

1 Disentangling bacterial invasiveness from
2 lethality in an experimental host-pathogen
3 system

4
5 Tommaso Biancalani¹ and Jeff Gore¹⁺

6 ¹ Physics of Living Systems, Department of Physics, Massachusetts Institute of Technology, Cambridge, MA, 02139, USA

7 ⁺Corresponding author: gore@mit.edu

8 ABSTRACT

9 Quantifying virulence remains a central problem in human health, pest control, disease ecology,
10 and evolutionary biology. Bacterial virulence is typically quantified by the *LT50* (*i.e.* the time
11 taken to kill 50% of infected hosts), however, such an indicator cannot account for the full
12 complexity of the infection process, such as distinguishing between the pathogen's ability to
13 colonize vs. kill the hosts. Indeed, the pathogen needs to breach the primary defenses in order to
14 colonize, find a suitable environment to replicate, and finally express the virulence factors that
15 cause disease. Here, we show that two virulence attributes, namely pathogen lethality and
16 invasiveness, can be disentangled from the survival curves of a laboratory population of
17 *Caenorhabditis elegans* nematodes exposed to three bacterial pathogens: *Pseudomonas*
18 *aeruginosa*, *Serratia marcescens* and *Salmonella enterica*. We first show that the host population
19 eventually experiences a constant mortality rate, which quantifies the lethality of the pathogen.
20 We then show that the time necessary to reach this constant-mortality rate regime depends on the
21 pathogen growth rate and colonization rate, and thus determines the pathogen invasiveness. Our
22 framework reveals that *Serratia marcescens* is particularly good at the initial colonization of the
23 host, whereas *Salmonella enterica* is a poor colonizer yet just as lethal once established.
24 *Pseudomonas aeruginosa*, on the other hand, is both a good colonizer and highly lethal after
25 becoming established. The ability to quantitatively characterize the ability of different pathogens
26 to perform each of these steps has implications for treatment and prevention of disease and for
27 the evolution and ecology of pathogens.

28

29 I - INTRODUCTION

30 Quantifying virulence is challenging because the mortality induced by a pathogen is determined
31 by a complex series of interactions between the pathogen and the host. The virulence of a
32 pathogen will depend upon the level of *invasiveness*, governed by the pathogen's ability to
33 colonize and grow within the host, as well as the level of *lethality*, governed by the mortality that
34 is induced following colonization due to factors such as toxicity [1]. Moreover, both the
35 invasiveness and lethality of a pathogen will depend upon host characteristics such as age,
36 immune system, and microbiome [2].

37

38 Despite these results, there is still no consensus on how to disentangle the various pathogen
39 attributes from the survival curves of a host-pathogen system. In the majority of experimental
40 studies, survival curves are merely boiled down to the phenomenological indicator *LT50*, which
41 denotes the median lethal time (*e.g.* in pest control [3] and toxicology [4]). The usage of *LT50* is
42 often justified on grounds of simplicity, despite the fact that this indicator suffers from being
43 highly sensitive to experimental conditions (as shown later). Most importantly, *LT50* does not
44 describe a specific characteristic of the pathogen but rather provides a rough account of all
45 factors that cause virulence. Hence, it is not possible to use *LT50* to disentangle whether the
46 hosts are dying because of a highly invasive pathogen or a highly lethal one. The question can
47 also be posed conversely: what pathogen attributes need to be known to fully determine the
48 survival curves of the hosts?

49

50 Here, we use an experimentally tractable host-pathogen model system to disentangle how
51 pathogen invasiveness and lethality lead to pathogen virulence. We study the dynamics of a
52 laboratory population of hosts (the nematode *C. elegans*) exposed to three bacterial human
53 pathogens that also cause mortality in *C. elegans*: *P. aeruginosa*, *S. marcescens* and *S. enterica*.

54 This experimental system, especially with the pathogen *P. aeruginosa*, has been used to
55 investigate molecular mechanisms of virulence [5] [6], animal immunity [7], and mechanisms for
56 pathogen aversion [8]. Quantitative analysis of survival curves of worms exposed to all three
57 pathogens revealed that the worms eventually experienced a pathogen-specific per-capita
58 mortality rate. This pathogen-specific mortality rate was independent of pathogen exposure,
59 indicating that it reflects the intrinsic lethality of the pathogen against this host. A theoretical
60 model incorporating host colonization and pathogen growth predicts that the constant host
61 mortality rate emerges from pathogen load saturating within the host, and this prediction is
62 confirmed experimentally. The time necessary to reach this exponential phase where the host
63 experiences a constant-mortality rate reflects the pathogen invasiveness, due to the pathogen
64 colonization rate and growth rate within the host. Our integrated experiments and modeling
65 approach therefore allows us to disentangle the invasiveness from the lethality and to see how
66 each quantitatively depends upon the pathogen colonization rate, the growth rate within the host,
67 and the pathogen lethality.

68 II - RESULTS

69

70 II-A Experiments show that survival curves display an exponential phase

71

72 Our initial aim was to analyze how the *C. elegans* host survival curves are affected by exposing
73 the hosts to the same pathogen at different pathogen densities. On agar plates with rich media,
74 we spread a lawn of *P. aeruginosa* and incubated for either 4 hr (low), 24 hr (mid), or 48 hr
75 (high), so that the pathogen density can reach different densities (*e.g.* high = high pathogen
76 density). On each agar plate, we then added a population of approximately fifty *C. elegans* adult
77 nematodes, which are same-age, reproductive sterile and initially germ-free. The nematodes feed
78 on the pathogens, which colonize the worm gut and disrupt the epithelium provoking the death of
79 the host. Using standard worm picking protocols we monitor the fraction of worms surviving
80 over time [9] (see *Materials and Methods*).

81

82 As expected from previous experimental results [10], the worms die due to bacterial infection
83 over the course of a few days, whereas in the absence of the pathogen the worms would live for a
84 few weeks. Consistent with the expectation that higher bacterial densities will be more virulent,
85 we find that the lethal time for 50% of worms to die (*LT50*) is approximately 95 hr for worms
86 fed at low bacterial density (that is, pre-incubated for 4 hr) and 55 hr for worms fed at high
87 bacterial density (pre-incubated for 48 hr) (**Fig. 1-A**). The measured *LT50* therefore depends not
88 only on the particular pathogen and host being studied, but also on the details of the experimental
89 protocol, in this case the pre-incubation time of the pathogen.

90

91 It would be ideal if there were some feature in the survival curves that was independent of the
92 pathogen lawn density, as this would indicate an attribute that was intrinsic to the pathogen and
93 its host. Encouragingly, the survival curves plotted on a semi-log scale show a linear regime,
94 indicating that over longer times the worms are dying at a constant (per capita) rate (**Fig. 1-A**). In
95 this regime, the fraction of worms surviving decays exponentially, thus we refer to this regime as
96 the exponential phase. The slopes of the survival curve lines, δ_s , correspond to host mortality
97 rates. Unlike *LT50*s, we find that the slopes are the same for the different initial pathogen

98 densities, leading to mortality rates of $\delta \sim 0.055 \text{ hr}^{-1}$ (70% of the host population dies every
99 day). Our experimental observation that the mortality rate in the exponential phase is
100 independent of pathogen densities suggests that the mortality rate is a reflection of the intrinsic
101 lethality of the pathogen.

102
103 We next tested whether pathogen-induced mortality with (eventual) constant rate occurs
104 ubiquitously across pathogens. We repeated the experiment under identical conditions but using
105 pathogens *P. aeruginosa* (*Pa*), *S. marcescens* (*Sm*), and *S. enterica* (*Se*) (**Fig. 1-B**). All survival
106 curves exhibited an exponential phase, although the slopes of the lines are different for the three
107 pathogens. In *Pa*, we confirm the result found in the previous experiment, whereas in *Sm* and *Se*
108 we find $\delta_{Sm} \sim 0.02 \text{ hr}^{-1}$ (~40% population death rate per day) and $\delta_{Se} \sim 0.03 \text{ hr}^{-1}$ (~50%). We
109 also confirmed that the lethalties δ_{Sm} and δ_{Se} are independent of the lawn pathogen density
110 (**Fig. S1**), as we already showed for δ_{Pa} . These results indicate that exponential death occurs in
111 our experimental system for many different pathogens, and that the lethality δ is a characteristic
112 indicator of the host-pathogen interaction.

113
114 We note that ranking the pathogens by their lethalties is not consistent with the ranking obtained
115 by their *LT50*s (**Fig. 1-B inset**). In fact, $LT50_{Sm} \sim 70 \text{ hr}$ and $LT50_{Se} \sim 120 \text{ hr}$, which suggests
116 that *Sm* is more virulent than *Se* whereas the slopes δ_{Sm} and δ_{Se} indicate the converse. This
117 discrepancy arises because to fully understand survival curves, we also need to consider the time
118 taken to enter the exponential phase (henceforth denoted by τ), in addition to the lethality δ .
119 Indeed, *LT50* is strongly correlated to the time τ , whereas the lethality δ is not (**Fig. 1-C**). In *Se*,
120 the time required to enter exponential phase is twice the time in *Sm* ($\tau_{Se} \sim 102 \text{ hr}$ and
121 $\tau_{Sm} \sim 50 \text{ hr}$), although the exponential phase in *Se* is characterized by a sharper decline ($\delta_{Se} >$
122 δ_{Sm}). This signifies that *Sm* kills the hosts with a higher rate than *Se* at the early stages of the
123 infection but is then surpassed by *Se* as the infection progresses. Therefore, the indicator pair
124 (τ, δ) provides a more comprehensive description of the host survival curves than *LT50*.

125

126 II-B Theoretical model disentangles pathogen invasiveness and lethality from the survival 127 curve of host population

128

129 To explain the previous results, we use a simple population dynamics model that incorporates a
130 pathogen colonization rate c , pathogen growth rate r , and saturating population size K within the
131 host. We assume that the pathogen population size within a worm, denoted by N , follows

132

$$133 \quad (1) \quad \frac{dN}{dt} = (rN + c) \left(1 - \frac{N}{K}\right).$$

134

135 This simple model ensures that the pathogen population saturates at carrying capacity K (as
136 compared to the similar choice for the right-hand side of equation (1) $rN(1 - N/K) + c$, but this
137 choice does not lead to any significant difference in our conclusions). We also make the simplest
138 assumption for host mortality, namely that host mortality is linearly proportional to the pathogen
139 load. In this case the fraction of worms surviving $w(t)$ will change according to:

140

$$141 \quad (2) \quad \frac{dw}{dt} = -\delta w \frac{N}{K}$$

142 where the constant δ is the lethality of the pathogen at saturation. Although models of wild
143 disease are complex [11], we find that this exceedingly simple model suffices in our setting. In
144 fact, our model predicts that the survival curves enter an exponential phase as the pathogen load
145 reaches carrying capacity K (**Fig. 2**). When $N(t) = K$, equation (1) reduces to $\dot{w} = -\delta w$,
146 indicating that the per capita death rate, \dot{w}/w , is given by the constant δ . Moreover, equations
147 (1) and (2) with our initial conditions can be exactly solved, and yield predictions for the survival
148 curve w and the pathogen load N in terms of the pathogen colonization rate, growth rate and
149 lethality (see *Supplementary Material: Theoretical Model*).

150
151 We next analyzed the host survival curve and extract the time taken to enter the exponential
152 phase, τ , which we refer to as the invasion time. This time can be formally defined as the
153 abscissa of the intersection point between the lines $w = 1$ and the asymptote of the exponential
154 phase (see Fig. 2). The invasion time can be written as:

$$156 \quad (3) \quad \tau = \frac{1}{r} \log \left(1 + K \frac{r}{c} \right).$$

157
158 We note that the invasion time τ does not depend on the lethality δ , but rather provides a
159 mechanistic summary of the pathogen's ability to invade the host, which is a combination of the
160 ability to colonize and grow within the host. Indeed, a prediction of our model is that the time
161 that it takes for the host mortality curves to reach the exponential phase (invasion time)
162 corresponds also to the time that it takes the pathogen population to reach saturation within the
163 host, which we will refer to as the saturation time. The quantity τ^{-1} therefore measures the
164 pathogen invasiveness.

166 II-C Survival curves enter exponential phase as the pathogen load is at carrying capacity

167
168 Next, we tested the model prediction that the time taken to enter the exponential phase
169 corresponds to the pathogen load growing to carrying capacity in the host population. The model
170 also provides expressions for the survival curve and the pathogen load curves in terms of the
171 pathogen colonization rate c , the growth rate r and the lethality δ (see *Supplementary Material:*
172 *Theoretical Model*). Fitting the theoretical predictions to experimental data would indicate that
173 these three parameters determine the host-pathogen dynamics.

174
175 Our initial objective was to measure the pathogen load at a certain point in time. To do so, we
176 collected approximately 10 worms, washed their cuticles to remove the external bacteria,
177 grounded the sample population using a motorized pestle, and finally estimated the content of
178 their intestines by colony counting (**Fig. 3-A**). Following this protocol, we measured the in-host
179 growth curves for the three pathogens, the survival curves of which we already showed in Fig. 1-
180 B (**Fig. 3-B**). After exposing the hosts for a day to a fully-grown lawn, we measure significantly
181 different number of cells: $N_{Se}(24 \text{ hr}) \sim 5 \cdot 10^3$ cells, $N_{Sm}(24 \text{ hr}) \sim 10^4$ cells and
182 $N_{Pa}(24 \text{ hr}) \sim 2 \cdot 10^4$ cells. This variation at early times reflects the varied colonization abilities
183 of the pathogens, such as their survival rates as they pass through the grinder, the *C. elegans*
184 tooth-like structure that crushes most bacteria prior to digestion [12]. After a few days, all
185 pathogen loads saturated to carrying capacity which differ up to an order of magnitude:
186 $K_{Se} \sim 1.7 \cdot 10^6$ cells, $K_{Sm} \sim 1.1 \cdot 10^5$ cells and $K_{Pa} \sim 2.8 \cdot 10^5$ cells. We note that *Se* is the

187 slowest colonizer but reaches the largest carrying capacity. In general, we found that the three
188 pathogens exhibit different colonization abilities and carrying capacities within the hosts.

189
190 We then normalized the pathogen load curves by their carrying capacities to visualize these
191 curves against the survival curves. In this way, we could test whether the entrance in the
192 exponential phase occurs as the pathogen load curve plateaus (**Fig. 3-C**). We confirm that this is
193 the case for the three pathogens: the times taken to enter exponential phase τ , were markedly
194 distinct in the three cases (from 50 hr to 110 hr), yet the invasion times are approximately equal
195 to the saturation times, as predicted by our model (**Fig. 3-D**). Indeed, we observed that the
196 pathogen load curves neatly plateaued as the survival curves reached the regime of constant
197 death rate (**Fig. 3-C**). We tested if our model quantitatively fit the pathogen load and survival
198 curve. We already measured the death rate δ and carrying capacity K for the three pathogens,
199 and we determined the colonization rates c and the growth rates r by inspection. We found
200 similar values for the growth rates of the three species ($r \sim 0.08 \text{ hr}^{-1}$), whereas the colonization
201 abilities of *Pa* and *Sm* ($\sim 250 \text{ cells / hr}$) are better than *Se* ($\sim 40 \text{ cells / hr}$). The agreement
202 between theory and data indicates that pathogen-induced mortality increases approximately
203 linearly with pathogen load. We note that this did not have to be the case, as the pathogens could
204 express virulence factors at any time during the infection process.

205

206 II-D Invasion time τ can be separated into colonization and replication time intervals

207

208 Next, we use our model and experiments to show that the invasion time τ can be split between a
209 time period that is colonization dominated and a time period that is replication dominated: $\tau =$
210 $\tau_c + \tau_r$ (**Fig. 4-A**). In the first-time interval, τ_c , the pathogen influx is mostly due to external
211 colonization rather than replication, since the hosts are initially sterile; in the second interval τ_r ,
212 colonization becomes negligible and the internal pathogen growth is the dominant effect. From
213 our model, equation [2], we can show that (see *Supplementary Material: Theoretical Model*).

214

$$215 \quad (4) \quad \tau_c = \frac{1}{r} \log(2),$$

216

217 which, interestingly, shows that the time interval in which colonization dominates corresponds to
218 the pathogen doubling time and is therefore independent of the colonization rate (although we
219 have assumed $r \gg c$ in the derivation of equations (3) and (4), which is always the biological
220 case). During time τ_c , the pathogen abundance grows until it reaches $N^* = c/r$, after which
221 replication becomes dominant. The invasion time τ can be rewritten as $\tau = \tau_c + \tau_r =$
222 $r^{-1} \log(1 + K/N^*)$ which also shows that τ_r depends on N^* . Therefore, the time in which
223 colonization dominates is largely independent of the lawn pathogen density (which, in our
224 experiment, determines the colonization rate c).

225

226 From our data, we can estimate the colonization time scale τ_c , the replication time scale τ_r and
227 the killing time scale δ^{-1} , for the three pathogens (**Fig. 4-B**). Since the pathogens have similar
228 growth rates, the colonization time intervals τ_c are approximately equal. In contrast, τ_r (and
229 hence τ) is much larger in *Se* compared to those of *Pa* and *Sm*, due to the difference in carrying
230 capacity. The killing time scale of *Se* (δ^{-1}) is greater than the killing time scale of *Sm* which,
231 again, is due to the difference in carrying capacity (in fact, *Sm* has a greater lethality per cell). It

232 is also worth noting that the $LT50$ s for these three pathogens falls in the killing time interval
233 (which following the invasion phase), meaning that more than 50% of the hosts die in the
234 exponential phase.

235
236 Finally, we inquired whether our model retains its predictive power when the colonization rates
237 are varied experimentally. We analyzed the *Pa* pathogen load curves obtained for different
238 pathogen incubation times (Fig. 1-A) and found that, as expected, only the colonization rate c
239 needs to be varied to fit the three curves (**Fig. 4-C**). These results illustrate how the dynamics of
240 pathogen colonization and growth are determined by the underlying processes by which the
241 pathogen invades the host.

242 III – DISCUSSION

243
244 In this study we have demonstrated that integrating quantitative analysis of survival curves with
245 mathematical modeling allows one to determine how the dynamics governing pathogen invasion
246 of the host lead to the different timescales associated with host mortality. In particular, we find
247 that the pathogen invasion time τ and lethality δ provide a better assessment of virulence than
248 using $LT50$. Extracting τ and δ from the survival curves allow us to disentangle whether the
249 salient pathogen characteristic is to be a good invader or a good killer, which is not possible to
250 determine just by using $LT50$. These indicators also inform us about the shape of the survival
251 curve according to the pathogen attributes. Skillful invaders exhibit a survival curve that drops
252 rapidly into the exponential phase. Lethal pathogens are characterized by a survival curve that
253 sharply declines once the pathogen reaches carrying capacity, suggesting that the host mortality
254 rate is greater in the late stages of the infection. These results contribute to demonstrate that *C.*
255 *elegans* is an excellent model system for unravelling simple quantitative laws in biology, as
256 already recently proved in other fields such as aging [13] and eco-evolutionary dynamics [14].
257

258 Disentangling virulence into its causal attributes is necessary to understand the ecology and
259 evolution of host-pathogen systems. Historically, it has been proposed that virulence attributes
260 are controlled by evolutionary trade-offs [15]. Plant-pathogen systems provide the longest-
261 standing example that pathogens excel at a certain attribute in spite of others (e.g. [16]), an effect
262 that results from co-evolution [17]. Indeed, hosts can cope with pathogens by diminishing their
263 invasiveness (host resistance) or by decreasing their lethality (host tolerance) [18]. Such trade-
264 offs have been observed in plants [17] and animals [19], and theoretical studies have
265 demonstrated that they constrain the evolutionary [20] and ecological [21] fate of the host-
266 pathogen system. However, there was no obvious trade-off between lethality and invasiveness
267 among our three pathogens (**Fig. 4B**, **Fig. S3-left**). Host resistance (K^{-1}) and tolerance ($K \delta^{-1}$),
268 defined as in [19], shows that hosts are more resistant to *Sm* and *Pa*, but they have higher
269 tolerance for *Se* (**Fig. S3-right**). These two indicators, however, do not account for how fast the
270 pathogen invades, which is also important for host survival and for the epidemiology of a
271 disease, especially in light of recent findings where pathogen strategies in human disease are
272 disentangled [22].
273

274 A key observation in this study is that survival curves enter an exponential death phase, namely,
275 the hosts eventually experience a constant mortality rate. Although to our knowledge this is the

276 first report in an experimental host-pathogen system, exponential laws are already well-
277 established in many other areas of biology. It is textbook knowledge that microbes die
278 exponentially after stress or prolonged starvation [23], even though density-dependent deviations
279 have been observed [24], which can be due to cell memory effects [25]. The most frequent
280 example of exponential statistics is perhaps found in the logistic growth of microorganisms,
281 where the exponential face is usually preceded by a lag phase [23]. Interestingly, the expression
282 that relates the lag time is remarkably similar to our equation (3) [26], which highlights a parallel
283 between microorganismal growth and host decay [27]. In this respect, our pathogen lethality δ
284 plays the analog of the bacterial growth rate, which has recently shown to be one of the few key
285 fundamental parameters determining the state of the cell [28].

286
287 We found that *C. elegans* reaches an exponential phase after exposure to three well-studied
288 pathogens, although when the hosts are exposed to non-pathogenic bacteria such as
289 *Pseudomonas chlororaphis* we find that there is no exponential phase (**Fig. S2**). Indeed, even
290 after ten days of feeding on *P. chlororaphis* the host mortality rate had not yet stabilized. In
291 contrast, the bacterial load saturates after 3 days, remains stable for the successive three days,
292 then starts growing again. This effect might be due to the fact that the bacterial carrying capacity
293 increases as the worm ages [29]. Further work will be required to clarify whether our framework
294 has a more general applicability. With recent technological advances [30], it might be possible to
295 adopt automatized protocols that would allow to repeat our investigations with higher-
296 throughput.

297
298 Finally, our investigations have been limited to single pathogens in sterile host populations, thus
299 neglecting the interaction between the pathogen and the host microbiome. Recent works have
300 demonstrated that *C. elegans* is a suitable model system for microbiome investigations [31][32].
301 For example, it is known that nematodes previously colonized with certain microbes exhibit
302 enhanced survivability during a pathogen infections [33] [34] [35]. With our framework, we can
303 quantify how each pathogen attribute is affected as a function of the microbiome composition,
304 thus formulating novel hypotheses for elucidating interspecies mechanics. It is our hope that
305 simple quantitative laws of host-pathogen dynamics may provide insight into pathogenesis in
306 more complex host-pathogen systems.

307 IV – MATERIALS AND METHODS

308 All chemicals were purchased from Sigma Aldrich (St. Louis, MO) if not stated otherwise.
309

310 Worm and bacterial strains

311 The bacterial strains used in the paper are *Pseudomonas aeruginosa* PA14 (from Ausubel's lab,
312 Harvard), *Serratia marcescens* Db10 (Caenorhabditis Genetics Center, CGC), *Salmonella*
313 *enterica* LT2 (CGC), *Escherichia coli* OP50 (CGC) and *Pseudomonas chlororaphis* (ATCC
314 9446). Throughout the work, we used *Caenorhabditis elegans* strain SS104 (*glp-4(bn2)*)
315 obtained from CGC. Due to the *glp-4* mutation, this strain is able to reproduce at 15°C but is
316 reproductive sterile at 25°C; use of this strain prevented the worms from producing progeny
317 during experiments, ensuring that the only changes in worm population were due to pathogen-
318 induced mortality.

319

320 Preparation of worm cultures

321 Synchronized (*i.e.* same age) worm cultures were obtained using standard protocols [36]. For
322 propagation of worms, SS104 cultures were maintained at 15°C on NGM agar plates with lawns
323 of the standard food organism *E. coli* OP50. For synchronization, worms from several nearly
324 starved plates were washed with sterile distilled water and treated with a bleach-sodium
325 hydroxide solution; the isolated eggs were placed in M9WB overnight to hatch, then transferred
326 to NGM + OP50 plates at the sterility-inducing temperature (25°C) for 2 days to obtain
327 synchronized adults. Worms were then washed from plates using M9 worm buffer + 0.1% Triton
328 X-100 (Tx), then rinsed with M9 worm buffer. Worms were then transferred to S medium + 100
329 µg/mL gentamicin + 5X heat-killed OP50 for 24 hours to kill any OP50 inhabiting the intestine,
330 resulting in germ-free synchronized worms. These 3-day-old synchronized adult worms were
331 then rinsed in M9PG (M9WB + 0.1% PEG; PEG is used to prevent the worms from sticking to
332 the pipette tip), washed via sucrose flotation to remove debris, and rinsed 3X in M9PG worm
333 buffer to remove sucrose before use in experiments.

334

335 Survival curves assay

336 To generate the data of Fig 1A and 1B we used a variation of a previously published protocol
337 [9]. For a single condition (*i.e.* a survival curve on *Pa*), we run our assay in a 6-well plate where
338 each well (4 cm diameter) represents a technical replicate. Each well is filled with 4 mL of SK
339 agar media (recipe in [16]). To prevent worms from exiting the plate, we add 15 µL of palmitic
340 acid (10 mg/mL in EtOH) to each well border. Pathogen monocultures are grown for 24 hr at
341 30°C in LB, then 7 µL of culture is pipetted to the center of each plate and spread using a small
342 metal cell spreader to create a pathogen lawn. The 6-well plate is then parafilmmed to prevent
343 evaporation and incubated at 25°C for the desired time (Fig. 1A: 4 hr, low density; 24 hr, mid
344 density; 48 hr, high density. Fig. 1B: 48 hr). To each well, we then add off-lawn a population of
345 ~50 adult reproductive sterile germ-free worms suspended in M9PG, as described in the previous
346 section. As the buffer is quickly absorbed by the plate, the worms start feeding on the pathogen
347 lawn. We keep the 6-well plate parafilmmed and incubated at 25°C throughout the whole
348 experiment, and we monitor the number of worm surviving using standard worm picking
349 protocols [9].

350

351 Pathogen load assay

352 Our protocol is a variation of previously published protocols [29] [33] [37]. Briefly, we use two
353 buffers: TXLV (M9WB with 1% Triton-X + 50 mM levamisole) and TXAB (TXLV +
354 gentamicin and carbenicillin ~210 µg/mL). We prepare 6-well plates as described in the previous
355 section. Since measurement of the pathogen load is destructive, we could not use the same worm
356 population to measure both the pathogen load and the survival curve. Thus, we prepared a group
357 of plates under identical conditions and separated them into two groups: we estimated the
358 survival curve from one group and the pathogen load from the other, thus assuming that the two
359 groups possess equal average dynamics. To measure the pathogen load, we collected each day
360 >30 alive worms and washed them four times in TXLV. Due to levamisole, worm peristalsis is
361 interrupted and the mouth and anus of the worm remains shut, thus preventing the internal
362 bacteria to be flushed out. We then resuspended the worm population in TXAB and incubated for
363 1 hr at 25 °C with gentle shaking. Every 20 mins, we washed and resuspended the population in
364 fresh TXAB. The purpose of the incubation is to remove the external bacteria attached to the
365 worm cuticle. We then washed the worms 3-4 times in TXLV to remove the antibiotic and

366 transfer the population to a small Petri dish. Using a dissecting scope, we removed the worms
 367 that were not fully paralyzed. We then split the worm population into three Kontes tubes so that
 368 each tube contains 50 μ L TXLV and 10 worms. Each tube constitutes a technical replicate. We
 369 homogenize the worms in a tube with a pellet pestle for 1 min continuously. We then dilute the
 370 solution in M9WB, and plate to LB agar plates for colony counting. Pathogen load is reported as
 371 the mean +/- sem of the three technical replicates.

372

373 Statistical analysis

374 Details of our statistical analysis are provided as a supplementary *R* notebook.

375

376 Figure 1

377 Markers in Fig. 1-A and 1-B represent mean survival curve averaged across 6 technical
 378 replicates. Corresponding *LT50*s were determined graphically from mean survival curve. Solid
 379 lines in Fig. 1-A and 1-B were obtained by fitting the mean survival curve using a linear model.

380

381 In Fig. 1-C, we reported lethalties of different pathogens, estimated for different initial densities.
 382 The corresponding survival curves are shown in Fig. 1-A and Fig. S1. To estimate lethalties, we
 383 determined the fitting region from the mean survival curve, then fitted each technical replicate
 384 using a linear model. Next, we computed average lethality and their standard errors obtaining the
 385 values in the tables below. These values are consistent with those in *Supplementary Table 1*,
 386 which were obtained with a non-linear fit, as described later.

387

	<i>Pa</i> (high)	<i>Pa</i> (mid)	<i>Pa</i> (low)
average lethality δ (hr^{-1})	0.057	0.053	0.052
standard error (hr^{-1})	0.009	0.003	0.006

388

	<i>Sm</i> (high)	<i>Sm</i> (mid)	<i>Sm</i> (low)
average lethality δ (hr^{-1})	0.034	0.031	0.028
standard error (hr^{-1})	0.005	0.004	0.004

389

	<i>Se</i> (high)	<i>Se</i> (mid)	<i>Se</i> (low)
average lethality δ (hr^{-1})	0.035	0.031	0.034
standard error (hr^{-1})	0.005	0.003	0.005

390

391

392 In Fig. 1-B we repeated the experiment with a single incubation time but using 12 technical
 393 replicates for each pathogen. We estimated lethalties following same procedure described
 394 above. We find the following values:

395

	<i>Pa</i>	<i>Sm</i>	³⁹⁶ <i>Se</i>
average lethality δ (hr ⁻¹)	0.058	0.022	0.033
standard error (hr ⁻¹)	0.004	0.002	0.006

397

398 Figure 4-C / Supplementary Table 1

399 Markers correspond to mean pathogen loads averaged over 3-4 technical replicates obtained for
400 different initial pathogen densities. Pathogen loads are rescaled by the carrying capacity $K_{Pa} =$
401 $2.8 \cdot 10^5$ cells, which we determined by inspection. The mean pathogen loads are fitted using the
402 solution of equation (1) (see *Supplementary Material: Theoretical Model*). To carry out the
403 fitting procedure, we imposed the same growth rate r for the three conditions, but different
404 colonization rates: c_{high} , c_{mid} and c_{low} . For each condition, we computed the sum-of-square
405 error between the solution of equation (1) and the mean pathogen load data. We estimated the
406 four parameters r , $c_{Pa,48}$, $c_{Pa,24}$ and $c_{Pa,4}$ by minimizing the sum of the errors for the three fits.
407 To provide errors for the fitted parameters, we bootstrapped the pathogen loads data and repeated
408 the fitting procedure, hence obtaining different values for r , $c_{Pa,48}$, $c_{Pa,24}$ and $c_{Pa,4}$. We repeated
409 the fit 500 times and computed the standard deviations of the four parameters, which represent
410 our errors on the fitted data. Values and errors are shown in the *Supplementary Table 1*.

411

412 Figure 3 / Supplementary Table 2

413 Figure 1-B show mean pathogen load data averaged over 3-4 technical replicates for each
414 pathogen. These curves are used to determine (by inspection) the carrying capacities (see
415 *Supplementary Table 2*). We then fit the model solutions of equations (1) and (2) (explicit
416 formulae in *Supplementary Material: Theoretical Model*) to the normalized pathogen load data
417 and the corresponding survival curves in Fig. 1-B. Our fitting procedure is similar to that in
418 Figure 4-C. We estimated the parameters r , c , and δ for the three pathogens by minimizing the
419 sum of the square-errors for the pathogen load and the growth curve. Errors to these parameters
420 are given by bootstrapping. Values and errors are shown in the *Supplementary Table 2*.

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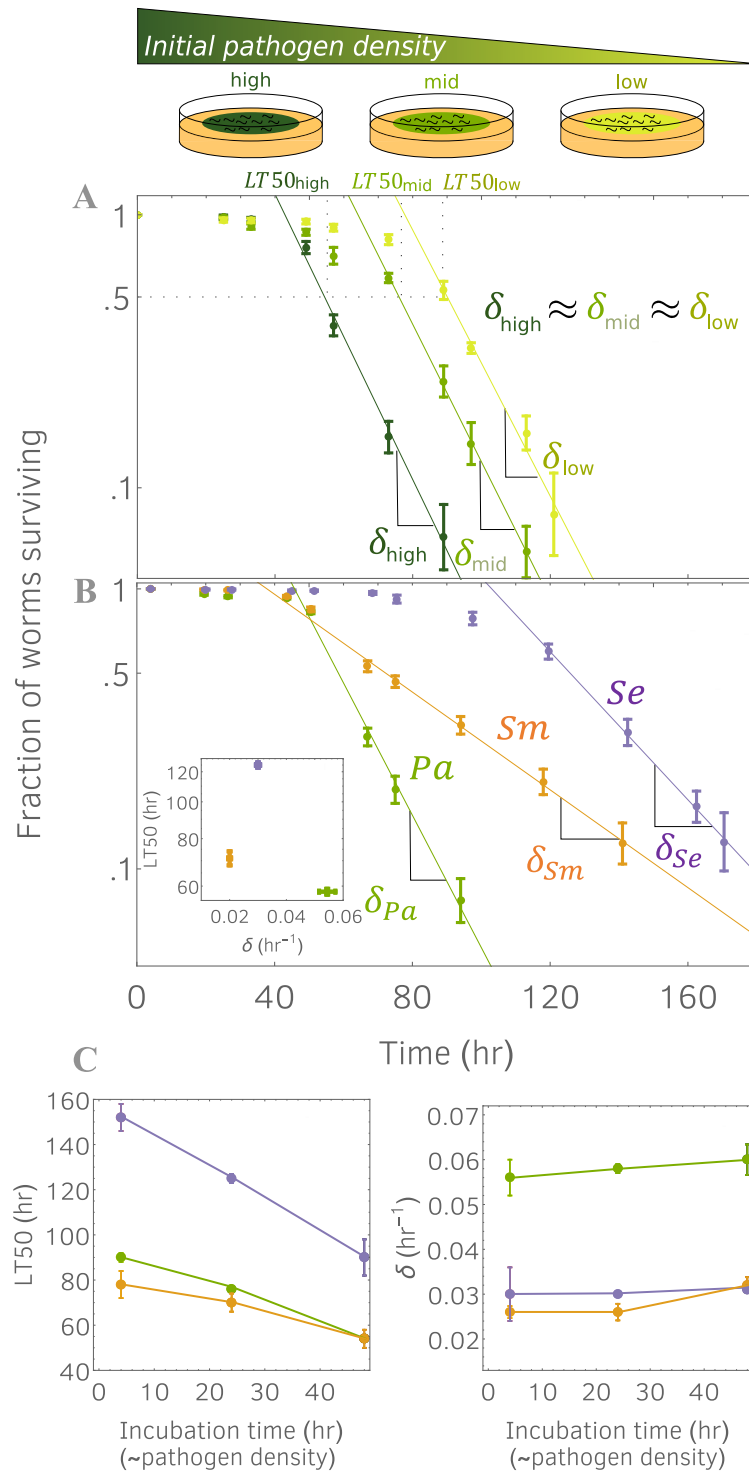


Figure 1: Host survival curves enter an exponential death phase (visualized as a line in semi-log scale), whose slope δ is characteristic of pathogen lethality. (A): Survival curves obtained by exposing a population of *C. elegans* nematodes to population *P. aeruginosa*. Shade of green corresponds to different initial pathogen densities (high, mid and low), obtained by pre-incubating the pathogen lawn for different times prior to adding the hosts. Markers correspond to experimental data averaged across six technical replicates. Error bars correspond to standard errors. Survival curves enter an exponential phase with a slope independent of the initial pathogen densities. **(B):** Survival curves obtained for bacteria *P. aeruginosa* (*Pa*), *S. marcescens* (*Sm*) and *S. enterica* (*Se*). For each pathogen, we report an exponential phase with a characteristic lethality. **(B-inset):** $LT50$ does not correlate with the lethality δ . **(C):** $LT50$ s (left) and δ s (right) of different pathogens (colors matched Fig. 1B and Fig. S1), are plotted against the incubation time of the pathogen lawn. Unlike $LT50$, the lethality δ does not change with the initial pathogen density.

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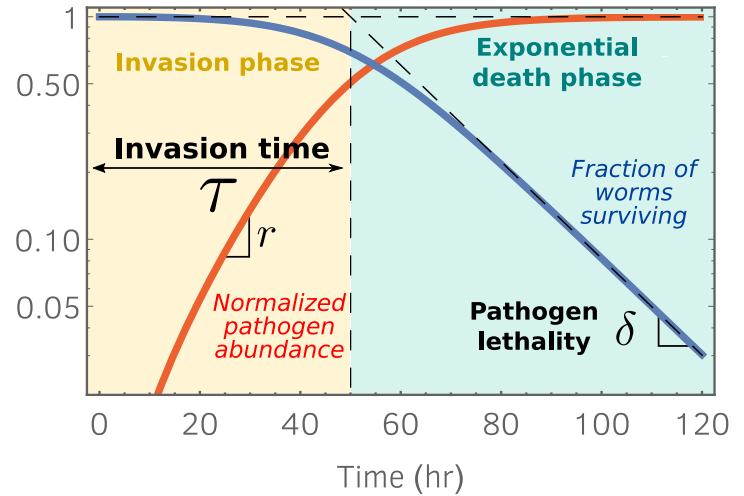
Fraction of worms surviving w

$$\underbrace{\frac{\dot{w}}{w}}_{\text{worm per capita death rate}} = - \underbrace{\delta \frac{N}{K}}_{\text{pathogen killing rate}}$$

Normalized pathogen abundance $\frac{N}{K}$

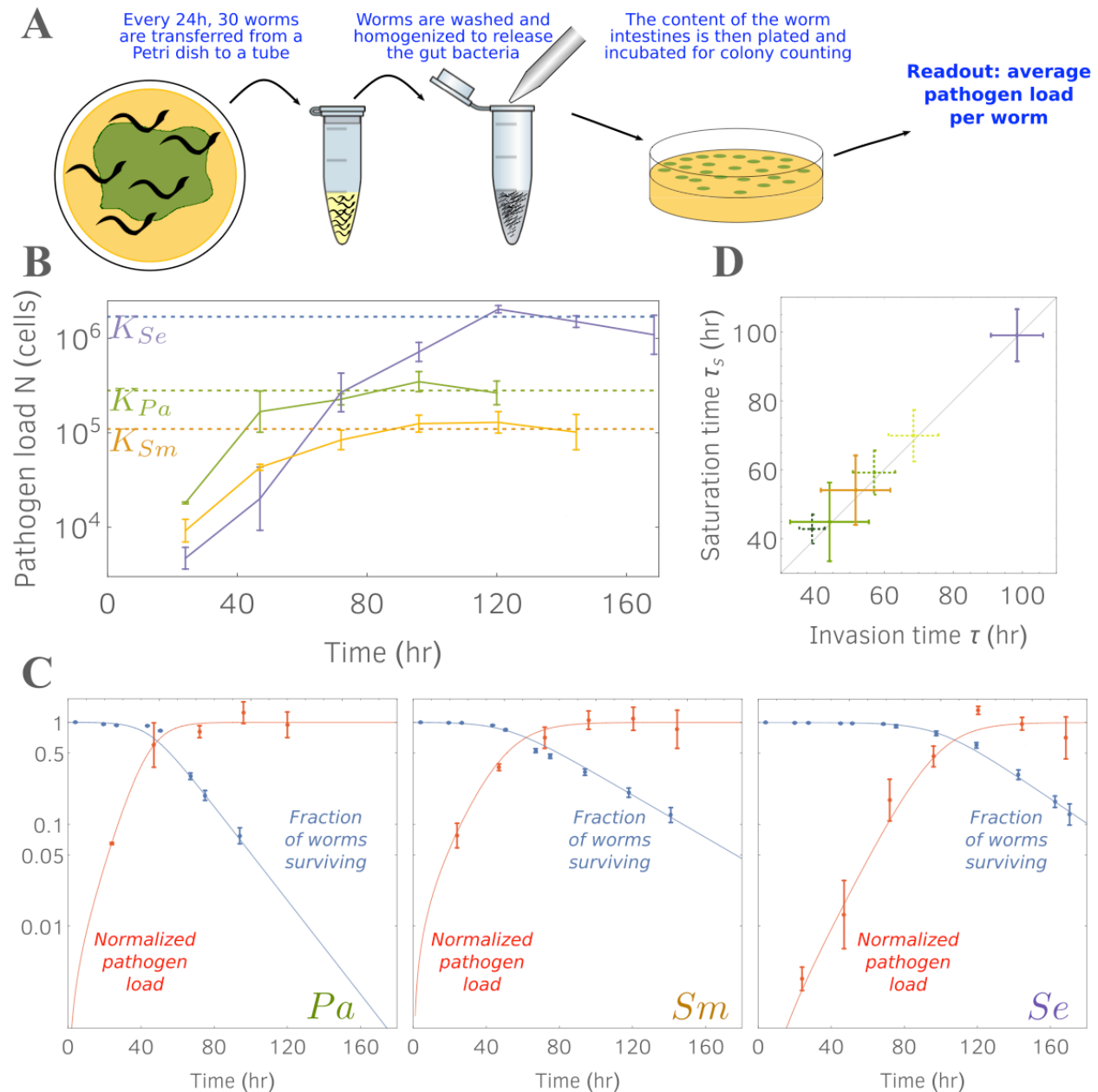
pathogen grows and colonizes saturates at carrying capacity

$$\dot{N} = (rN + c) \left(1 - \frac{N}{K}\right)$$



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429

43 **Figure 2: A theoretical model predicts that the survival curves enters the exponential phase as the pathogen abundance inside the hosts (*i.e.* pathogen load) reaches carrying capacity.** Our model formulates predictions for the survival curve w and the pathogen load N , starting from the pathogen growth rate inside the host r , the pathogen colonization rate c , the pathogen lethality δ , and the pathogen carrying capacity K . We use the initial conditions $N(0) = 0$ and $w(0) = 1$. The right panel shows the fraction of worms surviving (solid blue line) and the per capita pathogen load normalized by the carrying capacity, N/K (solid red line). The model disentangles the invasion phase, in which the worm mortality rate increases over time, from the exponential phase, where host mortality occurs with constant (maximum) rate δ . The time taken to enter the exponential phase τ is given by the intersection between the exponential phase asymptote (diagonal dashed line) and $w = 1$ (horizontal dashed line).



431

Figures 3: Experiments confirm that hosts enter the exponential death phase at the same time that the pathogens saturate within the host. (A): Illustration of the protocol used to measure the pathogen load within the hosts. **(B):** Per worm pathogen growth curves. Pathogen abundances data are obtained by averaging 3 or 4 replicates, each replicate consisting of a population of 10 worms. Error bars denote standard errors; dashed lines correspond to estimated pathogen carrying capacities. **(C):** Pathogen abundance data normalized by their carrying capacity (red markers) are plotted against the survival curve data (blue markers) of Fig. 1-B. Data are fitted by our model predictions (solid red and blue curves; parameter values in *Supplementary Table 2*; see *Material and Methods* section for fitting procedure). **(D):** As predicted by our model, the time taken by the pathogen population to saturate within the hosts is equal to the time necessary for the hosts to enter the exponential death phase (invasion time defined in Fig. 2). Solid lines: saturation and invasion times estimated from data in Fig. 1B and 3B. Dashed lines: times estimated from data in Fig. 1A and 4C.

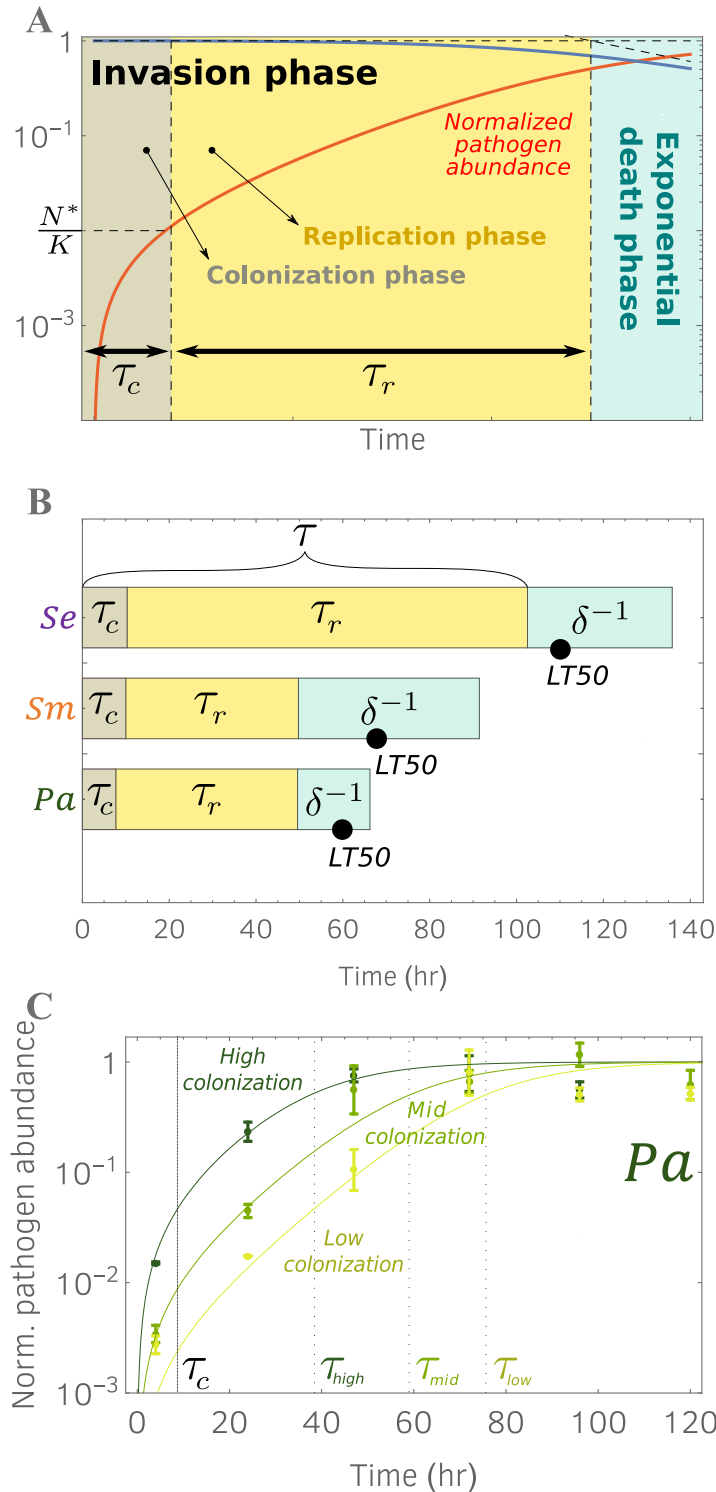


Figure 4: Invasion phase can be further disentangled into a colonization phase and a replication phase. (A): Invasion phase (see Fig. 2) is initially dominated by colonization for a time $\tau_c = r^{-1} \ln 2$. As the pathogen load reaches $N^* = c/r$, replication is the leading effect for the remaining time τ_r . The difference between colonization phase and replication phase results in a slope change in the normalized pathogen abundance (solid red line). **(B):** Timescales for the three pathogens (parameters values in *Supplementary Table 2*). **(C):** *Pa* growth curves corresponding to survival curves shown in Fig. 1-A. Solid lines are model predictions for normalized pathogen abundances, obtained for same parameter values but different colonization rates (*Supplementary Table 1*). Invasion times for different colonization rates are shown.

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