing quantities such as infectivity and invasion efficiency (invaded bacteria / total bacteria). We hypothesize that these rates are universal to a specific system - in this study, *Salmonella* invading HeLa cells.

Timelapse Image Analysis

To test our model and determine the HR fraction at different MOI, fluoresence microscopy images were taken of Salmonella invading HeLa cells over time by Jennifer Fredlund at the Institut Pasteur (lab of Jost Enninga).



Cells and bacteria were segmented using the CellProfiler analysis software [3]. An example of a cell with HR bacteria is shown here at different times post infection.

To systematically estimate if a cell is HR, the following criteria is used:

1. Fold growth (from 1.5 to 5.5 HPI) > 5
2. Net intensity growth > threshold
3. Net area growth > threshold
4. Growth fits exponential growth + background: $n(t) = n(0)\, e^{\gamma t} + c$
5. Growth exponent is positive within uncertainty: $\gamma - \sigma_\gamma > 0$

The first three criteria are chosen conservatively to catch any appreciable growth, while the 4th and 5th filter out slow growing bacteria.

Parameters

MOI $\equiv m$
Confluency $\equiv c$
Incubation time $\equiv t_{\max}$
Primary attachment rate (per bacterial density) $\equiv \Gamma_a$
Ruffle attachment rate (per bacterial density) $\equiv \Gamma_b$
Ruffle formation rate (per attached bacteria) $\equiv \Gamma_r$
Vacuolar invasion rate (per attached bacteria) $\equiv \Gamma_v$
Cytosolic invasion rate (per attached bacteria) $\equiv \Gamma_c$
Max invaded bacteria per cell $\equiv b_{\max}$

Dynamical variables

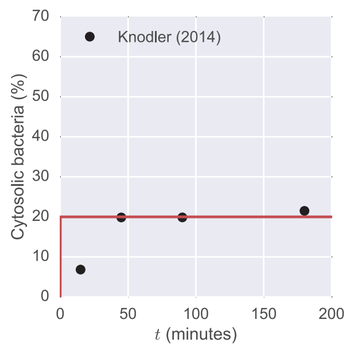
Host cells with attached bacteria $\equiv h_a(t)$
Host cells with vacuolar bacteria $\equiv h_v(t)$
Host cells with cytosolic bacteria $\equiv h_c(t)$
Host cells with ruffles $\equiv h_r(t)$
Ruffles per host cell $\equiv r(t)$
Attached bacteria $\equiv b_a(t)$
Vacuolar bacteria $\equiv b_v(t)$
Cytosolic bacteria $\equiv b_c(t)$
Swimming (unattached) bacteria $\equiv b(t)$
Invaded bacteria $\equiv b_x(t) = b_v + b_c$

Dynamical equations

$$\dot{h}_a = (1 - h_a)\Gamma_a m b c$$
$$\dot{h}_v = (h_a - h_v)\Gamma_v m b_a / h_a$$
$$\dot{h}_c = (h_a - h_c)\Gamma_c m b_a / h_a$$
$$\dot{h}_r = (h_a - h_r)\Gamma_r m b_a / h_a$$
$$\dot{r} = \Gamma_r m b_a / h_r$$
$$\dot{b}_a = \Gamma_a b c + r h_r \Gamma_b b c - b_a(\Gamma_v^* + \Gamma_c^*)$$
$$\dot{b}_v = b_a \Gamma_v^* = b_a \Gamma_v \left(1 - \tfrac{(b_x/h_x)}{b_{\max}}\right)$$
$$\dot{b}_c = b_a \Gamma_c^* = b_a \Gamma_c \left(1 - \tfrac{(b_x/h_x)}{b_{\max}}\right)$$

Data Analysis

There is a wealth of literature on the subject of *Salmonella* invasion into HeLa cells to parameterize our model. Experiment-specific parameters (MOI, incubation time, confluency) were generally available, and used as input into our model to extract rates. The **solid lines** below were calculated from our model using the rates that best fit all of the experimental data.

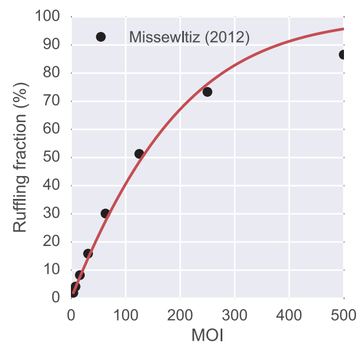


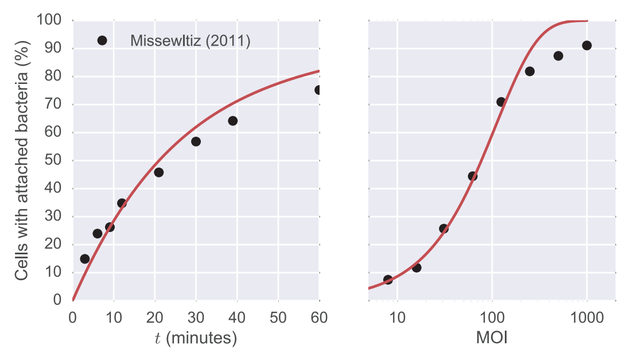
Knodler et al [4] quantified the proportion of cytosolic bacteria versus time post infection and found it rose quickly to ~20% and stayed until 3 hours. This suggests that the rates of internalization follow a ratio of

$$\Gamma_c \approx 4\Gamma_v$$

Another simple result came from Misselwitz et al [5], who calculated the ruffling fraction (fraction of cells with ruffles) versus MOI. Fitting the model gives us a ruffle formation rate of

$$\Gamma_r \approx 0.015 \text{ ruffles/min}$$



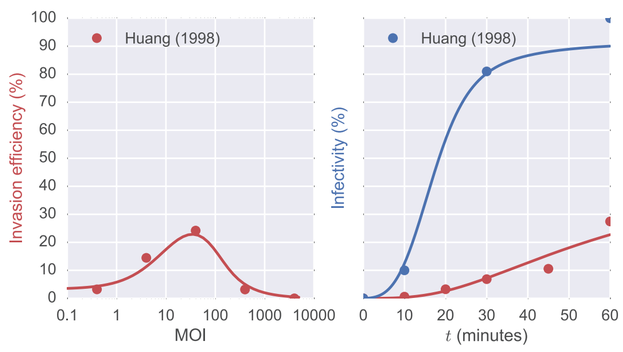


The same group in 2011 studied *Salmonella* binding to HeLa cells [6], and calculated the percentages of cells with attached bacteria versus MOI and time.

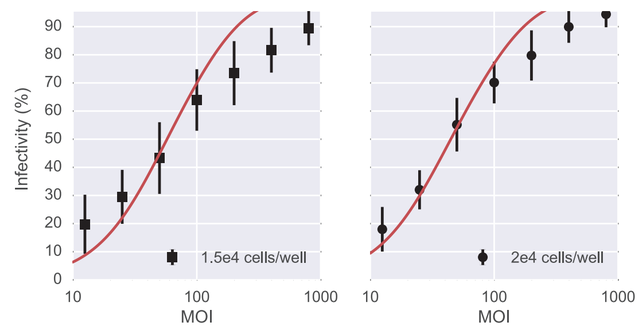
$$\Gamma_a \approx 0.002,\ \Gamma_b \approx 0.02 \text{ bacteria/min}$$

In 1998, Huang et al [7] found a strict physical limitation on bacterial entry at MOIs ≥ 40, affirming the need for a maximum number of internalized bacteria per cell, $b_{\max}$.

$$\Gamma_c + \Gamma_v \approx 0.013 \text{ bacteria/min}$$
$$b_{\max} \approx 20 \text{ bacteria}$$



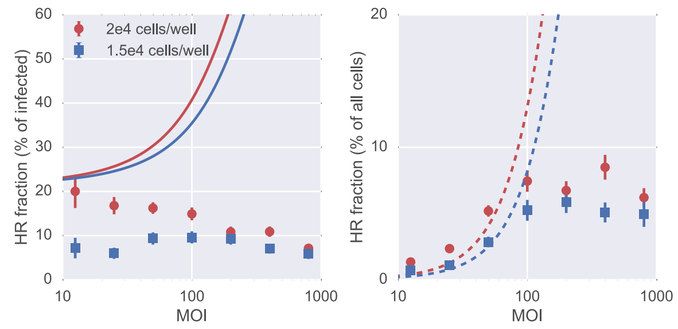
Now with a general idea of model parameter values, we can look at our own data. HeLa cells were infected for 25 minutes for a large range of MOI, and images were taken at multiple time points between 1.5 and 5.5 HPI to determine infectivity and HR fraction. Two independent experiments were performed at different confluencies (1.5e4 cells/well and 2e4 cells/well). Each data point is the mean ± st. dev. of 2-4 wells (17 fields each).



At MOIs > 100, the model prediction begins to overshoot the data. This high MOI overshoot is also seen in the literature (ruffling fraction [4] and attached bacteria [5]), which could indicate an MOI dependence in the primary attachment rate.

A simple assumption of our model is that any cytosolic bacterium goes on to hyper-replicate. Since bacteria become cytosolic ~20% of the time, the fraction of cells with cytosolic bacteria quickly rises with MOI (**lines** below).

Conversly, the experimental HR fraction either stays at, or drops to 5-10% with MOI. It is interesting, however, that the HR fraction of all cells rises until about 100 MOI before seeming to plateau.



Conclusions

A mean field model was developed to describe the invasion of *Salmonella* into HeLa cells. Data from a number of sources was used to find rates that are universal to the system, although there are still discrepancies at high MOI to be explained, and possibly incorporated into the model. HR fraction was estimated at MOIs from 12.5 to 800, and stayed between 5 and 20%. This suggests that not all cytosolic bacteria hyper-replicate. Other possibilities are that the success of HR bacteria is cell-dependent (i.e. some host cells are able to defend from HR) or load-dependent (i.e. at high load, there is competition between bacteria to grow).

[1] Ray, Katrina, et al. "Life on the inside: the intracellular lifestyle of cytosolic bacteria." Nature Rev Microbiol 7.5 (2009): 333-340.
[2] Knodler, Leigh A., et al. "Dissemination of invasive Salmonella via bacterial-induced extrusion of mucosal epithelia." Proc Natl Acad Sci USA 107.41 (2010): 17733-17738.
[3] Carpenter, Anne E., et al. "CellProfiler: image analysis software for identifying and quantifying cell phenotypes." Genome biology 7.10 (2006): R100.
[4] Knodler, Leigh A., Vinod Nair, and Olivia Steele-Mortimer. "Quantitative assessment of cytosolic Salmonella in epithelial cells." (2014): e84681.
[5] Misselwitz, Benjamin, et al. "Near surface swimming of Salmonella Typhimurium explains target-site selection and cooperative invasion." (2012): e1002810.
[6] Misselwitz, Benjamin, et al. "Salmonella enterica serovar Typhimurium binds to HeLa cells via Fim-mediated reversible adhesion and irreversible type three secretion system 1-mediated docking." Infect Immun 79.1 (2011): 330-341.
[7] Huang, Xiao-Zhe, et al. "Physical Limitations on Salmonella typhi Entry into Cultured Human Intestinal Epithelial Cells." Infect Immun 66.6 (1998): 2928-2937.