

Could the human papillomavirus vaccines drive virulence evolution?

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Abstract

The human papillomavirus (HPV) vaccines hold great promise for preventing several cancers caused by HPV infections. Yet, little attention has been given to whether HPV could respond evolutionarily to the new selection pressures imposed on it by the novel immunity response created by the vaccine. Here, we present and theoretically validate a mechanism by which the vaccine alters the Transmission-Recovery trade-off that constrains HPV's virulence such that higher oncogene expression is favoured. With a high oncogene expression strategy the virus is able to increase its viral load and infected cell population before clearance by the vaccine, thus improving its chances of transmission. This new rapid cell-proliferation strategy is able to circulate between hosts with medium-to-high turnover rates of sexual partners. We also discuss the importance of better quantifying the duration of challenge infections and the degree to which a vaccinated host can shed virus. The generality of the models presented here suggests a wider applicability of this mechanism and thus, highlights the need to investigate viral oncogenicity from an evolutionary perspective.

Keywords

Human papillomavirus, virulence evolution, oncogenes, transmission-recovery trade-off, within-host model

1 Introduction

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3 There is considerable excitement surrounding the Human papillomavirus (HPV) vaccines
4 due to their innovative virus-like-particles (VLP) technology and the very high efficacy rates
5 found in clinical trials ^{1,2}. The HPV vaccine is hailed as a very effective preventative measure
6 against the several cancers (cervical, penile, anal, head-and-neck) that are caused by this very
7 common sexually transmitted virus. Since HPV is a double-stranded DNA (dsDNA) virus, it is
8 often argued that it is unlikely that escape mutants could evolve to evade the VLP-induced
9 immunity against the virus's L1 surface protein, as is common in RNA virus evolution ^{2,3}.
10 Lacking in these discussions of potential HPV vaccination response (vaccine escape or type
11 replacement) is the idea that viruses can respond to vaccines by increasing their virulence ^{4,5}. An
12 important example of which to note is the vaccine-induced evolution of Marek's Disease virus
13 (MDV), which is also a dsDNA oncovirus. Unexpectedly, MDV has evolved increased virulence
14 and escape mutants in response to several vaccination campaigns ^{6,7}. Here, we heed this
15 cautionary tale and are the first to investigate the potential of HPV to evolve higher virulence in
16 response to the vaccine immunity.

17 In many infections, the within-host density of the infectious agent is the appropriate
18 measure of virulence. For example, Antia et al. define a lethal quantity of a parasite as a natural
19 choice for the maximal level of virulence and they show that within-host dynamics select for a
20 quantity that is just below lethal ⁸. However, HPV is mostly avirulent and asymptomatic and is
21 carried at low within-host densities. Only after several years of persistence do HPV infections
22 become deadly by the transformation of host cells that have become malignant after the infection
23 has stopped being productive for the virus ^{9,10}. Thus, the classic definition of virulence as a
24 consequence of high nearly lethal parasite dose as a strategy that benefits the virus does not
25 readily apply to natural HPV infections.

26 Defining HPV's virulence requires understanding the selection pressures that shape less
27 virulent pathogens, and specifically, oncoviruses. HPV exists as dozens of different types (i.e.
28 strains) with differing pathologies; the most clinically relevant being the high-risk (HR) types
29 which have oncogenes (E5, E6, E7) that interfere with the cell's growth cycle¹¹. Despite the
30 cancer-centric name, the main function of the oncogenes is to stimulate cell cycle re-entry in the

31 mid-epithelial layers in order to allow genome amplification ¹¹. As a result, the virus cannot
32 replicate without the oncogenes. There are two main additional beneficial functions of these
33 genes in HR types. First, the oncogenes interfere with the innate immune system (e.g. inhibition
34 of interferon synthesis and receptor signaling ^{11,12}), thus delaying the activation of the adaptive
35 immune response¹³. Second, the oncogenes inactivate the host's cell cycle regulators (proteins
36 p53 and pPB) in order to stimulate cell proliferation¹⁴. This increases the number of infected
37 cells without having to infect new cells or to increase the intrinsic replication rate of the virus.
38 Both of these oncogene functions improve the chances of transmission by increasing the duration
39 of the infection, and by increasing the amount of viruses transmitted per host-to-susceptible
40 contact. Nevertheless, it has been found that these oncogenes are not expressed at high levels
41 during acute infections because the early viral protein E2 suppresses oncogene expression^{10,15}. If
42 the oncogenes are very beneficial, then why are they not expressed in higher quantities?

43 It is believed that the cost of stimulating the growth of a large density of infected cells is
44 rapid detection by the immune system. Indeed, low-risk (LR) types that create genital warts are
45 cleared faster than HR types^{11,16} because most HR lesions begin flat and inconspicuous and only
46 with time does the extra cell proliferation they induce becomes noticeable to immune agents¹¹.
47 Clearance after immune detection, then, appears to be a major factor affecting HPV's life
48 history. Therefore, we and Orlando et al. believe that the main trade-off that affects this virus is
49 the Transmission-Recovery trade-off^{17,18}, and not the classic Transmission-Virulence trade-off
50 that constrains more virulent pathogens¹⁶ (we are unaware of studies that suggest the contrary).
51 The Transmission-Recovery trade-off posits that host recovery is the main limitation on
52 pathogen replication because if recovery happens before transmission then the pathogen's R_0 is
53 less than 1 and it cannot persistently circulate. Generally, the Transmission-Recovery trade-off is
54 believed to be the main selection pressure constraining less virulent pathogens¹⁷.

55 Vaccinated hosts are a new environment in which the vaccine-induced immune response
56 will act as a strong, novel selective pressure. A unique feature of the immunity induced by the
57 HPV vaccines is that it triggers a large antibody response, one that is at least two orders of
58 magnitude larger than the natural response¹⁹. Also distinct from natural immunity is the duration
59 of infection. Vaccine efficacy trials have shown that 99 % of vaccinated hosts clear challenge
60 infections with targeted types within 6 months²⁰. We postulate that since the immune response in

61 vaccinated hosts will always be triggered by memory cells and will always mount quickly, then
62 the current “lay low” strategy that HR vaccine-targeted types use to stay longer inside a host
63 ceases to be effective. We investigated whether altering the Transmission-Recovery trade-off in
64 vaccinated hosts could drive vaccine-targeted HR types to increase their virulence by changing
65 their oncogene production. Using an evolutionary ecology modelling approach, we find that,
66 indeed, higher oncogene expression is favoured in vaccinated hosts, which subsequently
67 increases the chances of transmission before clearance by the vaccine.

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72 **Methods**

73 We developed a within-host model to represent an HPV infection in an unvaccinated
74 host, which was then modified to represent a vaccinated host. These models were then linked to
75 epidemiological functions (similar to ^{8,17,21}) because selection pressures happen at both the
76 within- and between-host levels. Note that parameter estimates for both within- and between-
77 host models were taken from the literature (see Tables 1 and 2).

78 ***Within-host models***

79 The population of free virions, V , come into contact with uninfected cells, X , and infect
80 them at a rate ψ making infected cells, Y_1 . See the Appendix for the reduction that allows us to
81 not explicitly include X in the model. The first term of Y_1 encapsulates the creation of newly
82 infected cells by the interaction of uninfected cells with free virions, where N represents the total
83 population of all epithelial cells and ϕ is a half-growth constant. The infected cells can either
84 continue their life cycle or they can become self-proliferating cells, Y_2 . These cells have a higher
85 expression of the oncogenes, E6 and E7, which drive the cells to divide more in the mid-layer of
86 the epithelium before terminating and dying. Let ε represent the rate of oncogene expression of

87 the HPV type once in an infected cell. The rate of oncogene expression controls the conversion
 88 of Y_1 cells into becoming self-proliferating cells Y_2 . Self-proliferating infected cells grow at a
 89 rate $r\varepsilon$, proportional to their own density, and is dependent on oncogene expression (i.e. the
 90 higher the oncogene expression the more cell division). Both types of infected cells contribute to
 91 the overall population of free virions, V , by differing virion production rates, k_i . Since HPV is a
 92 non-lytic virus both kinds of infected cells die at the same rate, μ , and their viral production rates
 93 are adjusted by the infected cell death rate, μk_i . Free virions are cleared at a rate δ and the
 94 antibody response is captured implicitly by this viral clearance rate.

95 Finally, we assume that the cytotoxic T-cell (CTL) response, Z , is only initiated by the
 96 growth of Y_2 , and proliferates at a rate ω . The reason for this is two-fold. First, HPV infection is
 97 exclusively intraepithelial which causes no viremia and also hides antigen¹¹, therefore extra cell
 98 growth is a signal to the immune system that something is wrong^{11,16}. Second, the cell-mediated
 99 response needed for clearance is predominantly against the oncogene E6^{12,13,22}. Note that for
 100 simplicity we assume that the CTL kill both groups of infected cells with equally efficiency, with
 101 the killing rate a . Altogether then the **unvaccinated host** model is,

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$$\begin{aligned}
 \frac{dY_1}{dt} &= \psi V \left(\frac{N - Y_1}{\phi + (N - Y_1)} \right) - \varepsilon Y_1 - \mu Y_1 - a Y_1 Z \\
 \frac{dY_2}{dt} &= \varepsilon Y_1 + r \varepsilon Y_2 - \mu Y_2 - a Y_2 Z \\
 \frac{dV}{dt} &= \mu (k_1 Y_1 + k_2 Y_2) - \delta V \\
 \frac{dZ}{dt} &= \omega Y_2 Z
 \end{aligned} \tag{1}$$

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105 It should be noted that we considered a simpler model with only one infected cell population,
 106 and we also considered differential CTL killing rates (see Appendix).

107 In order to represent vaccinated hosts, several changes were made to this model: (i) The
 108 vaccine causes a strong antibody response, therefore, δ is increased to δ_{vac} ; (ii) proliferation of
 109 the CTL is now initiated by the vaccine-created memory response, not the innate response, so

110 only a very small amount of virus present (in Y_1 , Y_2 or V) will trigger the memory response
 111 targeting L1 epitopes to activate the adaptive response to invade, thus this changes the Z equation
 112 and Z 's initial conditions; (iii) the antibodies that flood the infection site help prevent newly
 113 produced free virions from infecting new cells, thus δ_{vac} scales down the infection rate of new
 114 cells, ψ . Together, this gives the model for a **vaccinated host**,

$$\begin{aligned}
 \frac{dY_1}{dt} &= \psi V \left(\frac{N - Y_1}{\phi + (N - Y_1)} \right) - \varepsilon Y_1 - \mu Y_1 - a Y_1 Z \\
 \frac{dY_2}{dt} &= \varepsilon Y_1 + r \varepsilon Y_2 - \mu Y_2 - a Y_2 Z \\
 \frac{dV}{dt} &= \mu (k_1 Y_1 + k_2 Y_2) - \delta_{vac} V \\
 \frac{dZ}{dt} &= \omega_{vac} Z
 \end{aligned} \tag{2}$$

116 where, now Z_0 is set to a value that initiates the Z equation once the infection is started. This is
 117 equivalent to having a very low threshold, such that a very small amount of the virus triggers the
 118 response, which is equivalent to being triggered by the mere presence of the virus, and not viral
 119 growth dependent as it is in the unvaccinated host (see ‘auto-pilot’ immune response²³ and refs
 120 therein). The CTL in a vaccinated host proliferate at a higher rate, ω_{vac} .

121

122 ***Within-host viral fitness***

123 Viral load is a measure of the virus’ reproductive output inside a particular host
 124 environment. The total amount of virus it is able to produce during the course of the infection
 125 represents the fitness of the virus for that particular within-host environment. We are interested
 126 to see how oncogene production changes viral output, so we want to determine the optimal
 127 oncogene strategy, ε^* , which is defined as the oncogene expression that maximizes the total viral
 128 output of a host. To determine this we first find the total viral output, V_{Total} , of a host²⁴, by
 129 finding the integral of the viral load curve, V ,

$$V_{Total}(\varepsilon) = \int_0^{\infty} V(\varepsilon, t) dt \tag{3}$$

130

131 then, we find the maximum with respect to ε , which gives ε^* . We can then compare the ε^*
 132 selected for in distinct within-host environments (vaccinated vs. unvaccinated). Note that
 133 because the model cannot be solved analytically, equation 4 was computed numerically, which is
 134 also true for the equations that follow. The maxima were computed numerically using the
 135 function *NMaximize* in *Mathematica*.

136

137 ***Transmission and between-host fitness***

138 Next we consider the effects of transmission. An optimal strategy at the within-host level
 139 might not be optimal for between-host transmission²⁵. We consider, then, how linking these
 140 within-host models to a different transmission function that represent the relationship between
 141 viral load and transmission (similar to^{8,21}). We considered a linear but scaled down rate of
 142 transmission, where α is $0 < \alpha < 1$.

$$\beta(V) = \alpha V \quad (4)$$

143 Since HPV is for the most part avirulent (virus produces almost no mortality), we equate
 144 the reproductive number, R_0 , to the number of new infections caused by an infected host before
 145 clearing the virions (similar to²⁴). To find an expression for R_0 we consider an equation that
 146 represents the number of hosts infected by the focal infected individual,

$$I(t+dt) = I(t) + mg(t)\beta(t)dt \quad (5)$$

148 where m is the rate of sexual acts, $g(t)$ is the probability that the partner is susceptible, given a
 149 sex act, and $\beta(t)$ is the probability of transmission given a sex act with a susceptible partner.
 150 From this equation we get an expression for the total number of infected hosts an individual can
 151 cause, such that

$$R_0(\varepsilon) = \int_0^{\infty} m \cdot g(t) \cdot \beta(V(\varepsilon, t)) dt \quad (6)$$

153 We include ε since we are interested in how oncogene expression can affect the R_0 of the
 154 infected host. It is important to consider $g(t)$ because humans are fairly monogamous, so

155 transmission to a new host happens only after switching to a new sexual partner, the chance of
156 which goes up in time. This changes the value of each contact event putting more weight on later
157 sexual contacts. Thus the state of partnership affects transmission of a sexually transmitted
158 pathogen like HPV. We modeled $g(t)$ explicitly using a model of three different states that the
159 infected individual can be in with respect to sexual partnerships,

$$\begin{aligned} \dot{b} &= m\beta(t)g - \sigma b \\ \dot{s} &= \sigma g + \sigma b - \rho s \\ \dot{g} &= \rho s - \sigma g - m\beta(t)g \end{aligned} \tag{7}$$

161 where ρ is the rate of new partner acquisition and σ is the rate of partner break up. Here $g(t)$ is
162 the probability the individual is in a partnership with a susceptible, $s(t)$ is the probability of them
163 being single, and $b(t)$ is the probability that their partner is also infected. Note that a host can
164 only be in one of these states and thus at any given time $g(t) + s(t) + b(t) = 1$. The initial
165 conditions were $\{b, s, g\} = \{1, 0, 0\}$. The focal host, then, begins by being in a partnership with the
166 host who gave them the infection, and then, we assume that they become single before forming a
167 new partnership, $b \rightarrow s$. We assume that the host does not form partnerships with hosts that have
168 the same infection. At rate $m\beta(t)g$ the focal host infects their new partner and they, again, are in
169 a partnership with an infected host, $g \rightarrow b$. An analytic solution for $g(t)$ is not easily found
170 because \dot{g} is non-autonomous and so $g(t)$ was calculated numerically.

171

172 ***Host Heterogeneity: Immune status***

173 HPV vaccine efficacy in immunocompetent patients is very high, where most vaccinated
174 individuals clear challenge infections within 6 months²⁶. The effect of the HPV vaccine in
175 immunocompromised patients should be diminished and overall, the strength of the immune
176 response will vary among individuals. It is believed that immunocompromised patients can build
177 a vaccine-induced humoral response because the HPV VLPs used in the vaccine are highly
178 immunogenic²⁷. For instance, HIV-positive men without low CD4+ counts have shown to
179 successfully seroconvert after vaccination²⁸ though at lower titres than HIV-negative patients
180^{29,30}. Immunocompromised individuals with low CD4+ counts or B-cell deficiencies will have

181 trouble building the adaptive response needed to clear the HPV infection and so, at the very least,
182 vaccinated immunocompromised patients should clear a challenge HPV infection slower than
183 vaccinated immunocompetent patients. Unfortunately, HPV vaccine efficacy and immunological
184 studies in immunocompromised patients are few²⁹. Here, we considered how impairment to the
185 adaptive response affects the results by investigating results when CTL proliferations rates, ω ,
186 and the initial densities of CTL were one order of magnitude lower the parameter estimate and
187 initial conditions of the natural case considered.

188

189 *Host Heterogeneity: Sexual behaviour*

190 Sexual behaviour varies between hosts and with age. The host's sexual partnership
191 switching behaviour is important to the transmission of the virus. Hosts that are celibate or do
192 not change sexual partners within the duration of the infection are "dead ends" for the virus,
193 signifying that the R_0 of that individual is less than 1 and, thus, only the formation of a new
194 partnership can lead to transmission³¹. We classified sexual behaviours into four groups (see
195 Table 1): 'long partnerships' to represent individuals who are in long-term serial monogamous
196 relationships; 'short partnerships' on average have 2 to 5 partners per year; 'casual relationships'
197 have even higher partner turnover; and 'superspreaders', such as sex workers, who have 20+
198 partners per year. Partner acquisition, break-up, and sex act rates were obtained from the
199 literature for these groups and all these rates increase with increased partnership turnover (Table
200 1). Also note that the per-partnership transmission probability is 0.6 for HPV³², and its R_0 is 2,
201 though higher for core-group individuals (e.g. superspreaders)³³.

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208 **Results**

209 *Unvaccinated host results*

210 The viral-immunity dynamics were represented using a within-host model. For various
211 values of oncogene expression, the unvaccinated model shows that CTL invasion is triggered if
212 the virus drives many infected cells to divide quickly, thus shortening the duration of the
213 infection (Fig. 1). The model, then, captures the recovery constraint that we expect. The amount
214 of oncogene expression that is favoured under this constraint is the one that generates the
215 maximal viral output within the duration of the infection (maximum of V_{total} , equation 4). For an
216 infection of 1.5 years (HPV-16 is cleared between 0.5 - 4.9 years³⁴ and on average before two
217 years) we find that the optimal oncogene expression, ε^* , is below 0.2 (Fig. 3 a). This model, thus,
218 depicts the HR HPV type strategy of producing few extra self-dividing infected cells in order to
219 have lesions that are fairly flat on the surface during acute infections¹⁶.

220 After calibrating the free parameter α to be 6×10^{-6} such that the short partnership group
221 had an R_0 of 2³³, we then estimated the R_0 of the other sexual behaviour groups. The R_0 was 2.9
222 for the casual group, < 1 for the long partnership group and 9.3 for superspreaders (Fig. 3b),
223 which is realistic though a bit low considering the high partnership turnover rates of
224 superspreaders.

225

226 *Vaccinated host results*

227 Unlike the unvaccinated host, the vaccinated within-host environment does not select for
228 low oncogene expression. Instead, oncogene expression can be very high since the total viral
229 load, V_{total} , grows monotonically with higher ε values (Fig. 3c), suggesting that the cost of growth
230 via cell division is removed in vaccinated hosts. For strains with low oncogene expression
231 strategies, the total viral output is sufficiently low that the vaccine is able to clear them
232 effectively (see Fig. 2 where Y_1 , Y_2 and V decay to zero for ε values below 0.7); suggesting then
233 that a high antibody response is an effective method to decrease viral replication. However, for
234 higher ε , this no longer holds and the exponential growth of V_{total} ($\varepsilon > 0.7$; Fig. 3c) can be
235 explained by Figure 2 where the Y_1 , Y_2 and V curves grow before clearance. Therefore, higher ε -

236 driven growth allows the virus to produce a high viral load before the inevitable clearance by the
237 vaccine. Note also that vaccinated immunodeficient hosts with high ε ($\varepsilon > 0.5$) produce higher
238 viral loads than vaccinated immunocompetent hosts with the same ε (Fig. 3c). As another
239 measure of virulence, comparing the populations of Y_2 cells shows that vaccinated hosts have
240 less Y_2 cells than the unvaccinated host for $\varepsilon < 0.9$, however, for $\varepsilon > 0.9$ the Y_2 populations in
241 vaccinated hosts reach a higher peak (compare Y_2 curves in Fig. 1 and Fig. 2).

242 We determined the between-host fitness of the higher ε strategies by checking that the
243 viral loads are high enough for transmission within a population (equation 6). Since there is no
244 longer a maximum in the vaccinated host that defines the optimal oncogene expression, we
245 instead determine where $R_0 = 1$ and define ε_{vac}^* as the oncogene expression necessary for a strain
246 to persist in a population (Fig. 3d). We find that the $R_0(\varepsilon)$ curve of the long partnership group
247 does not reach $R_0 = 1$ within any reasonable ε value; implying that even with very high viral
248 loads, there is not enough partner-switching to allow for transmission within the infection
249 window. The other three groups (short, casual and superspreaders) do reach $R_0 = 1$ when $\varepsilon = 3.3$,
250 1.6, and 1.3 respectively (Fig. 3d). We find that the shape of the vaccinated $R_0(\varepsilon)$ curve rose for
251 higher values of ε , which is not possible in unvaccinated hosts because of the Transmission-
252 Recovery trade-off (compare Fig. 3 b and d). This implies that removing the ability of the virus
253 to delay effector cell invasion allows types with higher oncogene expression to have R_0 values
254 higher than 1, and thus can spread in the population. Consequently, the vaccine lifts the
255 constraint that is most likely keeping HPV virulence low. Finally, comparing Figure 3a and c,
256 this shows that in vaccinated immunocompetent superspreaders this new ε -strategy requires a
257 lower minimum viral load, of $< 10^7$, for persistent transmission.

258 Since the vaccine's main response is humoral, we considered how increasing the strength
259 of the antibody response affected ε_{vac}^* . In Figure 4a, we see that as δ_{vac} is increased to 100 times
260 the natural antibody clearance rate, a higher ε_{vac}^* is needed for a strain to persist. Thus, the
261 vaccine response selects for high oncogene expression. The strains in the shaded regions that are
262 above all three curves have ε -values above ε_{vac}^* and could out-compete strains with lower ε_{vac}^*
263 because they can circulate in all three kinds of hosts (Fig. 4a). In Figure 4b, we plotted the
264 derivative at ε_{vac}^* for different strengths of the humoral response (for increasing δ_{vac}) as a
265 measure of the strength of the selection for ε_{vac}^* . Selection for ε_{vac}^* is faster when the humoral

266 response is weaker ($\delta_{vac} < I$) and it is also faster in immunodeficient hosts (Fig. 4b), suggesting
267 that immunodeficient patients provide a better environment for the emergence of more virulent
268 strains.

269 Note that the long partnership group is not included in the analysis in Figure 4 because
270 this group does not reach $R_0 = 1$ (as explained above). This implies that hosts engaged in longer
271 partnerships and who have contracted a challenge infection lasting up to 150 days have $R_0 < 1$.
272 These hosts, then, do not contribute to the persistent circulation of strains with higher oncogene
273 expression.

274 Finally, Figure 4c shows how the duration of infection in a vaccinated host affects the
275 ε_{vac}^* . High initial Z values, Z_0 , equates to faster invasion by the adaptive response. As the
276 duration of the infection shrinks due to the faster clearance by CTL, a higher ε_{vac}^* is needed for
277 persistence. Note, however, that if the CTL-invasion happens within less than 50 days ($Z_0 > 1$),
278 then the vaccine is able to clear all infections in all groups, regardless of the level of oncogene
279 expression.

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283 Discussion

284 The evolutionary responses of viruses to vaccines are of serious concern, and they may
285 appear several years after the introduction of such control measures³⁵. In a review, Read and
286 Mackinnon contrast successful vaccines that stimulated natural immunity to novel vaccines
287 which stimulate new responses that differ considerably from natural immunity. They warn that
288 imposing new effector mechanisms can create very different selection pressures, with potentially
289 unwanted consequences⁵. Our findings appear to coincide with this scenario, in that the novel
290 vaccine immunity favours increased virulence in order to allow for transmission during the short
291 window of time before vaccine-induced clearance.

292 The HPV vaccines change the within-host ecology encountered by the virus in three main
293 ways. First, the vaccine-targeted types experience a strong antibody response that is unnaturally
294 high³⁰, and which we find drives the oncogene expression necessary for persistent circulation up
295 further. Second, the vaccine-induced effector cells invade faster, and invasion can no longer be
296 delayed through strategies using slow viral replication and signalling interference. We show that
297 this effect changes the Transmission-Recovery trade-off such that low oncogene expression
298 strategies are no longer favoured.

299 Finally, the vaccine adaptive response now exclusively targets epitopes of the surface
300 protein L1³⁰, which is distinct from natural responses that target the early proteins, E2, E6 and
301 E7, for clearance^{13,22}. Since the L1 is a late gene whose epitopes are expressed in the upper
302 layers of epithelium or are exposed on the capsids¹¹, the vaccine-induced effectors will mainly
303 target free virions and these terminating cells. However, infected cells of the mid- and lower-
304 levels of the epithelium express the early proteins, and so should be targeted less readily by the
305 vaccine response. Though this detail is not present in our models, we expect that it could
306 augment the effect we found, by selecting against the re-infection strategy and favouring the self-
307 proliferation strategy. In this new environment, variants of the vaccine-targeted types exhibiting
308 higher than average cell proliferation would have an advantage.

309 Discussions of HPV evolutionary responses have been scant and have focused on the
310 potential of L1 neutralization escape³⁶. We believe that we are the first to suggest this kind of
311 evolutionary response in HPV types targeted by (or cross-reactive with) the vaccine. The main
312 form of vaccine “leakiness” that has been addressed in the HPV literature is that of type-
313 specificity and whether it can result in type replacement^{37,38}. A “leak” that has not been
314 considered, and what we find here to be important, is what happens when the vaccine does not
315 block infection and viral shedding? Given that challenge infections by vaccine-targeted types
316 were detectable in vaccinated women²⁶ during HPV vaccine trials, we argue that the vaccine
317 does not always fully block viral shedding. Indeed, a humoral response may not always provide
318 perfect protection from viral challenge³⁹. Since HPV is transmitted mechanically through the
319 shedding of both free virions and dead infected keratinocytes from the epithelial surface⁴⁰, it is
320 possible then, that even if the antibody response lowers the free virion population significantly,
321 a vaccinated host could still transmit the virus by shedding infected keratinocytes. For

322 comparison, consider once again the oncogenic MDV example in which shedding of epithelial
323 cells was also involved in transmission. Indeed, the MDV vaccines are leaky because they do not
324 block infection and viral shedding (though this leak is more pronounced compared to the HPV
325 vaccine's stronger prophylactic effect), which has played an important role in the subsequent
326 virulence evolution of MDV^{6,7}. In light of this, we strongly encourage studies of challenge
327 infections in vaccinated hosts, their frequency, their duration, and to what degree they shed
328 infected cells. Cross-sectional epidemiological studies or longitudinal time-points separated 6
329 months apart will often lack the resolution to address these questions, especially if the challenges
330 are short lived.

331 Our model assumes that the high antibody response is instantaneous (δ_{vac} is a constant),
332 and thus it captures the prophylactic effect of high neutralizing antibody titres the vaccine is
333 intended to create. Locally, however, there should be lower levels of neutralizing antibodies (e.g.
334 in cervicovaginal secretions)¹³ and there should be a lag from the time of first challenge until the
335 time the memory B cell induced antibodies, and subsequent cellular response, invade at full
336 force. We have not seen empirical estimates of how many days this takes, though their timing
337 could have considerable consequences on the evolution of the virus and its transmission.

338 To demonstrate the essential ingredients of the phenomenon, our conceptual model had to
339 idealize the viral replication process by neglecting many of its known details. So, though we
340 demonstrate that virulence evolution is possible, we cannot determine with this study whether it
341 is probable. It has been argued that accelerated carcinogenesis is not adaptive because cells in
342 higher grade lesions do not produce fully assembled virions⁴¹. However, given that animal
343 models can be infected with DNA plasmids to produce robust, productive infections^{42,43}, then,
344 how infectious are keratinocytes containing HPV DNA? Even if cancer cells themselves are not
345 infectious, how infectious are the cells in the lesions leading up to cancer? Experiments, then, are
346 needed to assess to what degree oncogene expression can rise while maintaining viable viral
347 production, infectiousness and transmission. Furthermore, following several challenges to the
348 prevailing view of slow dsDNA virus evolution (where mechanisms such as recombination are
349 possible⁴⁴⁻⁴⁸), there is a need for more direct investigations into the evolutionary potential of
350 HPV variants.

351 In a recent study, Orlando et al. found that HR types are best suited for transmission in
352 long partnerships (because HR infections last longer) while shorter partnerships with higher
353 turnover rates allow for the persistence of LR types (because LR types are cleared faster)¹⁶. We
354 show here that by artificially shortening the infection duration, targeted HR types can more
355 strongly adopt the strategy of cell proliferation (a strategy that was costly in natural conditions)
356 in order to increase their chance of transmission, thus, adopting a similar strategy to LR types.
357 Yet, oncogenes of HR types have stronger cell transforming abilities, and expression at higher
358 levels should more readily cause cellular genetic instabilities and lead to faster progression
359 towards cancer.

360 Our study does not contain a full population model of interacting hosts, so we cannot
361 investigate the conditions needed for a host population to maintain an emergent vaccine-adapted
362 type. Heterogeneity of hosts plays an important role in the emergence of strains⁴⁹, and indeed we
363 found variation in the optimal oncogene expression required of the virus to persist in different
364 sexual activity groups. For instance, superspreaders required lower viral loads for persistent
365 transmission, and in a highly sexually active core group this could favour the emergence of a
366 variant with higher oncogene expression. Emergence happens in stuttering transmission chains,
367 potentially in small groups of individuals, and certain host groups are more likely to be carriers
368 and superspreaders⁵⁰⁻⁵². Therefore, future studies should consider how pockets of core-group
369 individuals (the causal and superspreader groups in this study) or of immunodeficient individuals
370 may contribute to the emergence and circulation of new variants.

371 In conclusion, the uniqueness of the HPV vaccines lies in that they target a virus that is
372 avirulent for the majority of hosts but has strong cell transformation properties. Other
373 oncoviruses have similar features to HPV, making it likely that this vaccination program may be
374 emulated in the future. Given that virulence is not a fixed trait in any pathogen, it is in our best
375 interest to understand how we are changing the ecological landscape and the selection pressures
376 acting on the virus, in order to more confidently declare a vaccine's evolutionary robustness.

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385

MANUSCRIPT

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Table and Figure Captions

Table 1. Within-host parameter estimates. The vaccine parameters ω_{vac} and δ_{vac} were set to be 100 times¹⁹ the unvaccinated estimates listed in this table.

Table 2. Sexual behaviour groups and between-host parameters from literature.

Figure 1. Time-series of unvaccinated within-host model for various oncogene expression levels. Warm to cool colours represent time-series runs for different ε values from 0 to 1. Lower ε gives slower growth of Y_1 and Y_2 (e.g. orange-red). Note that Y_1 and Y_2 infected cells produce the V curves. The invasion of Z is delayed at lower levels of ε , thus faster growth of Y_2 , due to higher ε , leads to faster clearance.

Figure 2. Time-series of vaccinated within-host model for various oncogene expression levels. At lower levels of oncogene expression the virus is cleared effectively by the CTL (decay of Y_1 , Y_2 , and V for $\varepsilon < 0.7$) but if higher, then viral load increases due to an increase in self-dividing infected cells. Note that Z appears at the same time regardless of oncogene expression. The range of ε shown is from 0 to 1.2.

Figure 3. Unvaccinated host plots. **a) V_{total} of both immunocompetent and -deficient hosts.** The ϵ^* that is selected for by within-host processes is low, which demonstrates that recovery is the cost to rapid growth inside the host. Immunodeficient hosts can select for a slightly higher optimal oncogene expression. *Unvaccinated immunodeficient parameters: $\omega = 0.0001$, $Z_0 = 10^{-5}$.* **b) R_0 with respect to oncogene expression for various sexual behaviours.** Immunocompetent only. Superspreaders (yellow) and individuals with casual partnerships (purple) have higher R_0 values (maximum) above the average (short partnerships, red), and individuals with long partnerships (blue) are below 1. Including the sexual behaviour model does not change the ϵ^* away from the within-host optimal, thus all three groups select for the same ϵ^* . **Vaccinated host plots.** **c) V_{total} of both immunocompetent and immunodeficient hosts.** No maximum is achieved, instead higher oncogene expression allows for higher viral loads. Immunodeficient hosts have steeper curves implying they reach higher viral loads with lower ϵ values. *Vaccinated immunodeficient parameters: $\omega = 0.01$, $Z_0 = 10^{-5}$.* **d) R_0 with respect to oncogene expression for various sexual behaviours.** Immunocompetent only. The ϵ values where the curves cross $R_0 = 1$ is the minimum value of ϵ needed for the virus to circulate, ϵ_{vac}^* . Superspreaders need a lower oncogene expression (ϵ_{vac}^*) to maintain circulation of the virus, than casual and short partnerships (higher ϵ_{vac}^* on purple and red curves respectively). Long partnerships (blue) do not rise fast enough to cross $R_0 = 1$.

Figure 4. Effect of vaccine humoral response on optimal epsilon. Sexual behaviour groups: superspreaders (yellow), casual (purple), and short (red). **a)** The oncogene expression needed for persistent circulation, ϵ_{vac}^* , with respect to the strength of the antibody response, δ_{vac} . Generally, ϵ_{vac}^* increases with a stronger humoral response. Note that above each line are ϵ values that can also circulate (with R_0 values > 1). **b)** The derivative at ϵ_{vac}^* for various δ_{vac} . The strength of selection for higher epsilon is stronger in immunodeficient hosts (dashed lines) in both casual and superspreader groups. Higher δ_{vac} implies slower selection towards ϵ_{vac}^* . **c) The effect of vaccine-induced clearance time on optimal epsilon.** Each line represents the oncogene expression needed for persistent circulation, ϵ_{vac}^* , in a particular sex group, thus the shaded region above are ϵ values that have R_0 values higher than 1. The oncogene expression needed for ϵ_{vac}^* in the vaccinated host depends on how quickly vaccine-induced clearance happens. At $Z_0 = 10^{-4}$ the vaccinated host sheds virus for about 150 days, and at $Z_0 = 1$ the vaccinated host shed the virus for 50 days. For all three sexual behaviour groups, if the challenge infection is cleared quickly (high Z_0) then a higher ϵ_{vac}^* is favoured, but if the infection is cleared in under 50 days then even high oncogene expression cannot help the virus from escaping the vaccine.

Figures

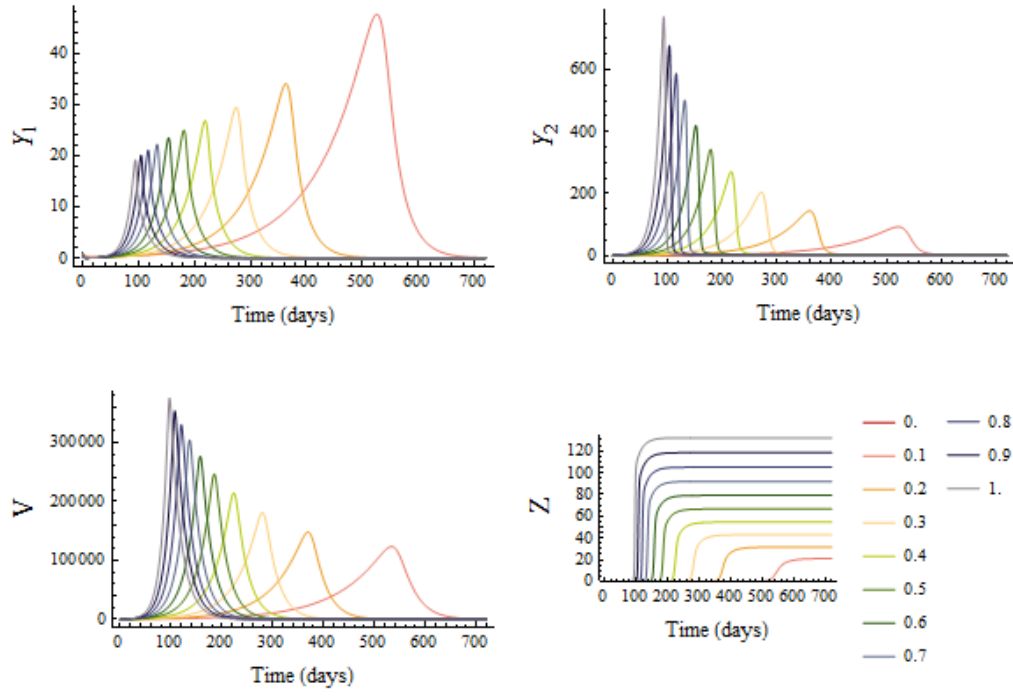


Figure 1.

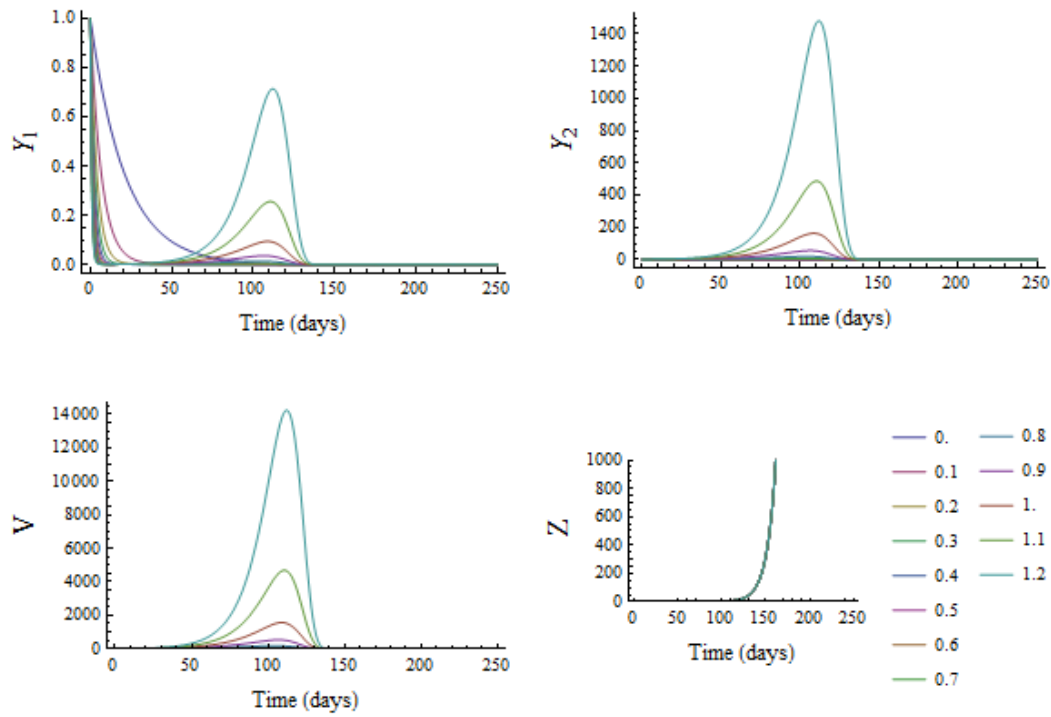


Figure 2.

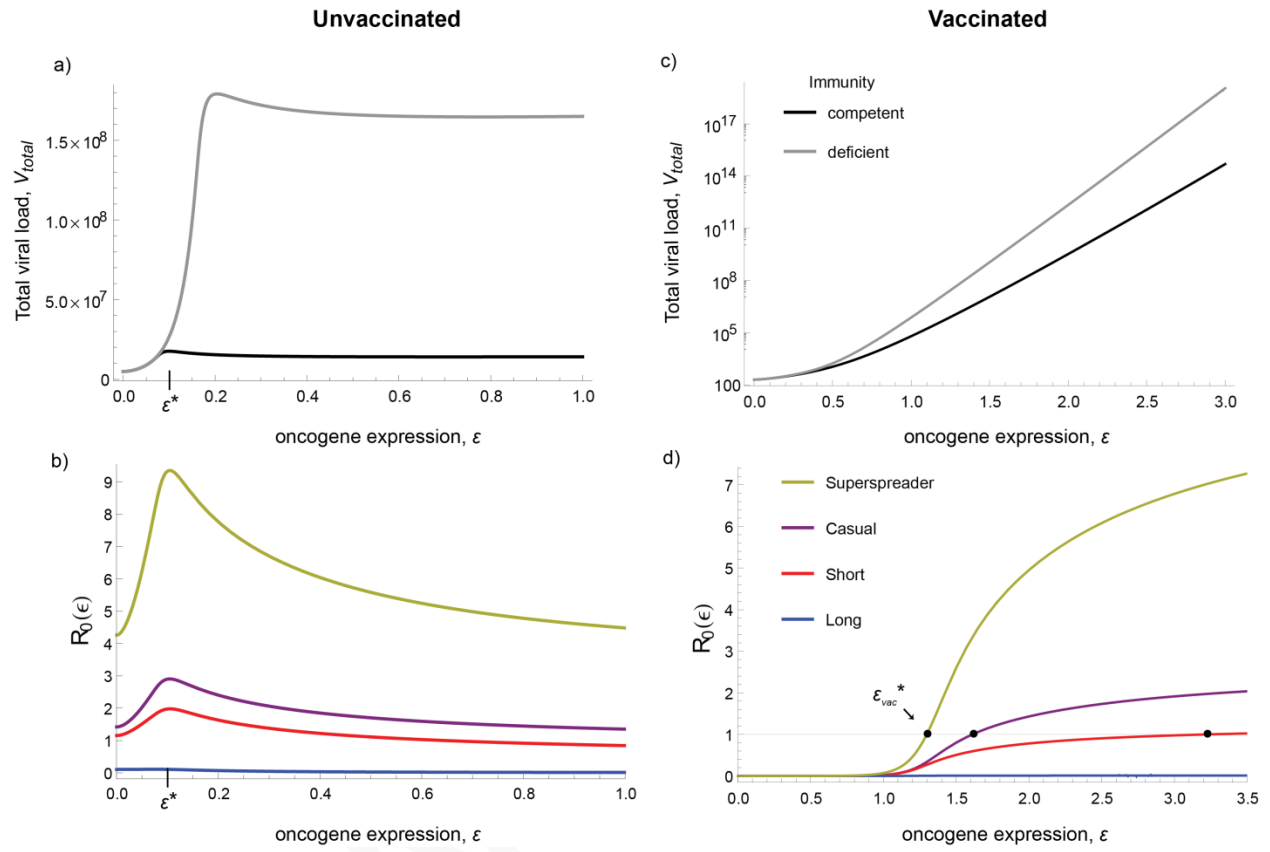


Figure 3.

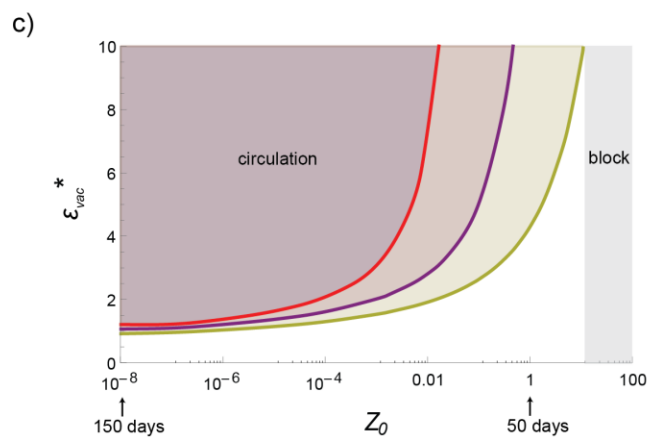
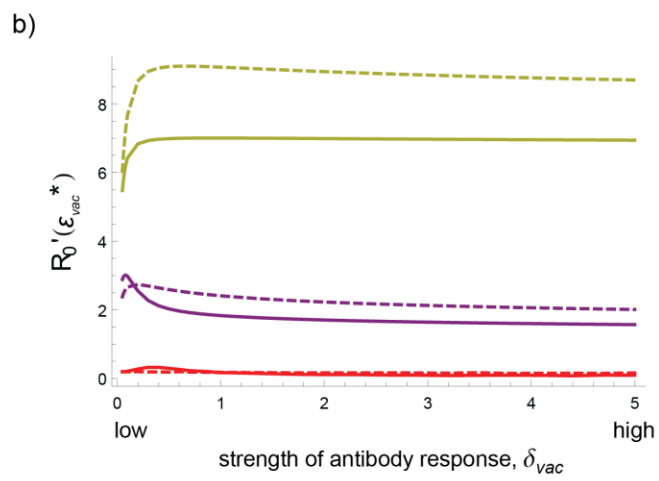
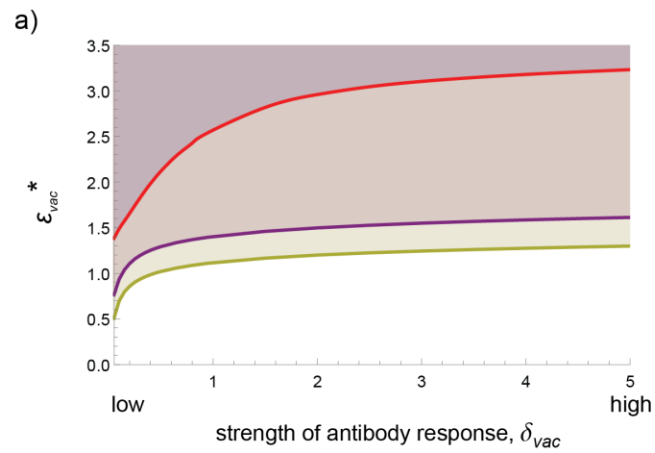


Figure 4.

Tables

Parameter		Estimate	References
ψ	infection rate of uninfected cells	0.0067 day ⁻¹	53
μ	death rate of cells	0.048 day ⁻¹	54
k	burst size	1000 virions/cell	54
ω	proliferation rate of CTL	0.001 day ⁻¹	55
a	killing rate of CTL	0.01 day ⁻¹	56
δ	decay rate of free virions	0.05 day ⁻¹	57
r	self-division rate of infected cells	0.1 day ⁻¹	fixed
N	total population of available cells	10000	fixed
ϕ	half-growth constant	10 ⁶	fixed

Table 1.

Group	Average number of partners/year	Rates (in days)	References	Comments
Long partnerships	1	$\rho = 0.0027$ $\sigma = 0.0004$ $m = 0.356$	58,59 60 61,62	e.g. marriage/common-law, serial monogamy * partnership lasts 6 years
Short partnerships	2-5	$\rho = 0.0096$ $\sigma = 0.05$ $m = 0.43$	59 median 63* 64	e.g. dating *considered dissolution within 20 days to 12 weeks
Casual relationships	6-8	$\rho = 0.019$ $\sigma = 0.1$ $m = 0.43$	65 median 60* 64**	e.g. single, dating, hook-ups * dissolution within 10 days ** 3 / week
Superspreader	20 +	$\rho = 0.068$ $\sigma = 0.44$ $m = 1.44$	58 61* 66**	e.g. sex workers, bathhouse frequenters, etc. * dissolution within 2.3 days * estimate 11 /week for 48 weeks

Table 2.

Appendix

1.1 The unvaccinated model

The population of uninfected basal epithelial cells that HPV targets are represented by the variable X , and they are born at a rate $\lambda(t)$ and die naturally at rate μ . The population of free virions, V , come into contact with uninfected cells, X , and infect them at a rate ψ making infected cells, Y_1 . Infection of new uninfected cells is limited by the fact that most cells are hidden under the epithelium and so abrasions are needed in order for HPV virions to reach them. For this reason we have slowed down the interaction between V and X by making their relationship grow hyperbolically (using a type-II functional response). Thus we assign the constant ϕ to be the density of uninfected cells at which the rate of growth of the Y_1 population is half-maximal.

These infected cells become self-replicating cells, Y_2 , depending on the rate of oncogene expression, ε . Infected cells, Y_1 and Y_2 , are killed by the CTL response, Z . The full model which includes all the assumptions mentioned in the methods is,

$$\begin{aligned}\frac{dX}{dt} &= \lambda(t) - \mu X - \psi V \left(\frac{X}{\phi + X} \right) \\ \frac{dY_1}{dt} &= \psi V \left(\frac{X}{\phi + X} \right) - \varepsilon Y_1 - \mu Y_1 - a Y_1 Z \\ \frac{dY_2}{dt} &= \varepsilon Y_1 + r \varepsilon Y_2 - \mu Y_2 - a Y_2 Z \\ \frac{dV}{dt} &= \mu (k_1 Y_1 + k_2 Y_2) - \delta V \\ \frac{dZ}{dt} &= \omega Y_2 Z\end{aligned}\tag{A.1}$$

To reduce this model, we assumed that birth rate of the uninfected cells, $\lambda(t)$, maintains the total population size of epithelial cells at a constant population size of N and $\frac{d(X + Y_1)}{dt} = 0$, thus X can be replaced by $X = N - Y_1$. Thus, the Y_1 equation becomes

$$\frac{dY_1}{dt} = \psi V \left(\frac{N - Y_1}{\phi + (N - Y_1)} \right) - \varepsilon Y_1 - \mu Y_1 - a Y_1 Z\tag{A.2}$$

as seen in model 1 in the methods.

1.2 Simplified model

We considered a simpler version of this model that only contained one class of infected cells,

$$\begin{aligned}\frac{dX}{dt} &= \lambda(t) - \mu X - \psi V \left(\frac{X}{\phi + X} \right) \\ \frac{dY}{dt} &= \psi V \left(\frac{X}{\phi + X} \right) + r\epsilon Y - \mu Y - aYZ \\ \frac{dV}{dt} &= \mu kY - \delta V \\ \frac{dZ}{dt} &= \omega YZ\end{aligned}\tag{A.3}$$

Here, the Y equation grows either by the infection of uninfected cells (first term) or from its own self-division (second term). The results of this model were very similar to the one in the text, with two main exceptions. The unvaccinated immunity does not select for a low oncogene expression (Fig. A1 a) but when connected to the partnership model, the transmission constraints select for a low oncogene expression (Fig. A1 b). This shows how within- and between-host selection pressures can be at odds, and, in this case, the between-host selection pressure determines the optimal strategy. The other main difference is that the vaccinated short partnership behavior group requires significantly higher oncogene expression (than the super-spreaders and causal groups) to allow persistent circulation in this sexual behavior group (Fig. A1 d).

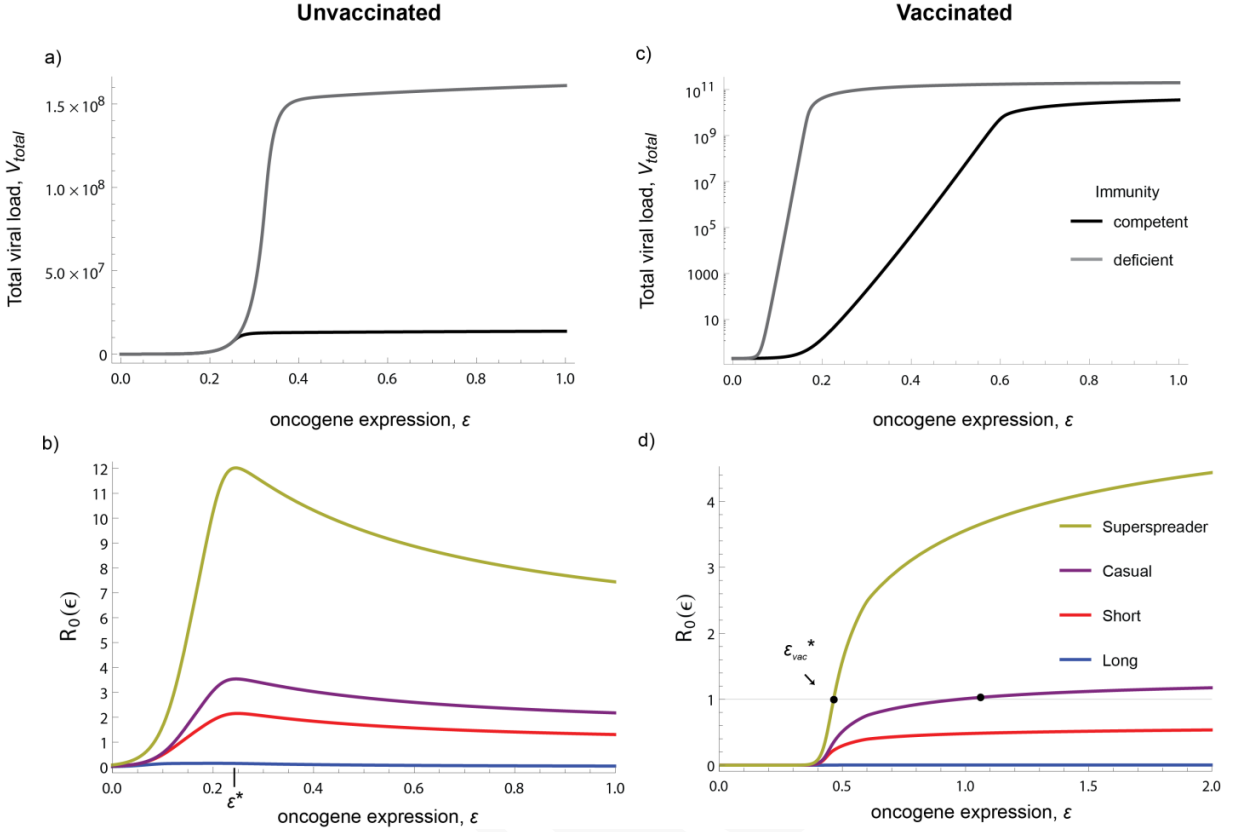


Figure A1. Unvaccinated and vaccinated within-host V_{total} (a and c respectively) and unvaccinated and vaccinated between-host selection for optimal oncogene expression (b and d respectively).

1.3 Sensitivity to parameter values

Attack rates

As an initial simplifying assumption, we considered the CTL attack rates against both Y_1 and Y_2 infected cell populations to be equal in strength (where Z removes either Y_i at a rate, a). However, to study the situation where CD8 T-cells attack the infected cell populations differentially, we considered slight alterations of models 1 and 2 such that a in equation dY_1/dt became a_1 and in equation dY_2/dt the attack rate specific to Y_2 cells become a_2 . A biological reason for the natural immune response to exhibit differential attack rates would be that the increased oncogene expression in Y_2 were differentially targeted (otherwise, the two infected cell groups behave similarly). Indeed, the cell-mediated immune response needs to target E6 epitopes for effective clearance [1,2]. In this case, a_2 should be larger than a_1 because Y_2 cells maintain a higher oncogene expression. We considered this scenario, and found that even if a_2 was increased by 3 orders of magnitude (in relation to a_1) little changed. For instance, in the time-series the infected cells and viral load peak lower, a smaller population of CD8 T-cells are

needed to clear the infection and that the timing and the shapes of the curves remained the same (Fig. A2). Likewise, the ε^* values found by V_{total} and R_0 do not change compared to the scenario where the attack rates are the same (Fig. A3). The same can be said for the case where $a_1 > a_2$ (not shown), though a biological reason for this scenario is not apparent. Since the vaccine-induced immunity targets the L1 late protein, the two infected cell groups should be targeted at the same intensity by effector cells (as we considered in the main text). Nonetheless, we considered differential attack rates in vaccinated hosts for completeness. In the time-series, when $a_1 > a_2$ the Y_1 peak for the higher oncogene expression is lowered, while the rest of the curves stay almost the same (not shown). When $a_2 > a_1$, then all Y_1 curves decay and the growth of the Y_2 nearly instantaneous for higher oncogene expression values, thus the “increase rapid cell division before clearance” effect is more pronounced (Fig. A4). In both vaccine cases, less effector cells (lower Z) are needed to clear the infection and the V_{total} and R_0 give the same ε^* as when the attack rates are equal.

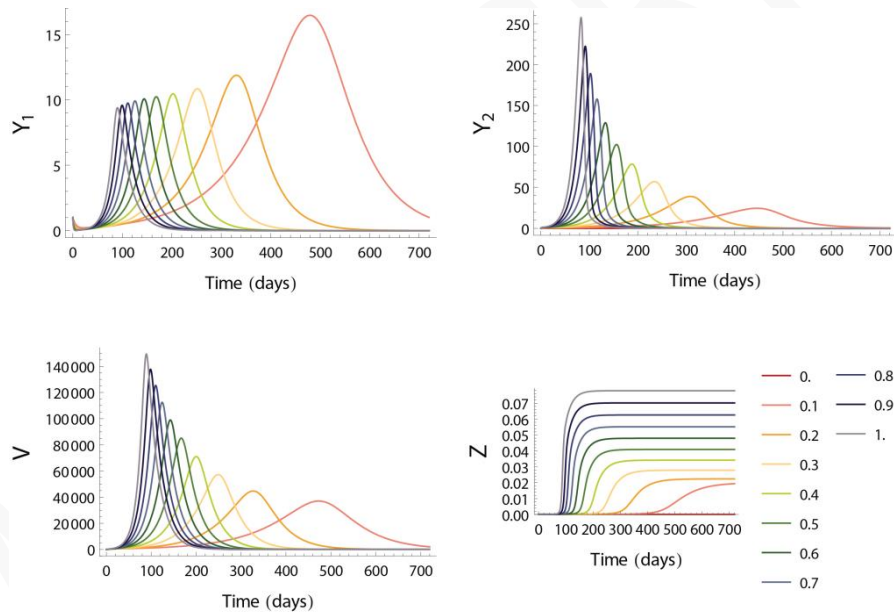


Figure A2. Unvaccinated time-series with $a_2 \gg a_1$ ($a_2 = 10$ and $a_1 = 0.01$), for various ε values (from 0 to 1).

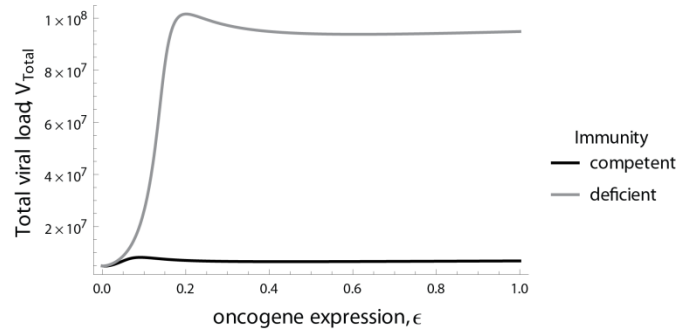


Figure A3. Unvaccinated V_{total} with $a_2 \gg a_1$ ($a_2 = 10$ and $a_1 = 0.01$).

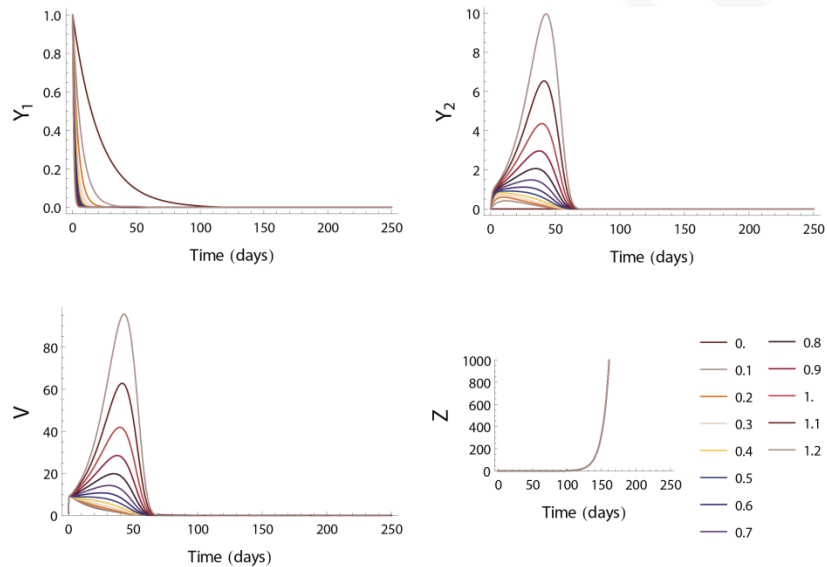


Figure A4. Vaccinated time-series with $a_2 \gg a_1$ ($a_2 = 10$ and $a_1 = 0.01$), for various ϵ values (from 0 to 1.2).

Sexual behaviour parameters

It should be noted that partnership length and turnover can vary throughout a host's life. Therefore, HPV prevalence in different age groups should be linked to higher proportions of short or casual individuals in these age groups which permit more transmission of HPV. The average partner turnover at different age demographic groups (e.g. in 20s, 30s, or 40s) is cultural (and gender-specific), which might help explain variations in HPV prevalence in the same age groups across the world [3]. Indeed, we find that the parameter that most affects the host's R_0 is

partnership acquisition, ρ , which demonstrates that increasing the number of new partners (even when old partnerships have not broken up) increases the transmission of the virus (e.g. fig. A5).

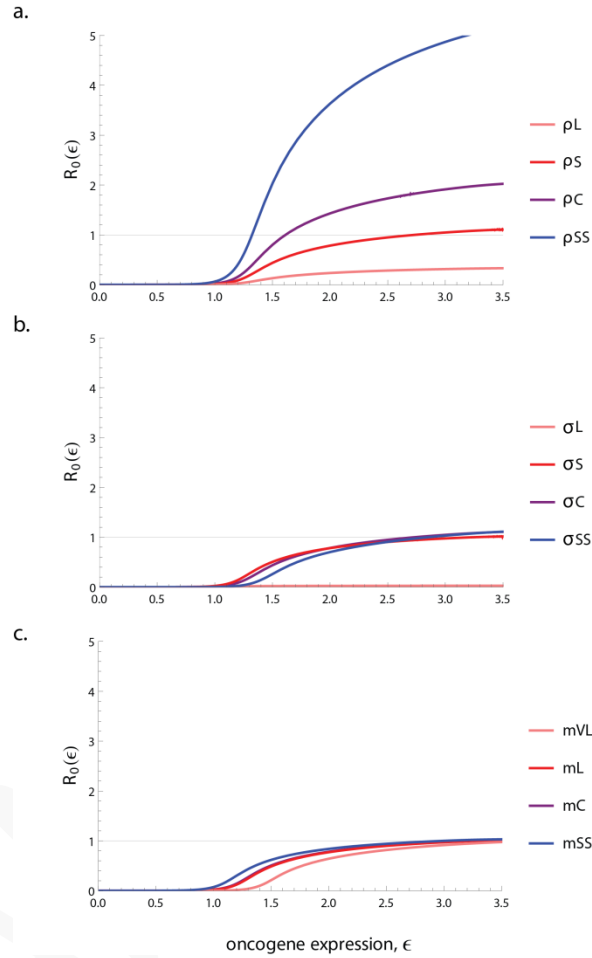


Figure A5. Vaccinated hosts: oncogene expression needed for transmission, ϵ_{vac}^* , is the point where the curve crosses $R_0 = 1$, and is most affected by acquisition of new partners rate, ρ . **a)** parameters $\sigma = 0.05$ and $m = 0.43$ are held constant, and ρ is varied ($\rho L = 0.0027$, $\rho S = 0.0096$, $\rho C = 0.019$, $\rho SS = 0.068$). **b)** parameters $\rho = 0.0096$ and $m = 0.43$ are held constant and σ is varied ($\sigma L = 0.0004$, $\sigma S = 0.05$, $\sigma C = 0.1$, $\sigma SS = 0.44$). **c)** parameters $\rho = 0.0096$ and $\sigma = 0.05$ are constant and m is varied ($mVL = 0.033$, $mL = 0.356$, $mC = 0.43$, $mSS = 1.45$).

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