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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  $\lambda_i$  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:

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

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- $\alpha$  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- $\alpha$  are given  
216 by:

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

218 Interferon- $\alpha$  can modify viral within-host dynamics in a number of ways. One way is for  
219 interferon- $\alpha$  to reduce the rate of viral production from infected cells. Another way is for  
220 interferon- $\alpha$  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  $\beta HV$  (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- $\alpha$  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 
$$\frac{dR}{dt} = \phi FH \quad (15)$$

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

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

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  $\phi$  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  $3.5 \times 10^{11}$  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  $\lambda$ , the per-particle cellular mortality rate  $\alpha$ , and the dispersion parameter  $k$  to be the same  
264 across infected ponies. We let the viral clearance rate  $\eta$ , the viral infectivity rate  $\beta$ , 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- $\alpha$ , 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  $\alpha$ ,  $\phi$ ,  $\lambda$ ,  $d$ , 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  $\eta$ ,  
305  $\beta$ ,  $q$ , 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- $\alpha$  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- $\alpha$ , 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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473

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.

	$\beta_{max}$	$\beta_{max}/\beta_{00}$	$\beta_{00}$	$\beta$	$\beta'$	$\beta''$	$\beta'''$	$\beta''''$	$R_0$
	est.	(95% CI)	est.	(95% CI)	(95% CI)	(95% CI)	(95% CI)	(95% CI)	est.
1	0.0001	(0.00005, 0.00015)	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
2	0.0001	(0.00005, 0.00015)	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
3	0.0001	(0.00005, 0.00015)	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
4	0.0001	(0.00005, 0.00015)	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
5	0.0001	(0.00005, 0.00015)	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
6	0.0001	(0.00005, 0.00015)	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

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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).

	$\beta_{max}$	$\beta_{max}/\beta_{00}$	$\beta_{00}$	$\beta$	$\beta'$	$\beta''$	$\beta'''$	$\beta''''$	$R_0$
	est.	(95% CI)	est.	(95% CI)	(95% CI)	(95% CI)	(95% CI)	(95% CI)	est.
1	0.0001	(0.00005, 0.00015)	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
2	0.0001	(0.00005, 0.00015)	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
3	0.0001	(0.00005, 0.00015)	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
4	0.0001	(0.00005, 0.00015)	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
5	0.0001	(0.00005, 0.00015)	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
6	0.0001	(0.00005, 0.00015)	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

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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.

505

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- $\alpha$  (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- $\alpha$  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].





