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Modelling phage therapy dynamics of MRSA on ex vivo pig skin experiments

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ModAH, Nantes

August, 29th 2024

Speaker: Alfredo Acosta

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PHAGE-EX

Project

Use of phage applications to combat MRSA at the sow-piglet interface to reduce exposure of staff and contamination of the environment

ENVIRONMENT | INTERVENTIONS | TRANSMISSION

Research Project: 2022-01-04 - 2025-03-31

https://www.jpiamr.eu/projects/phage-ex/

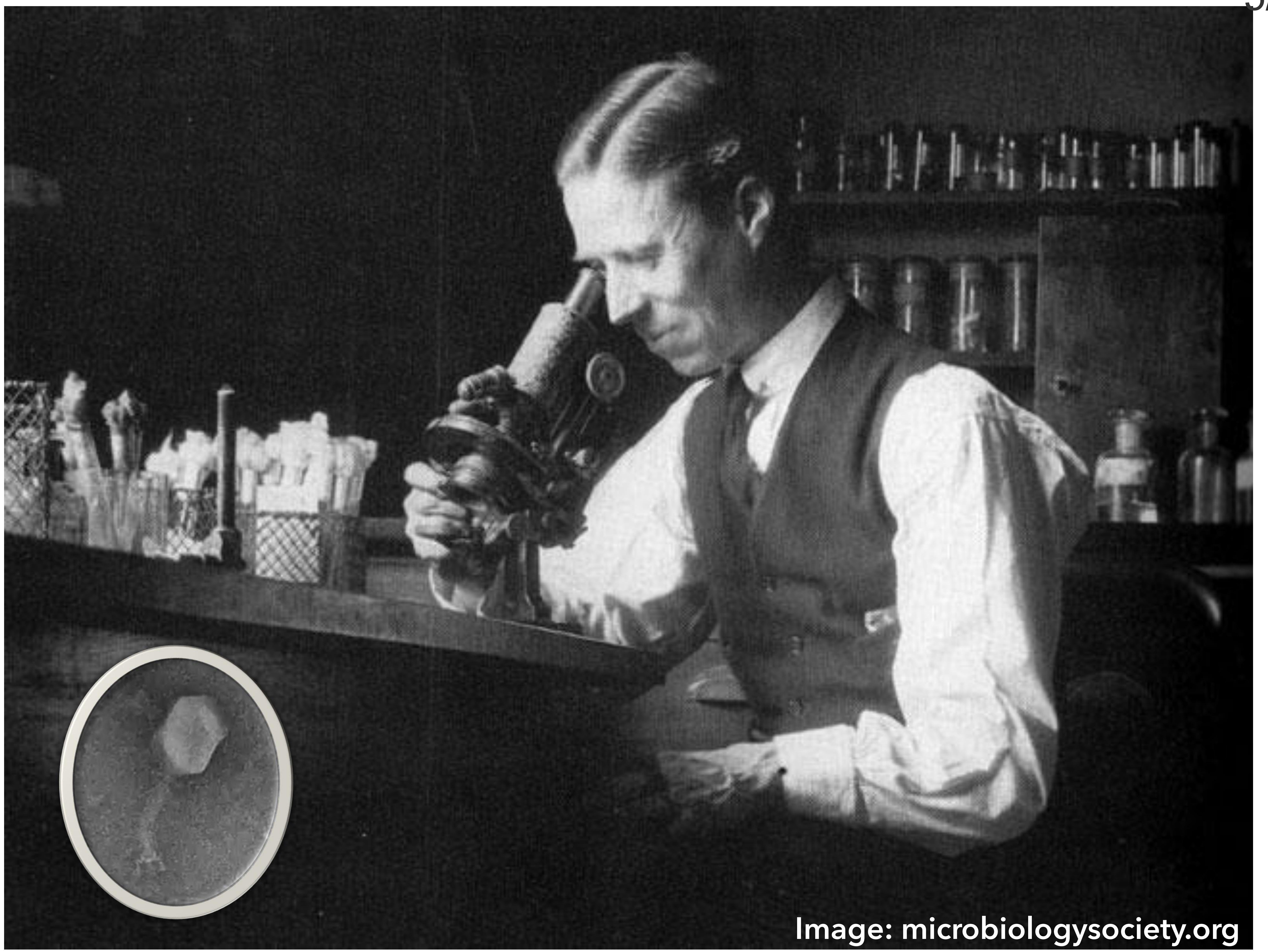
Background

Alexander Fleming, 1928.
The age of antibiotics


THE

LANCET.

Feb 13, 1915
Volume 185, Number 4772, p309-358
Originally published as Volume 1, Issue 4772



William Twort, 1918.

https://microbiologysociety.org/publication/past-issues/world-war-i/article/frederick-william-twort-not-just-bacteriophage.html

LA-MRSA

- Pigs are common carriers of livestock-associated methicillin resistant Staphylococcus aureus LA-MRSA.
- MRSA is Resistant to several common antibiotics.
- zoonotic.
- Initially hospital-associated, its prevalence have augmented since 1990s.

Angen, Ø., et al., 2019. Controlling Transmission of MRSA to Humans During Short-Term Visits to Swine Farms Microbiol. 9. https://doi.org/10.3389/fmicb.2018.03361

Phage-MRSA Interactions

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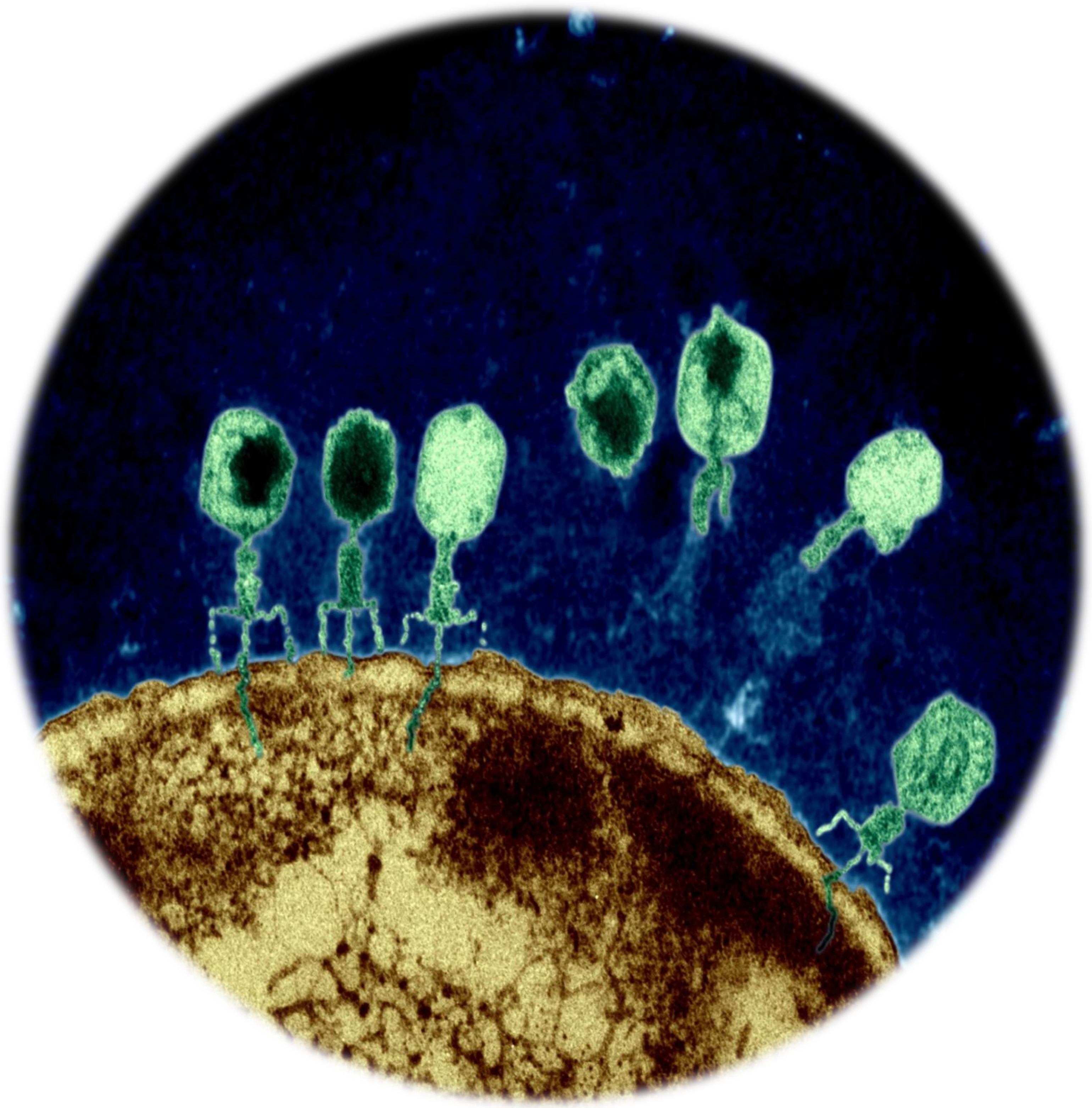
Infection rate of MRSA by bacteriophages depends by chance and various types of parameters, usually:

a) Affinity of the phage to irreversibly bind with the receptor.

b) Rate of diffusion of phage particles in the infection medium.



Sinha, S., et al, 2018. Modeling Bacteria–Phage Interactions and Its Implications for Phage Therapy
https://doi.org/10.1016/bs.aambs.2018.01.005

Multiplicity of Infection

Behavior of suscesive phage applications.

Same phage different MOI.

"Multiplicity of infection literally means the ratio of phages to bacteria"

MOI 0.1 = 10^5 CFU/ml and 10^4 PFU/ml (less phages than bacteria)

MOI 1 = 10^5 CFU/ml and 10^5 PFU/ml (same concentration)

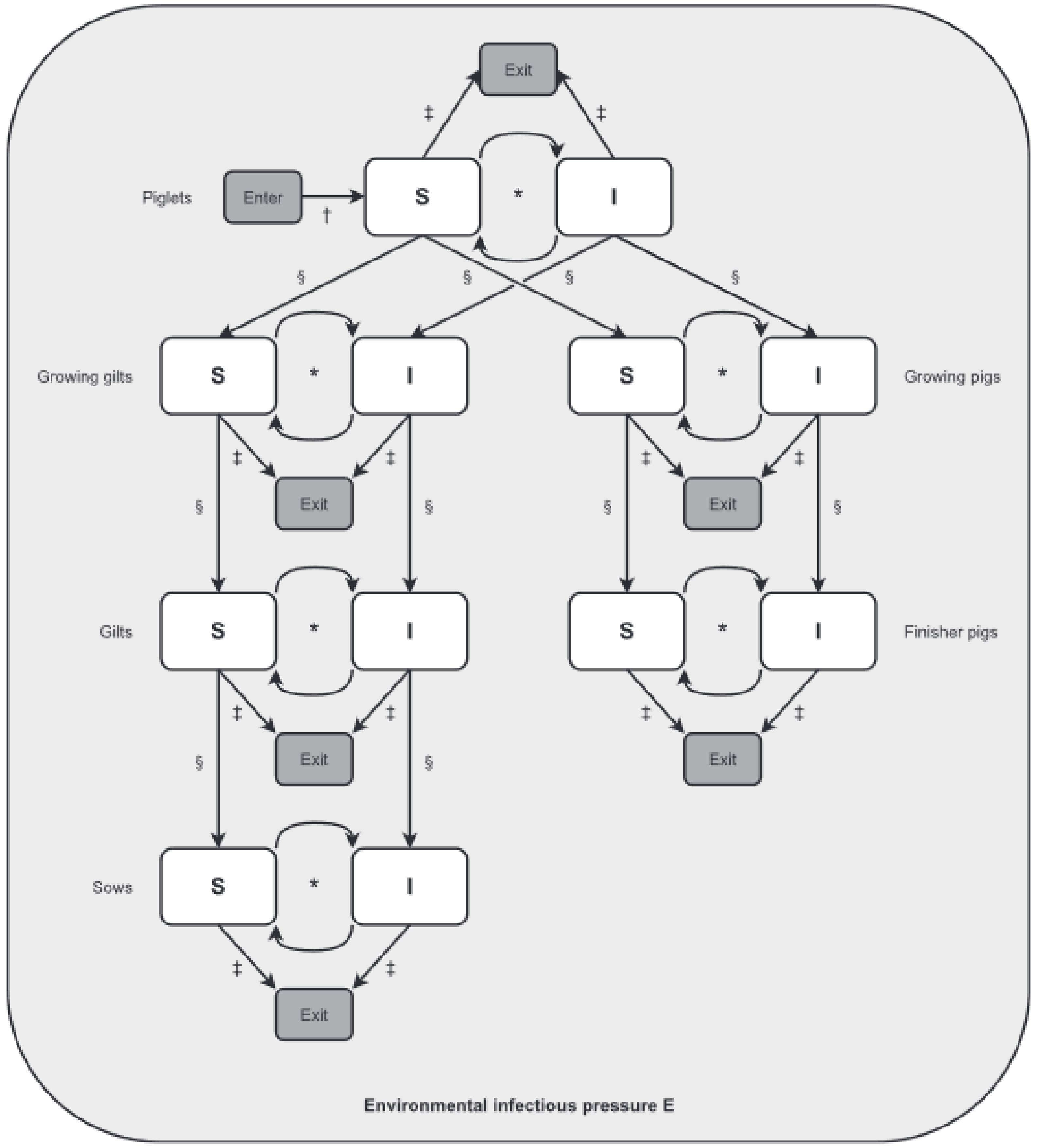
MOI 10 = 10^5 CFU/ml and 10^6 PFU/ml (more phages than bacteria)

Abedon, S.T., 2016. Phage therapy dosing: The problem(s) with multiplicity of infection (MOI). Bacteriophage 6, e1220348. https://doi.org/10.1080/21597081.2016.1220348

Previous work

Testing control mesures against MRSA

Control measure	Mean time (days) to elimination	Probability of elimination (%)
Single control measures		
BS+	559	0.01
Biweekly[1]	587	0.07
Test[2] gilts	300	2.96
Clean AIAO	1 158	0.02
Combined control measures		
Test G + S, clean CF and AIAO, BS+, M–	365	100.00
Test G + S, clean CF and AIAO, M–	536	100.00
Test G + S, clean AIAO, BS+, M–	533	99.99
Test G + S, clean AIAO, BS+	492	99.98
Test G + S, clean AIAO, M–	946	94.04
Test G + S, clean AIAO	920	94.33
Test G + S, BS+, M–	565	23.7
Test G + S	291	3.26
Test gilts, clean AIAO, BS+, M–	868	54.39
Test gilts, clean AIAO, BS+	780	63.31
Test gilts, clean AIAO, M–	660	18.92
Test gilts, clean AIAO	648	23.63
Test sows, clean AIAO, BS+, M–	977	99.1
Test sows, clean AIAO, BS+	931	99.39
Test sows, clean AIAO, M–	1 600	18.67
Test sows, BS+, M–	1 109	0.02
Clean AIAO, BS+, M–	1 510	1.46
Clean CF and AIAO	1 370	73.02



Modelling environmental spread of MRSA

Tuominen, K.S., Sternberg Lewerin, S., Jacobson, M., Rosendal, T., 2022. Modelling environmentally mediated spread of livestock-associated methicillin-resistant Staphylococcus aureus in a pig herd. Animal 16, 100450. https://doi.org/10.1016/j.animal.2021.100450

Tuominen, K.S., Sternberg Lewerin, S., Widgren, S., Rosendal, T., 2023. Assessment of control measures against livestock-associated methicillin-resistant Staphylococcus aureus in a farrow-to-finish pig herd using infectious disease modelling. animal 17, 100840. https://doi.org/10.1016/j.animal.2023.100840

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Objetive

- Determine the viability of phage therapy to combat livestock-associated methicillin-resistant Staphylococcus aureus (LA-MRSA);
- Reduce transmission within and between pig herds, exposure of farm staff and the environment.

Materials

- Sample preparation

- 2 ml of:

  MRSA strain 19ST269

  Isolated from pig barn swab sample

  10^5 CFU/ml per skin sample

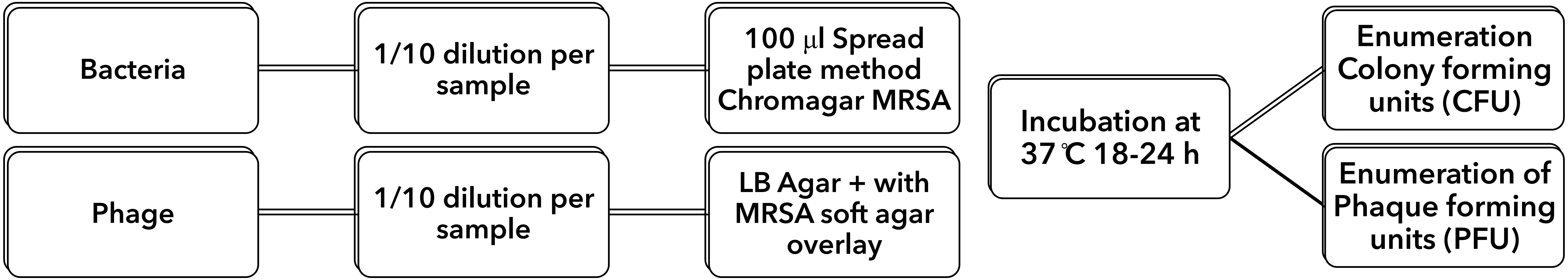
- Phage P19ST269-22

  Isolated from pig slaughter wastewater

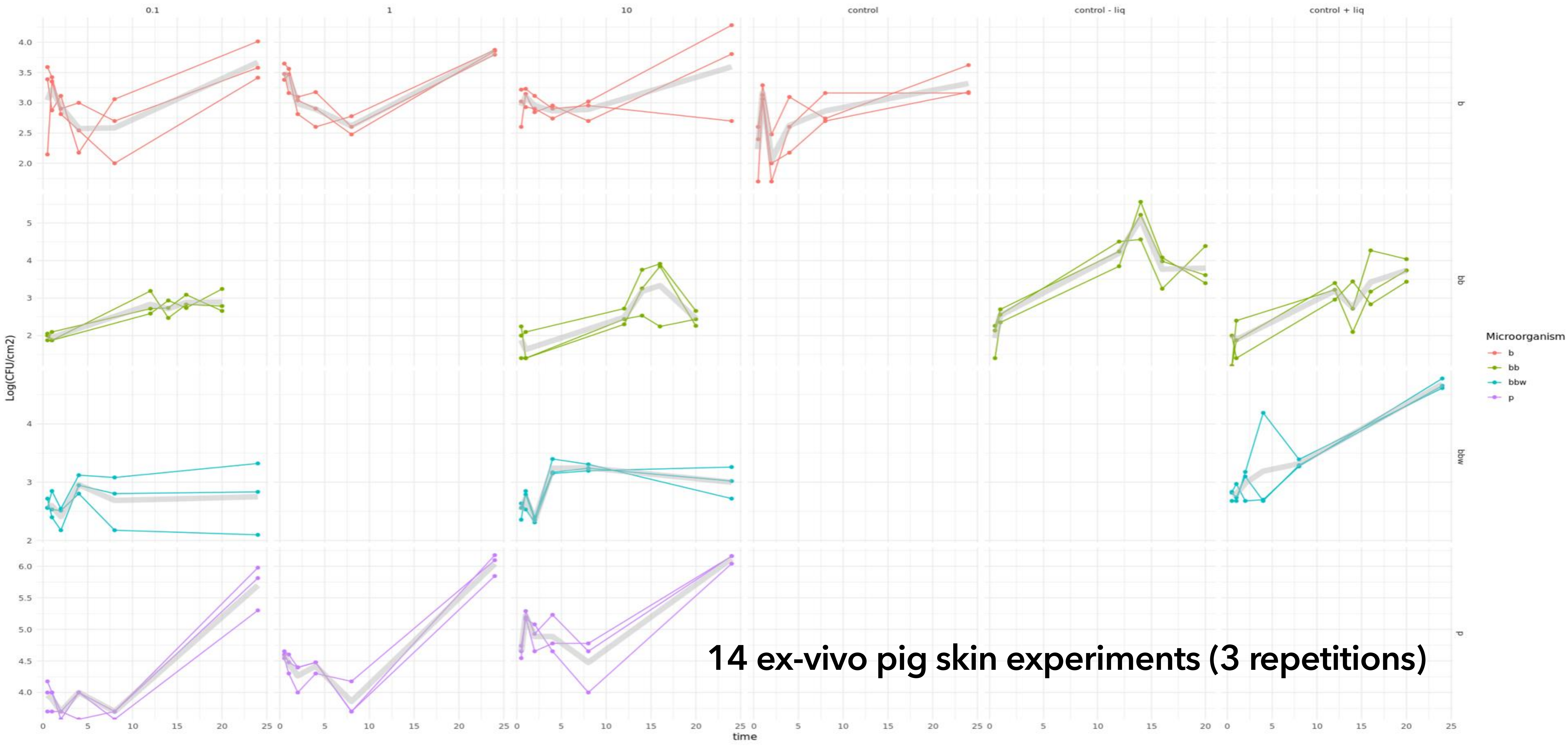
- Sampling

Die cutter 5 cm2 → 3 biological replicates per time point → Dilution in 100 ml SB buffer → Homogenization (120s at 4000 rpm)

Materials


Bacteria
1/10 dilution per sample
100 µl Spread plate method Chromagar MRSA
Phage
1/10 dilution per sample
LB Agar + with MRSA soft agar overlay
Incubation at 37℃ 18-24 h
Enumeration Colony forming units (CFU)
Enumeration of Phaque forming units (PFU)

Datasets



14 ex-vivo pig skin experiments (3 repetitions)

Methods

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Only bacteria counts are used / frequency dependency

$$\frac{dS}{dt} = \alpha S$$

$$\frac{dS}{dt} = \alpha S - \beta SP/N$$

$$\frac{dS}{dt} = \alpha S - \beta SP/N$$
$$\frac{dP}{dt} = \beta SP/N$$

$$\frac{dS}{dt} = \alpha S - \beta SP/N$$
$$\frac{dI}{dt} = \beta SP/N - \gamma I$$
$$\frac{dP}{dt} = hI$$

$$\frac{dS}{dt} = \alpha S - \beta SP/N - \delta SP/N$$
$$\frac{dI}{dt} = \beta SP/N - \gamma I$$
$$\frac{dR}{dt} = \alpha R + \delta SP/N$$
$$\frac{dP}{dt} = hI$$

Parameter	Symbol	Units	Reference
alpha (α)	Growth rate of bacteria	h^{-1}	Modelled, (Cairns et al., 2009)
beta (β)	Binding rate of phages	ml CFU^{-1} h^{-1}	Modelled
gamma (γ)	Latency period	h^{-1}	Modelled,
delta (δ)	Resistance of bacteria	h^{-1}	Modelled,
h	Burst size at lysis	PFU^{-1}	Santos (2014)

abc

Distance function : $\bar{x}((log(model) - log(expected))^2)$
Priors: Modell parameter : $\alpha, \beta, \gamma, \delta$, h
N particles: 100 - 2000
Ninit: 500 - 25.000

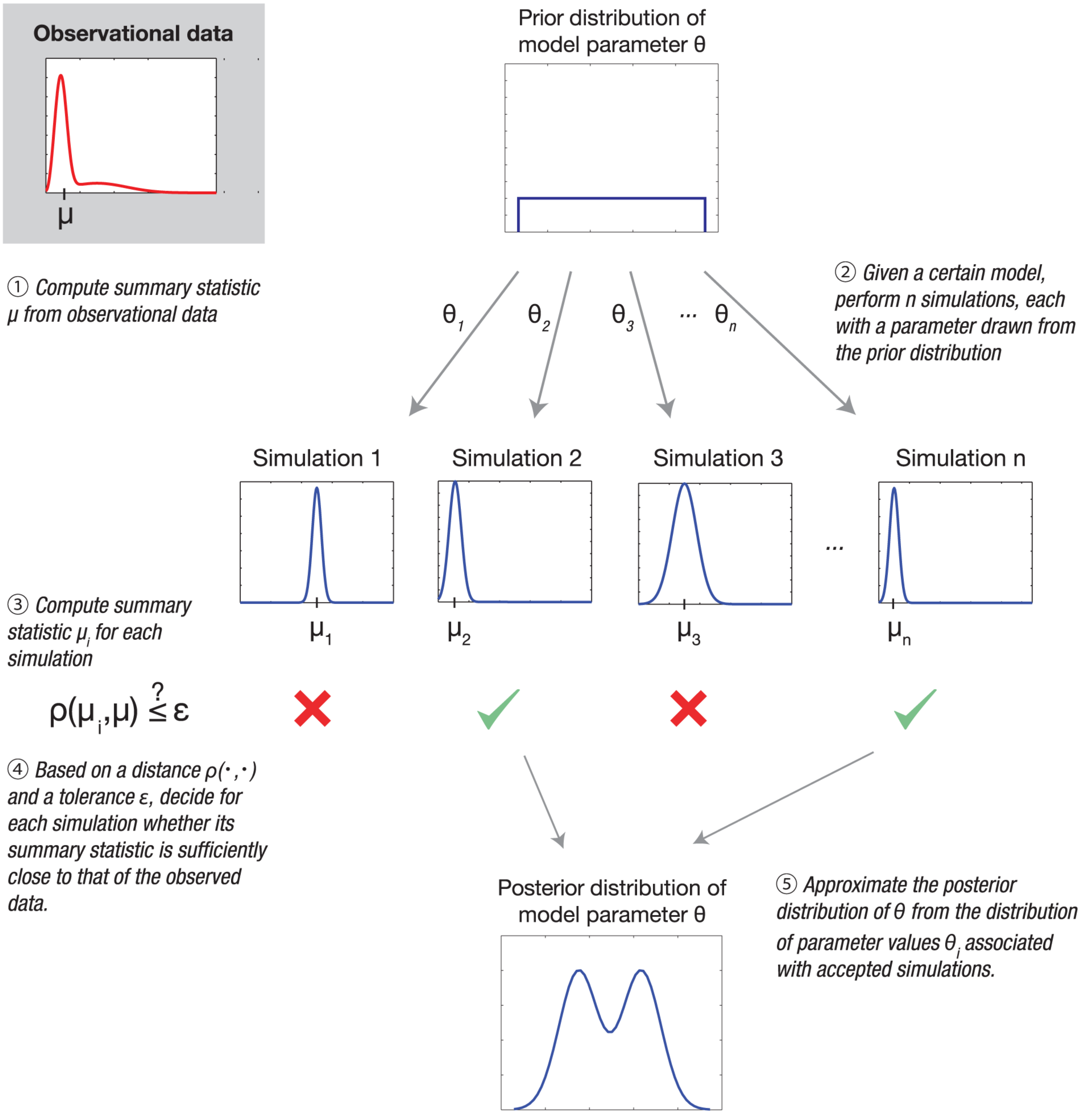

Image: Sunnåker, 2013.

SimInf

Widgren, S.,et al., 2019. SimInf: An R Package for Data-Driven Stochastic Disease Spread Simulations. Journal of Statistical Software 91, 1–42. https://doi.org/10.18637/jss.v091.i12

Sunnåker, M., 2013. M., Approximate Bayesian Computation. PLOS Computational Biology 9, https://doi.org/10.1371/journal.pcbi.1002803

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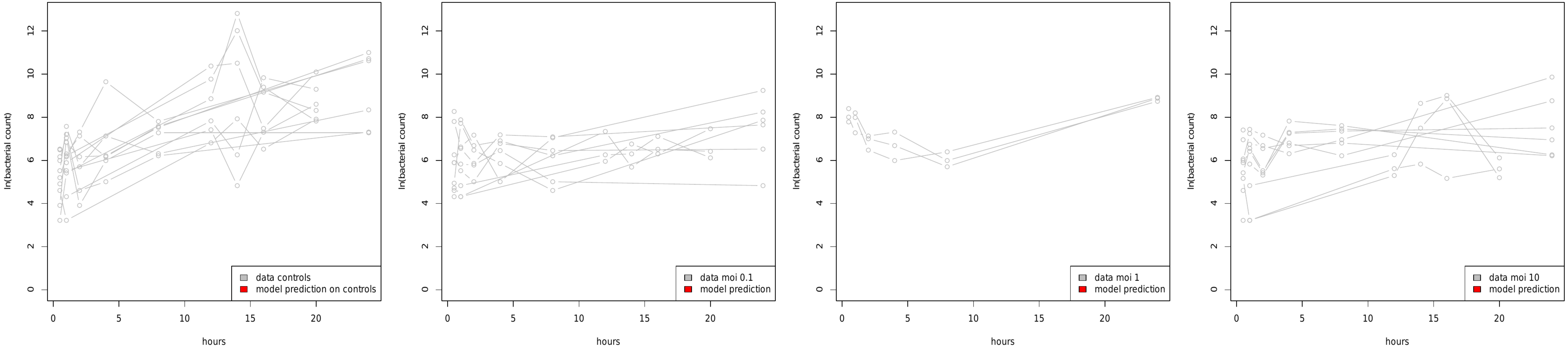
Results

[1] Growth rate of bacteria (α) glm



Growth rate

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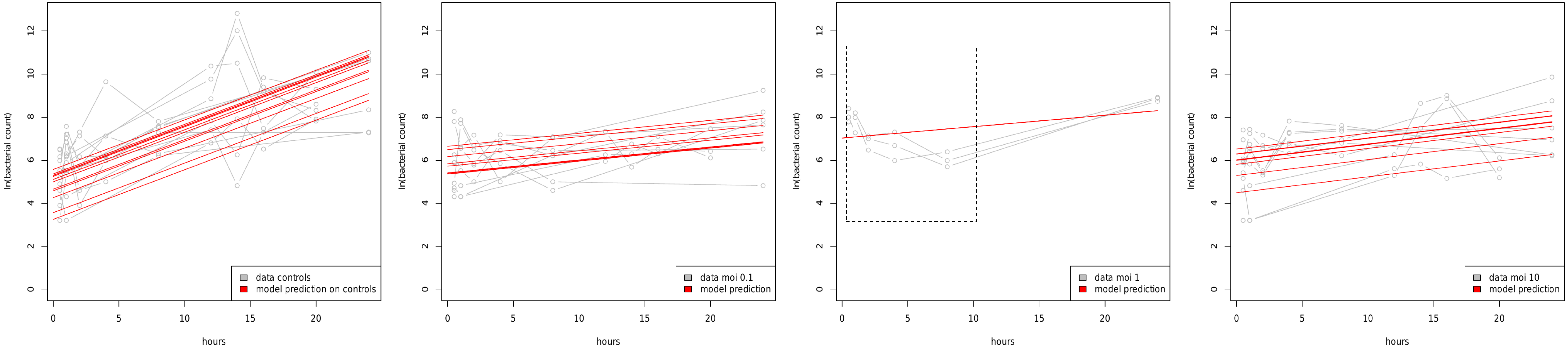
[1] Growth rate of bacteria (α) glm



α = 0.23 | α = 0.06 | α = 0.05 | α = 0.07

Growth rate

[1.1] Growth rate of bacteria (α) glm
First 8 hours

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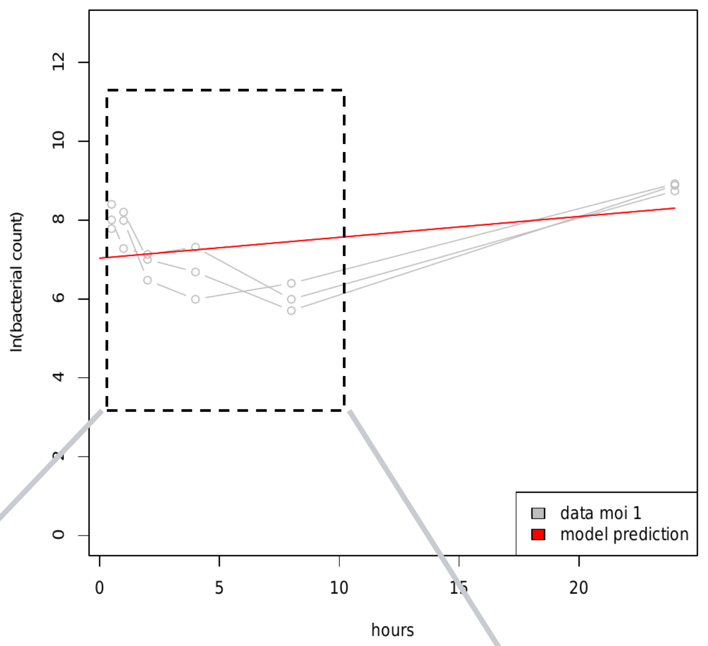
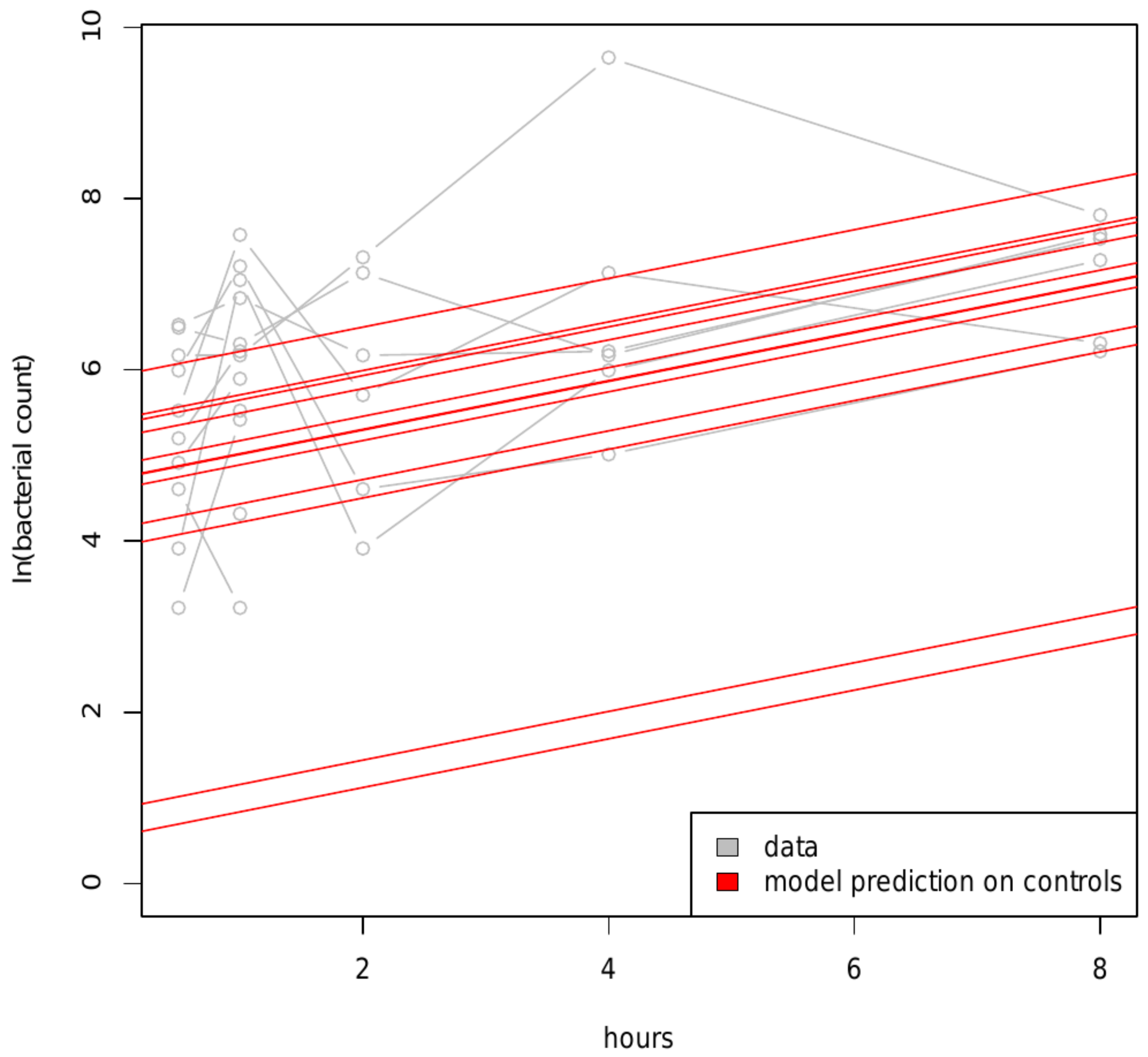
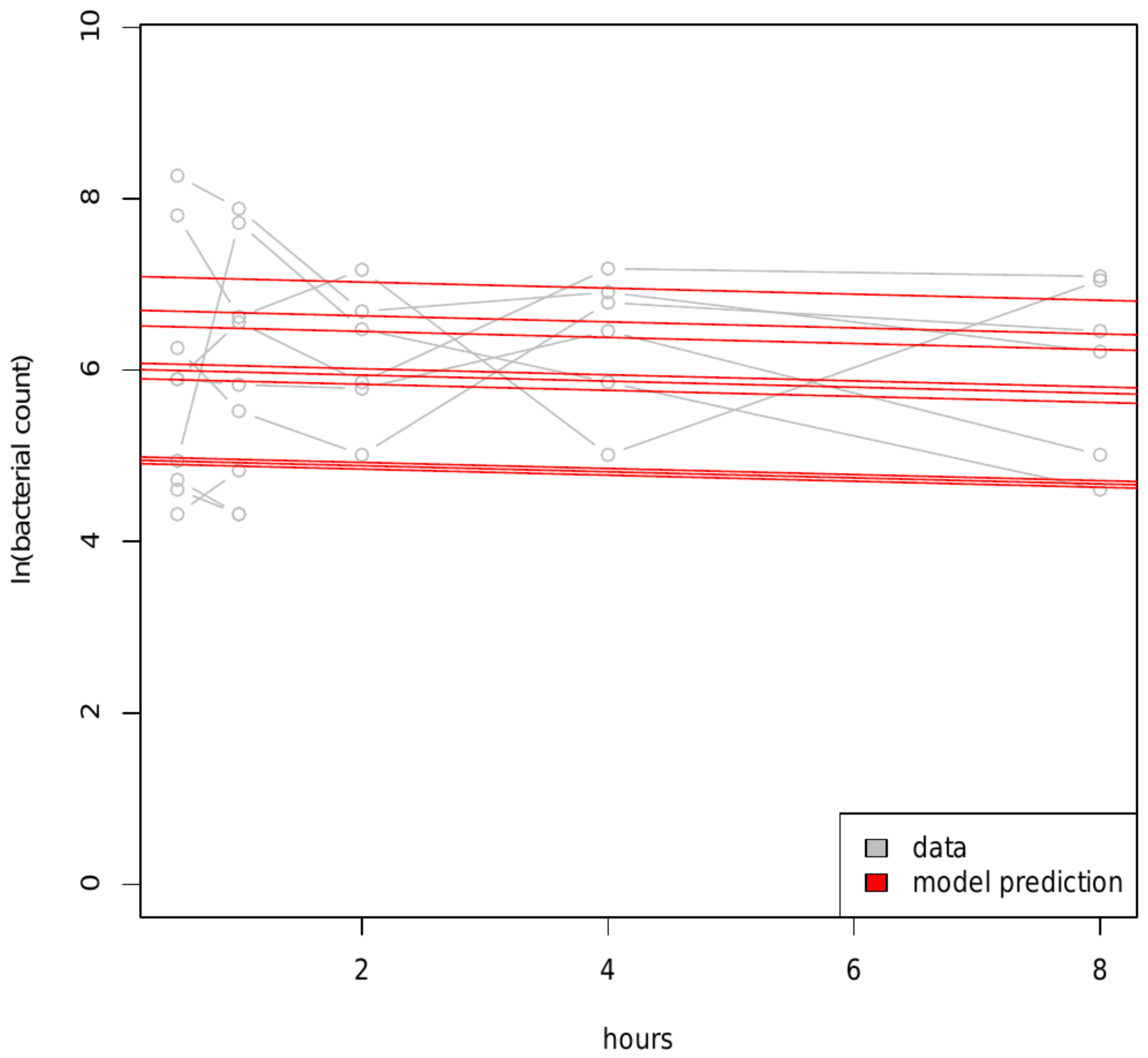
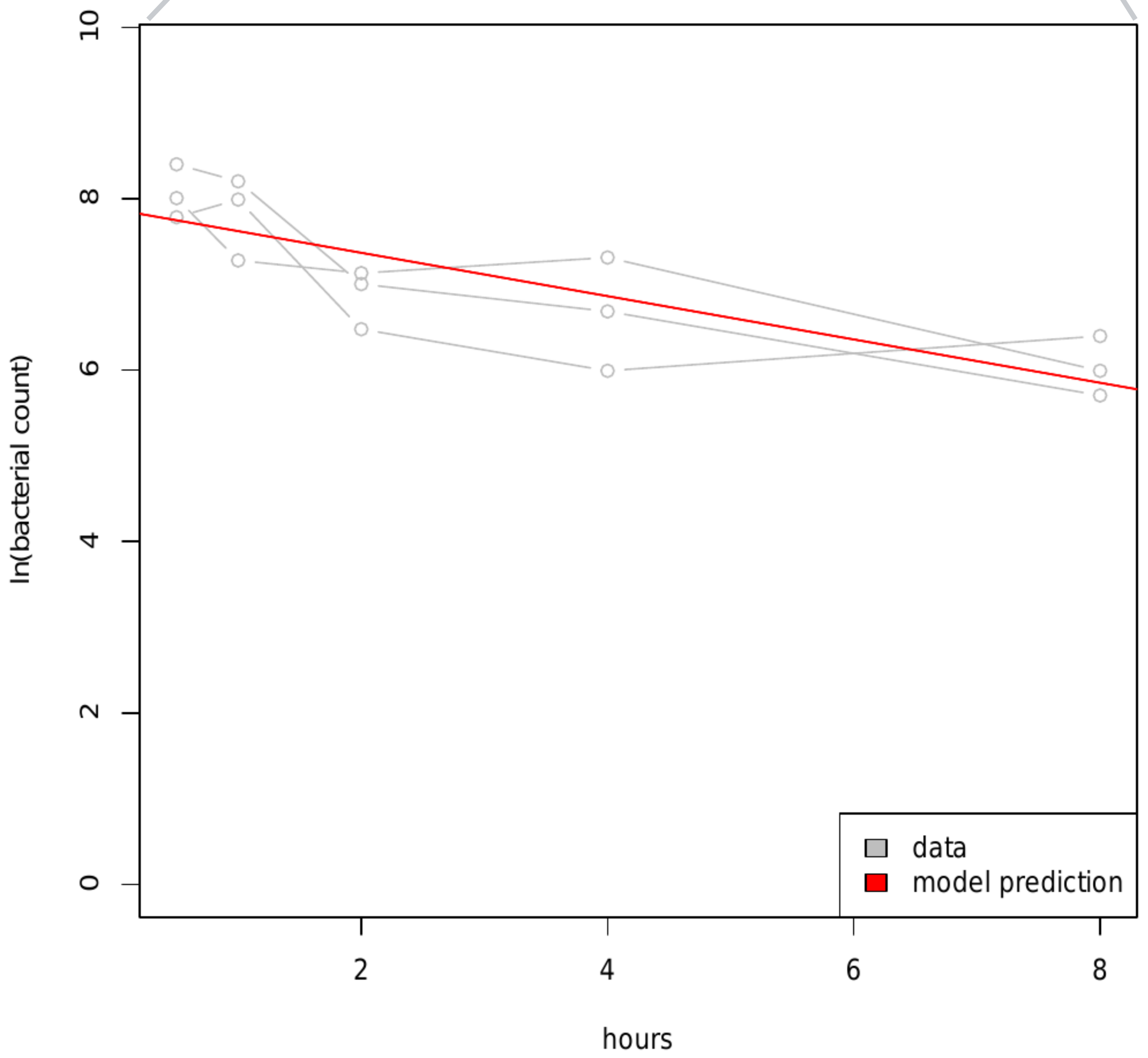
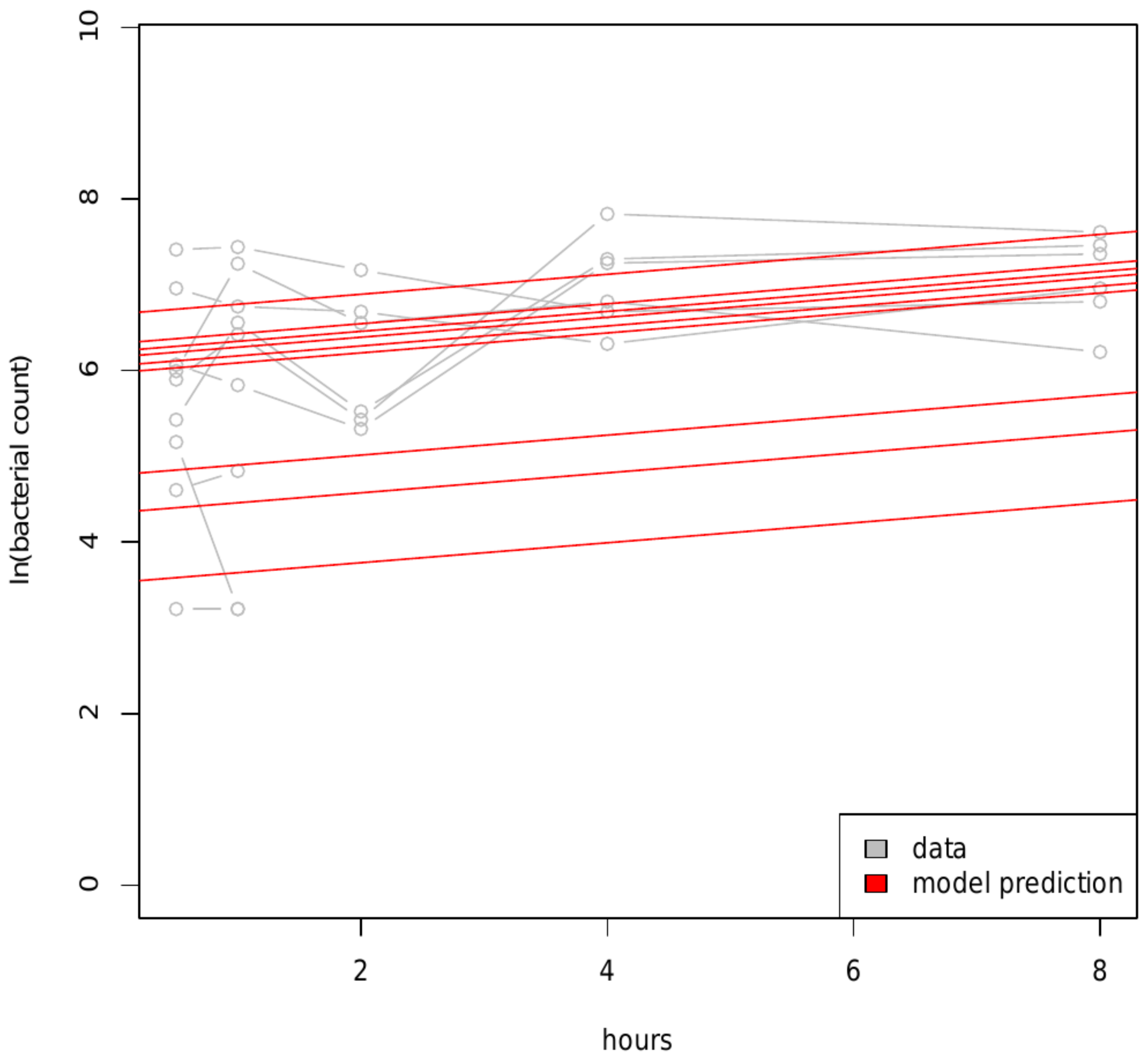




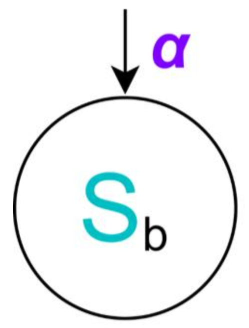






8h	α = 0.28	α = -0.04	α = -0.25	α = 0.12
24h	α = 0.23	α = 0.06	α = 0.05	α = 0.07

Growth rate



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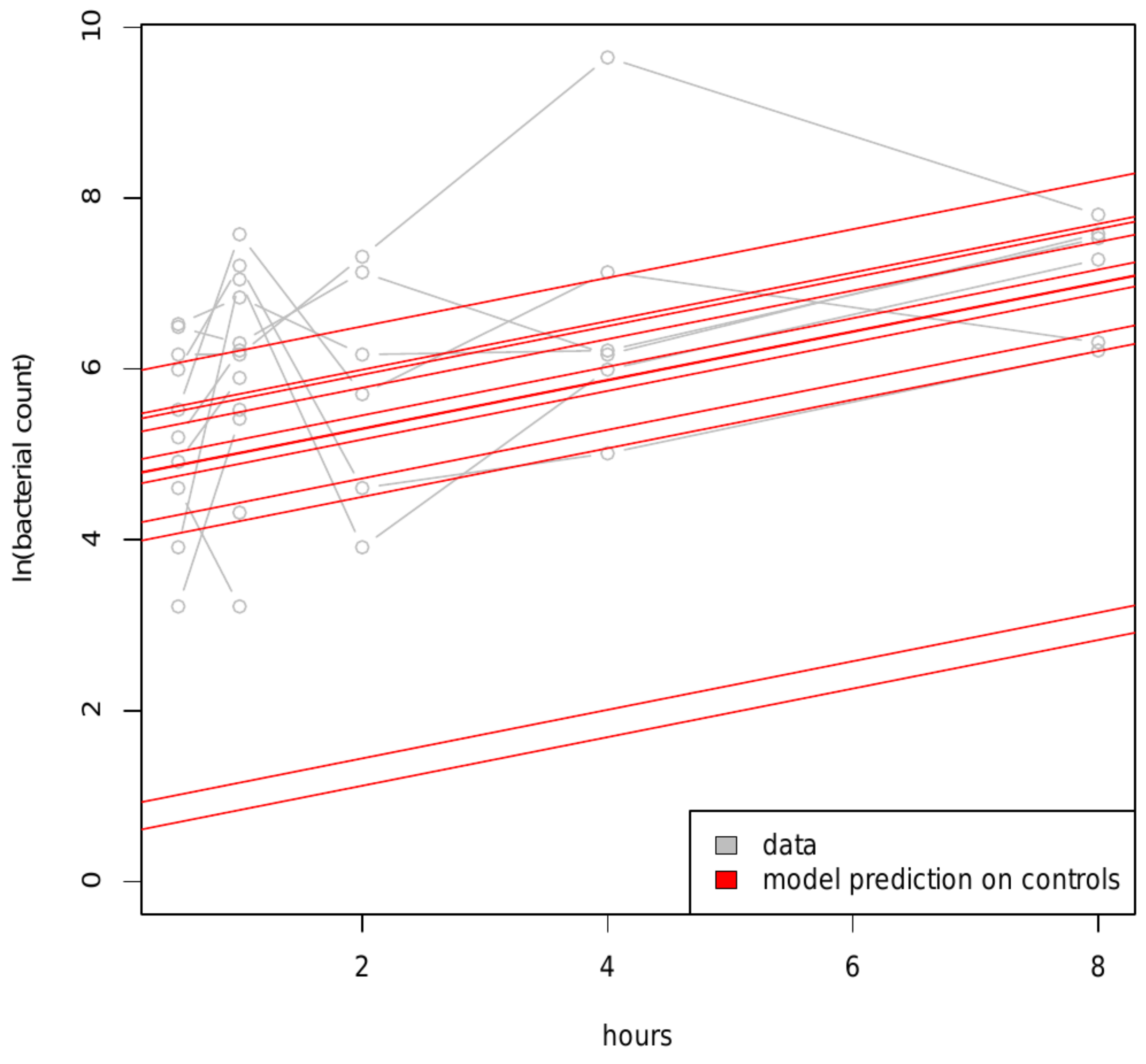
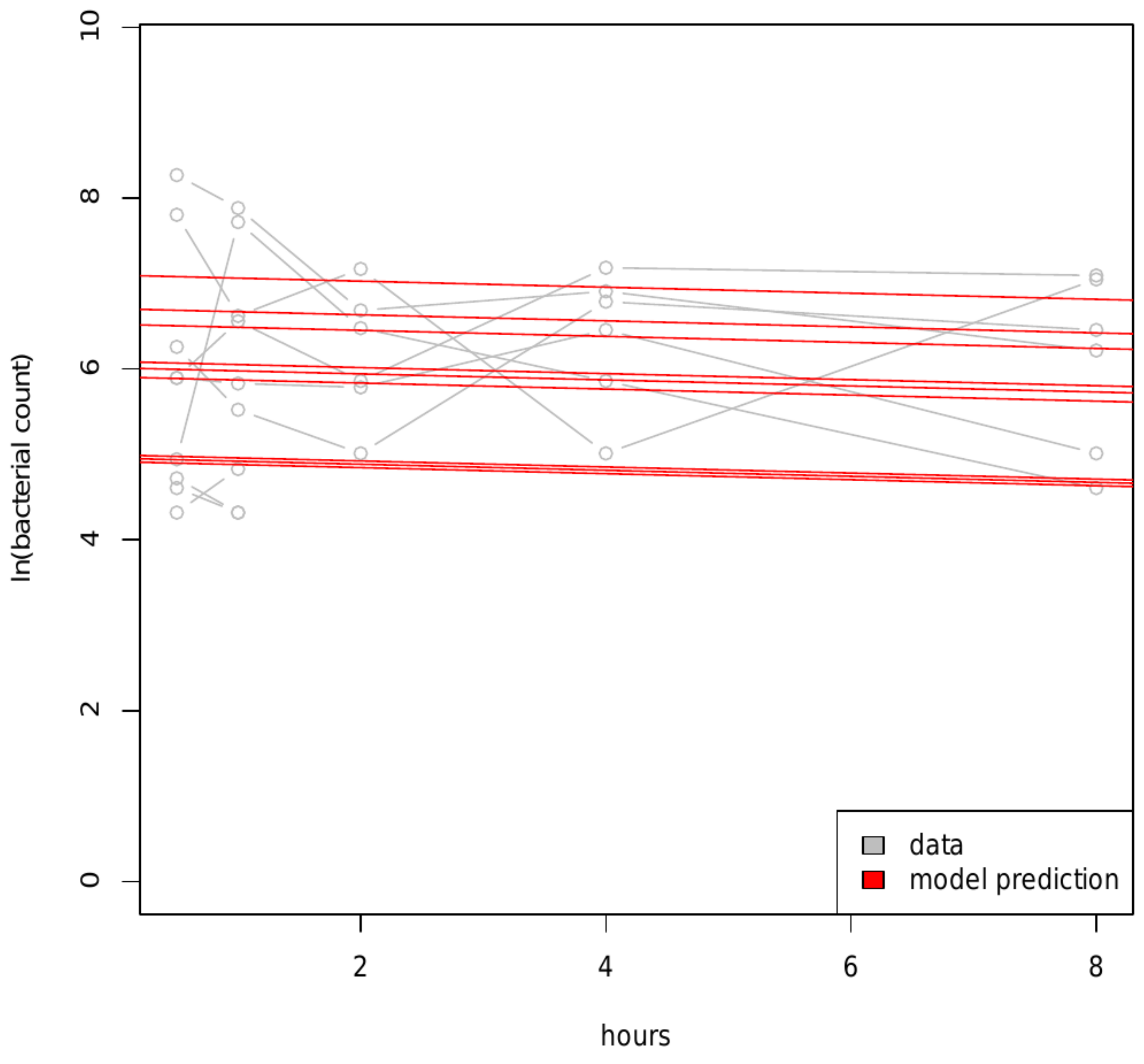
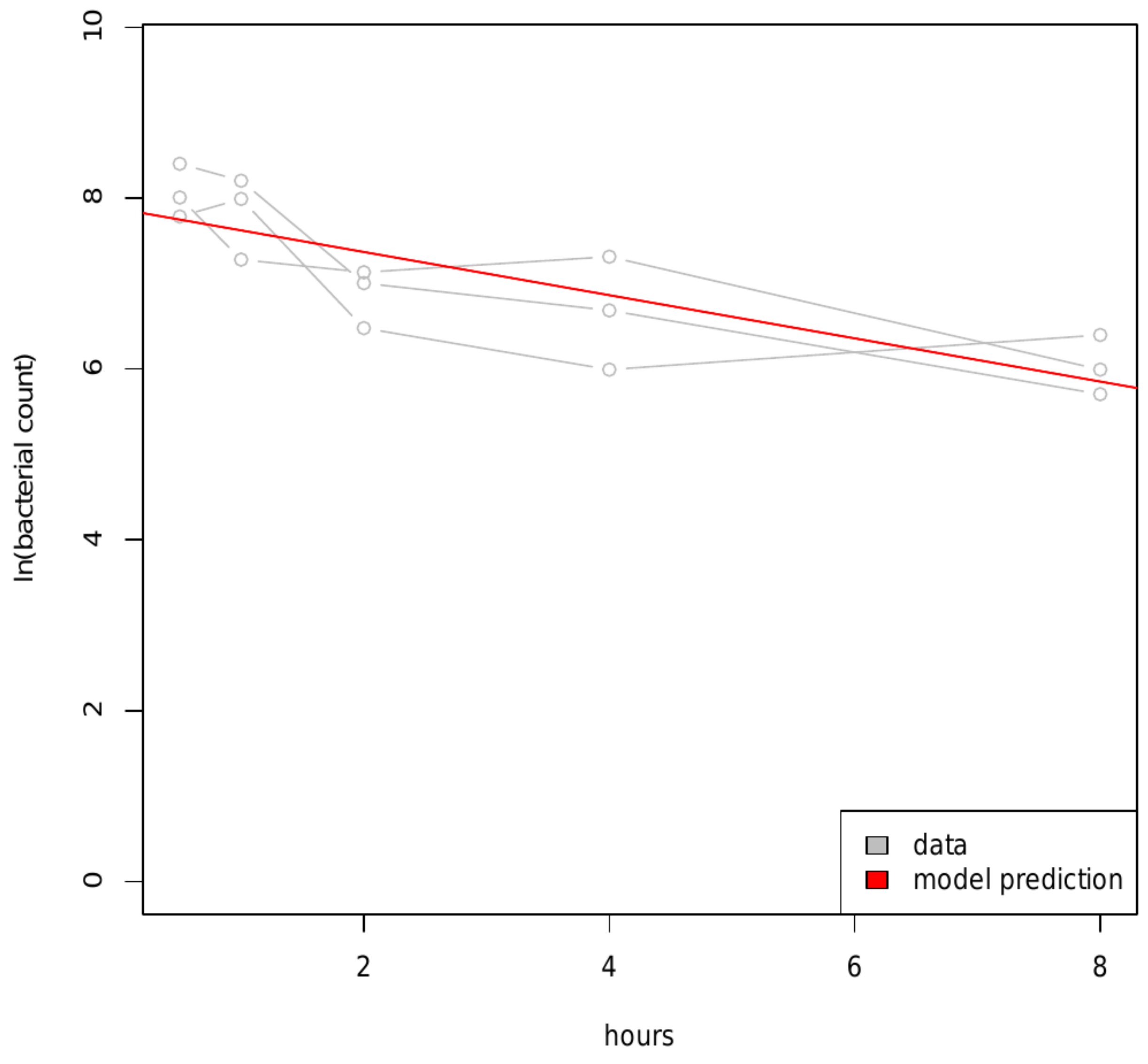
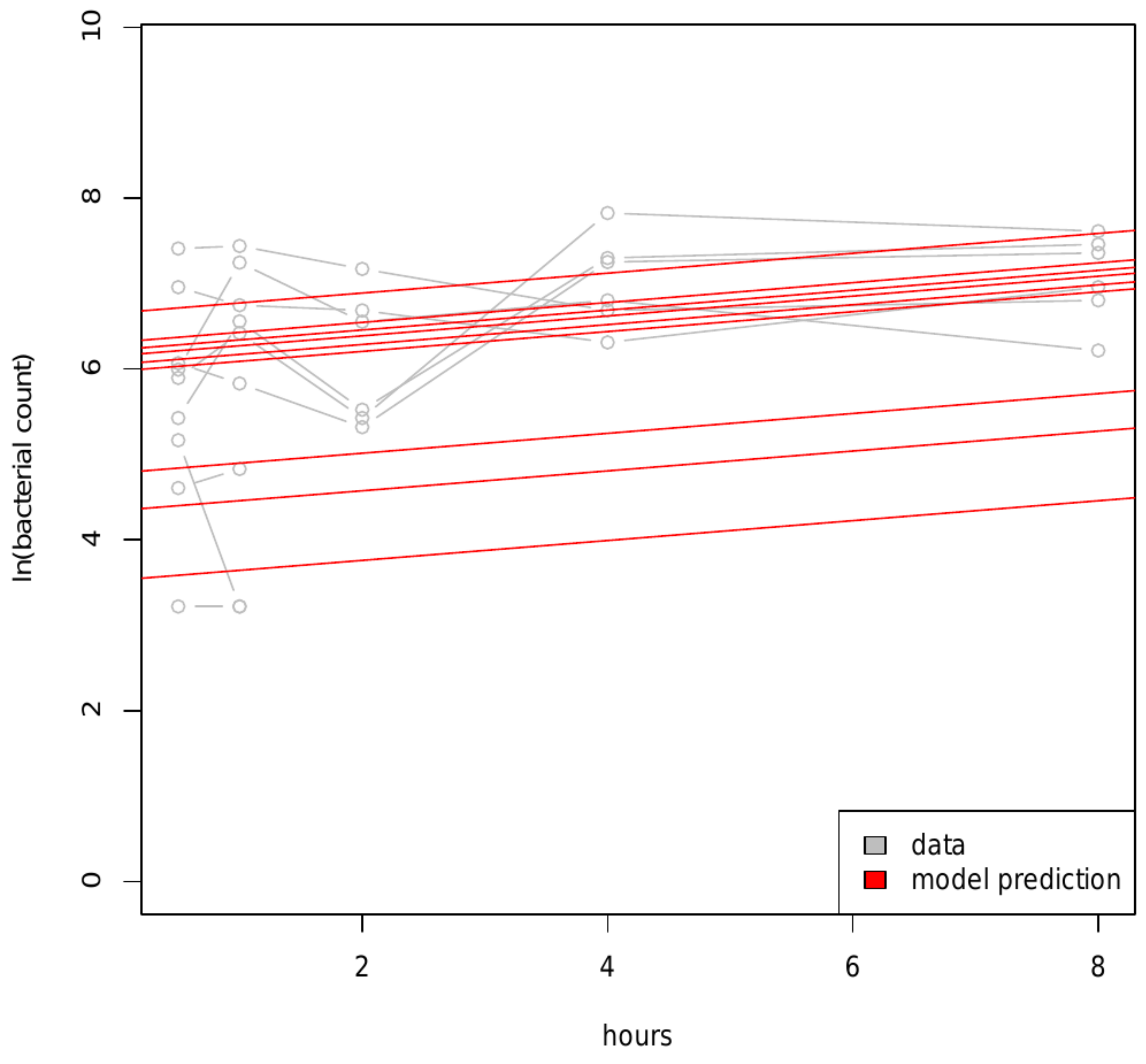
[2] Compartment model: Grow rate









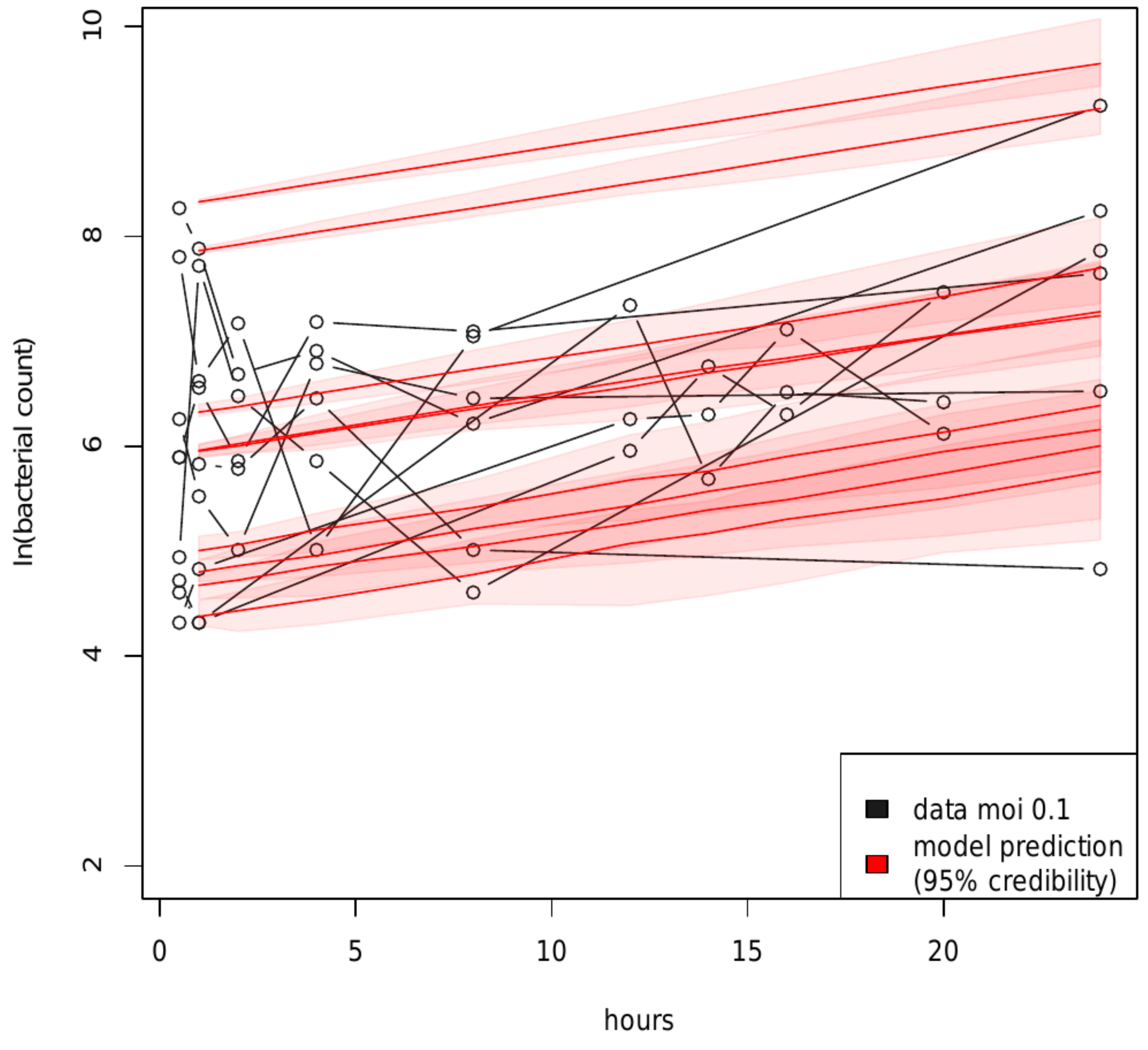
α = 0.26 | α = 0.066 | α = 0.050 | α = 0.092

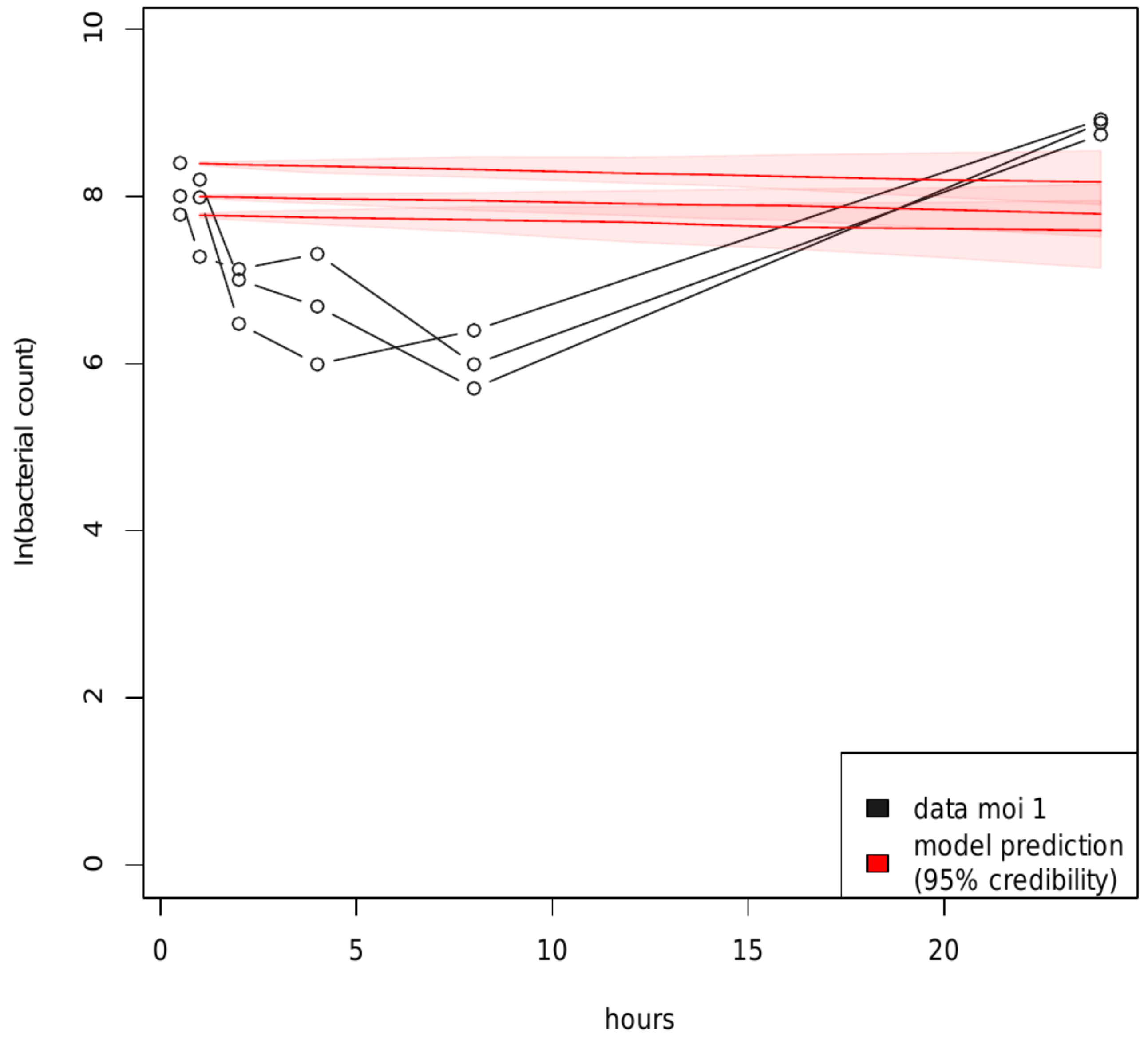
glm

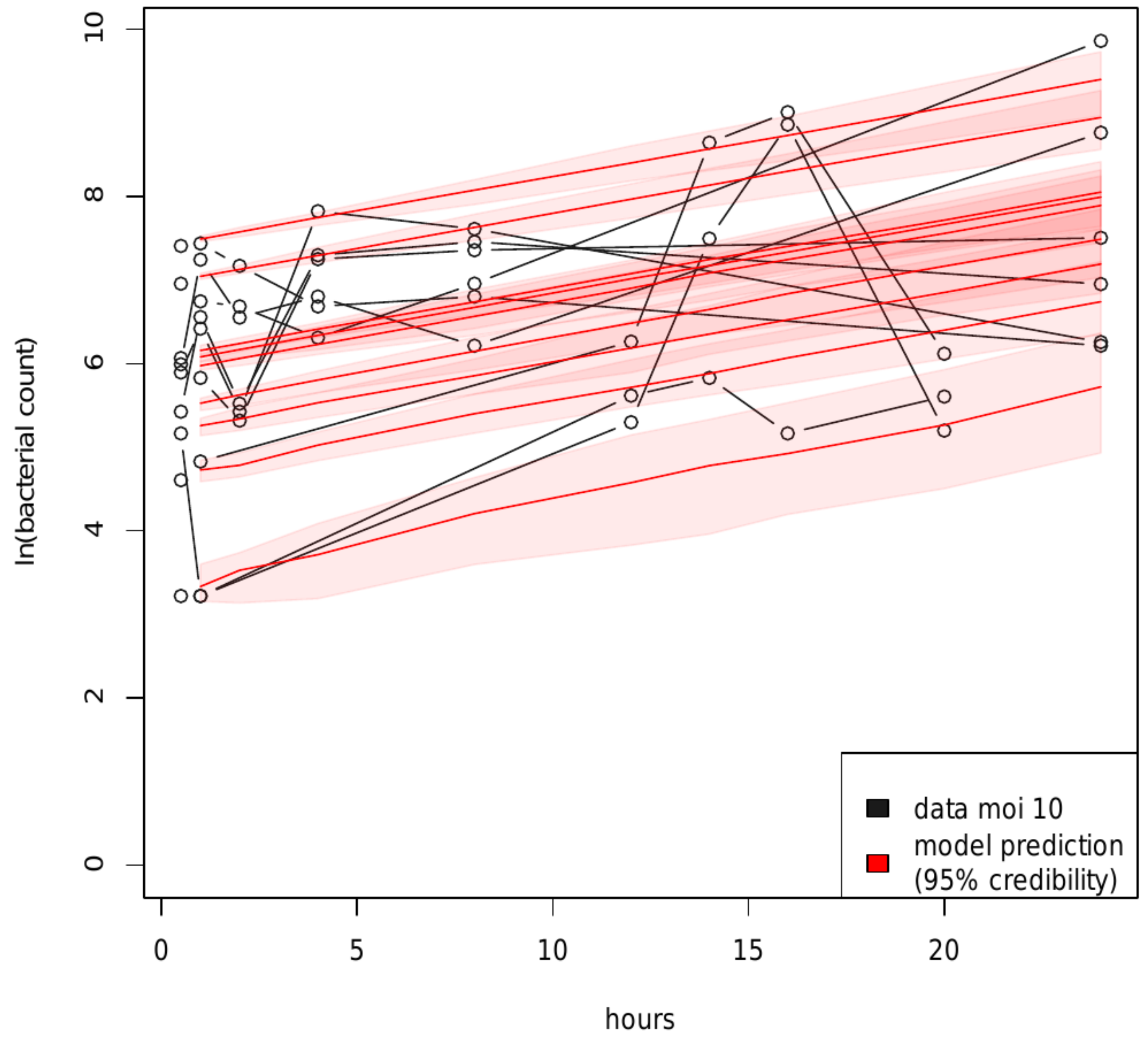
α = 0.23 | α = 0.06 | α = 0.05 | α = 0.07

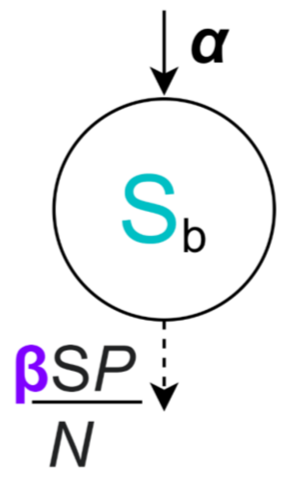
Force of infection

[3] Compartimental model with phages
Concentration is constant between S and P









β = 1.98

β = 0.26

= 0.017

Force of infection

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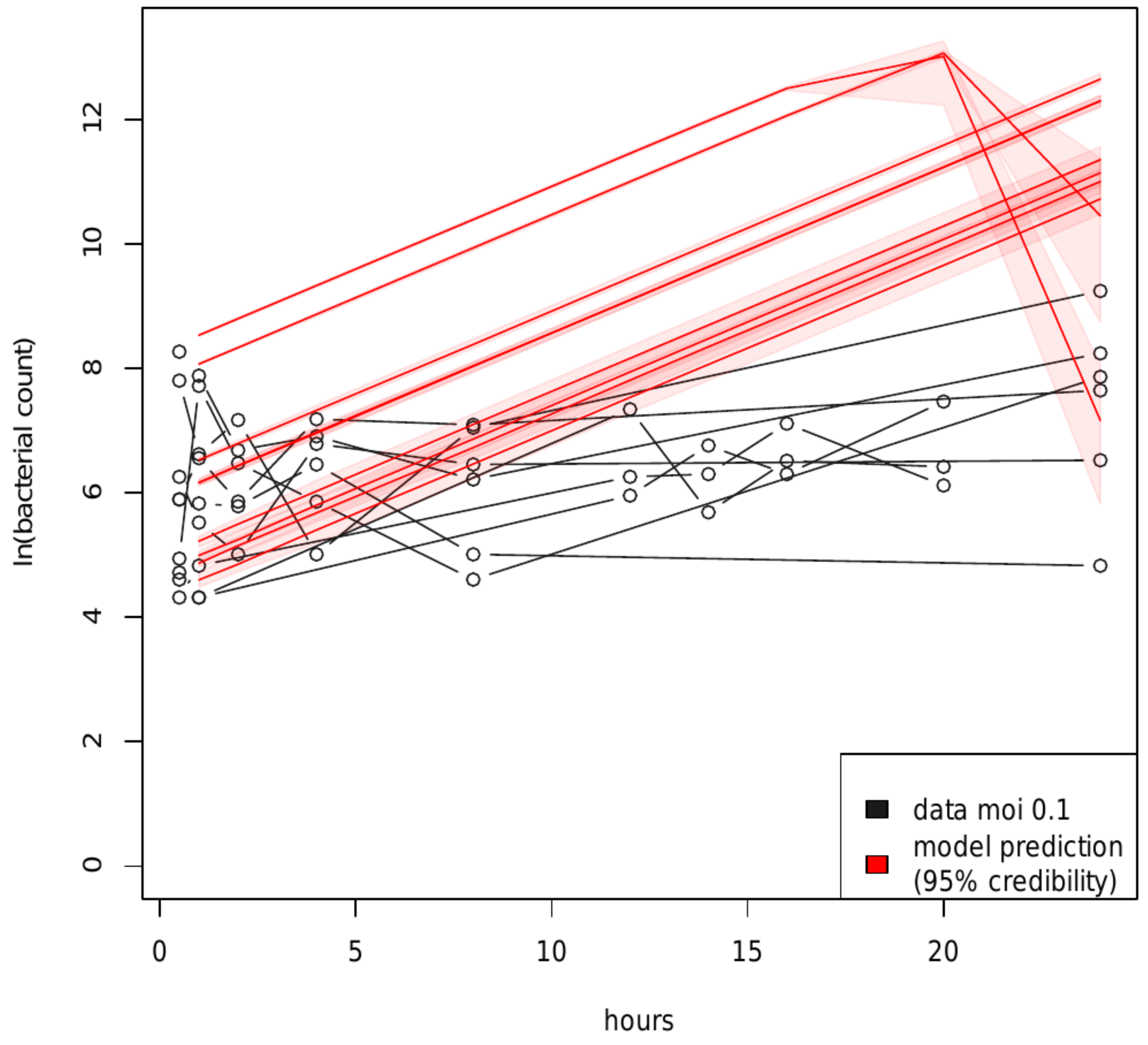
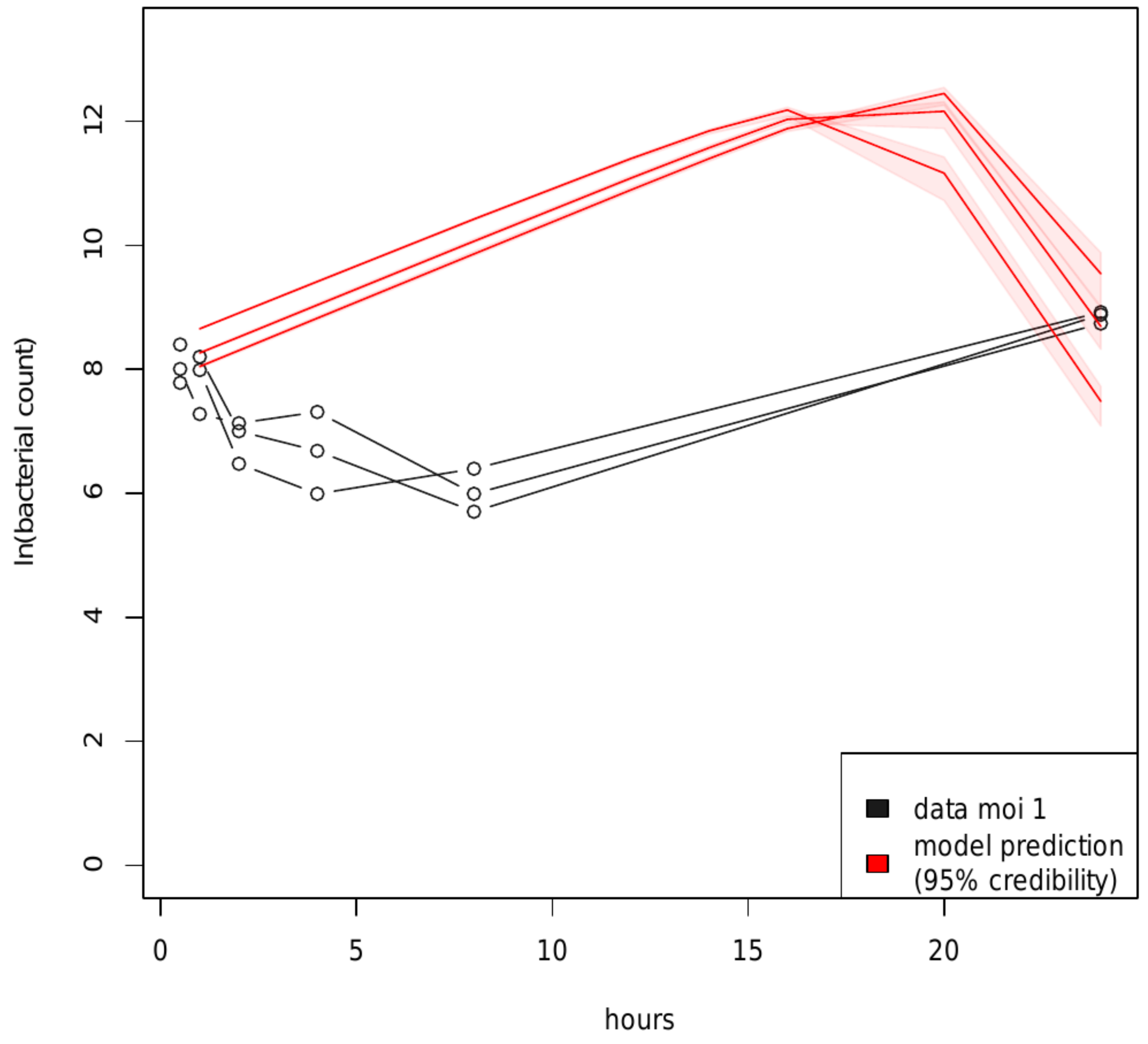
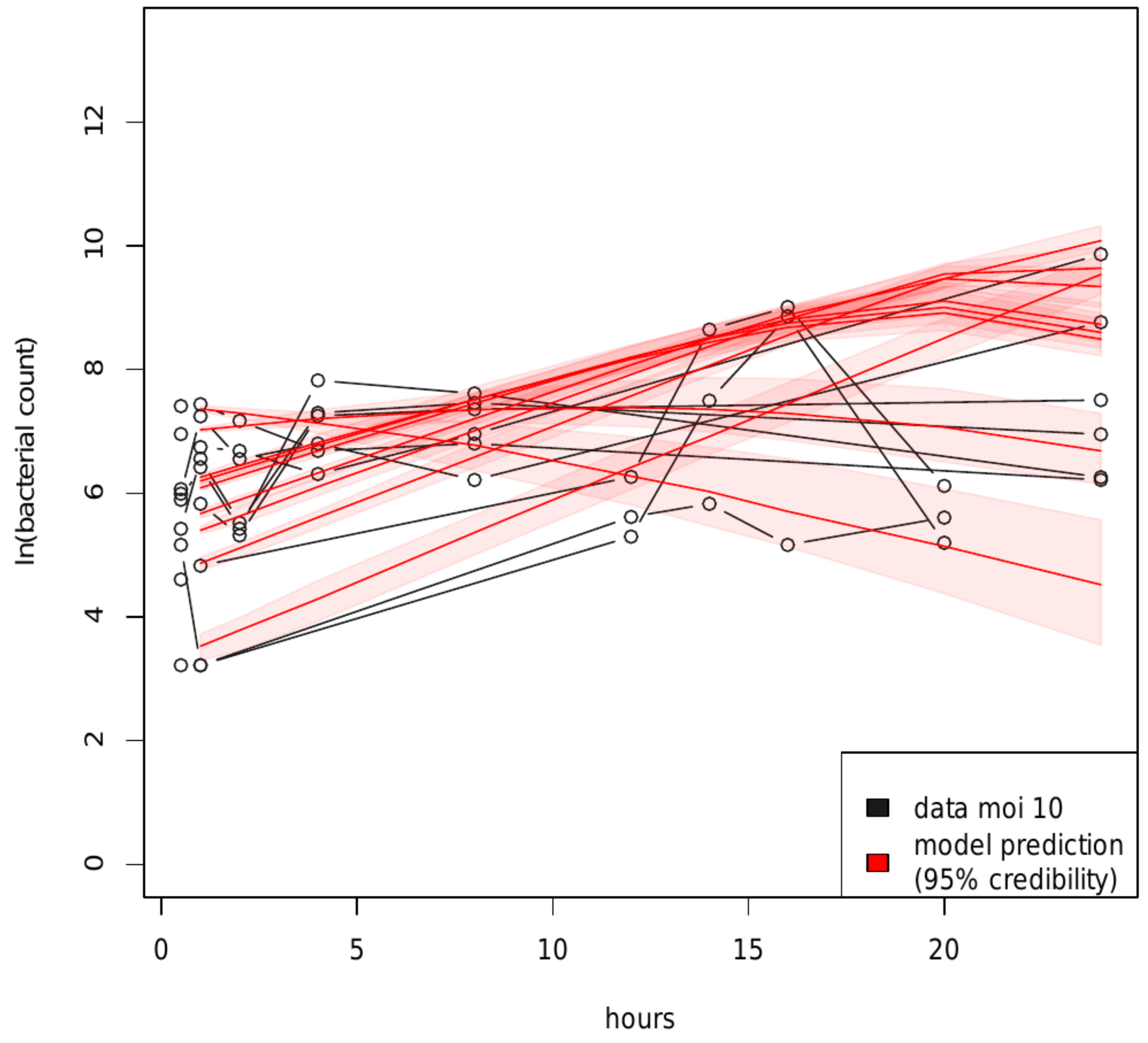
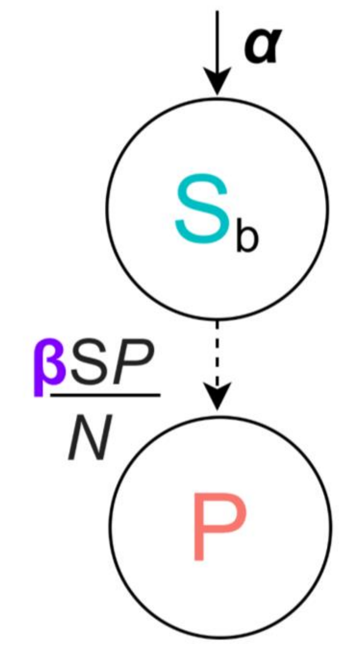
[4] Compartimental model with Growing phages









β = 2.5e-6

β = -12.78

= 1.19e-5

Force of infection & burst size

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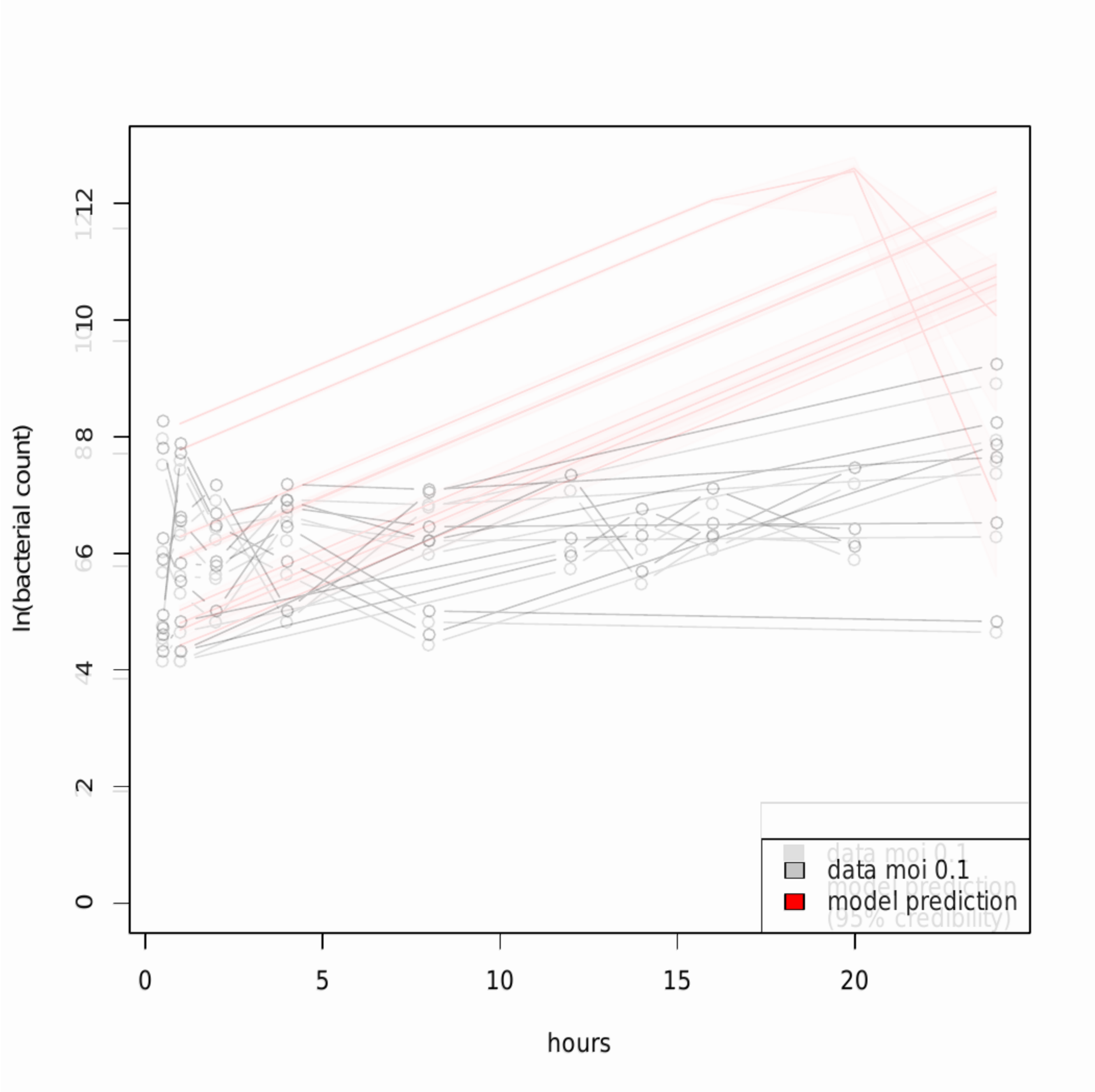
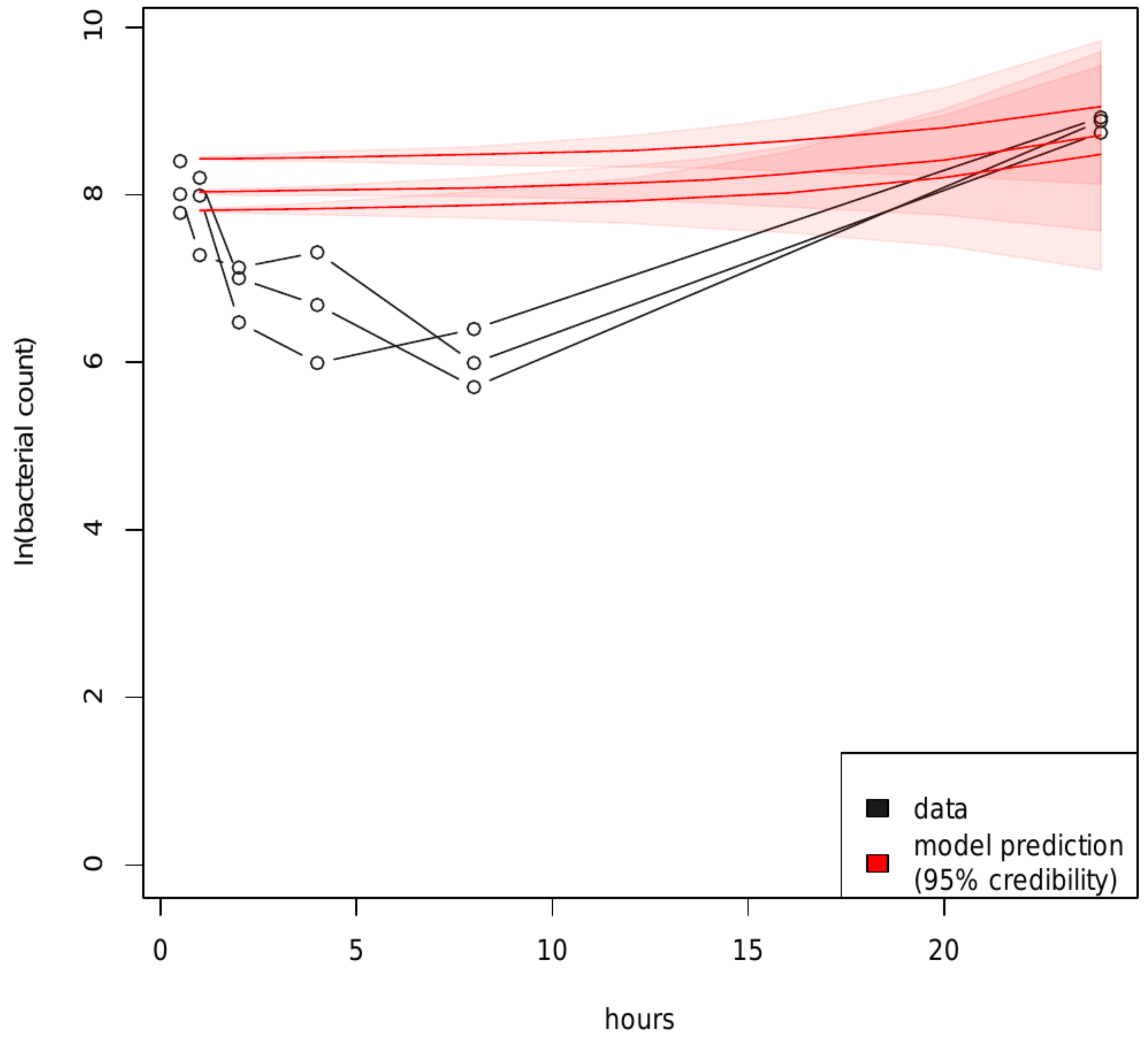
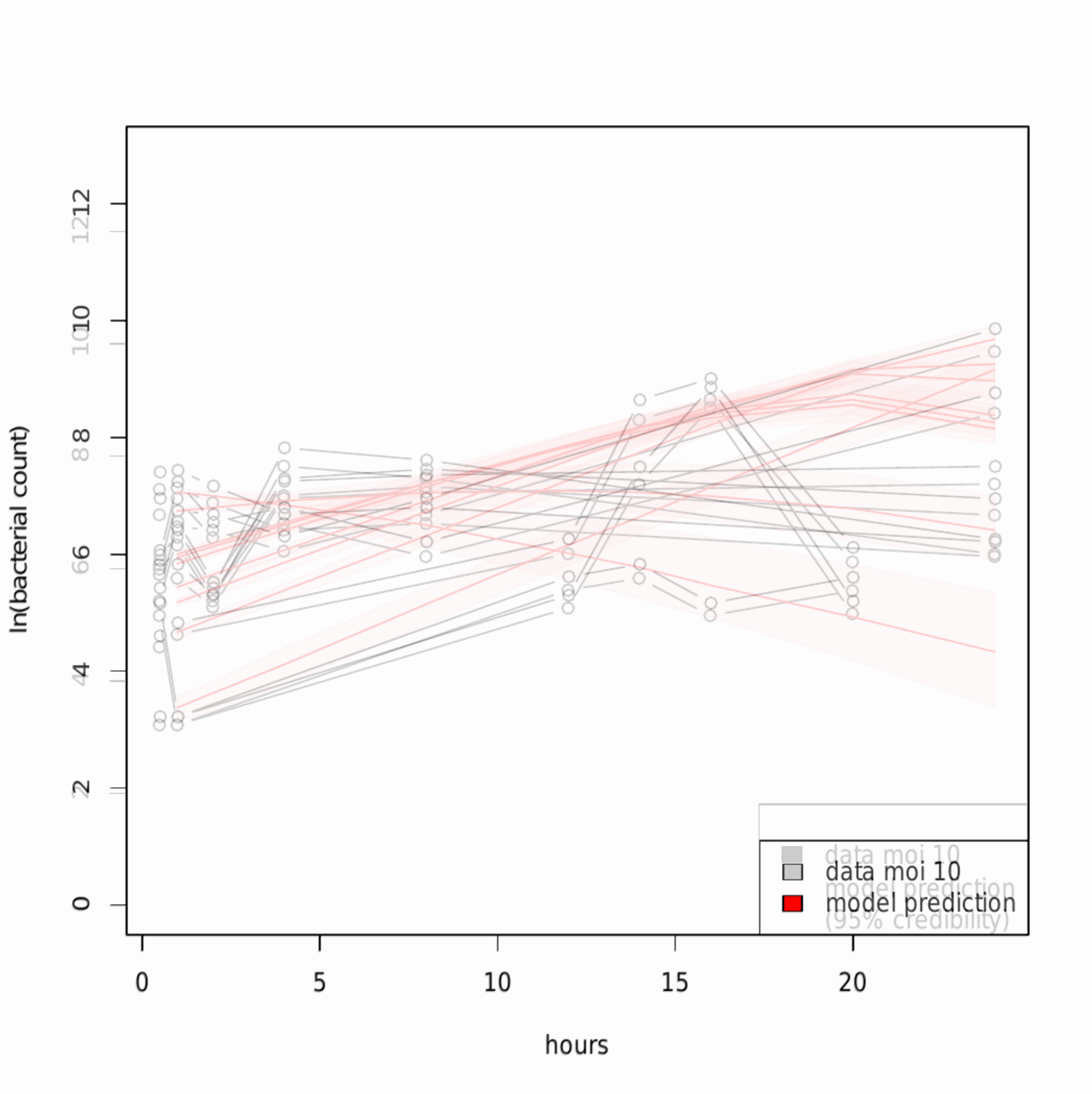
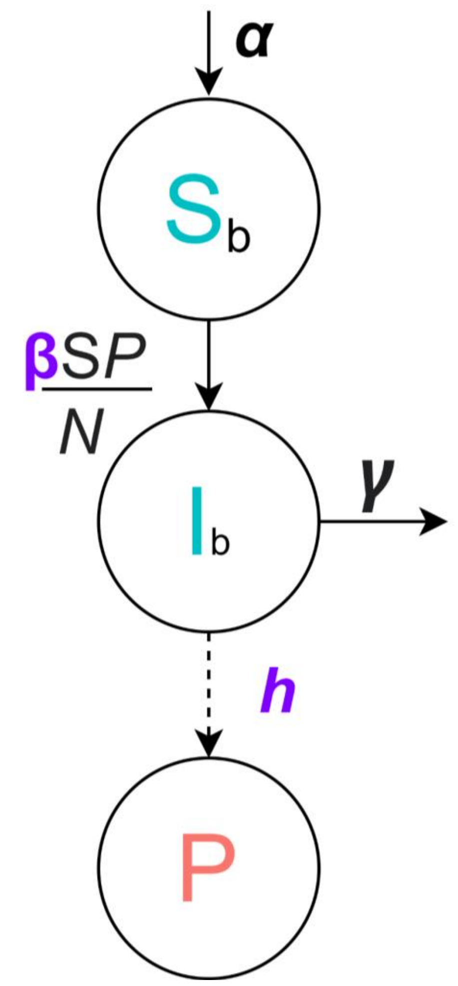
[5] Compartimental model + Infected compartment









β = 4.19e-06

β = 0.53

= 1.19e-05

h = 11.97

h = 7.90e-07

h = 47.5

Mutation rate

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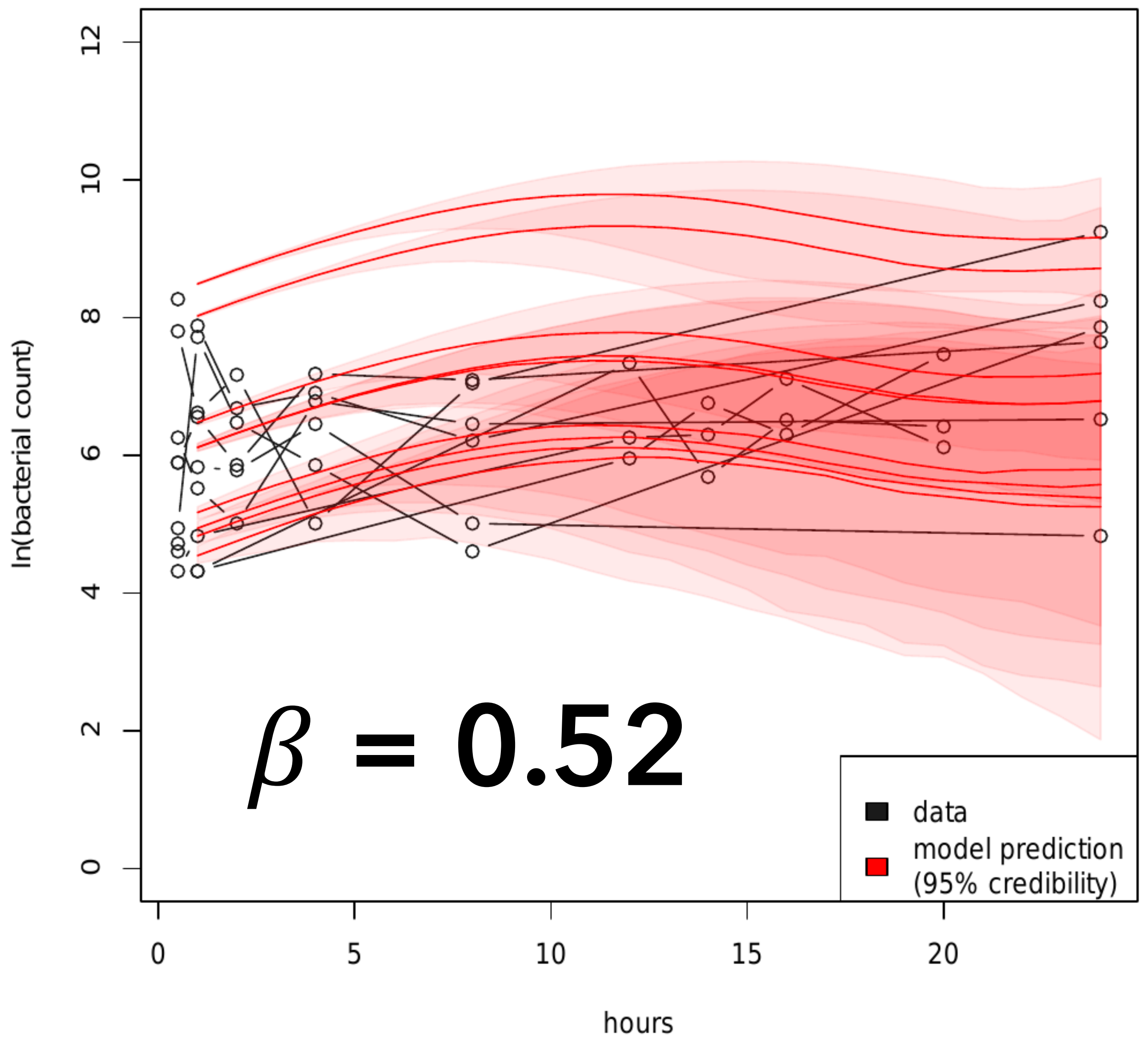
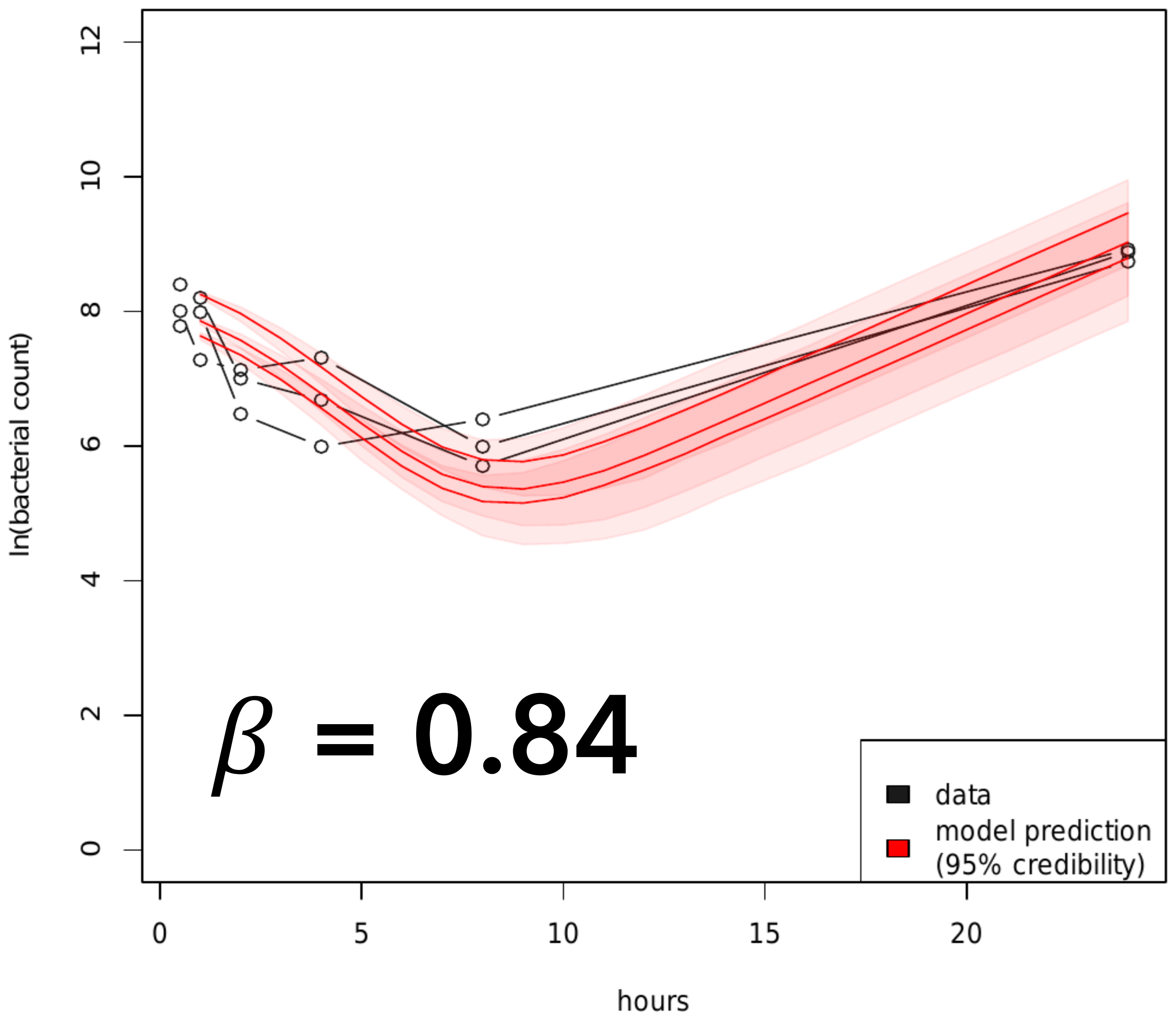
[6] Resistence development

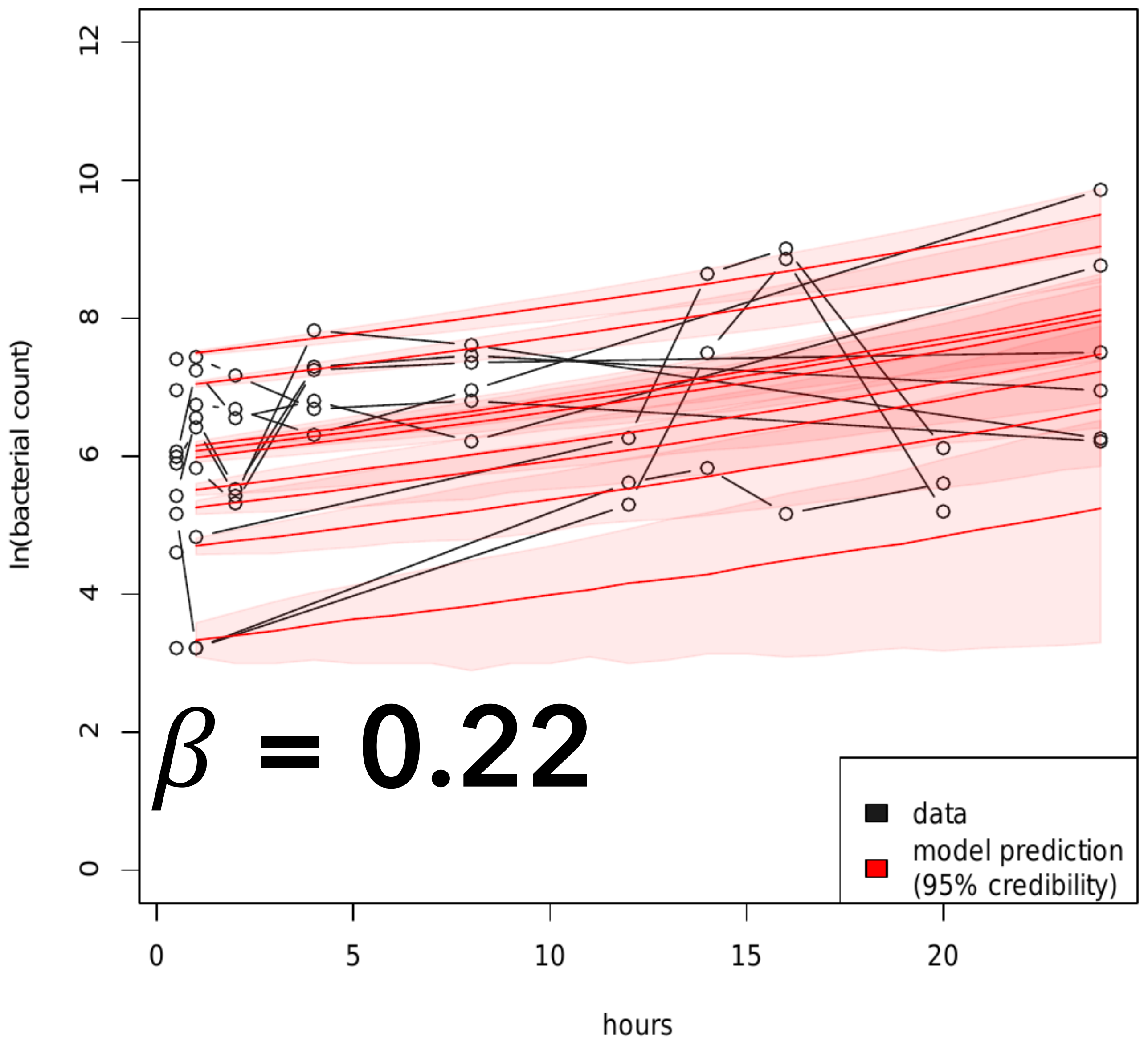
δ = 1.06e-3

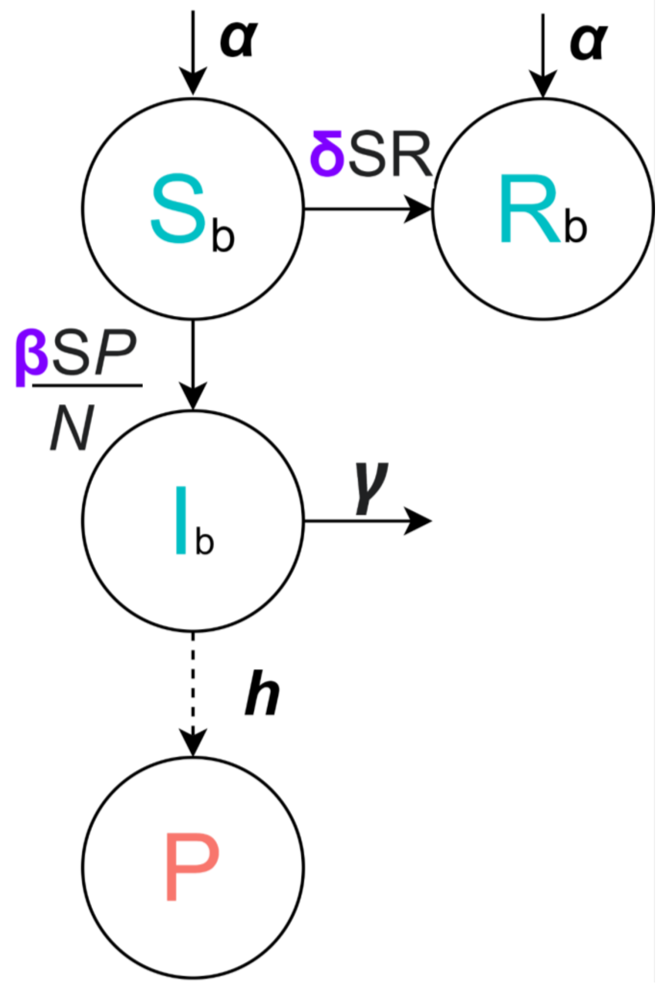


δ = 3.9e-03



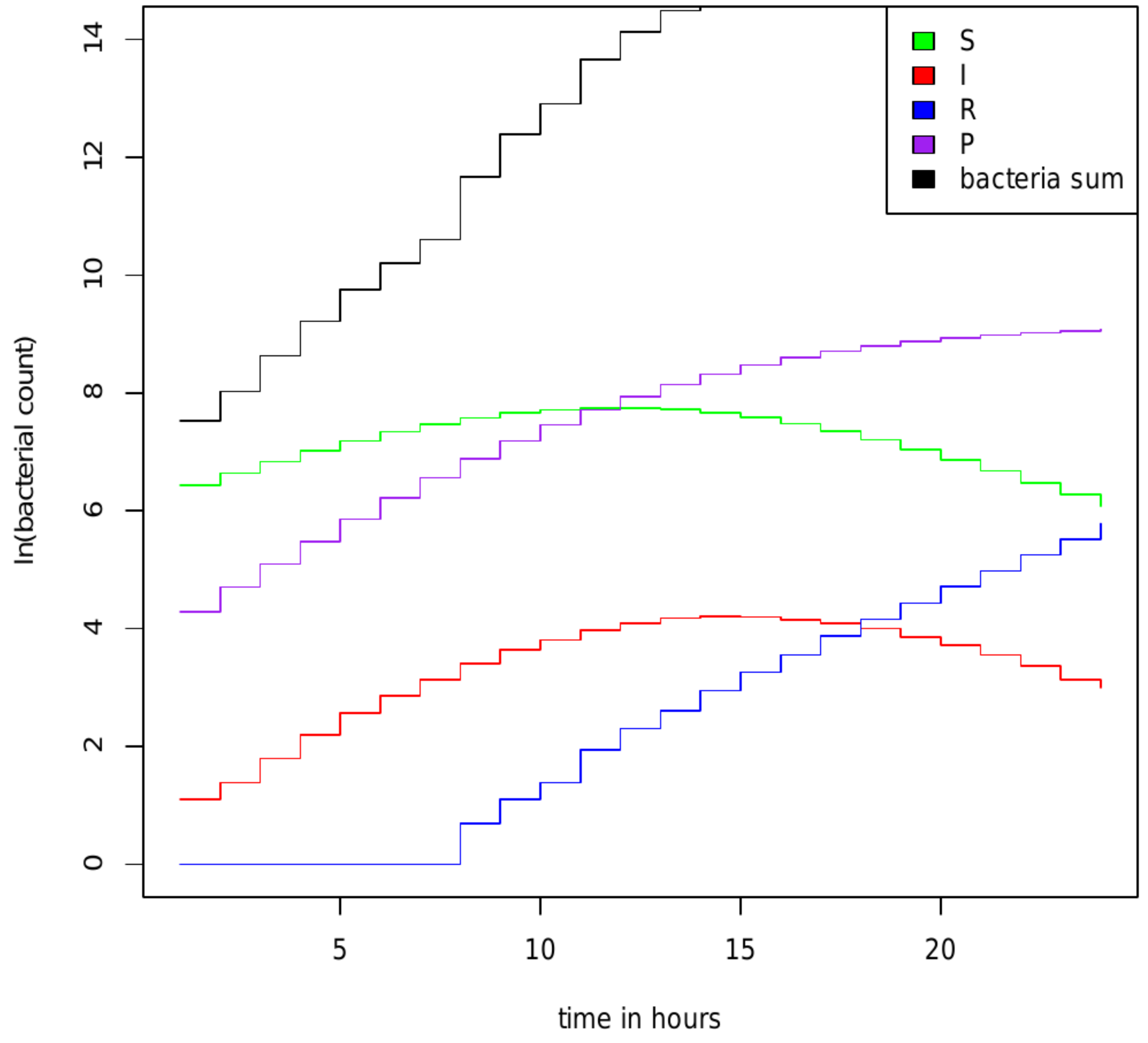
δ = 7.3e-5

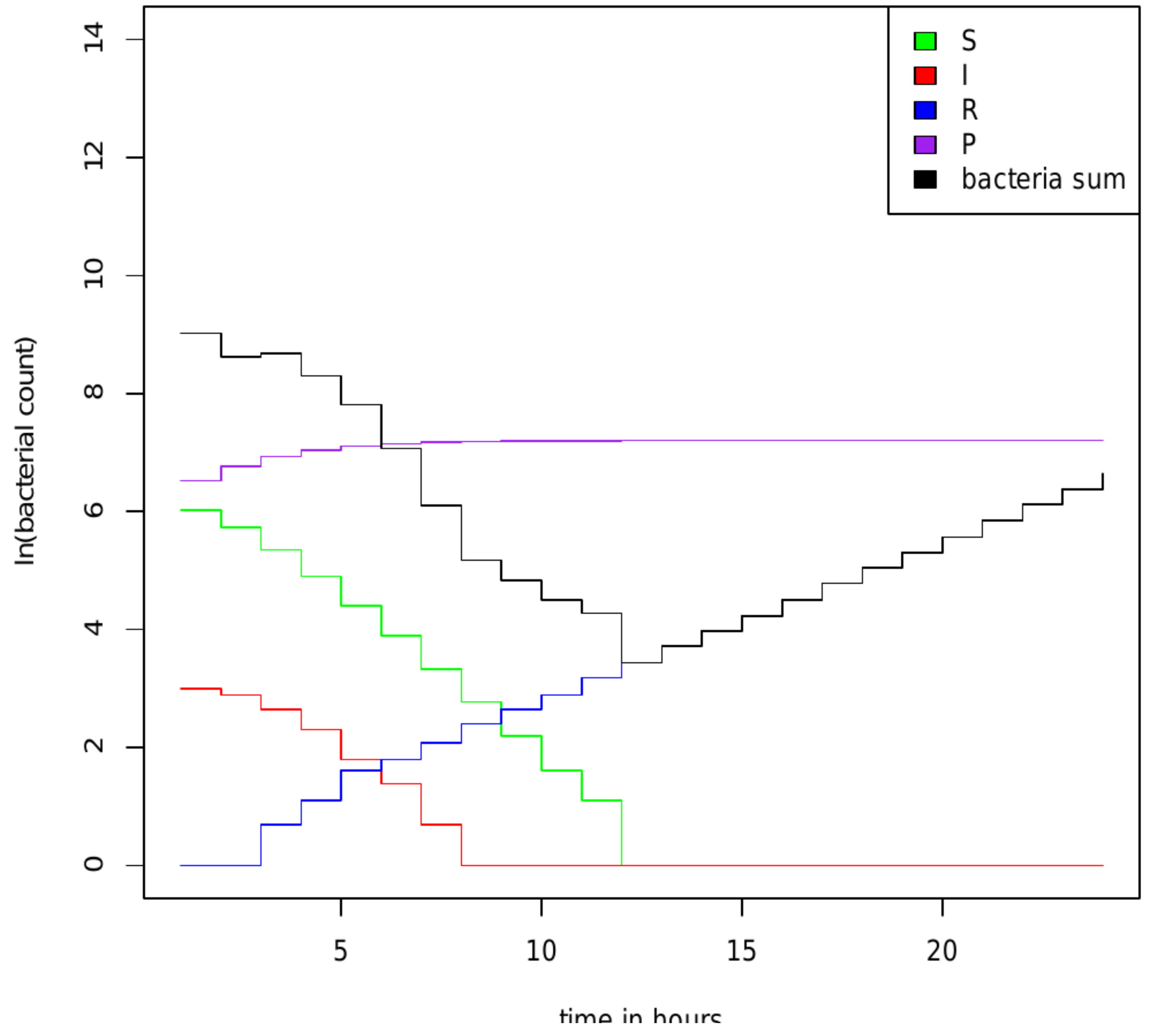


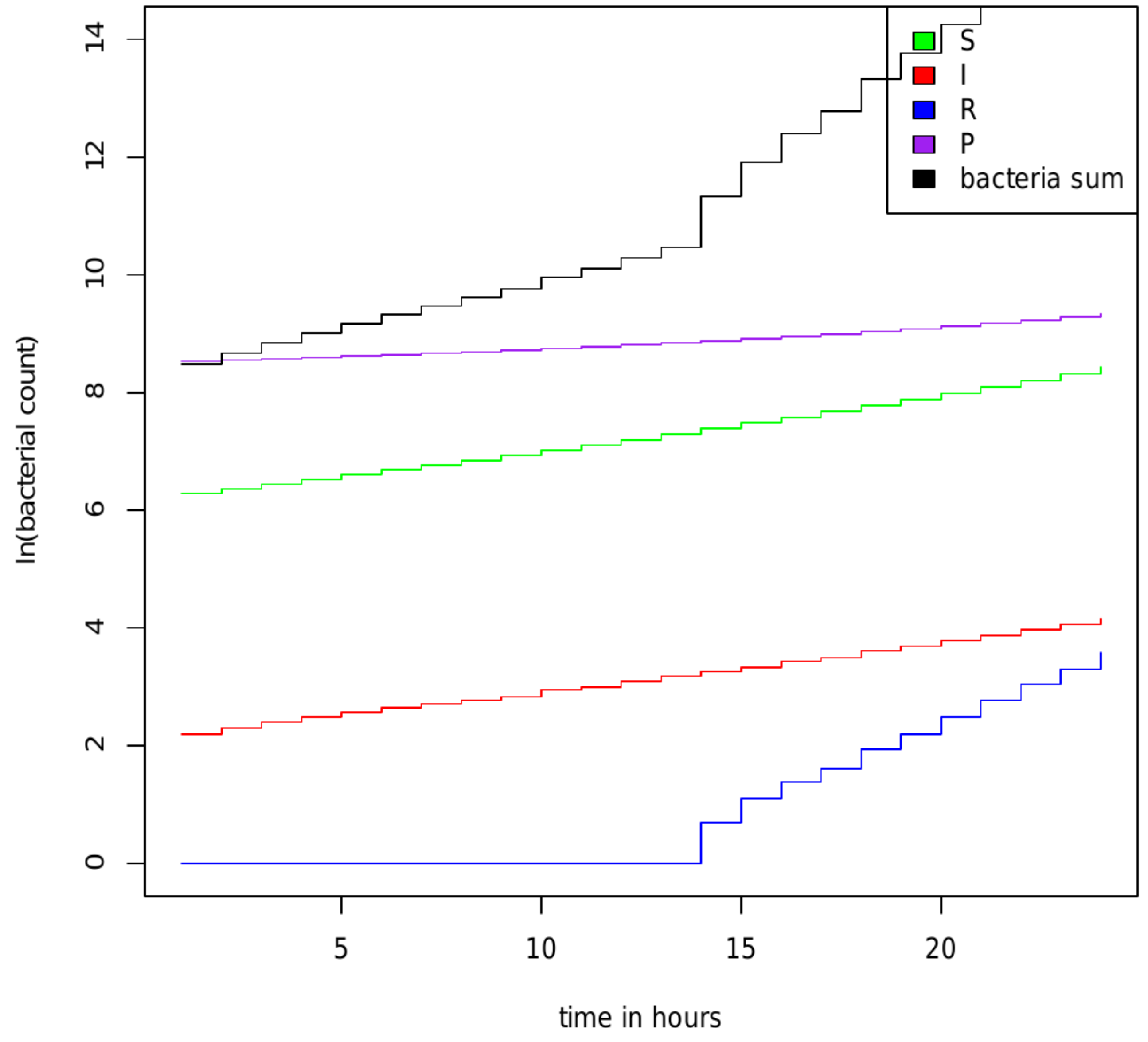


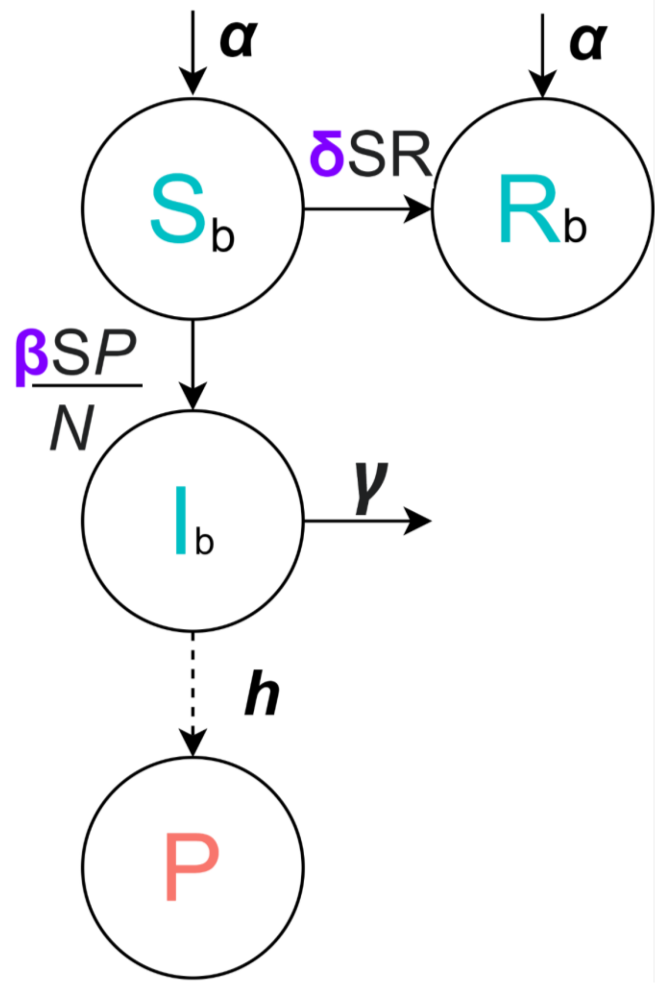
Mutation rate

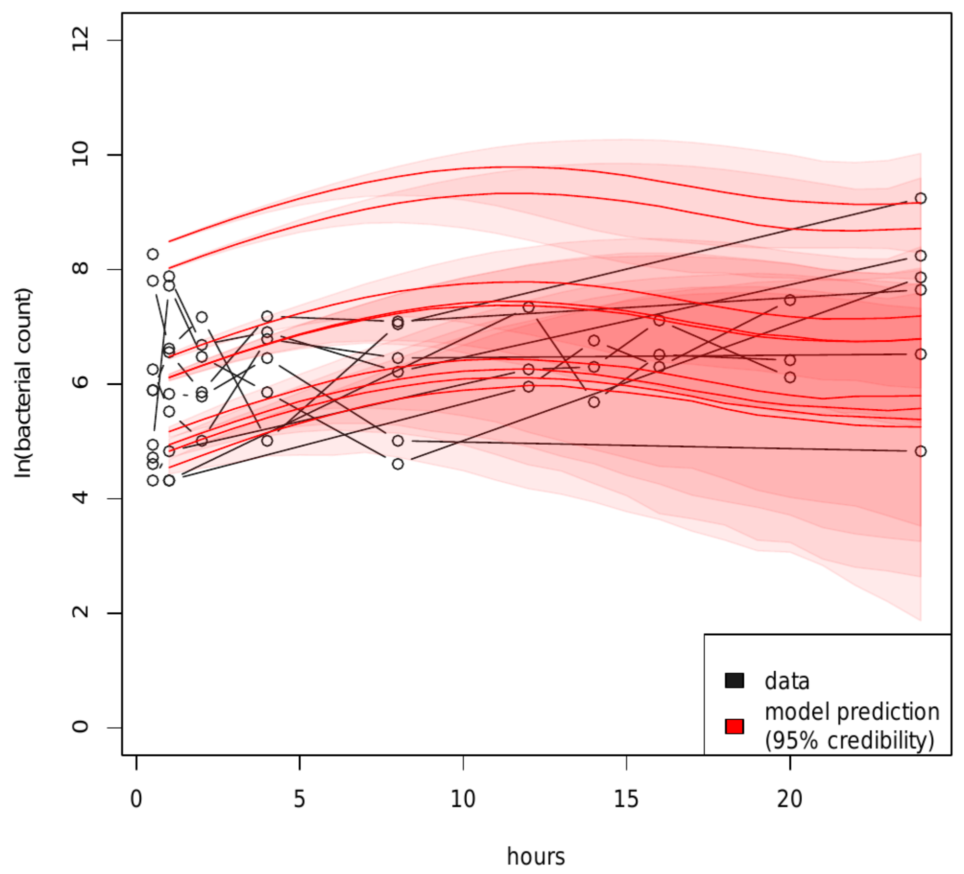
[6] Resistence development

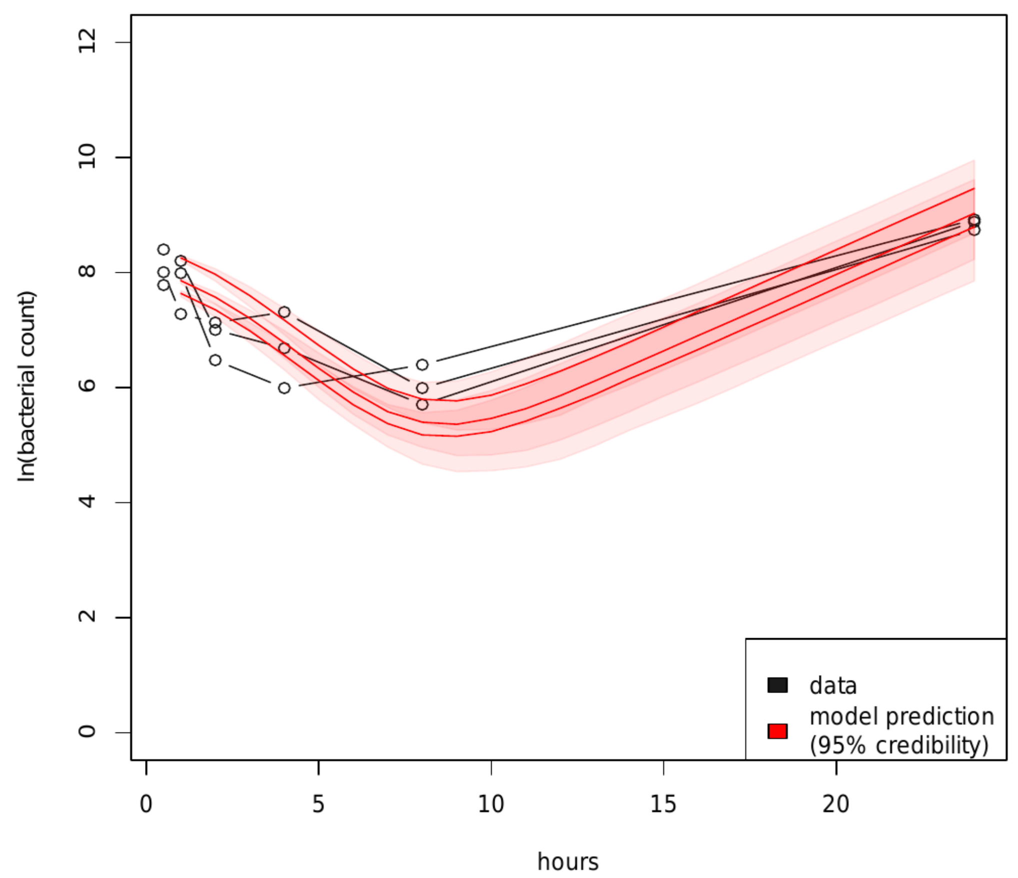


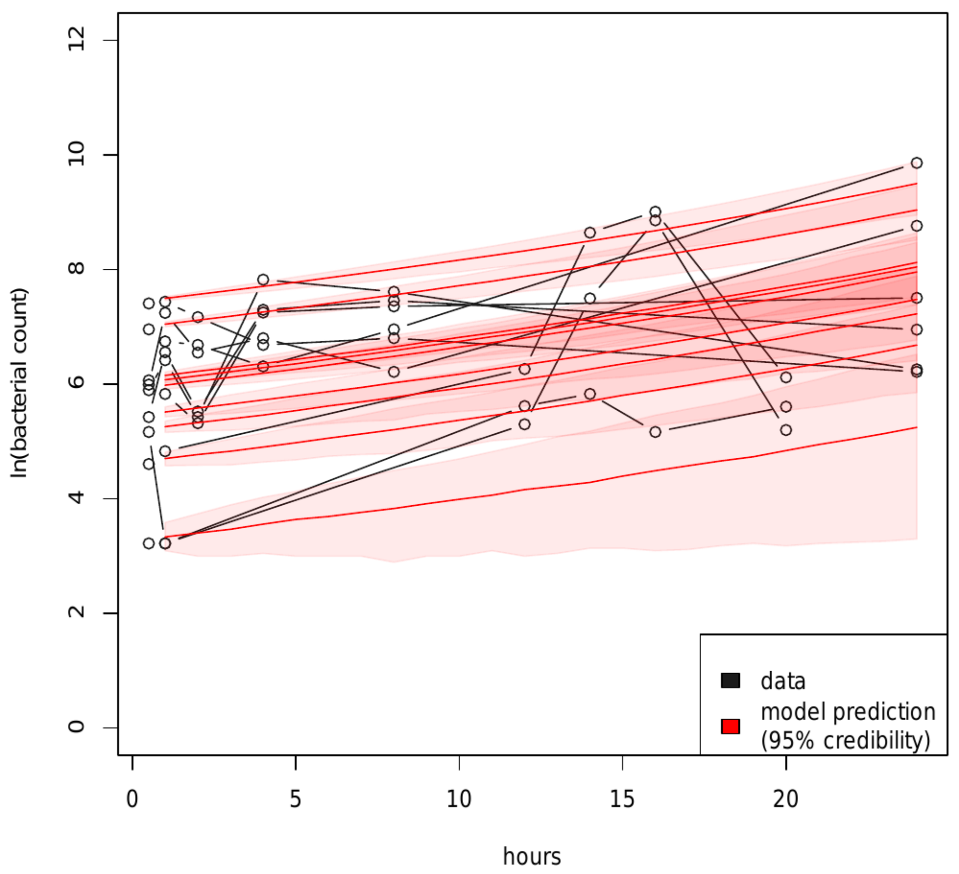




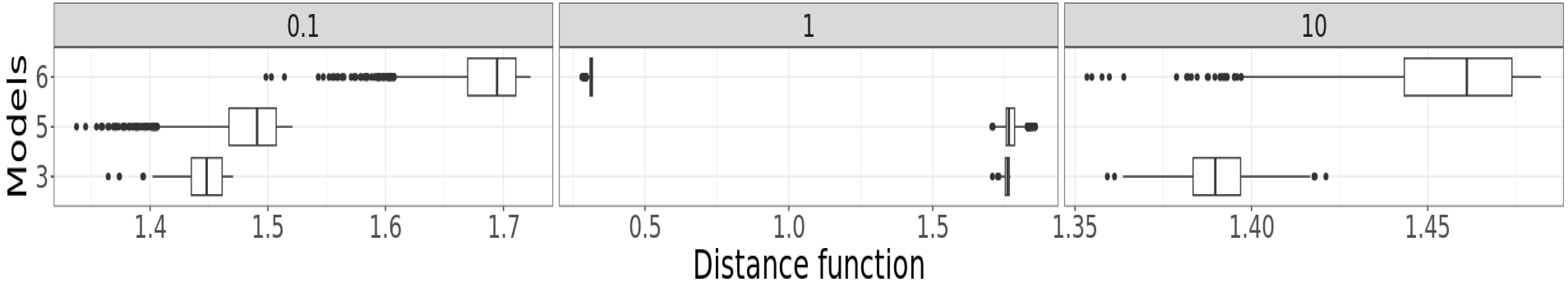


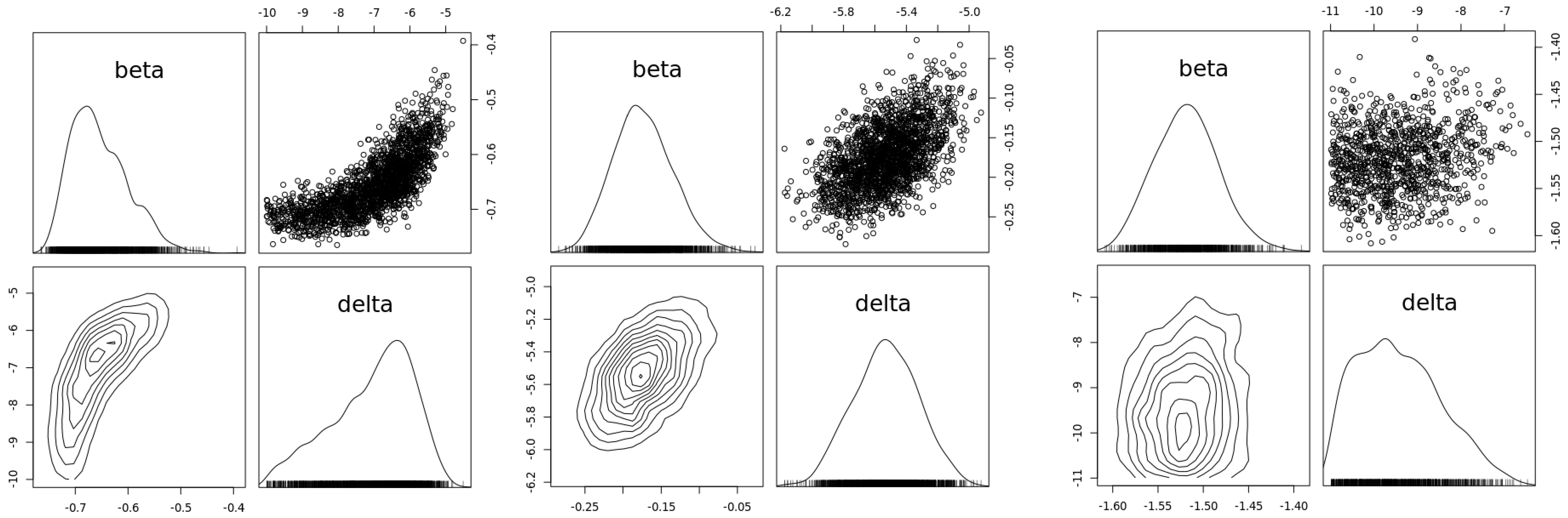






Model evaluation





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Sensitivity analysis

Partial rank correlation coeficient

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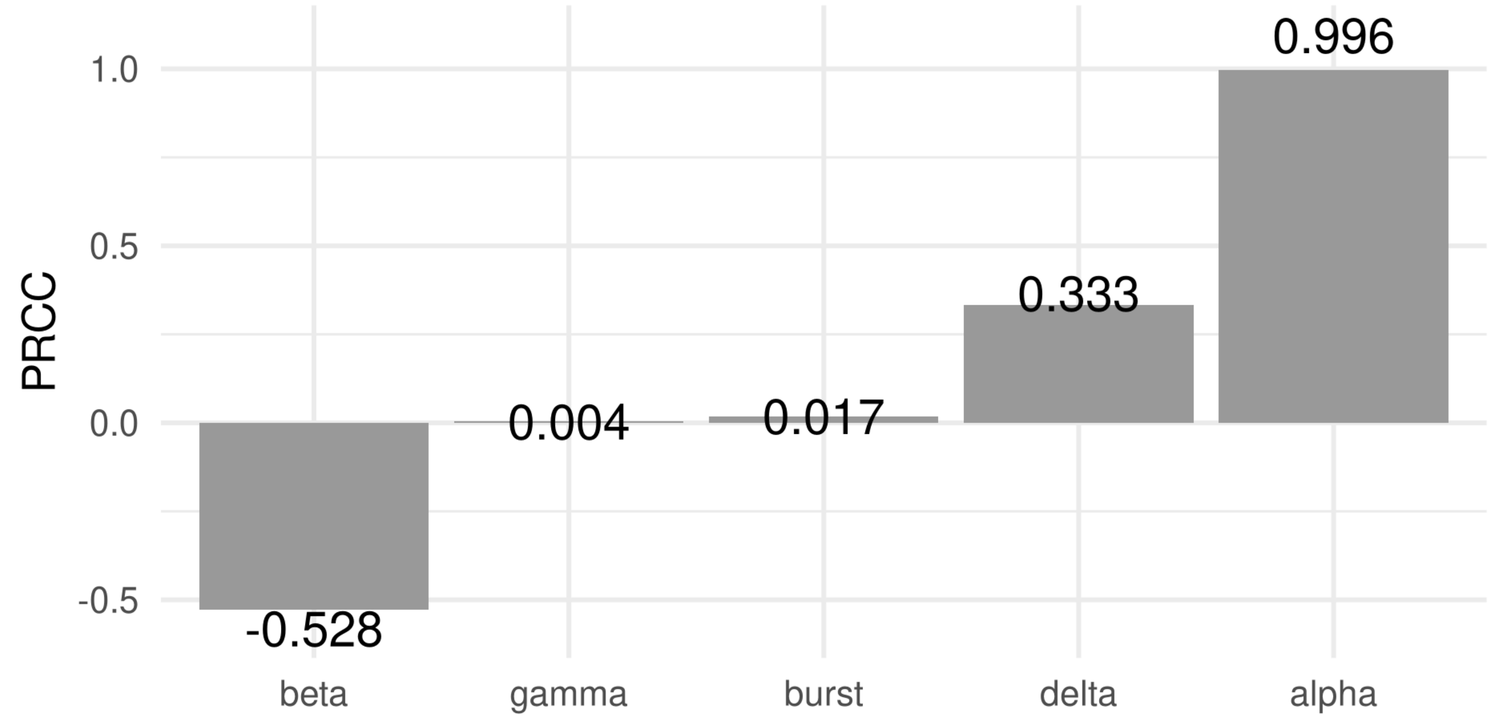
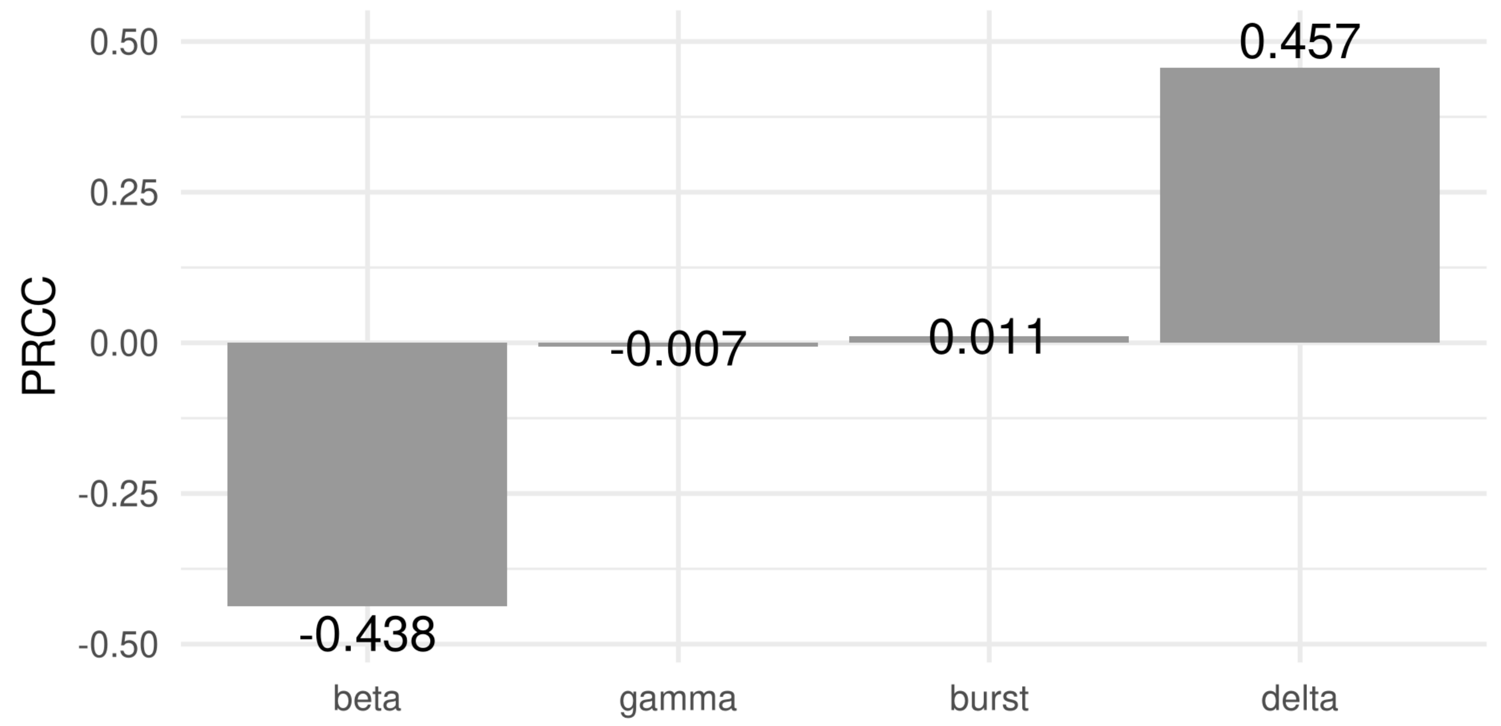
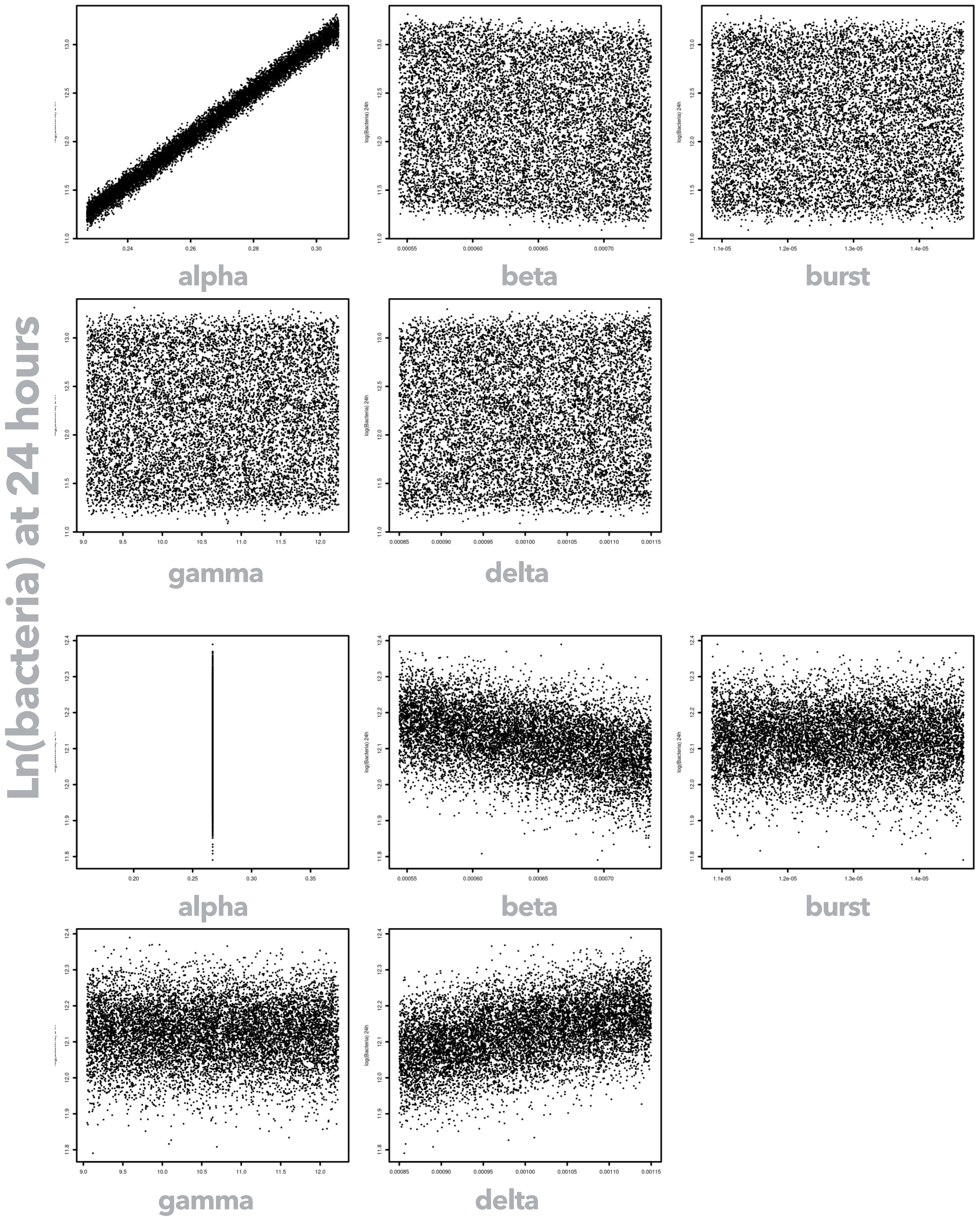
+/- 0.15



+/- 0.1 - 10





Take-home message

- Reduction in the bacterial growth rate suggest an effect of phages combating LA-MRSA (2.7 and 3.5 log units' reduction).

- Phages effectivity decrease with time (~8h).

- Multiplicity of infection drives the mechanism of bacterial infection (changes in beta).

  -Lower MOI behave better.

- The inclusion of the resistance mechanism on the model improved the fit and explained the biological process.

We are not driven by the mere desire to satisfy curiosity…

Phage therapy

Future work

- Include phage counts in the fit.

- Quantification of resistant bacteria (lab bfR).

- Testing therapeutical potential in silica:

  Add new phages. (when (t), how much (MOI)).

- How much the force of infection is needed to extinguish LA-MRSA.

Thanks to our partners

BfR
German Federal Institute for Risk Assessment

Veterinærinstituttet
Norwegian Veterinary Institute

jpiamr

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Stefan Widgren

Thomas Rosendal


Anna. D. Knipper

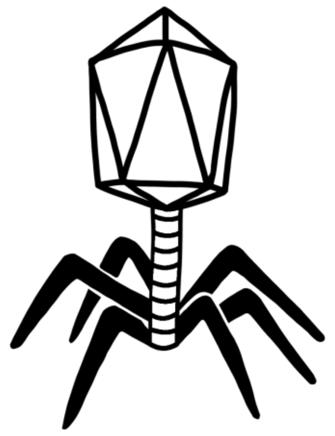
Kausrud Kyrre

Bernd Tenhagen

Jens Hammerl

Tobias Lienen

https://www.jpiamr.eu/projects/phage-ex/



All models are wrong…

sva.se/en/what-we-do/research-at-sva/researchers-at-sva/researchers/alfredo-acosta/

alfredo.acosta@sva.se

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