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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

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Introduction

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

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

Defining HPV's virulence requires understanding the selection pressures that shape less virulent pathogens, and specifically, oncoviruses. HPV exists as dozens of different types (i.e. strains) with differing pathologies; the most clinically relevant being the high-risk (HR) types which have oncogenes (E5, E6, E7) that interfere with the cell's growth cycle[11]. Despite the cancer-centric name, the main function of the oncogenes is to stimulate cell cycle re-entry in the

mid-epithelial layers in order to allow genome amplification [11]. As a result, the virus cannot replicate without the oncogenes. There are two main additional beneficial functions of these genes in HR types. First, the oncogenes interfere with the innate immune system (e.g. inhibition of interferon synthesis and receptor signaling [11,12]), thus delaying the activation of the adaptive immune response[13]. Second, the oncogenes inactivate the host's cell cycle regulators (proteins p53 and pPB) in order to stimulate cell proliferation[14]. This increases the number of infected cells without having to infect new cells or to increase the intrinsic replication rate of the virus. Both of these oncogene functions improve the chances of transmission by increasing the duration of the infection, and by increasing the amount of viruses transmitted per host-to-susceptible contact. Nevertheless, it has been found that these oncogenes are not expressed at high levels during acute infections because the early viral protein E2 suppresses oncogene expression[10,15]. If the oncogenes are very beneficial, then why are they not expressed in higher quantities?

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

Vaccinated hosts are a new environment in which the vaccine-induced immune response will act as a strong, novel selective pressure. A unique feature of the immunity induced by the HPV vaccines is that it triggers a large antibody response, one that is at least two orders of magnitude larger than the natural response[19]. Also distinct from natural immunity is the duration of infection. Vaccine efficacy trials have shown that 99 % of vaccinated hosts clear challenge infections with targeted types within 6 months[20]. We postulate that since the immune response in

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

Methods

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

Within-host models

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. See the Appendix for the reduction that allows us to not explicitly include X in the model. The first term of Y_1 encapsulates the creation of newly infected cells by the interaction of uninfected cells with free virions, where N represents the total population of all epithelial cells and ϕ is a half-growth constant. The infected cells can either continue their life cycle or they can become self-proliferating cells, Y_2. These cells have a higher expression of the oncogenes, E6 and E7, which drive the cells to divide more in the mid-layer of 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, $\mu\, k_i$. 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 antigen [11], 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,

102

$$
\begin{aligned}
\frac{dY_1}{dt} &= \psi V\left(\frac{N-Y_1}{\phi+(N-Y_1)}\right) - \varepsilon Y_1 - \mu Y_1 - aY_1Z \\
\frac{dY_2}{dt} &= \varepsilon Y_1 + r\varepsilon Y_2 - \mu Y_2 - aY_2Z \\
\frac{dV}{dt} &= \mu(k_1Y_1 + k_2Y_2) - \delta V \\
\frac{dZ}{dt} &= \omega Y_2 Z
\end{aligned}
\qquad (1)
$$

103

104

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

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

$$\begin{aligned}
\frac{dY_1}{dt} &= \psi V\left(\frac{N-Y_1}{\phi+(N-Y_1)}\right) - \varepsilon Y_1 - \mu Y_1 - aY_1Z \\
\frac{dY_2}{dt} &= \varepsilon Y_1 + r\varepsilon Y_2 - \mu Y_2 - aY_2Z \\
\frac{dV}{dt} &= \mu(k_1Y_1 + k_2Y_2) - \delta_{vac}V \\
\frac{dZ}{dt} &= \omega_{vac}Z
\end{aligned} \qquad (2)$$

where, now Z_0 is set to a value that initiates the Z equation once the infection is started. This is equivalent to having a very low threshold, such that a very small amount of the virus triggers the response, which is equivalent to being triggered by the mere presence of the virus, and not viral growth dependent as it is in the unvaccinated host (see 'auto-pilot' immune response [23] and refs therein). The CTL in a vaccinated host proliferate at a higher rate, ω_{vac}.

Within-host viral fitness

Viral load is a measure of the virus' reproductive output inside a particular host environment. The total amount of virus it is able to produce during the course of the infection represents the fitness of the virus for that particular within-host environment. We are interested to see how oncogene production changes viral output, so we want to determine the optimal oncogene strategy, ε^*, which is defined as the oncogene expression that maximizes the total viral output of a host. To determine this we first find the total viral output, V_{Total}, of a host [24], by finding the integral of the viral load curve, V,

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

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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 transmission [25]. 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 $0 < \alpha < 1$.

$$\beta(V) = \alpha V \tag{4}$$

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 to [24]). To find an expression for R_0 we consider an equation that
146 represents the number of hosts infected by the focal infected individual,

147 $$I(t+dt) = I(t) + mg(t)\beta(t)dt \tag{5}$$

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

152 $$R_0(\varepsilon) = \int_0^\infty m \cdot g(t) \cdot \beta(V(\varepsilon, t))\, dt \tag{6}$$

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

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

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

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

Host Heterogeneity: Immune status

HPV vaccine efficacy in immunocompetent patients is very high, where most vaccinated individuals clear challenge infections within 6 months [26]. The effect of the HPV vaccine in immunocompromised patients should be diminished and overall, the strength of the immune response will vary among individuals. It is believed that immunocompromised patients can build a vaccine-induced humoral response because the HPV VLPs used in the vaccine are highly immunogenic [27]. For instance, HIV-positive men without low CD4+ counts have shown to successfully seroconvert after vaccination [28] though at lower titres than HIV-negative patients [29,30]. Immunocompromised individuals with low CD4+ counts or B-cell deficiencies will have

trouble building the adaptive response needed to clear the HPV infection and so, at the very least, vaccinated immunocompromised patients should clear a challenge HPV infection slower than vaccinated immunocompetent patients. Unfortunately, HPV vaccine efficacy and immunological studies in immunocompromised patients are few [29]. Here, we considered how impairment to the adaptive response affects the results by investigating results when CTL proliferations rates, ω, and the initial densities of CTL were one order of magnitude lower the parameter estimate and initial conditions of the natural case considered.

Host Heterogeneity: Sexual behaviour

Sexual behaviour varies between hosts and with age. The host's sexual partnership switching behaviour is important to the transmission of the virus. Hosts that are celibate or do not change sexual partners within the duration of the infection are "dead ends" for the virus, signifying that the R_0 of that individual is less than 1 and, thus, only the formation of a new partnership can lead to transmission [31]. We classified sexual behaviours into four groups (see Table 1): 'long partnerships' to represent individuals who are in long-term serial monogamous relationships; 'short partnerships' on average have 2 to 5 partners per year; 'casual relationships' have even higher partner turnover; and 'superspreaders', such as sex workers, who have 20+ partners per year. Partner acquisition, break-up, and sex act rates were obtained from the literature for these groups and all these rates increase with increased partnership turnover (Table 1). Also note that the per-partnership transmission probability is 0.6 for HPV [32], and its R_0 is 2, though higher for core-group individuals (e.g. superspreaders) [33].

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Results

Unvaccinated host results

The viral-immunity dynamics were represented using a within-host model. For various values of oncogene expression, the unvaccinated model shows that CTL invasion is triggered if the virus drives many infected cells to divide quickly, thus shortening the duration of the infection (Fig. 1). The model, then, captures the recovery constraint that we expect. The amount of oncogene expression that is favoured under this constraint is the one that generates the maximal viral output within the duration of the infection (maximum of V_{total}, equation 4). For an infection of 1.5 years (HPV-16 is cleared between 0.5 - 4.9 years[34] and on average before two years) we find that the optimal oncogene expression, ε^*, is below 0.2 (Fig. 3 a). This model, thus, depicts the HR HPV type strategy of producing few extra self-dividing infected cells in order to have lesions that are fairly flat on the surface during acute infections[16].

After calibrating the free parameter α to be 6×10^{-6} such that the short partnership group had an R_0 of 2 [33], we then estimated the R_0 of the other sexual behaviour groups. The R_0 was 2.9 for the casual group, < 1 for the long partnership group and 9.3 for superspreaders (Fig. 3b), which is realistic though a bit low considering the high partnership turnover rates of superspreaders.

Vaccinated host results

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

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

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

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

response is weaker ($\delta_{vac} < 1$) and it is also faster in immunodeficient hosts (Fig. 4b), suggesting that immunodeficient patients provide a better environment for the emergence of more virulent strains.

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

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

Discussion

The evolutionary responses of viruses to vaccines are of serious concern, and they may appear several years after the introduction of such control measures[35]. In a review, Read and Mackinnon contrast successful vaccines that stimulated natural immunity to novel vaccines which stimulate new responses that differ considerably from natural immunity. They warn that imposing new effector mechanisms can create very different selection pressures, with potentially unwanted consequences[5]. Our findings appear to coincide with this scenario, in that the novel vaccine immunity favours increased virulence in order to allow for transmission during the short window of time before vaccine-induced clearance.

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The HPV vaccines change the within-host ecology encountered by the virus in three main ways. First, the vaccine-targeted types experience a strong antibody response that is unnaturally high[30], and which we find drives the oncogene expression necessary for persistent circulation up further. Second, the vaccine-induced effector cells invade faster, and invasion can no longer be delayed through strategies using slow viral replication and signalling interference. We show that this effect changes the Transmission-Recovery trade-off such that low oncogene expression strategies are no longer favoured.

Finally, the vaccine adaptive response now exclusively targets epitopes of the surface protein L1[30], which is distinct from natural responses that target the early proteins, E2, E6 and E7, for clearance[13,22]. Since the L1 is a late gene whose epitopes are expressed in the upper layers of epithelium or are exposed on the capsids[11], the vaccine-induced effectors will mainly target free virions and these terminating cells. However, infected cells of the mid- and lower-levels of the epithelium express the early proteins, and so should be targeted less readily by the vaccine response. Though this detail is not present in our models, we expect that it could augment the effect we found, by selecting against the re-infection strategy and favouring the self-proliferation strategy. In this new environment, variants of the vaccine-targeted types exhibiting higher than average cell proliferation would have an advantage.

Discussions of HPV evolutionary responses have been scant and have focused on the potential of L1 neutralization escape[36]. We believe that we are the first to suggest this kind of evolutionary response in HPV types targeted by (or cross-reactive with) the vaccine. The main form of vaccine "leakiness" that has been addressed in the HPV literature is that of type-specificity and whether it can result in type replacement [37,38]. A "leak" that has not been considered, and what we find here to be important, is what happens when the vaccine does not block infection and viral shedding? Given that challenge infections by vaccine-targeted types were detectable in vaccinated women[26] during HPV vaccine trials, we argue that the vaccine does not always fully block viral shedding. Indeed, a humoral response may not always provide perfect protection from viral challenge[39]. Since HPV is transmitted mechanically through the shedding of both free virions and dead infected keratinocytes from the epithelial surface[40], it is possible then, that even if the antibody response lowers the free virion population significantly, a vaccinated host could still transmit the virus by shedding infected keratinocytes. For

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

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

To demonstrate the essential ingredients of the phenomenon, our conceptual model had to idealize the viral replication process by neglecting many of its known details. So, though we demonstrate that virulence evolution is possible, we cannot determine with this study whether it is probable. It has been argued that accelerated carcinogenesis is not adaptive because cells in higher grade lesions do not produce fully assembled virions[41]. However, given that animal models can be infected with DNA plasmids to produce robust, productive infections[42,43], then, how infectious are keratinocytes containing HPV DNA? Even if cancer cells themselves are not infectious, how infectious are the cells in the lesions leading up to cancer? Experiments, then, are needed to assess to what degree oncogene expression can rise while maintaining viable viral production, infectiousness and transmission. Furthermore, following several challenges to the prevailing view of slow dsDNA virus evolution (where mechanisms such as recombination are possible[44–48]), there is a need for more direct investigations into the evolutionary potential of HPV variants.

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

Our study does not contain a full population model of interacting hosts, so we cannot investigate the conditions needed for a host population to maintain an emergent vaccine-adapted type. Heterogeneity of hosts plays an important role in the emergence of strains [49], and indeed we found variation in the optimal oncogene expression required of the virus to persist in different sexual activity groups. For instance, superspreaders required lower viral loads for persistent transmission, and in a highly sexually active core group this could favour the emergence of a variant with higher oncogene expression. Emergence happens in stuttering transmission chains, potentially in small groups of individuals, and certain host groups are more likely to be carriers and superspreaders[50–52]. Therefore, future studies should consider how pockets of core-group individuals (the causal and superspreader groups in this study) or of immunodeficient individuals may contribute to the emergence and circulation of new variants.

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

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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 times [19] 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.

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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:* ω = 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*. V**accinated 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:* ω = 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

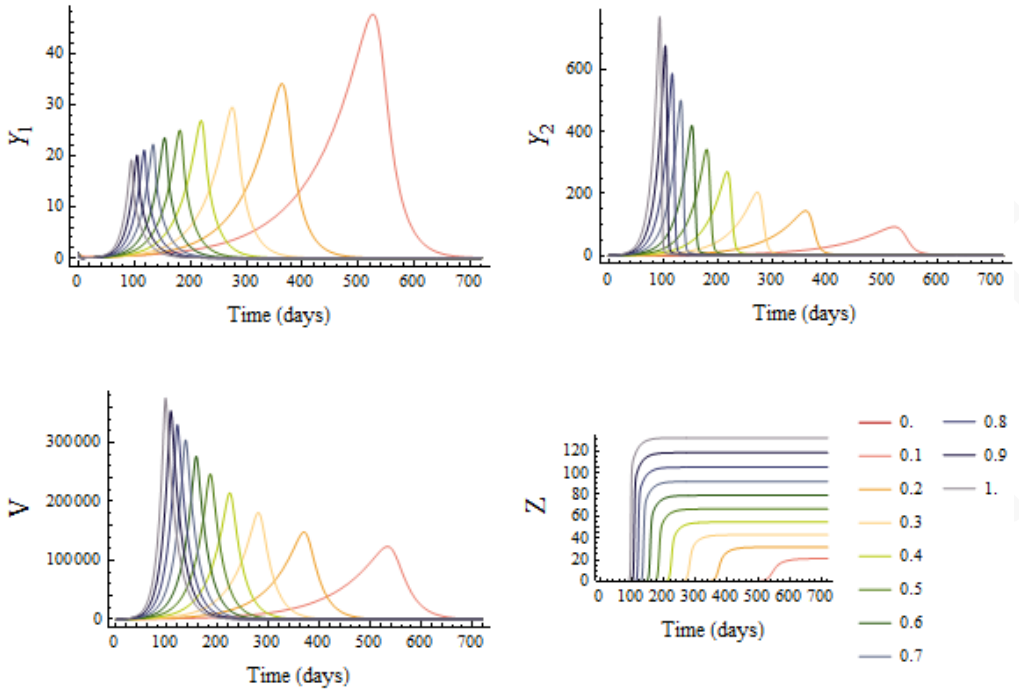

Figure 1.

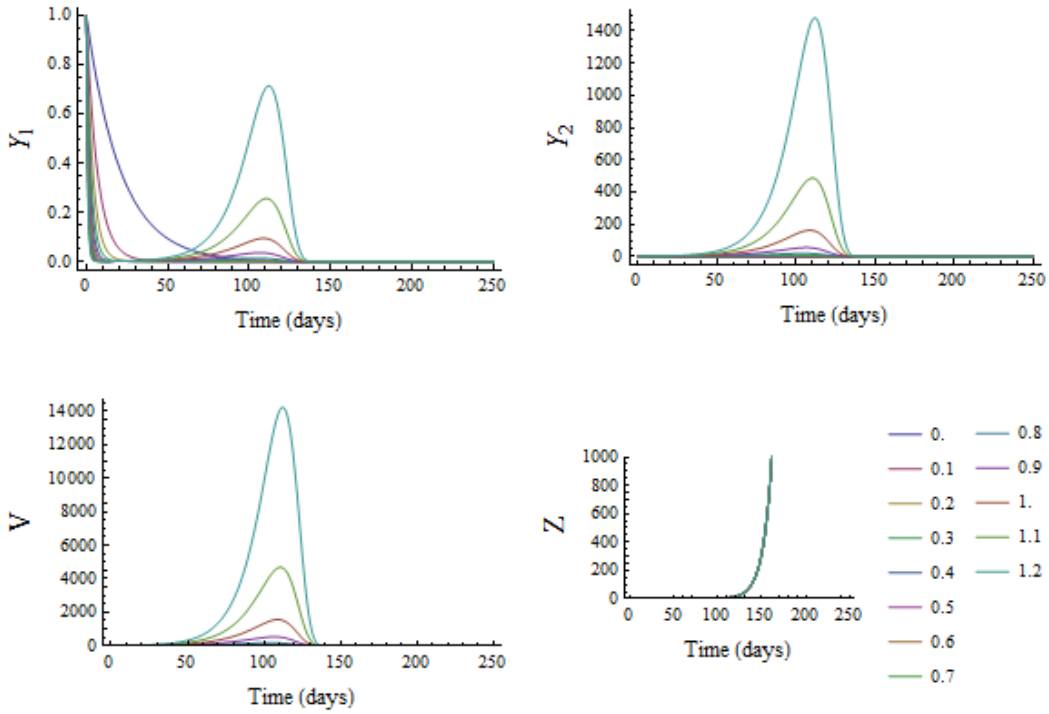

Figure 2.

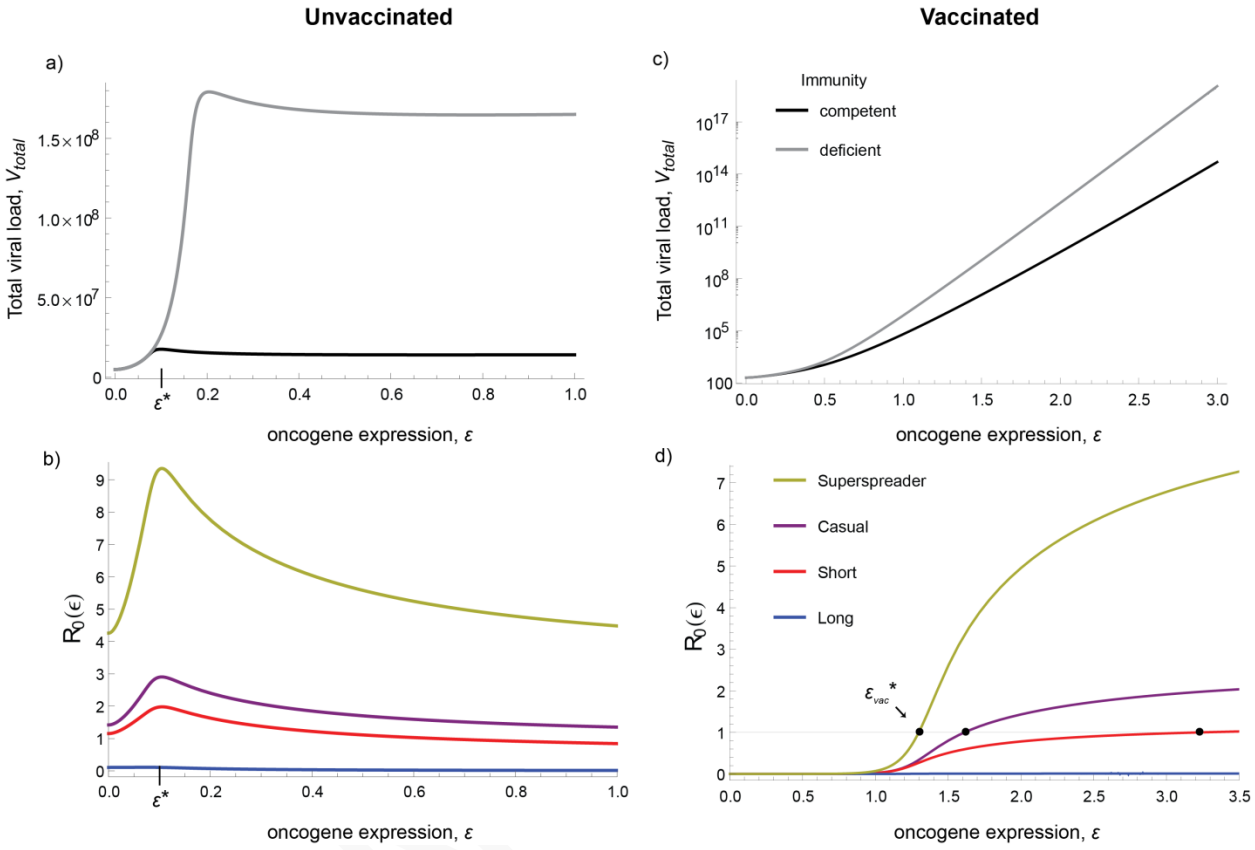
Unvaccinated
Vaccinated
a)
Total viral load, V_{total}
1.5×10^8
1.0×10^8
5.0×10^7
0
0.0
ε^*
0.2
0.4
0.6
0.8
1.0
oncogene expression, ε
b)
$R_0(\epsilon)$
oncogene expression, ε
c)
Immunity
competent
deficient
Total viral load, V_{total}
10^{17}
10^{14}
10^{11}
10^8
10^5
100
0.0
0.5
1.0
1.5
2.0
2.5
3.0
oncogene expression, ε
d)
Superspreader
Casual
Short
Long
$R_0(\epsilon)$
ε_{vac}^*
oncogene expression, ε

Figure 3.

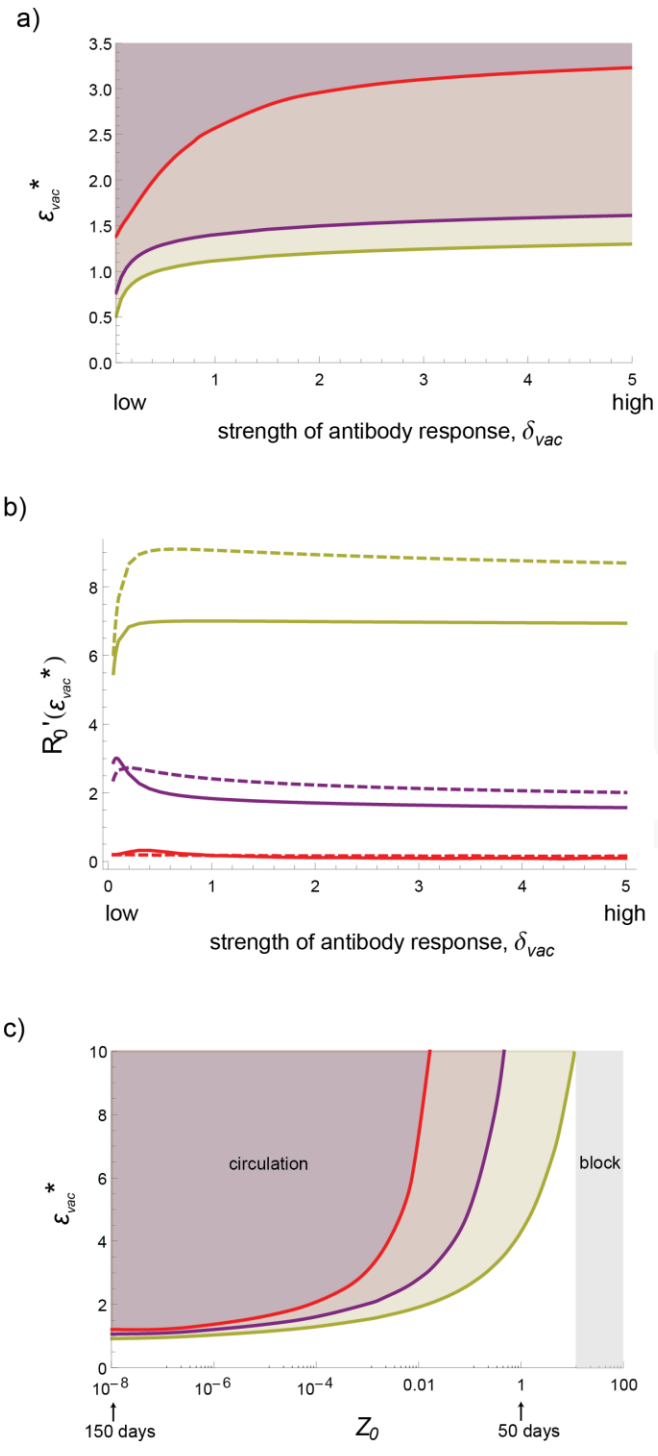
a)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ε_{vac}^*
1
2
3
4
5
low
high
strength of antibody response, δ_{vac}
b)
8
6
4
2
0
$R_0'(\varepsilon_{vac}^*)$
0
1
2
3
4
5
low
high
strength of antibody response, δ_{vac}
c)
10
8
6
4
2
0
ε_{vac}^*
circulation
block
10^{-8}
10^{-6}
10^{-4}
0.01
1
100
150 days
Z_0
50 days

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.

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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,

$$\begin{aligned}
\frac{dX}{dt} &= \lambda(t) - \mu X - \psi V\left(\frac{X}{\phi + X}\right) \\
\frac{dY_1}{dt} &= \psi V\left(\frac{X}{\phi + X}\right) - \varepsilon Y_1 - \mu Y_1 - aY_1Z \\
\frac{dY_2}{dt} &= \varepsilon Y_1 + r\varepsilon Y_2 - \mu Y_2 - aY_2Z \\
\frac{dV}{dt} &= \mu(k_1Y_1 + k_2Y_2) - \delta V \\
\frac{dZ}{dt} &= \omega Y_2 Z
\end{aligned} \qquad \text{(A.1)}$$

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

$$\frac{dY_1}{dt} = \psi V\left(\frac{N - Y_1}{\phi + (N - Y_1)}\right) - \varepsilon Y_1 - \mu Y_1 - aY_1Z \qquad \text{(A.2)}$$

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,

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

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).

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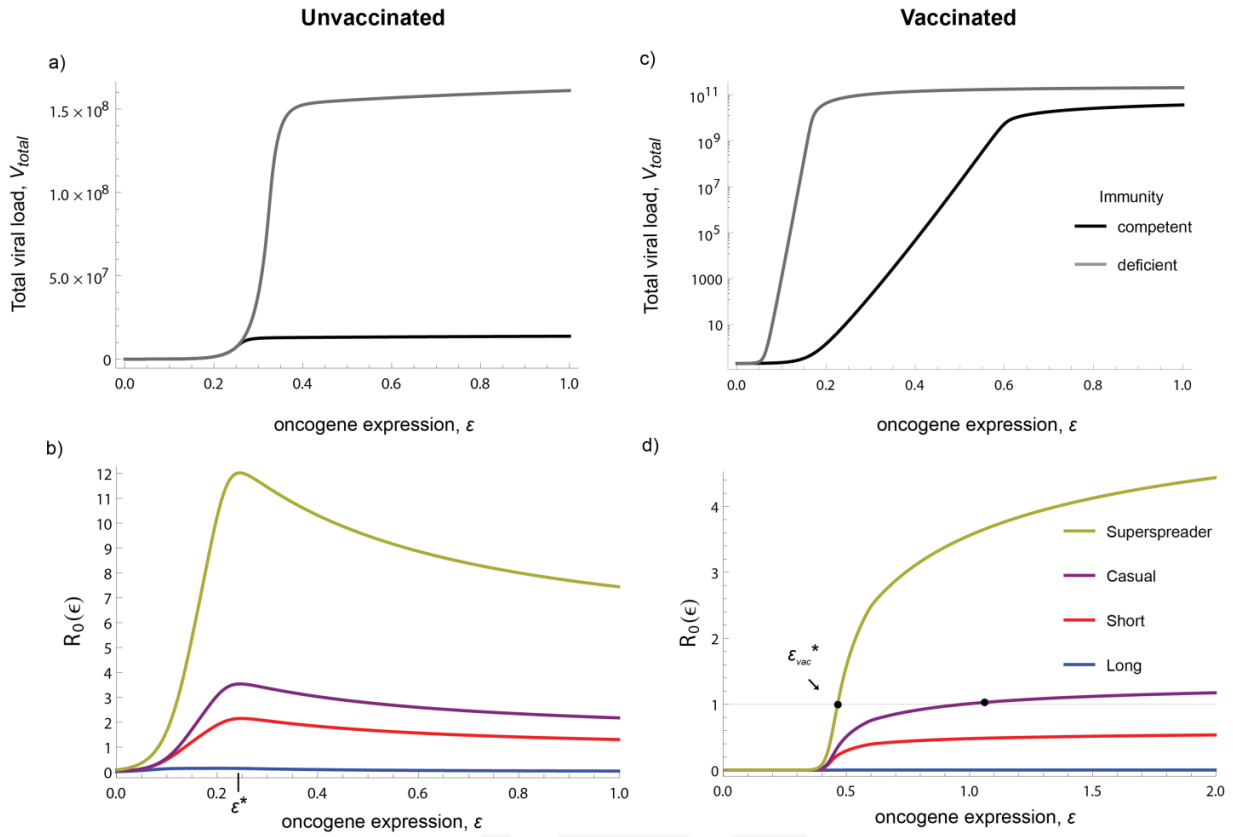

Figure A1. Unvaccinated and vaccinated within-host V_{total} (a and c respectively) and unvaccinated and vaccinated between-host selection for optimal oncogene expression (b and d respectively).

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.

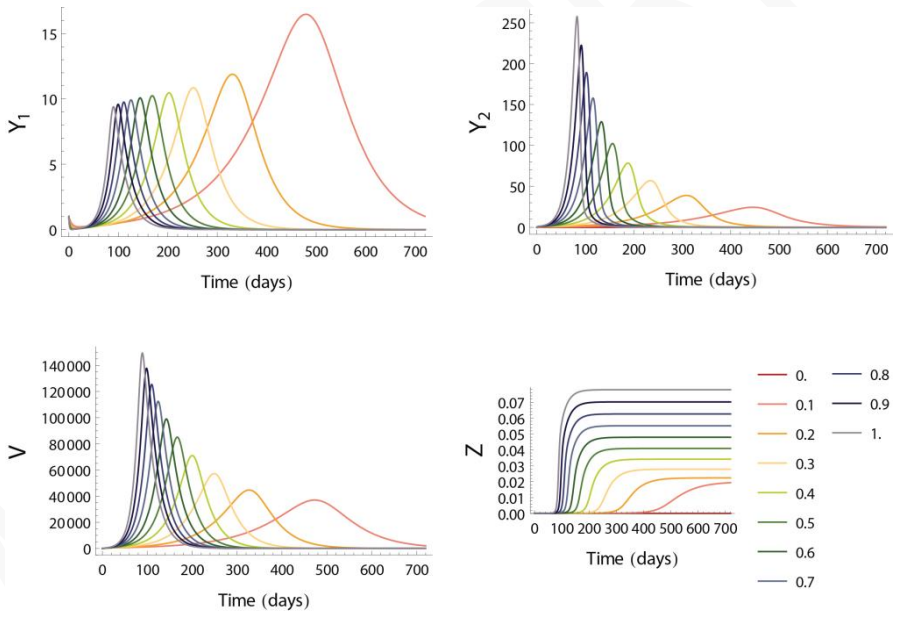

Figure A2. Unvaccinated time-series with $a_2 >> a_1$ ($a_2 = 10$ and $a_1 = 0.01$), for various ε values (from 0 to 1).

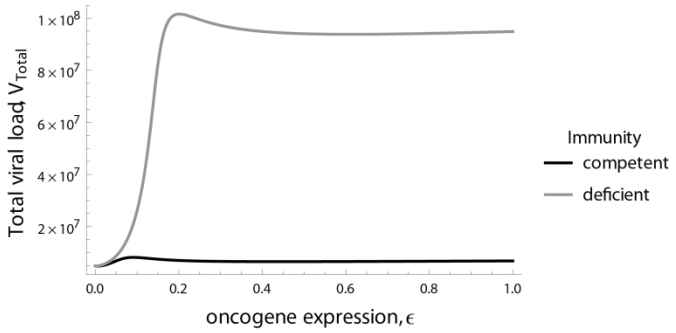

Figure A3. Unvaccinated V_{total} with $a_2 >> a_1$ (a_2 = 10 and a_1 = 0.01).

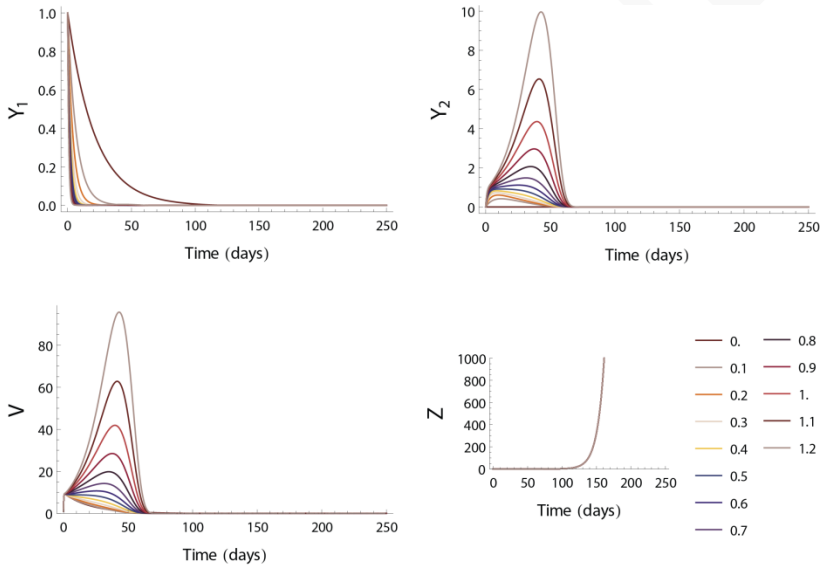

Figure A4. Vaccinated time-series with $a_2 >> a_1$ (a_2 = 10 and a_1 = 0.01), for various ε values (from 0 to 1.2).

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).

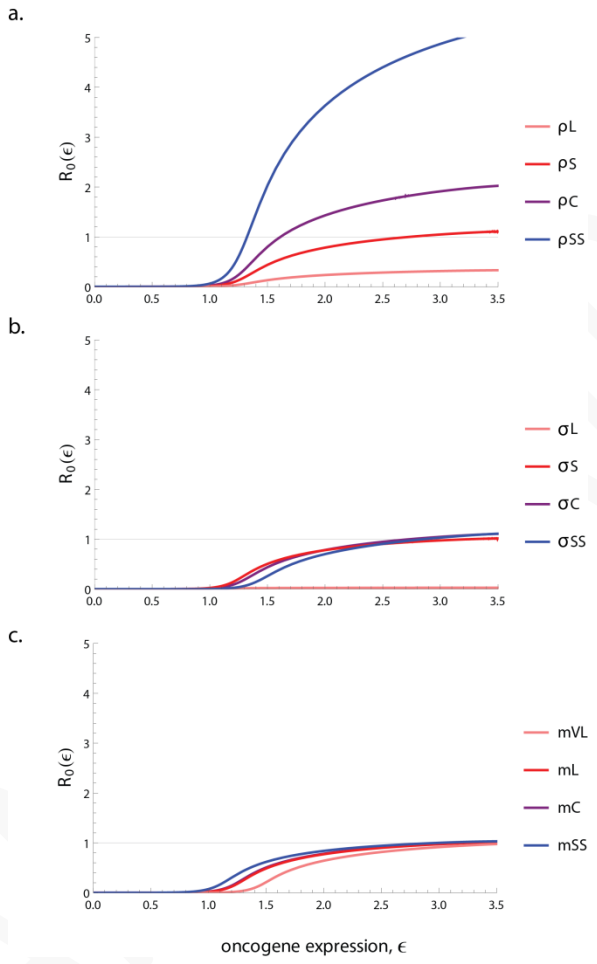

Figure A5. Vaccinated hosts: oncogene expression needed for transmission, ε_{vac}*, is the point where the curve crosses $R_0 = 1$, and is most affected by acquisition of new partners rate, ρ. **a)** parameters $\sigma = 0.05$ and $m = 0.43$ are held constant, and ρ is varied ($\rho L = 0.0027$, $\rho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