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Within-host infectious disease models accommodating cellular coinfection, with an application to influenza

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27 **Abstract**

28 Within-host models are useful tools for understanding the processes regulating viral load
29 dynamics. While existing models have considered a wide range of within-host processes, at their
30 core these models have shown remarkable structural similarity. Specifically, the structure of
31 these models generally consider target cells to be either uninfected or infected, with the
32 possibility of accommodating further resolution (for example, cells that are refractory to infection
33 and cells that are in an eclipse phase). Recent findings, however, indicate that cellular coinfection
34 is the norm rather than the exception for many viral infectious diseases, and that cells with high
35 multiplicity of infection are present over at least some duration of an infection. The reality of
36 these cellular coinfection dynamics is not accommodated in current within-host models although
37 it may be critical for understanding within-host dynamics. This is particularly the case if
38 multiplicity of infection impacts infected cell phenotypes such as their death rate and their viral
39 production rates. Here, we present a new class of within-host disease models that allow for
40 cellular coinfection in a scalable manner by retaining the low-dimensionality that is a desirable
41 feature of many current within-host models. The models we propose adopt the general structure
42 of epidemiological ‘macroparasite’ models that allow hosts to be variably infected by parasites
43 such as nematodes and host phenotypes to flexibly depend on parasite burden. Specifically, our
44 within-host models consider target cells as ‘hosts’ and viral particles as ‘macroparasites’, and
45 allow viral output and infected cell lifespans, among other phenotypes, to depend on a cell’s
46 multiplicity of infection. We show with an application to influenza that these models can be
47 statistically fit to viral load and other within-host data, that they can reproduce notable features
48 of within-host viral dynamics, and that important *in vivo* quantities such as the mean multiplicity
49 of cellular infection can be easily quantified with these models once parameterized. The within-
50 host model structure we develop here provides an alternative approach for modeling within-host
51 viral load dynamics and allows for a new class of questions to be addressed that consider the
52 effects of cellular coinfection, collective viral interactions, and viral complementation in within-
53 host viral dynamics and evolution.

54

55 Introduction

56 In part through the development and analysis of mathematical models, the processes driving the
57 within-host dynamics of viral infections have been increasingly well understood over the last two
58 decades. Statistical fitting of models to within-host data such as viral load measurements and
59 immune response data have yielded estimates of within-host basic reproduction numbers for
60 various viral pathogens, including HIV [1], influenza [2–4], measles [5], and dengue [6,7]. These
61 fits have further characterized the roles of the innate immune response [2,3,7], and, particularly
62 in secondary infections, the adaptive immune response [7,8] in regulating within-host viral
63 dynamics. The structure of these within-host models have generally mirrored the structure of
64 epidemiological ‘microparasite’ models, with cells being considered either uninfected or
65 infected. In some models [2,3], uninfected cells have been further categorized as either
66 susceptible or refractory to infection, again, mirroring hosts who are either susceptible or
67 immune to infection in epidemiological models.

68 While these within-host models capture many of the important features of within-host viral
69 processes, the majority of them implicitly assume that cellular coinfection does not occur [9] or
70 that cellular coinfection, if it occurs, does not affect the phenotypes of infected cells [10,11]. Yet
71 several experimental findings point towards cellular multiplicity of infection having the potential
72 to impact cellular phenotypes such as the rate at which infected cells produce viral output [12,13]
73 and the probability of initiating an interferon response [14]. The implicit assumption that a cell’s
74 multiplicity of infection does not impact its phenotypes is hard-wired into ‘microparasite’-
75 structured models because these models generally only consider a single class of infected cells,
76 regardless of cellular multiplicity of infection. With increasing genomic evidence that cellular
77 coinfection frequently occurs in chronic viral infections such as HIV [15] and HCV [16], as well as
78 in acute viral infections such as influenza [17–19], a few notable models have been developed
79 that have accommodated the possibility of cellular coinfection [10,11,20–22]. However, these
80 models either remain high dimensional [11] or have made the assumption that host cell resources
81 are limiting, such that viral output is independent of the extent of cellular coinfection [20]. While
82 this assumption may be warranted for some viruses, it is likely not met in the case of many other
83 viral pathogens.

84 Here, we develop a new class of low-dimensional within-host models whose structure flexibly
85 allows for cellular coinfection. We base this new class of models on the structure of
86 epidemiological ‘macroparasite’ models [23]. Development of these powerful epidemiological
87 models started in the 1970’s [24,25], and they are now being commonly used to study how
88 macroparasites such as nematodes spread through host populations [26]. They have further been
89 used to assess the effect of control strategies on disease burden and host mortality [27]. We
90 specifically develop this class of within-host ‘macroparasite’ models in the context of acute viral
91 infections, although their structure can also easily accommodate the within-host dynamics of
92 chronic infections. Finally, to demonstrate the usability of these models, we fit specific instances
93 of these models to within-host equine influenza data.

94 **Methods**

95 The structure of the within-host viral dynamic models we propose is based on a close analogy to
96 population-level macroparasite models that are well-established and frequently used in the field
97 of disease ecology and epidemiology (Figure 1). In Supplemental Section ‘Epidemiological
98 macroparasite models’, we briefly review the derivation of the canonical structure of these
99 population-level macroparasite models. Co-opting this canonical formulation for within-host
100 viral dynamics allows us to flexibly model cells that have become infected with 0, 1, ... n viral
101 particles, while maintaining a low-dimensional set of equations.

102 *Target-cell limited ‘macroparasite’ models.* The simplest version of the within-host
103 ‘macroparasite’ model is a target-cell limited model. In its most general form, this model is given
104 by:

$$105 \quad \frac{dH}{dt} = a - bH - H \sum_{i=0}^{\infty} \alpha_i p_i, \quad (1)$$

$$106 \quad \frac{dV}{dt} = H \sum_{i=0}^{\infty} \lambda_i p_i - \eta V - \beta HV \quad (2)$$

$$107 \quad \frac{dP}{dt} = \beta HV - bH \sum_{i=0}^{\infty} i p_i - H \sum_{i=0}^{\infty} i \alpha_i p_i. \quad (3)$$

108 The variable H quantifies the total number of target cells, which includes both uninfected and
109 variably infected cells. In this model, both uninfected and infected cells can be targets of further

110 infection, so this variable differs from the variable representing uninfected target cells in
111 traditional within-host ‘microparasite’ models. In equation (1), a is the constant rate of target cell
112 production and b is the per capita background mortality of target cells. In the absence of
113 infection, the target cell population equilibrates to $H=a/b$. In an acute viral infection such as
114 influenza, the rate of target cell production a and the background rate of cell mortality b are
115 frequently assumed to be sufficiently small to be ignored. The third term, $-H\sum_{i=0}^{\infty}\alpha_i p_i$ is the
116 decrease in the number of target cells due to virus-induced mortality, with p_i being the proportion
117 of target cells that are infected with a cellular multiplicity of infection (MOI) of i .

118 The variable V quantifies the amount of free (extracellular) virus, and is analogous to the free
119 virus variable generally modeled in traditional within-host ‘microparasite’ models. The first term,
120 $H\sum_{i=0}^{\infty}\lambda_i p_i$, quantifies the overall rate at which free virus is produced from target cells, where
121 λ_i is the rate at which cells infected with an MOI of i produce free virus. The second term
122 quantifies the rate of viral clearance, and the third term captures loss of free virus from its entry
123 into target cells. This third term is often lost in traditional within-host ‘microparasite’ models,
124 with an argument that loss of free virus from cell entry is negligible relative to loss of free virus
125 through viral clearance [9].

126 The variable P quantifies the total amount of internalized virus across all target cells H and does
127 not have an analogue in traditional within-host ‘microparasite’ models. This variable is related
128 to, but distinct from, cellular multiplicity of infection (MOI), or equivalently, ‘cellular input’. While
129 cellular MOI quantifies the number of virions a single cell has internalized, the variable P
130 quantifies the number of internalized virions across all existing target cells. Mathematically, P can
131 also be written as the product of the mean cellular MOI and the number of target cells H .

132 The first term in equation (3) captures the increase in the number of internalized virions from the
133 entry of free virus V into target cells H . The second term, $bH\sum_{i=0}^{\infty}i p_i$, captures loss of internalized
134 virus through background mortality of target cells, with the loss of a cell with an MOI of i leading
135 to the loss of i internalized virions. The third term, $H\sum_{i=0}^{\infty}i\alpha_i p_i$, captures the loss of internalized

136 virus through virus-induced mortality, with, again, the loss of a cell with an MOI of i leading to
137 the loss of i internalized virions.

138 At this point, we can further simplify the model by adopting assumptions that may or may not be
139 empirically supported. For instance, we can make analogous assumptions to the ones made in
140 epidemiological ‘macroparasite’ models, e.g., that the viral production rate is linearly related to
141 cellular MOI ($\lambda_i = \lambda i$), and that the cell mortality rate scales linearly with cellular MOI ($\alpha_i = \alpha i$).
142 Applying these assumptions, equations (1-3) become the following for an acute infectious
143 disease:

$$144 \quad \frac{dH}{dt} = -\alpha P, \quad (4)$$

$$145 \quad \frac{dV}{dt} = \lambda P - \eta V - \beta HV \quad (5)$$

$$146 \quad \frac{dP}{dt} = \beta HV - \alpha H \sum_{i=0}^{\infty} i^2 p_i \quad (6)$$

147 To further simplify equation (6), we can adopt an analogous assumption to one that is present in
148 epidemiological ‘macroparasite’ models (see Supplemental Section ‘Epidemiological
149 macroparasite models’): that the distribution of cellular MOIs is given by a negative binomial
150 distribution with mean P/H and dispersion parameter k in the range of $(0, \infty)$. This assumption
151 simplifies equation (6) to:

$$152 \quad \frac{dP}{dt} = \beta HV - \alpha P - \alpha \frac{(1+k)P^2}{kH}. \quad (7)$$

153 The negative binomial distribution allows for the possibility of cellular MOI overdispersion (low
154 k), while still allowing for a Poisson distribution of cellular MOIs when $k = \infty$. Overdispersion of
155 cellular MOIs *in vivo* is highly likely for several reasons. First, some target cells might be more
156 susceptible to infection than others due to variation in the number and types of receptors.
157 Second, given spatial aspects of within-host viral spread, there is likely considerable variation in
158 the rate at which cells are exposed to virus. Third, variation in the time cells remain in their eclipse
159 phase can under certain conditions produce overdispersion of cellular MOIs (see Supplemental
160 Section ‘Negative binomial distribution for multiply infected cells’).

161 We can define the basic reproduction number R_0 for the target-cell limited model given by
 162 equations (4), (5), and (7) as the number of new viral particles generated by a single viral particle
 163 at the onset of an individual's infection when the overwhelming majority of target cells are
 164 uninfected. To derive R_0 for this model, we can first make a fast viral dynamics assumption, such
 165 that $\frac{dV}{dt} \approx 0$ and $V \approx \frac{\lambda P}{\eta + \beta H}$. Plugging this expression into equation (7), and recognizing that the
 166 ratio $\frac{P^2}{H} \approx 0$ at the onset of infection, yields: $\frac{dP}{dt} = \frac{\lambda H}{\eta/\beta + H}P - \alpha P$. From this expression, it is clear

167 that $R_0 = \frac{\lambda \left[\frac{H_0}{\eta/\beta + H_0} \right]}{\alpha}$, where H_0 is the number of target cells present at the beginning of the infection.

168 While we assume fast viral dynamics in the derivation of R_0 , we model within-host viral dynamics
 169 under the target-cell limited version of this model using all three variables (H , V , and P) because
 170 it is uncommon to assume fast viral dynamics in within-host models and retaining V in the model
 171 allows for a more straightforward interface with viral load data.

172 Since little is known about how viral production rates and cellular mortality rates scale with
 173 cellular input, alternative assumptions can also be made that would still allow for a simplification
 174 of equations (1)-(3). For example, it could be assumed that viral production rates are independent
 175 of cellular input, as long as a cell is infected. This assumption would implicitly assume that host
 176 cell machinery is the limiting factor governing viral production from a cell. This assumption would
 177 lead to equation (2) becoming:

$$178 \quad \frac{dV}{dt} = \lambda H \left(1 - \left(\frac{k}{k + P/H} \right)^k \right) - \eta V - \beta HV \quad (8)$$

179 where the term $\left(1 - \left(\frac{k}{k + P/H} \right)^k \right)$ provides the probability that a target cell has internalized at least
 180 one viral particle. It could also be assumed that the cellular mortality rate is independent of
 181 cellular input (as long as there is some input), and equations (1) and (3) would be modified
 182 accordingly:

$$183 \quad \frac{dH}{dt} = -\alpha H \left(1 - \left(\frac{k}{k + P/H} \right)^k \right), \quad (9)$$

184
$$\frac{dP}{dt} = \beta HV - \alpha P. \quad (10)$$

185 Under the assumption that virions are internalized independently of a cell's MOI, the dispersion
186 parameter $k = \infty$ and equations (8)-(9) become:

187
$$\frac{dV}{dt} = \lambda H(1 - e^{-P/H}) - \eta V - \beta HV \quad (11)$$

188
$$\frac{dH}{dt} = -\alpha H(1 - e^{-P/H}). \quad (12)$$

189 Defining the number of currently infected target cells as $I = H(1 - e^{-P/H})$ allows one to expand
190 equation (12) into uninfected (T) and infected (I) target cell classes: $\frac{dT}{dt} = -\beta TV$ and $\frac{dI}{dt} = \beta TV - \alpha I$
191 , respectively. Neglecting the third term, this definition also allows us to simplify equation (11) to
192 $\frac{dV}{dt} = \lambda I - \eta V$. The variable P can be excluded if it assumed to be in equilibrium with V . As such,
193 it is clear that with the assumption of Poisson-distributed cellular MOIs, an MOI-independent
194 viral production rate, and an MOI-independent mortality rate of infected cells, the within-host
195 'macroparasite' model folds into the traditional within-host 'microparasite' model. This finding is
196 consistent with findings from a previous, high dimensional model for HIV that accommodated
197 multiply-infected cells. An analysis of that model showed that it simplified to the structure of a
198 within-host 'microparasite' model that had all infected cells belonging to a single infected class I
199 when viral production rates (and infected cell mortality rates) were independent of the number
200 of internalized virions [20].

201 The 'true' relationship between cellular input and the rate of viral production likely depends on
202 virus and host cell type, and needs to be empirically addressed when applying the within-host
203 'macroparasite' model to a specific viral infection. Similarly, little is known about how cellular
204 mortality rate scales with cellular input and experimental studies need to be performed to
205 address this knowledge gap.

206 *Within-host 'macroparasite' models incorporating the host's immune response.*

207 Within-host models of viral infections frequently incorporate the host's immune response, since
208 it is this response that is thought to play a critical role in regulating and ultimately clearing viral

209 infections [7,25]. Minimally, the host's immune response can be incorporated by considering only
210 the innate immune response, which can, again at minimum, be captured by a single additional
211 variable. This variable can encompass the activity of interferons and cytokines, as well as cells of
212 the innate immune response such as natural killer (NK) cells. In many models, the dynamics of
213 specifically interferon- α have been included, with interferon production occurring from infected
214 cells and decaying at a constant rate [2,3,7]. If we assume that cells produce interferon at a rate
215 (or probability) that scales linearly with cellular MOI, then the dynamics of interferon- α are given
216 by:

$$217 \quad \frac{dF}{dt} = qP - dF \quad (13)$$

218 Interferon- α can modify viral within-host dynamics in a number of ways. One way is for
219 interferon- α to reduce the rate of viral production from infected cells. Another way is for
220 interferon- α to decrease the susceptibility of cells to infection (or further infection). Both of these
221 mechanisms of action can be assumed to respond to immediate levels of interferon. In this case,
222 the viral production rate can be reduced from $H\sum_{i=0}^{\infty}\lambda_i p_i$ (equation 2) to $\frac{H}{1+\varepsilon F}\sum_{i=0}^{\infty}\lambda_i p_i$, or similar
223 (as in [28]), and the rate of viral entry into target cells can be reduced from βHV (equations 2
224 and 3) to $\frac{\beta}{1+\varepsilon F}HV$ or similar (as in [3]). Alternatively, cellular exposure to interferon could have
225 prolonged effects, with cells becoming refractory to infection (or further infection) for a period
226 of time (as in [2,3]) and infected cells reducing their viral output for a period of time following
227 exposure. A third, indirect effect of interferon- α is to facilitate the recruitment of innate effector
228 cells, which would act to clear infected target cells, leading to an overall effective increase in the
229 rate at which target cells decline. Here, for simplicity, we consider the two direct effects of
230 interferon: the effect of these molecules on reducing cell susceptibility to infection and on
231 reducing the rate of viral production from infected cells, assuming that interferon has prolonged
232 effects on cells. Our innate immune response model is given by equation (13) and the following
233 system of equations:

$$234 \quad \frac{dH}{dt} = -\alpha P - \phi FH \quad (14)$$

$$235 \quad \frac{dR}{dt} = \phi FH \quad (15)$$

$$236 \quad \frac{dV}{dt} = \lambda P - \eta V - \beta HV \quad (16)$$

$$237 \quad \frac{dP}{dt} = \beta HV - \alpha P - \alpha \frac{(1+k)P^2}{kH} - \phi FP \quad (17)$$

238 Here, H is the total number of *susceptible* target cells (including infected and uninfected target
239 cells), R is the total number of target cells that are *refractory* to further infection (including
240 uninfected cells and already infected cells), V is again the amount of free virus, and P is the total
241 number of viral particles across *susceptible* target cells. The parameter ϕ quantifies the per capita
242 rate at which interferon makes cells refractory to infection. This model formulation assumes that
243 all susceptible cells, whether uninfected or infected, become refractory to infection (or further
244 infection) at similar rates, that refractory cells stay permanently refractory, and that no virus is
245 produced from refractory cells. This latter assumption effectively reduces the overall rate at
246 which the total infected cell pool produces virus as a result of interferon exposure. Model
247 equations (13-17) assume analogous effects of interferon as the within-host ‘microparasite’
248 model presented in [3], while adopting assumptions of linear scaling between cellular MOI and
249 infected cell mortality rate, between cellular MOI and viral production rate, and between cellular
250 MOI and the probability of cellular interferon production.

251 **Results**

252 Here, we fit the within-host macroparasite models presented above to empirical within-host
253 data, highlighting biological insight that can be gained from these models that within-host
254 ‘microparasite’ models have difficulty providing. We first fit the target cell limited model given
255 by equations (4), (5), and (7) to influenza A/H3N8 viral load measurements from experimentally
256 infected ponies. Prior to fitting this model, we confirmed that all of its parameters are structurally
257 identifiable (Supplemental Section ‘Identifiability analysis’). However, due to the limited number
258 of data points for each pony, we did not attempt to estimate all model parameters for each pony
259 independently. Instead, based on previous analyses of these data [2,3], we set the initial number
260 of target cells (in our case, given by the variable H) to 3.5×10^{11} cells, and further set the initial
261 number of internalized virions P to 0. This latter assumption corresponds to the assumption made

262 in previous models of 0 initially infected cells. We further constrained the per-particle production
263 rate λ , the per-particle cellular mortality rate α , and the dispersion parameter k to be the same
264 across infected ponies. We let the viral clearance rate η , the viral infectivity rate β , and the initial
265 amount of free virus $V(0)$, differ across ponies, since in part these parameters reflect host-specific
266 characteristics or phenotypes related to a host's immune history. Under these constraints, all
267 parameters of this basic model were practically identifiable.

268 To estimate model parameters, we used a maximum likelihood approach that further
269 accommodates below the limit of detection measurements (Supplemental Section 'Statistical
270 estimation of model parameters'). Figure 2a shows the viral load measurements from the ponies,
271 along with the fit of the target cell limited macroparasite model (equations 4, 5, and 7). Table 1
272 lists the estimated model parameters. The model reproduces key features of the observed
273 within-host influenza dynamics. Most notably, the within-host macroparasite model can hit peak
274 viral loads and reproduce the biphasic viral declines that are observed in several of the ponies.
275 This stands in contrast to the within-host, target-cell limited 'microparasite' model (for example,
276 equations 1-3 in [4]), which cannot reproduce the biphasic viral decline and has trouble hitting
277 peak viral loads.

278 In addition to being able to better reproduce these two key features of the viral dynamics than
279 the within-host microparasite model, the within-host macroparasite model allows one to
280 calculate the time-varying mean multiplicity of infection (Figure 2b). The mean multiplicity of
281 infection in this model is simply given by the total number of internalized particles divided by the
282 total number of target cells: P/H . Plotting the mean multiplicity of infection over time allows us
283 to better understand the processes that are driving the biphasic viral declines projected by the
284 macroparasite model. As viral load increases, the mean multiplicity of infection increases
285 dramatically. Infected cells with high multiplicity of infection then experience high mortality
286 rates, leading to a very rapid decline in viral load and a rapid depletion of target cells H (Figure
287 2c). Mean cellular MOI drops as a result of this rapid depletion of cells with high MOI (Figure 2b).
288 It then remains low because of low levels of free virus V and thus little opportunity to internalize
289 more virus. The second phase of the viral decline comes about from the low mortality rate of
290 cells with low MOI.

291 One can also easily calculate the proportion of infected cells that are infected by more than one
292 viral particle (Figure 2d). This information may be useful for anticipating the extent to which viral
293 reassortment occurs *in vivo*, or for characterizing the landscape available to defective interfering
294 particles, which ‘cheat’ off of wild-type virus for their own replication.

295 More recent quantitative analyses and data have indicated that influenza viral dynamics are likely
296 regulated by the host immune response, particularly the innate immune response [2,3]. We
297 therefore also fit the innate immune response model, provided by equations (13)-(17), to the
298 available equine data, which now include viral load measurements, fold change measurements
299 in interferon- α , and an estimate that only ~27% of host cells were depleted by the end of an
300 infection (Supplemental Section ‘Statistical estimation of model parameters’; see [2,3] for
301 descriptions of the data). Again, we constrained a subset of the model parameters to be the same
302 across the ponies, while letting other parameters be pony-specific. Specifically, we constrained
303 parameters α , ϕ , λ , d , and k to be the same across the ponies, because these parameters quantify
304 infected cell phenotypes that we expect to be common across individuals. We let parameters η ,
305 β , q , and $V(0)$ differ across ponies, due to host-specific factors. Figures 3a and 3b shows that this
306 within-host macroparasite model can quantitatively reproduce features of the viral load and
307 interferon- α measurements, without depletion of target cells to unreasonable levels (Figure 3c).
308 Table 2 provides parameter estimates for this model. Here, the very rapid initial viral decline
309 results from several processes: the cellular input-dependent mortality rate of infected cells, the
310 rapid removal of susceptible target cells H through exposure to interferon- α , and the lack of viral
311 production from refractory cells. The second, slower phase of viral decline results from the slow
312 clearance of the remaining infected cells that have low multiplicity of infection.

313 Discussion

314 Here, we have developed a new class of within-host models for understanding the *in vivo*
315 dynamics of viral infections. This new class of models differs from existing within-host models in
316 that it allows for target cells to be variably infected and for the degree of cellular input to impact
317 the phenotypes of infected cells, such as their death rate and the rate at which they produce
318 virus. Despite allowing for cellular coinfection dynamics, these models remain low-dimensional.

319 This addresses existing concerns in the literature about the scalability of within-host models that
320 allow for cellular coinfection [11]. While we have developed and applied these within-host
321 models to acute viral infections, the structure of these models is immediately applicable to
322 chronic viral infections. When applied to chronic infections, the terms we lost for the
323 replenishment and natural death of target cells (see the Methods section) would simply need to
324 be reintroduced.

325 The structure of the within-host models we derived here co-opt the structure of epidemiological
326 ‘macroparasite’ models. Based on empirical data, those models generally assume that host death
327 rates scale linearly with macroparasite burden and that the rate of egg release from infected
328 hosts similarly scales linearly with macroparasite burden. The within-host models we fit similarly
329 adopt these scaling relationship assumptions, although other scaling assumptions can be
330 adopted while retaining the desirable low-dimensionality of the model equations. Clearly, the
331 structure of the within-host model should reflect empirically supported relationships between
332 cellular input and cellular phenotypes. To date, very few studies have attempted to empirically
333 quantify these relationships, making the appropriate choice of model structure difficult to decide
334 upon. For influenza, the studies that do exist have shown that the timing and amounts of viral
335 yield depend critically on cellular input, and that cumulative viral yield generally increases with
336 cellular input [12,13]. Intriguingly, these results stand in contrast to the current structure of the
337 model that we have presented. This is because, if we assume that both the viral production rate
338 and the cell death rate scale linearly with cellular input, the total cellular output of an infected
339 cell should be independent of its multiplicity of infection i , with total cellular output being given
340 by: $\lambda i / \alpha i = \lambda / \alpha$. The empirically determined relationship between higher cellular output with
341 higher cellular input therefore seems to indicate that cellular death rates must scale less than
342 linearly with cellular input and/or that viral production rates must scale faster than linearly with
343 cellular input. The latter relationship, if empirically supported, would provide tantalizing evidence
344 for viral cooperation within cells playing a role in within-host viral dynamics, an idea that has
345 recently gained traction [29–31].

346 A key feature of epidemiological macroparasite models is the possibility of nematode
347 overdispersion across hosts. Parasite overdispersion has considerable empirical support, with the

348 overwhelming majority of macroparasite distributions studied having a variance to mean ratio
349 exceeding 1 and an estimated dispersion parameter k of less than 1 [32]. A more recent analysis
350 further indicates that observed levels of parasite overdispersion can be attributed almost entirely
351 to host heterogeneity in parasite exposure or host heterogeneity in susceptibility to infection
352 [33]. Here, in the context of within-host viral dynamics, we also found statistical support for very
353 high levels of overdispersion, with a dispersion factor k estimate of 0.77 in the target-cell limited
354 model (Table 1) and an estimate of $k = 0.30$ in the innate immune response model (Table 2). This
355 overdispersion could similarly reflect variation in target cell susceptibility to infection. It could
356 also reflect heterogeneity in viral exposure, likely due to the intrinsically spatial aspect of
357 influenza virus spread within infected hosts. Finally, as described in Supplemental Section
358 ‘Negative binomial distribution for multiply infected cells’, overdispersion could also result from
359 the distribution of time that cells remain in the eclipse phase prior to becoming productively
360 infected. Regardless of the causes of viral overdispersion across target cells, the consequences
361 of viral overdispersion are that the majority of infected cells are multiply infected, at least over
362 some duration of the infection (Figure 2d). This would give rise to the expectation of considerable
363 levels of viral reassortment within infected hosts, consistent with findings from a guinea pig study
364 that found robust reassortment *in vivo* between phenotypically neutral strains that differed from
365 one another only by silent mutations [18]. In contrast, however, analysis of viral sequence data
366 from a human challenge study indicated very limited effective reassortment, perhaps because of
367 multiple initiating foci of infection [34].

368 In addition to its effects on viral population dynamics, viral overdispersion across target cells
369 would have important evolutionary consequences. First, reassortment between genetically and
370 phenotypically distinct strains could bring together beneficial mutations on different gene
371 segments or allow for a more effective purging of deleterious mutations. Second, viral
372 overdispersion effectively produces ‘collective infectious units’ [29]. The existence of these
373 collective infectious units will put selection pressures on a virus to evolve cooperative traits, or,
374 conversely, non-cooperative traits that would allow a virus to ‘cheat’. In either case, the
375 importance of quantifying cellular multiplicity of infection is clear, as multiplicity of infection will
376 determine the distribution of viral group sizes, which would in turn affect the types of ‘social

377 interactions' experienced by viral populations [30]. The within-host macroparasite models
378 presented here provide an approach for estimating the degree of viral overdispersion from fits
379 to viral data. More generally, these models allow for the reality of cellular coinfection dynamics
380 to be integrated into within-host disease models, in a scalable, low-dimensional fashion. While
381 their general formulation has been developed here, these models require assumptions to be
382 made between cellular input and various cellular phenotypes. Empirical studies examining the
383 structure of these relationships is the next critical step to the continued development of these
384 models, and towards their use in better understanding the within-host and evolutionary
385 dynamics of viral infections.

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387 **References**

- 388 1. Ribeiro RM, Qin L, Chavez LL, Li D, Self SG, Perelson AS. Estimation of the Initial Viral
389 Growth Rate and Basic Reproductive Number during Acute HIV-1 Infection. *J Virol.*
390 2010;84: 6096–6102. doi:10.1128/JVI.00127-10
- 391 2. Pawelek KA, Huynh GT, Quinlivan M, Cullinane A, Rong L, Perelson AS. Modeling Within-
392 Host Dynamics of Influenza Virus Infection Including Immune Responses. *PLoS Comput*
393 *Biol.* 2012;8: e1002588. doi:10.1371/journal.pcbi.1002588
- 394 3. Saenz RA, Quinlivan M, Elton D, MacRae S, Blunden AS, Mumford JA, et al. Dynamics of
395 influenza virus infection and pathology. *J Virol.* 2010;84: 3974–3983.
396 doi:10.1128/JVI.02078-09
- 397 4. Baccam P, Beauchemin C, Macken CA, Hayden FG, Perelson AS. Kinetics of Influenza A
398 Virus Infection in Humans. *J Virol.* 2006;80: 7590–7599. doi:10.1128/JVI.01623-05
- 399 5. Heffernan JM, Keeling MJ. An in-host model of acute infection: Measles as a case study.
400 *Theor Popul Biol.* 2008;73: 134–147. doi:10.1016/j.tpb.2007.10.003
- 401 6. Clapham HE, Tricou V, Van Vinh Chau N, Simmons CP, Ferguson NM. Within-host viral
402 dynamics of dengue serotype 1 infection. *J R Soc Interface.* 2014;11: 20140094–20140094.
403 doi:10.1098/rsif.2014.0094
- 404 7. Ben-Shachar R, Koelle K. Minimal within-host dengue models highlight the specific roles of
405 the immune response in primary and secondary dengue infections. *J R Soc Interface.*
406 2014;12: 20140886–20140886. doi:10.1098/rsif.2014.0886

- 407 8. Handel A, Longini IM, Antia R. Towards a quantitative understanding of the within-host
408 dynamics of influenza A infections. *J R Soc Interface*. 2010;7: 35–47.
409 doi:10.1098/rsif.2009.0067
- 410 9. Smith AM, Perelson AS. Influenza A virus infection kinetics: quantitative data and models.
411 *Wiley Interdiscip Rev Syst Biol Med*. 2011;3: 429–445. doi:10.1002/wsbm.129
- 412 10. Dixit NM, Perelson AS. Multiplicity of Human Immunodeficiency Virus Infections in
413 Lymphoid Tissue. *J Virol*. 2004;78: 8942–8945. doi:10.1128/JVI.78.16.8942-8945.2004
- 414 11. Phan D, Wodarz D. Modeling multiple infection of cells by viruses: Challenges and insights.
415 *Math Biosci*. 2015;264: 21–28. doi:10.1016/j.mbs.2015.03.001
- 416 12. White DO, Cheyne IM. Early events in the eclipse phase of influenza and parainfluenza
417 virus infection. *Virology*. 1966;29: 49–59.
- 418 13. White DO, Day HM, Batchelder EJ, Cheyne IM, Wansbrough AJ. Delay in the multiplication
419 of influenza virus. *Virology*. 1965;25: 289–302.
- 420 14. Gifford GE. Variation of interferon yield with multiplicity of infection. *Nature*. 1963;200:
421 91–92.
- 422 15. Onafuwa-Nuga A, Telesnitsky A. The Remarkable Frequency of Human Immunodeficiency
423 Virus Type 1 Genetic Recombination. *Microbiol Mol Biol Rev*. 2009;73: 451–480.
424 doi:10.1128/MMBR.00012-09
- 425 16. Ke R, Li H, Wang S, Ding W, Ribeiro RM, Giorgi EE, et al. Superinfection and Cure of
426 Infected Cells as Mechanisms for Hepatitis C Virus Adaptation and Persistence. *PNAS*.
- 427 17. Brooke CB, Ince WL, Wei J, Bennink JR, Yewdell JW. Influenza A virus nucleoprotein
428 selectively decreases neuraminidase gene-segment packaging while enhancing viral fitness
429 and transmissibility. *Proc Natl Acad Sci*. 2014;111: 16854–16859.
430 doi:10.1073/pnas.1415396111
- 431 18. Marshall N, Priyamvada L, Ende Z, Steel J, Lowen AC. Influenza Virus Reassortment Occurs
432 with High Frequency in the Absence of Segment Mismatch. Basler CF, editor. *PLoS Pathog*.
433 2013;9: e1003421. doi:10.1371/journal.ppat.1003421
- 434 19. Fukuyama S, Katsura H, Zhao D, Ozawa M, Ando T, Shoemaker JE, et al. Multi-spectral
435 fluorescent reporter influenza viruses (Color-flu) as powerful tools for in vivo studies. *Nat*
436 *Commun*. 2015;6. doi:10.1038/ncomms7600
- 437 20. Dixit NM, Perelson AS. HIV dynamics with multiple infections of target cells. *Proc Natl Acad*
438 *Sci*. 2005;102: 8198–8203. doi:10.1073/pnas.0407498102

- 439 21. Wodarz D, Levy DN. Multiple HIV-1 infection of cells and the evolutionary dynamics of
440 cytotoxic T lymphocyte escape mutants. *Evolution*. 2009;63: 2326–2339.
441 doi:10.1111/j.1558-5646.2009.00727.x
- 442 22. Wodarz D, Levy DN. Effect of different modes of viral spread on the dynamics of multiply
443 infected cells in human immunodeficiency virus infection. *J R Soc Interface*. 2011;8: 289–
444 300. doi:10.1098/rsif.2010.0266
- 445 23. Roberts MG, Smith G, Grenfell BT. Mathematical models for macroparasites of wildlife.
446 *Ecology of Infectious Diseases in Natural Populations*. pp. 177–208.
- 447 24. Anderson RM, May RM. *Infectious Diseases of Humans: Dynamics and Control*. Oxford
448 University Press; 1992.
- 449 25. Anderson RM, May RM. Regulation and Stability of Host-Parasite Population Interactions:
450 I. Regulatory Processes. *J Anim Ecol*. 1978;47: 219. doi:10.2307/3933
- 451 26. Hollingsworth TD, Pulliam JRC, Funk S, Truscott JE, Isham V, Lloyd AL. Seven challenges for
452 modelling indirect transmission: vector-borne diseases, macroparasites and neglected
453 tropical diseases. *Epidemics*. 2015;10: 16–20. doi:10.1016/j.epidem.2014.08.007
- 454 27. Truscott J, Hollingsworth TD, Anderson R. Modeling the Interruption of the Transmission
455 of Soil-Transmitted Helminths by Repeated Mass Chemotherapy of School-Age Children.
456 McCarthy JS, editor. *PLoS Negl Trop Dis*. 2014;8: e3323. doi:10.1371/journal.pntd.0003323
- 457 28. Canini L, Carrat F. Population modeling of influenza A/H1N1 virus kinetics and symptom
458 dynamics. *J Virol*. 2011;85: 2764–2770. doi:10.1128/JVI.01318-10
- 459 29. Sanjuán R. Collective Infectious Units in Viruses. *Trends Microbiol*. 2017;
460 doi:10.1016/j.tim.2017.02.003
- 461 30. Díaz-Muñoz SL, Sanjuán R, West S. Sociovirology: Conflict, Cooperation, and
462 Communication among Viruses. *Cell Host Microbe*. 2017;22: 437–441.
463 doi:10.1016/j.chom.2017.09.012
- 464 31. Brooke CB. Population diversity and collective interactions during influenza virus
465 replication and evolution. *J Virol*. 2017; JVI.01164-17. doi:10.1128/JVI.01164-17
- 466 32. Shaw DJ, Dobson AP. Patterns of macroparasite abundance and aggregation in wildlife
467 populations: a quantitative review. *Parasitology*. 1995;111 Suppl: S111-127.
- 468 33. Poulin R. Explaining variability in parasite aggregation levels among host samples.
469 *Parasitology*. 2013;140: 541–546. doi:10.1017/S0031182012002053

470 34. Sobel Leonard A, McClain MT, Smith GJD, Wentworth DE, Halpin RA, Lin X, et al. The
 471 effective rate of influenza reassortment is limited during human infection. Lauring AS,
 472 editor. PLOS Pathog. 2017;13: e1006203. doi:10.1371/journal.ppat.1006203

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474 **Tables**

475

476 **Table 1.** Parameter estimates for the target cell limited ‘macroparasite’ model, for each of the
 477 six ponies. The model is given by equations (4), (5), and (7). The within-host basic reproduction
 478 numbers for each pony are also listed, which are calculated from the listed parameter estimates
 479 according to the formula provided in the text.

	β	$\beta_{\text{eff}}(\text{day}^{-1})$	μ	λ	α	δ	σ	β	R_0
Unit	day ⁻¹ (copies ⁻¹)	individuals ⁻¹ day ⁻¹	day ⁻¹	day ⁻¹	day ⁻¹	day ⁻¹	day ⁻¹	($\beta \mu^{-1} \alpha^{-1} \delta^{-1} \sigma^{-1}$)	unitless
Mean	SD	CI	SD	CI	SD	CI	SD	CI	SD
1	1.0E+02	1.0E+02	1.0E+00	1.0E+00	1.0E+00	1.0E+00	1.0E+00	1.0E+00	1.0E+00
2	1.0E+02	1.0E+02	1.0E+00	1.0E+00	1.0E+00	1.0E+00	1.0E+00	1.0E+00	1.0E+00
3	1.0E+02	1.0E+02	1.0E+00	1.0E+00	1.0E+00	1.0E+00	1.0E+00	1.0E+00	1.0E+00
4	1.0E+02	1.0E+02	1.0E+00	1.0E+00	1.0E+00	1.0E+00	1.0E+00	1.0E+00	1.0E+00
5	1.0E+02	1.0E+02	1.0E+00	1.0E+00	1.0E+00	1.0E+00	1.0E+00	1.0E+00	1.0E+00
6	1.0E+02	1.0E+02	1.0E+00	1.0E+00	1.0E+00	1.0E+00	1.0E+00	1.0E+00	1.0E+00

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483 **Table 2.** Parameter estimates for the ‘macroparasite’ model incorporating the innate immune
 484 response, for each of the six ponies. The model is given by equations (13)-(17).

	β	$\beta_{\text{eff}}(\text{day}^{-1})$	μ	λ	α	δ	σ	β	R_0
Unit	day ⁻¹ (copies ⁻¹)	individuals ⁻¹ day ⁻¹	day ⁻¹	day ⁻¹	day ⁻¹	day ⁻¹	day ⁻¹	($\beta \mu^{-1} \alpha^{-1} \delta^{-1} \sigma^{-1}$)	unitless
Mean	SD	CI	SD	CI	SD	CI	SD	CI	SD
1	1.0E+02	1.0E+02	1.0E+00	1.0E+00	1.0E+00	1.0E+00	1.0E+00	1.0E+00	1.0E+00
2	1.0E+02	1.0E+02	1.0E+00	1.0E+00	1.0E+00	1.0E+00	1.0E+00	1.0E+00	1.0E+00
3	1.0E+02	1.0E+02	1.0E+00	1.0E+00	1.0E+00	1.0E+00	1.0E+00	1.0E+00	1.0E+00
4	1.0E+02	1.0E+02	1.0E+00	1.0E+00	1.0E+00	1.0E+00	1.0E+00	1.0E+00	1.0E+00
5	1.0E+02	1.0E+02	1.0E+00	1.0E+00	1.0E+00	1.0E+00	1.0E+00	1.0E+00	1.0E+00
6	1.0E+02	1.0E+02	1.0E+00	1.0E+00	1.0E+00	1.0E+00	1.0E+00	1.0E+00	1.0E+00

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493 **Figures**

494

495 **Figure 1.** A schematic showing parallels between epidemiological and within-host infectious
496 disease models. Epidemiological models fall into two groups: (A) models for microparasites and
497 (B) models for macroparasites, such as nematodes. Models for microparasites categorize
498 individuals as being infected or uninfected. Models for macroparasites consider the parasite
499 burden of infected individuals, as this burden affects the production rate of macroparasites from
500 infected hosts and the mortality rate of hosts. (C) General structure of current within-host disease
501 models. These models generally categorize cells as being infected or uninfected. (D) Schematic
502 of a within-host ‘macroparasite’ model, proposed here. Models of this type would consider the
503 multiplicity of cellular infection, as multiplicity of infection affects the rate of viral production and
504 the lifespan of infected cells, among other phenotypes.

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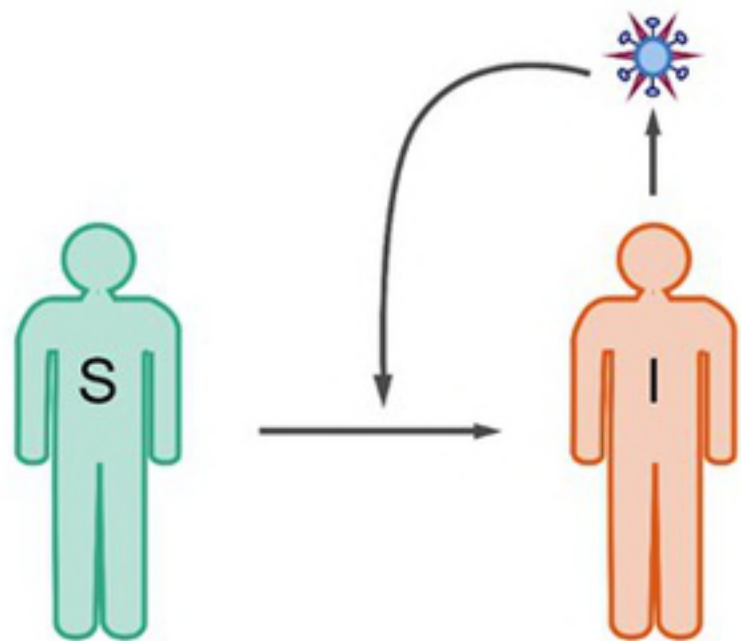
506 **Figure 2.** Target-cell limited within-host macroparasite model dynamics. (A) Within-host viral
507 dynamics, parameterized by fitting the model to influenza A/H3N8 viral load measurements from
508 experimentally infected ponies (black dots). The dashed black line shows the limit of detection,
509 and open circles show below the limit of detection measurements. Blue lines show maximum
510 likelihood fits of the within-host target-cell limited macroparasite model. Red lines show the
511 maximum likelihood fits of a within-host target-cell limited microparasite model (equations 1-3
512 in [4]). The macroparasite model fits the data considerably better than the microparasite model
513 (log likelihood = -49.76 vs. -57.87, with the same number of estimated parameters). (B) Mean
514 multiplicity of infection over time for each of the ponies shown in (A), as calculated from the
515 within-host ‘macroparasite’ model. Mean multiplicity of infection is calculated as the total
516 number of intracellular particles divided by the total number of target cells, $P(t)/H(t)$, where t is

517 time since infection. (C) The number of target cells over time for the within-host ‘macroparasite’
518 model (blue lines; H) and for the within-host ‘microparasite’ model (red lines; $T + I$). (D) The
519 proportion of infected cells that are infected by more than one viral particle, calculated from the
520 within-host ‘macroparasite’ model.

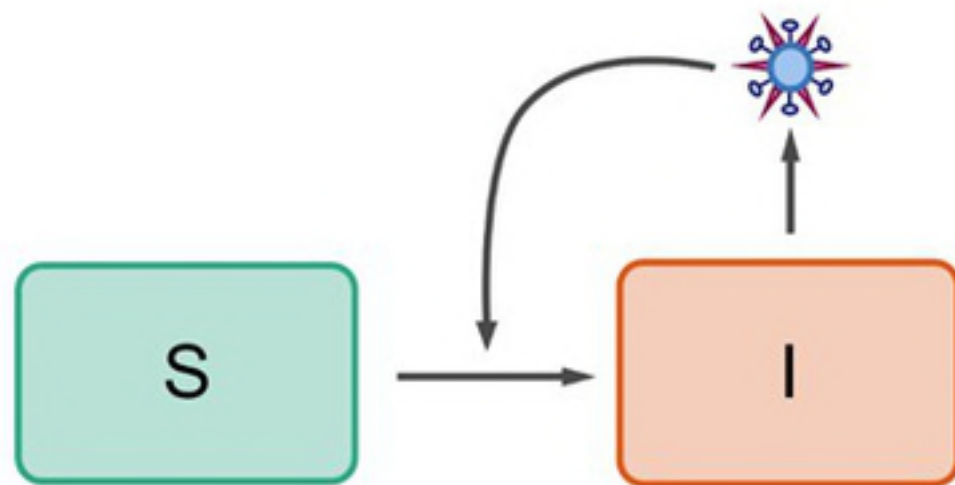
521

522 **Figure 3.** Within-host dynamics from the macroparasite model incorporating the innate immune
523 response. The model, given by equations (13)-(17) was parameterized by fitting to influenza
524 A/H3N8 viral load measurements (A) and fold change measurements of interferon- α (B) from
525 experimentally infected ponies. We further used an estimate of 73% of target cells remaining at
526 the end of infection (C) to parameterize the model. (A) Within-host viral dynamics, with data as
527 in Figure 2A. Blue lines show maximum likelihood fits of the within-host macroparasite model
528 incorporating the innate immune response. (B) Interferon- α dynamics. (C) Target cell dynamics.
529 Red lines show the dynamics of susceptible target cells H , green lines show the dynamics of
530 refractory target cells R , and dashed blue lines show the dynamics of all target cells ($H + R$).
531 Dashed black line shows an estimate for the final number of target cells, given by a 27% reduction
532 in the number of target cells [3].

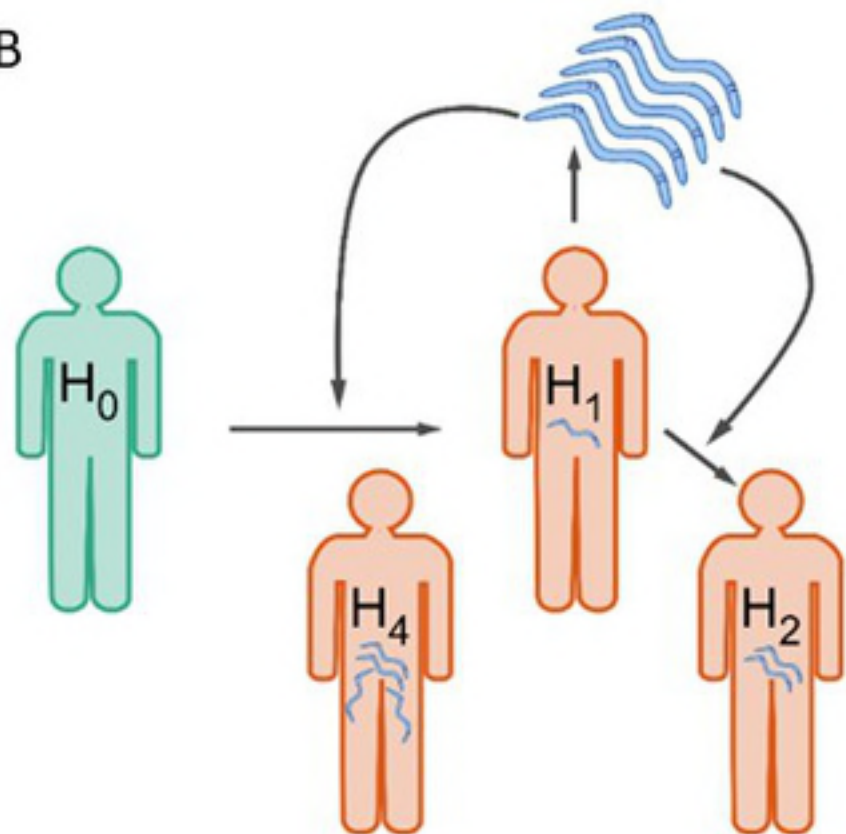
A



C



B



D

