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VACCINATION, WITHIN-HOST DYNAMICS, AND VIRULENCE EVOLUTION

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Abstract.—We explore the potential consequences of vaccination on parasite epidemiology and evolution. Our model combines a microscopic (within-host dynamics) and a macroscopic (epidemiological dynamics) description of the interaction between the parasite and its host. This approach allows relevant epidemiological traits such as parasite transmission, parasite virulence, and host recovery to emerge from a mechanistic model of acute infection describing the interaction between the parasite and the host immune system. We model the effect of a vaccine as an activator of immunity enhancing the replication rate of lymphocytes, their initial density at infection's initiation, their efficacy to kill the parasite, or their activation delay after infection. We analyze the evolution of the replication rate of parasites and show that vaccination may promote the evolution of faster replicating and, consequently, more virulent strains. We also show that intermediate vaccination coverage may lead to the coexistence of two different parasite strategies (a low-virulence strain adapted to naive hosts, and a high-virulence strain, more generalist, adapted to both naive and vaccinated hosts). We discuss the consequences of various vaccination strategies under different epidemiological situations using several distinct measures to evaluate the cost induced by the parasite on individuals and entire host populations.

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The rapid evolution of parasite populations is challenging the success of our battle against infectious diseases. Vaccine efficacy, in particular, may be strongly eroded by the emergence and the spread of antigenic variants (Lipsitch 1999; McLean 1999; Earn et al. 2002; Frank 2002). These mutations get a selective advantage from a modification of some qualitative property of the parasite (e.g., when the mutation alters a surface protein that may be the target of an immune cell or drug) that allows it to escape from being cleared. But other types of mutations may be beneficial in vaccinated hosts. In particular, faster replication rate within the host may be an alternative way to escape (at least temporarily) from the action of the immune system. In contrast with antigenic variants, this form of evolution will, as a by-product, increase the virulence of the parasite in unvaccinated hosts. Gandon et al. (2001, 2003) explored the consequences of imperfect vaccination on virulence evolution and showed that different types of imperfect vaccines can have qualitatively different evolutionary consequences. For example, vaccines that reduce within-host growth rate select for a faster exploitation strategy to compensate the loss of growth due to the vaccine and, consequently, for more virulent strains, when measured on naive nonvaccinated hosts. In contrast, vaccines that reduce the probability of infection may counteract the effect of antigrowth vaccines and select lower virulence strategies. However, in the above studies, the relationships among the different parasite's life-history traits (transmissibility, virulence, and recovery) in the different types of hosts are set by arbitrary functions that constrain the range of possible phenotypes. The influence of the shape of these functions could be explored, but it remains unclear what generates certain forms of constraints.

An alternative approach has been developed recently that relies on the explicit modeling of constraints in mechanistic models of infection. This requires taking into account the parasite dynamics within each individual host (Antia et al. 1994; Ganusov et al 2002; Gilchrist and Sasaki 2002; André et al. 2003; Alizon and van Baalen 2005). In these models the relationships among various parasite traits are not artificially imposed but emerge from the interaction between the parasite and the host immune system. For example, André et al. (2003) use a mechanistic model of parasite dynamics during acute infections. The host is characterized by the replication rate, initial density, and killing rate of its immune cells and the parasite by its within-host replication rate. These proximal traits yield within-host infectious dynamics that are then used by André et al. (2003) to calculate the epidemiological parameters of the infection. Transmissibility, virulence, and clearance are therefore governed by both parasite strategy and host immunity.

In epidemiological models of vaccination, a complication emerges from the fact that partial vaccination coverage yields a heterogeneous host population (i.e., naive and vaccinated hosts can be viewed as very different habitats for the parasite). The impact of host heterogeneity on virulence evolution has been studied in the absence of explicit parasite within-host dynamics (Regoes 2000; Gandon et al. 2001, 2003; Gandon 2004). Models that follow such within-host dynamics, however, mostly focused on situations where the host population is homogeneous. Ganusov et al. (2002) and Ganusov and Antia (2003) did study the impact of host heterogeneity on parasite evolution, but these models were not designed to model vaccination (with two types of hosts, naive and vac-

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cinated) and, most importantly, they did not incorporate the fact that such heterogeneity (the composition of the host population) may also depend on parasite's strategy.

Here we combine a microscopic description of the within-host dynamics (André et al. 2003) with a macroscopic description of the epidemiological dynamics when a fraction of the host population is vaccinated (Gandon et al. 2001, 2003). The microscopic model yields important epidemiological variables (transmission, virulence, recovery), which are then used at the macroscopic level to derive a measure of selection on the parasite in a heterogeneous host population. We use this model to analyze the consequences of different vaccination strategies on various measures of mortality, which help to evaluate the various components of the cost of parasitism for both individuals and populations.

The Model

Within-Host Dynamics

We model the within-host dynamics of microparasites facing a specific immune response during an acute infection. $P(\tau)$ and $I(\tau)$ are the densities of parasites (parasitemia) and specific immune cells (lymphocytes), respectively, at the stage τ, where τ measures the time since the start of the infection. Parasite dynamics are given by $dP/d\tau = (r - kI)P$, where r is the parasite replication rate and k the lymphocytes killing rate. The growth of the population of immune cells is assumed to be exponential, $dI/d\tau = \rho I$, where ρ is the replication rate of lymphocytes. Note that, in contrast with other models of within-host dynamics (Ganusov et al. 2002; Gilchrist and Sasaki 2002), we used the simplifying assumption that the immune response is independent of parasite density. This departure from the classical predator-prey framework is compatible with recent experimental evidence showing that the immune response quickly becomes independent of the antigen density, because a brief encounter with the antigen is sufficient to stimulate a program of extensive division and differentiation of effector immune cells (see Antia et al. 2003 and references therein). This assumption also allows solving for parasite density as a function of time (André et al. 2003):

$$P(\tau) = P_0 \exp\left[r\tau + \frac{kI_0}{\rho}(1 - e^{\rho\tau})\right], \qquad (1)$$

where P_0 and I_0 are the parasite and immune cells densities, respectively, at the beginning of the infection. P_0 is chosen as the unitary parasitemia ($P_0 = 1$) and the infection is assumed to be cleared when parasite density falls below one. Clearance time (T) is determined numerically by solving for $P(T) = 1$.

Parasitemia at stage τ is assumed to have a pleiotropic effect on both parasite transmission, $\beta(\tau)$, and host mortality, $m(\tau)$. We explored the effect of different functional relationships between these traits but, in the following, we will focus on a simple linear situation (we will mention the consequences of other possible relationships in the discussion). Parasite transmissibility is thus assumed to be linearly related to density, $\beta(\tau) = bP(\tau)$, where b, the parasite transmission ability, is a constant measuring the effect of a per capita increase in parasitemia on transmission. Host mortality is $m(\tau) = \delta + \alpha(\tau)$, where δ is the background host mortality (or emigration from the population) and $\alpha(\tau)$ is the virulence of parasites at stage τ of infection. The total infection's virulence is assumed to be the sum of the virulence effects of each parasite. It is therefore linearly related with parasitemia through the function $\alpha(\tau) = u(1 + r)^{\theta} \cdot P(\tau)$. The virulence effect of one unity of parasitemia, $u(1 + r)^{\theta}$, is depending on the rate of resource intake from the host and hence on the replication rate r of each parasite, where u is a proportionality constant and θ measures a synergistic effect (see Gilchrist and Sasaki 2002; André et al. 2003).

We are interested in the potential effects of natural immunization (after recovery from a previous infection) and artificial immunization (when the host immune system is stimulated by the inoculation of a vaccine) against the parasite. Both natural and artificial immunity emerge from the differentiation of memory cells, which alter the ability of the immune system to control the parasite. Different properties of the immune system can be altered, as shown by experimental works on immune memory (Veiga-Fernandes et al. 2000; Berard and Tough 2002; Blattman et al. 2002; Wherry et al. 2003). In our model higher initial density of lymphocytes, I_0, higher killing efficiency, k, or higher replication rate of lymphocytes, ρ, decrease the parasitemia and the duration of the infection. In particular, because the initial growth rate of the parasite population is $r - kI_0$, an increase of kI_0 above r would prevent the within-host growth of the parasite. This would yield perfect immunity because it would prevent infection. Because the efficacy of immunity (or of a vaccine) depends also on the parasite, immunity may be perfect against a given parasite and imperfect against a faster-replicating mutant. In this case, if a large proportion of the host population becomes immune, the mutant would replace the resident strain. Host immunity may thus impose strong selective pressures on the parasite population. In the following we study the evolution of the within-host replication rate as an adaptation against host immunity.

Epidemiological Dynamics

Let us now focus on the epidemiological dynamics when there are two types of hosts (naive and vaccinated) that can potentially be infected by a single parasite strain (the resident strain). Let $S_n(t)$ and $S_v(t)$ denote the densities of uninfected naive and vaccinated hosts respectively, and $I_n(\tau, t)$ and $I_v(\tau, t)$ the densities of infected hosts whose infection occurred τ time units earlier. Defining λ as a constant immigration rate of hosts, vaccinated with a probability p (vaccine coverage), the temporal dynamics of susceptibles can be described by the following differential equations:

$$\frac{dS_n}{dt} = (1 - p)\lambda - [\delta + h(t)]S_n + (1 - \kappa)I_n(T, t) \quad \text{and} \qquad (2a)$$

$$\frac{dS_v}{dt} = p\lambda - [\delta + h(t)]S_v + I_v(T, t) + \kappa \cdot I_n(T, t), \qquad (2b)$$

where $h(t)$ is the force of infection at time t, given by:

$$h(t) = \sum_{i=n,v} \int_0^{T_i} \beta_i(\tau) I_i(\tau, t)\, d\tau, \qquad (3)$$

where $\beta_i(\tau)$ is the transmissibility of infected hosts of type i ($i = n$ or v) at stage τ of their infection and T_i is the deterministic clearance time of infections in hosts of type i (defined as the stage at which the parasitemia falls below its initial value).

The temporal dynamics of infected hosts is given by the McKendrick-von Foerster equation (see Caswell 2001, pp. 194–196; Day 2001), simply expressing the fact that as time passes infected hosts either get older and eventually recover (stage τ increases), or die:

$$\frac{\partial I_n(\tau, t)}{\partial \tau} + \frac{\partial I_n(\tau, t)}{\partial t} = -m_n(\tau) I_n(\tau, t) \quad \text{and} \tag{4a}$$

$$\frac{\partial I_v(\tau, t)}{\partial \tau} + \frac{\partial I_v(\tau, t)}{\partial t} = -m_v(\tau) I_v(\tau, t), \tag{4b}$$

where $m_i(\tau) = \delta + \alpha_i(\tau)$ is the mortality rate of a host infected at stage τ (for a similar model see also Hastings and Wolin 1989). The boundary condition expresses the fact that newly infected hosts (stage 0) emerge after transmission from already infected hosts to susceptibles:

$$I_n(0, t) = S_n(t) \cdot h(t) \quad \text{and} \tag{5a}$$

$$I_v(0, t) = S_v(t) \cdot h(t). \tag{5b}$$

Note that the epidemiological dynamics can be expressed in a simpler way, provided the population of infections reaches a stable stage distribution (see Appendix 1, 2, available online only at http://dx.doi.org/10.1554/05-220.1.s1). This finding will be detailed and employed later, to perform numerical simulations of the model.

In equations (2a, b) host immunity can emerge from vaccination of newborns and naive immigrants, but also from natural immunization with a probability κ. In the following we will neglect natural immunization ($\kappa = 0$) because it simplifies the epidemiological dynamics (p controls the fraction of immunized hosts) without altering qualitatively the conclusions.

In the absence of the parasite the host reaches a density $\hat{S} = \hat{S}_n + \hat{S}_v = \lambda/\delta$ (the hat refers to the equilibrium situation in the absence of the parasite) with a fraction p of vaccinated hosts. From a stability analysis of the system (eqs. 2–5), adapted from Day (2001; see Appendix 3 online at http://dx.doi.org/10.1554/05-220.1.s1) to the case where two types of hosts are present, we show that a given parasite strain can invade such a virgin population if its basic reproductive ratio R_0, the expected number of secondary infections produced during an entire infectious period, is greater than one (Anderson and May 1991; Diekmann and Heesterbeek 2000). In such heterogeneous populations the basic reproductive ratio of a parasite is a weighted sum over the different types of hosts: $R_0 = \hat{S}_n B_n + \hat{S}_v B_v$, where B_i is the number of secondary infections per susceptible host available, produced during the entire infectious period of an infected host of type i (van Baalen and Sabelis 1995; André et al. 2003):

$$B_i = \int_0^{T_i} \beta_i(\tau)\sigma_i(\tau)\, d\tau, \tag{6}$$

where $\sigma_i(\tau) = \exp\{-\int_0^{\tau}[\delta + \alpha_i(s)]\, ds\}$ is the probability of a host surviving to stage τ. At endemic equilibrium, the variables B_i are the individual reproductive values of parasites infecting different types of hosts (Frank 1996; Gandon et al. 2001).

Evolutionary Dynamics

When the parasite can invade a virgin host population (i.e., $R_0 > 1$) the system will ultimately reach an endemic equilibrium where $\bar{S}_n$, $\bar{S}_v$, $\bar{I}_n$ and $\bar{I}_v$ are the equilibrium densities of uninfected and infected hosts of each type. These equilibrium densities depend on both the characteristics of the host (e.g., immune system, vaccine properties, and vaccination coverage) and the parasite (e.g., replication rate). Under the parameter values that we used this endemic equilibrium was always stable (we checked the stability of this equilibrium numerically). When the system is located far from this equilibrium and in situations where this equilibrium is unstable it is more appropriate to use the formalism developed by Day and Proulx (2004) and Day and Gandon (2005), where epidemiological and evolutionary dynamics can be tracked simultaneously.

Let a rare mutant with replication rate r^* appear in a resident parasite population (with replication rate r) at epidemiological equilibrium. The mutant has reproductive values $B_n(r^*)$ and $B_v(r^*)$ in naive and vaccinated hosts, respectively (eq. 6). The stability analysis of this system shows that a resident parasite population at endemic equilibrium can be invaded by a mutant if the mutant's basic reproductive ratio $R(r^*, r)$ is greater than one (adapted from Day 2001; see Appendix 4, online only at http://dx.doi.org/10.1554/05-220.1.s1). $R(r^*, r)$ is also called the mutant's per generation rate of increase (Mylius and Diekmann 1995; van Baalen and Sabelis 1995; Gandon et al. 2001; 2003; Dieckmann et al. 2002) and is equal to:

$$R(r^*, r) = B_n(r^*)\bar{S}_n(r) + B_v(r^*)\bar{S}_v(r), \tag{7}$$

where $\bar{S}_n(r)$ and $\bar{S}_v(r)$ are the equilibrium densities of naive and vaccinated susceptible hosts set by the resident parasite and, consequently, by its replication rate, r. Note that equation (7) neglects the effects of superinfection, which is consistent with the fact that we focus on acute infections (infections are short and the probability of multiple infection is low) with cross protection (when immunity is activated it will also clear strains that may infect already-infected hosts).

In the remainder of the paper the search for both epidemiological and evolutionary equilibria were performed numerically. Those simulations allowed us to check that there was always a single and stable endemic epidemiological equilibrium (not shown). Given that this equilibrium was stable, we used average epidemiological parameters derived above to avoid simulating two nested dynamic levels (within hosts and among hosts). This allowed us to describe the dynamics of a single parasite strain, two competing strains, or even a whole range of strains with various growth rates. We discuss at the end of the paper how to perform an evolutionary analysis in more complex situations (i.e., when the system is away from an endemic equilibrium).

Results

Recent experimental evidences show that accelerated T cell responses seen upon reexposure to an antigen (immunolog-

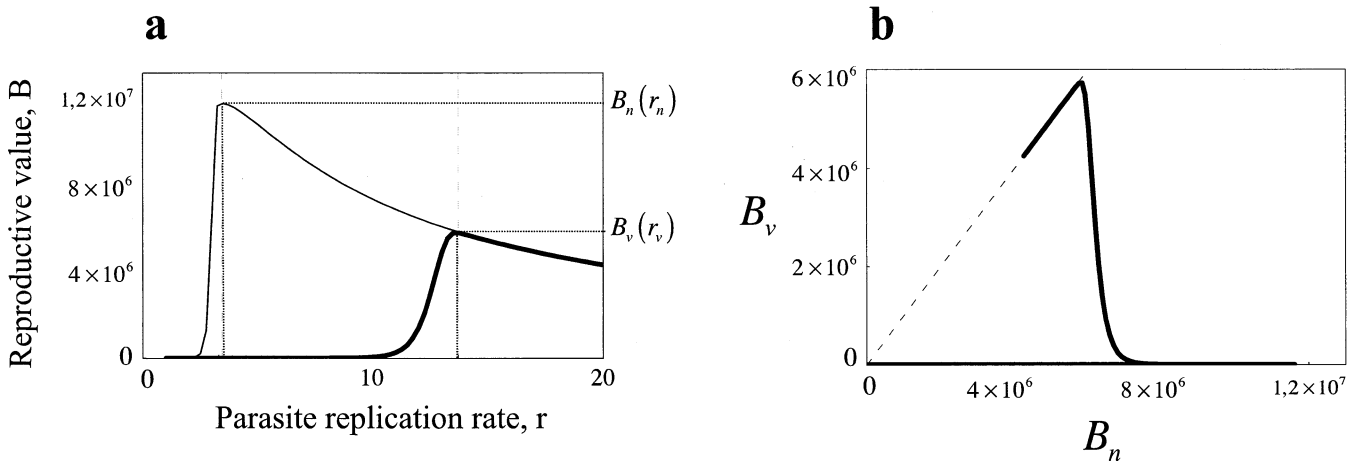

FIG. 1. (a) Parasite reproductive value in naive (B_n, thin line) or vaccinated hosts (B_v, thick line) as a function of parasite replication rate, r. The optimal replication rate in naive (vaccinated) hosts is $r_n(r_v)$ and maximizes B_n (B_v). (b) Parasite reproductive value in vaccinated hosts as a function of reproductive value in naive hosts. The dashed line indicates the points where $B_n = B_v$. Replication rate of immune cells $\rho_n = 1$ in naive hosts and $\rho_v = 5$ in vaccinated; initial killing efficiency of immunity $kI_0 = 10^{-3}$ in both hosts; parasite transmission ability $b = 10^{-11}$; constant relating replication rate to unitary virulence $u = 10^{-10}$ and $\theta = 1$; natural host mortality $\delta = 0.1$.

ical memory) are due to an increase in the density of antigen-specific T cells as well as qualitative changes in memory T cells that allow them to respond faster and develop into more efficient effector cells (Ahmed and Gray 1996; Gray 2000; Veiga-Fernandes et al. 2000; Berard and Tough 2002). In our model, host immunity can act both quantitatively through an increase of initial density of immune cells, I_0, but also qualitatively through modifications of two properties of memory cells: their replication rate, ρ, and/or their killing rate, k. We found that these different properties of induced immunity have qualitatively similar effects on parasite evolution. Thus, for the sake of clarity, we first focus on the analysis of the effects of a specific vaccine that only boosts the replication rate of immune cells. These effects will then be compared with those of other types of vaccines.

Reproductive Values: Evolution in Homogeneous Host Populations

Figure 1a presents the reproductive values of a parasite (eq. 6) in naive, $B_n(r)$ and vaccinated, $B_v(r)$, hosts as a function of its replication rate, r. In a homogeneous host population ($p = 0$ or 1), parasite evolution should ultimately yield a maximization of these reproductive values, $B_n(r)$ or $B_v(r)$ respectively (Levins 1962). Note that, as in classical models of virulence evolution (Frank 1996), parasite reproductive value is maximized for intermediate values of replication rate (Fig. 1a). This intermediate optimum results from the balance between the benefits (higher transmission, $\bar{\beta}$, and lower recovery, $\bar{\gamma}$) and the cost (higher virulence, $\bar{\alpha}$) associated with faster replication rate. These epidemiological parameters reflecting the costs and benefits of higher growth rate are derived as average parameters taken at stable stage distribution of infections (for details see Appendix 1 and 2 available online) and shown in Figures 2a–c. Optimal replication rate, however, may vary between naive and vaccinated hosts. Figure 1a thus shows that in a 100% vaccinated population ($p = 1$) the evolutionarily stable replication rate is higher than in a fully naive population ($p = 0$): $r_v > r_n$, where r_v and r_n are vaccine-adapted and naive-adapted replication rates, respectively (see André et al. 2003). This is consistent with the effect of antigrowth rate vaccine found by Gandon et al. (2001, 2003).

Heterogeneous Host Populations: A Geometrical View of Evolution

When the host population is heterogeneous ($0 < p < 1$) the maximization of $R(r^*, r)$ at $r = r^*$ yields the following evolutionary equilibrium condition (Regoes et al. 2000; Gandon et al. 2003):

$$\frac{dB_v(r^*)}{dB_n(r^*)} = -\frac{\bar{S}_n(r)}{S_v(r)}. \tag{8}$$

Note that the left side of equation (8) refers to the relative performance of the mutant in different hosts and depends only on the mutant strategy. In contrast, the right side refers to the relative proportion of naive and vaccinated hosts in the susceptible population; it is set by the resident strategy and does not depend on the rare mutant's strategy. Note that parasites infect randomly susceptible hosts, regardless of their immune status (naive or vaccinated). As a result, the only potential influence of the resident parasite on the composition of the susceptible population (right side of eq. 8) is through the return of infected hosts back to the susceptible class, after immune clearance.

Some insights into the evolutionary dynamics of the parasite can then be gained from the analysis of the situation where recovered individuals cannot be infected a second time (a classical susceptible-infected-recovered [SIR] model). It can be shown that in this situation the evolutionary equilibrium condition (eq. 8) still holds, while the ratio of the different types of susceptible hosts becomes independent of the resident strategy: $\bar{S}_n(r)/\bar{S}_v(r) = (1 - p)/p$. The fitness of the mutant thus becomes independent of the resident, and evolution simply maximizes $(1 - p)B_n(r^*) + pB_v(r^*)$, the sum of parasite reproductive values in the different hosts (the two curves in Fig. 1a), weighted by the proportion of the different

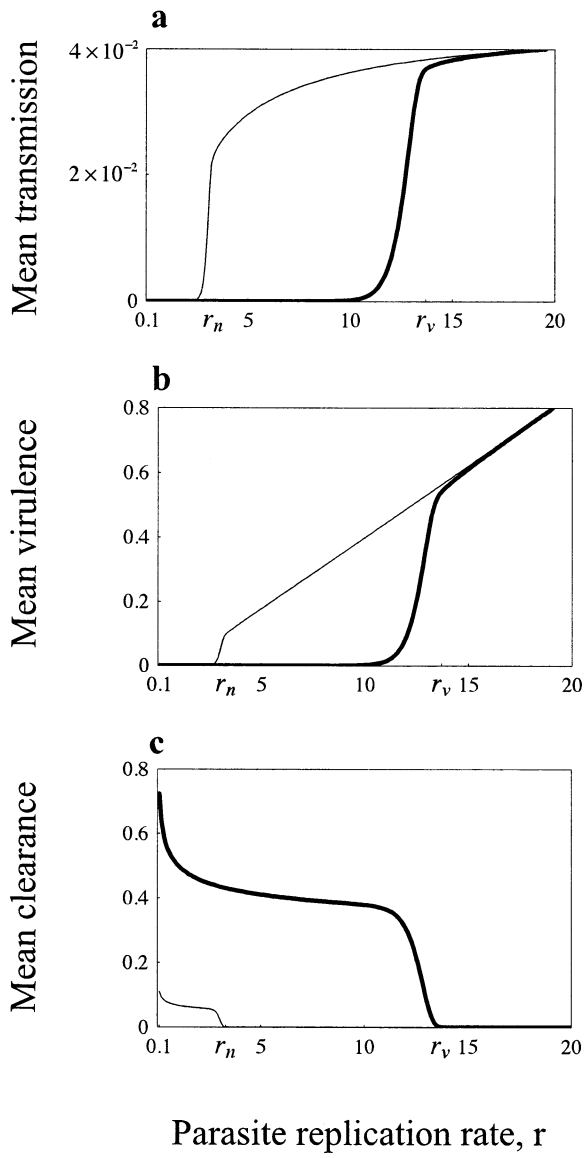

FIG. 2. Average transmissibility (a), virulence (b), and clearance (c) of infections taken at stable stage distribution ($\bar{\beta}$, $\bar{\alpha}$ and $\bar{\gamma}$, respectively), as a function of parasite replication rate within the host. Parameter values as in Figure 1.

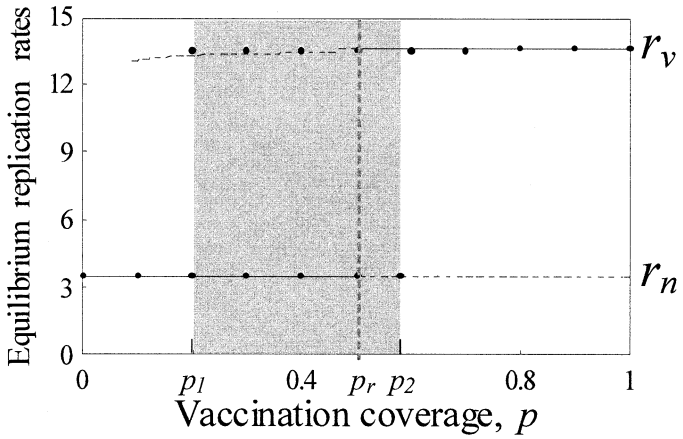

FIG. 3. Evolution of parasite replication rate against vaccination coverage. r_n and r_v are the evolutionary stable replication rates at 0% and 100% vaccination coverage, respectively. Lines show the evolutionary outcome when recovered hosts are fully resistant (susceptible-infected-recovered model). In this case, coexistence is impossible. A single strategy is globally evolutionary stable (full line), but a second strategy may be locally stable (dashed line). The threshold p_r is the coverage above which the globally stable strategy becomes a fast replication rate, close to r_v. The dots are simulation results of the case where recovered hosts are susceptible (without natural immunization). In these simulations, multiple parasite strategies emerge through mutation and compete against each other. In this case coexistence is possible (gray region). The threshold p_1 is the vaccination coverage above which a fast-replicating strain (close to r_v) becomes stable at evolutionary equilibrium. The threshold p_2 is the vaccination coverage above which the slow-replicating strain (close to r_n) is eliminated at equilibrium. Parameter values as in Figure 1, with the parasite transmission ability $b = 10^{-11}$ and the host's immigration rate $\lambda = 100$.

types of susceptibles (which depends only on vaccination coverage). In this case, a convenient representation is to plot $B_v(r^*)$ as a function of $B_n(r^*)$ (Fig. 1b). Equation (8) shows that the evolutionary equilibria are found at the points where the tangents to the set of reproductive values have the slope $-(1 - p)/p$. A similar condition is obtained when there is no recovery after infection (Gandon et al. 2003). This geometrical view of evolution is particularly useful when the set of reproductive values is convex (see fig. 2b in Gandon et al. 2003). Indeed, this convexity indicates that two evolutionary equilibria may exist. First, a globally stable equilibrium that cannot be invaded by any mutant strategy, that is, a global maximum of $(1 - p)B_n(r^*) + pB_v(r^*)$. Second, a locally stable but globally unstable equilibrium (i.e., nearby mutants cannot invade but sufficiently different mutants can). Figure 1b shows that such convexity of the set of reproductive values is also emerging in our mechanistic model of within-host dynamics. Both global and local equilibria are found by numerical maximization of $(1 - p)B_n(r^*) + pB_v(r^*)$ and are plotted in Figure 3 (full and dashed lines for global and local equilibria, respectively) as a function of vaccination coverage p. When coverage is low, a parasite strain with low replication rate is evolutionarily globally stable, but a faster-replicating strain may be locally stable (for intermediate coverage). When vaccination coverage is high, the fast-replicating strain becomes globally stable, but a slow-replicating strategy may be locally stable (for intermediate coverage). It is worth pointing out that the evolutionary equilibria are very close to the strategies r_n and r_v evolving in a 0% and 100% vaccinated populations. This is a direct consequence of the shape of the set of reproductive values. The strong convexity of this set (Fig. 1b) implies that the evolutionary equilibrium is not very sensitive to the slope given by the right side of equation (8). In other words, whatever this slope, the evolutionary equilibria will always be very close to the strategies r_n and r_v.

If we return to the assumption that recovered hosts can be reinfected (as is assumed in eq. 2), the ratio of the different types of susceptible hosts now depends on the resident parasite strategy. Consequently, the geometrical description illustrated in Figure 1b becomes less useful because the slope to the set of reproductive values now depends on parasite

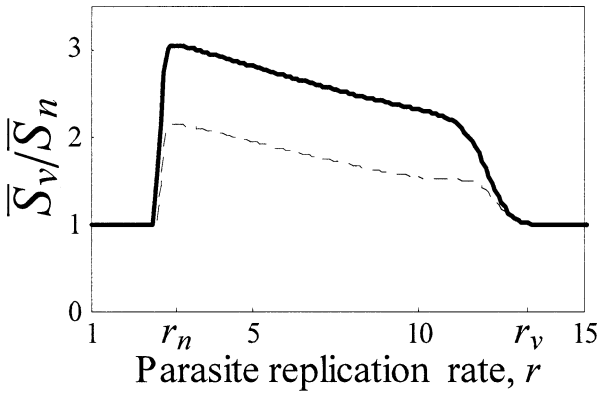

FIG. 4. Ratio of the density of vaccinated to naive hosts among susceptibles at demographic equilibrium as a function of the replication rate of the resident parasite. r_n and r_v are the evolutionarily stable replication rates at 0% and 100% coverage. Parameter values as in Figure 2, except the vaccination coverage held constant at $p = 0.5$ and the parasite transmission ability $b = 10^{-11}$ (full line) or $b = 5 \times 10^{-12}$ (dashed line). Note that the parasite effect on the composition of the susceptible population is stronger with high transmission ability.

strategy (i.e., the slope giving the evolutionary equilibrium changes as parasite strategy moves along the set; see De Mazancourt and Dieckmann 2004; Rueffler et al. 2004). However, the evolutionary equilibria can still be obtained, from numerical simulations describing the dynamics of a range of parasites strains in competition. The dots in Figure 3 present the outcome of simulations as a function of vaccination coverage. Note that a stable coexistence of two parasite strains is possible for intermediate vaccination coverage (gray region in Fig. 3). In addition, for the parameter values that we used (where vaccinated hosts have a much stronger immunity), all the parasite strains present at evolutionary equilibrium are still very close to the strategies r_n and r_v evolving in a 0% and 100% vaccinated populations (compare the dotted and solid lines in Fig. 3). This is particularly convenient because it means that the evolutionary dynamics can be approximated by the analysis of the competition between naive and vaccine-adapted strains (r_n and r_v, respectively). In the following, we focus on the outcome of this competition with numerical simulations.

Competition between Naive and Vaccine-Adapted Parasites

Unlike the SIR model analyzed above, when recovered hosts can be reinfected, the competition between r_n and r_v may result in three (instead of two) different outcomes. These outcomes occur under different vaccination coverage. Two vaccination coverage thresholds (p_1 and p_2) are delimiting these three different outcomes (Fig. 3). When vaccination coverage is either low (below p_1) or high (above p_2), we recover the result that naive or vaccine-adapted strategies, respectively, are globally stable. As shown by Figure 3 (dots), for intermediate vaccination coverage (between p_1 and p_2), however, the coexistence between these two parasite strategies becomes possible. Equation (8) can help us to understand why. As noted above, under our model's assumptions, the right side of equation (8) depends on the strategy of the resident parasite. Figure 4 shows how the relative proportion of the different types of susceptible hosts varies as a function of the resident replication rate. Because vaccinated hosts undergo weaker infections and recover more frequently than naive hosts, their relative density is increased by the presence of a parasite $\bar{S}_v/\bar{S}_n > p/(1 - p)$. This is amplified if parasite prevalence is large (i.e., large transmission ability in Fig. 4, cf. the plain and dashed lines). This effect then declines as the parasite replicates faster and kills most of its hosts (both naive and vaccinated) before recovery can occur. Because $\bar{S}_v/\bar{S}_n$ is also a measure of the relative weight associated with the selection in vaccinated hosts, coexistence can be explained by the negative-frequency dependent selection emerging from the fact that this ratio decreases with parasite replication. Indeed, for some intermediate vaccination coverage, a fast-replicating strain can be favored when it is rare (due to the high proportion of vaccinated hosts) without reaching fixation (because $\bar{S}_v/\bar{S}_n$ decreases as this strain becomes frequent).

Effect of demographic parameters

Various factors may affect the outcome of the competition between naive and vaccine-adapted parasites. For example, the transmission ability, b, of the parasite strongly interacts with the effect of vaccination coverage (Fig. 5a). If b is low, the vaccine-adapted strain, r_v, may be unable to invade because higher vaccination coverage will result in parasite eradication before parasite evolution. This will occur if, and only if, the vaccine-adapted strain is unable to maintain itself in an entirely susceptible population (naive or susceptible), that is, $R_0(r_v) < 1$. This is possible because the maximal reproductive value of parasites infecting vaccinated hosts is always lower than when they infect naive hosts (i.e., $B_v^v < B_n^n$, see Fig. 1). When the transmission ability of the parasite, b, increases it allows the vaccine-adapted parasite to invade and to escape vaccine-driven eradication. Note also that higher parasite transmission ability increases the range of vaccination coverage under which coexistence between the two parasites is possible (because p_1 is decreasing with b while p_2 remains constant).

Effect of vaccine type

Recall that our model allows us to assess the consequences of vaccine that increase either the replication rate of lymphocytes (ρ), their initial density (I_0), and/or their killing rate (k). Increasing both of the last two parameters is equivalent because they only appear as a product of each other in the parasite dynamics (see eq. 1). Some vaccines can also reduce the activation delay of immune cells (time between initiation of infection and activation of lymphocytes). We analyzed a modified version of our model to include such a delay, and we reached the conclusion that reducing the activation delay was very similar to an increase of kI_0 (not shown).

The quality and the quantity of the vaccine interact over the outcome of the competition (Fig. 5b). Vaccine efficacy (i.e., larger ρ and/or kI_0) increases the threshold values of vaccination coverage above which the vaccine-adapted strain may appear and exclude the naive-adapted strain (both the values of p_1 and p_2 increase with ρ). Figure 5b shows that very efficient vaccines may even lead to parasite eradication

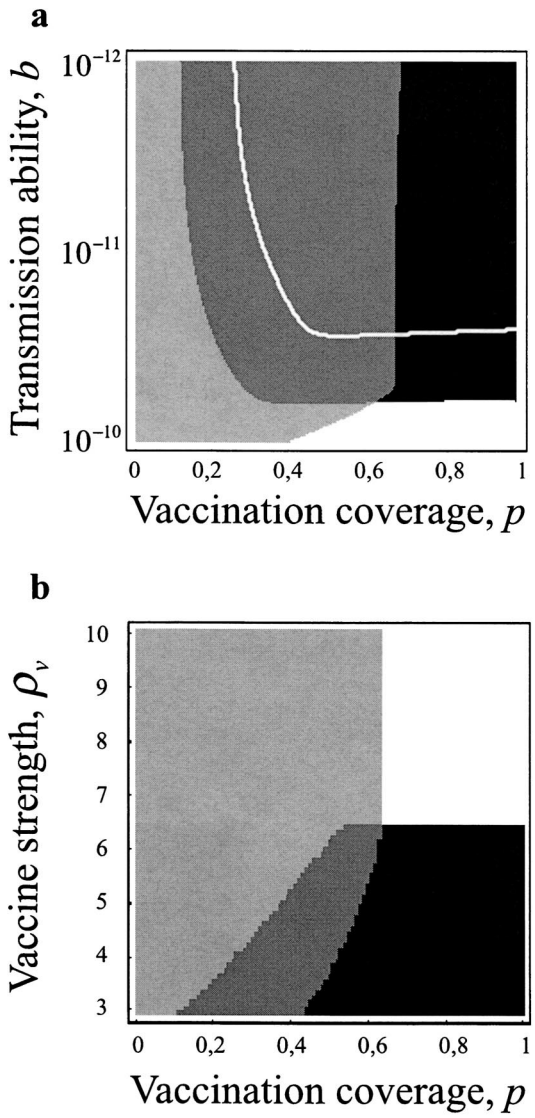

FIG. 5. Evolutionary outcome (a) as a function of vaccination coverage and parasite transmission ability and (b) as a function of vaccination coverage and vaccine strength. In white areas the parasite is absent at equilibrium; in light gray areas the naive-adapted strain (r_n) is solely present; in dark gray areas both strains coexist; in black areas the vaccine-adapted strain (r_v) is solely present. The dotted gray line in (a) indicates the eradication threshold in the absence of evolution (p_c): the vaccination coverage above which the naive-adapted strain becomes unable to maintain even in absence of the vaccine-adapted strain. Above the white line in (a) the average host mortality at equilibrium ($\overline{\overline{m}}$) is higher than in absence of vaccination. In (a) the replication rate of immune cells in vaccinated is $\rho_v = 5$. In (b) the transmission ability of parasites is $b = 2 \times 10^{-12}$. All other parameters are as in Figure 2.

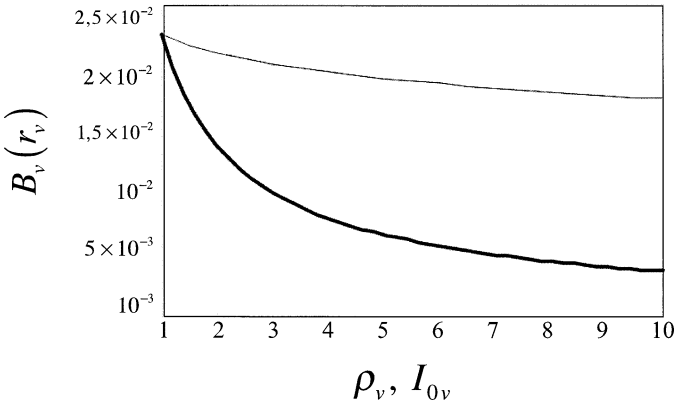

FIG. 6. Maximal reproductive value in vaccinated hosts B_v^v, as a function of vaccine strength. Note that the replication rate of the vaccine-adapted strain (r_v) is increasing with vaccine strength. In naive hosts, the replication rate of immune cells is $\rho_n = 1$ and their initial density I_{0n}. Thick line, replication rate of immune cells in vaccinated hosts increased from $\rho_v = 1$ to 10; thin line, initial density of immune cells in vaccinated increased from $I_{0v} = 1$ to 10. The per capita killing rate of immune cells is $k = 10^{-3}$. Other parameter values as in Figure 1.

without opportunity of invasion for the vaccine-adapted strain if the basic reproductive ratio of the vaccine-adapted strain, $R_0(r_v)$, is driven below one.

These effects are qualitatively similar over the different types of vaccines. For a more quantitative comparison among various vaccines, we can study the effects of these different vaccines on the maximal reproductive value in vaccinated hosts, B_v^v. Indeed, low B_v^v yields a high value of p_1 (the threshold vaccination coverage above which the virulence mutant may increase in frequency) and may thus prevent virulence evolution. In Figure 6 we plot B_v^v, as a function of vaccine strength, for ρ and kI_0 vaccines. This shows that kI_0 vaccines must be orders of magnitude stronger than the others to achieve the same reduction of B_v^v. Yet, immunization is likely to have a much stronger effect on I_0 than on qualitative properties of immune cells. Typically, a 5- to 100-fold increase in the frequency of antigen-specific T cells are observed (Ahmed and Gray 1996). In contrast, Veiga-Fernandes et al. (2000) showed that the increase in growth rate of memory cells was less dramatic (a 1.1- to 2-fold increase depending on the age of the cells). In summary, the qualitative change of memory cells is generally small but with dramatic consequences, while the increase in the quantity of memory cells can be large but with smaller consequences for the immune response. Consequently, it is likely that both qualitative and quantitative properties of memory cells may govern the efficacy of the vaccine.

Different Measures of Mortality

The above analysis yields ultimate epidemiological and evolutionary outcomes after a vaccination campaign. This could be used to derive practical suggestions to optimize the vaccination strategy. For example, it is clear that one should try to use very efficient vaccines and large coverage to lead the parasite toward extinction (see Fig. 5b). In many cases, however, eradication is very unlikely, for instance, when the parasite transmission ability is very large, the host population is dense, only poorly efficient vaccines are available, or large vaccination coverage is unattainable. In all these situations, we may use other criteria to measure the consequences of vaccination. In particular, it is important to note that the characteristics of vaccine-adapted strains vary with the efficacy of the vaccine (r_v increases with ρ and kI_0 in Fig. 6). It is thus necessary to weight the risk of parasite evolution

with the actual virulence of vaccine-adapted strains to fully evaluate the risk of virulence evolution. In the following, we use different measures of parasite-induced mortality to evaluate the long-term consequences (after parasite evolution) of different vaccination strategies.

The Cost of Parasitism for Infected Hosts

Virulence

Virulence, the parasite-induced host mortality rate, is classically used to measure the cost of infection. Here virulence is a dynamic parameter varying in the course of infection. However, it is convenient to use the virulence, $\bar{\alpha}$, averaged over stable age distribution (see Appendix 1, 2 available online). With all vaccine types, vaccination promotes the evolution of a faster replicating strain ($r_v > r_n$), which yields very high virulence when infecting a naive host. This virulence is noted $\bar{\alpha}_n^v$, where the subscript n refers to the type of host and v to the parasite strain. In contrast, the infection of a vaccinated host by a naive-adapted parasite (r_n) results in very low virulence ($\bar{\alpha}_v^n$), because r_n is too low to efficiently exploit a vaccinated host. Moreover, the virulence of the r_v strain in a vaccinated host is higher than the virulence of the r_n strain in a naive host ($\bar{\alpha}_v^v > \bar{\alpha}_n^n$). Indeed, as shown by André et al. (2003), in our model (with exponential immune dynamics), the maximal length of infection (T) is strongly limited by immunity, while the maximal attainable parasitemia is only loosely constrained. As a result, in hosts with strong immune system (e.g., vaccinated hosts), the only way for the parasites to gain a larger overall transmission is by increasing their maximal parasitemia and hence their damage to the host. In other words, the trade-off between transmission and virulence is changed by vaccination in a direction favoring higher virulence (André et al. 2003). This yields:

$$\bar{\alpha}_n^v > \bar{\alpha}_v^v > \bar{\alpha}_n^n > \bar{\alpha}_v^n. \tag{9}$$

The overall consequence of vaccination on a randomly chosen infected host can be evaluated by the overall virulence, $\bar{\bar{\alpha}}$, which is the sum of the four possible virulences ($\bar{\alpha}_n^n$, $\bar{\alpha}_n^v$, $\bar{\alpha}_v^n$, and $\bar{\alpha}_v^v$), weighted by the equilibrium frequency of each type of infection (see Fig. 7a). First, this overall virulence is reduced by vaccination at low coverage, because vaccinated hosts are protected from naive-adapted parasites. Second, the overall virulence increases at intermediate coverage, because the vaccine-adapted strain appears and rises in frequency. Finally, the overall virulence decreases again when the vaccine-adapted strain has reached fixation, because vaccination can protect the remaining naive hosts from very virulent infections. Note that if the vaccine-adapted strain appears, virulence is always higher after complete vaccination ($p = 1$) than before vaccination ($p = 0$) because $\bar{\alpha}_v^v > \bar{\alpha}_n^n$.

Case mortality

As pointed out by Day (2002) the case mortality, χ (i.e., the probability of parasite induced host death once infected), may be a more relevant measure of the cost of being infected because it takes into account both virulence and clearance. In the case of a dynamic infection ending deterministically

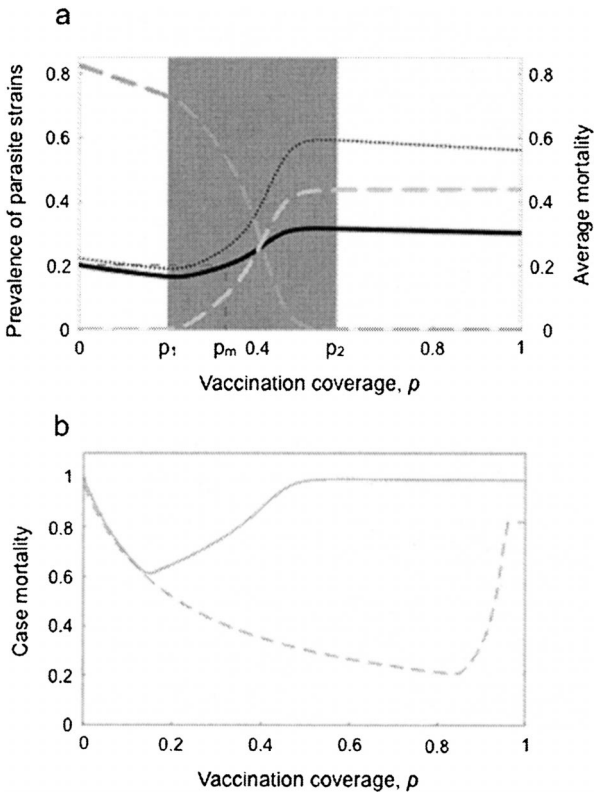

FIG. 7. Prevalence, average mortality, and case mortality at equilibrium against vaccination coverage. In (a) we plot the equilibrium prevalence of each parasite strain r_n and r_v (dashed gray and light gray lines, respectively), along with the average mortality among infected hosts (dotted line) and the average mortality among all hosts ($\bar{\bar{m}}$, solid line). The average parasite virulence is found as the average mortality among infected hosts minus the host natural death rate ($\bar{\alpha} = \bar{m} - \delta$). Above the threshold coverage p_m, the average host mortality among all hosts at equilibrium (solid line) is higher than in the absence of vaccination. Coexistence between the two parasite strains occurs in the gray region. All parameters are as in Figure 2. (b) The average case mortality of infections at equilibrium. All parameters are as in (a) except the parasite transmission ability $b = 10^{-4}$, the constant relating replication rate to unitary virulence $u = 10^{-5}$, and $\theta = 1$ (solid line) or $\theta = 5$ (dashed line). The beneficial effect of the vaccine on case mortality is amplified with large u and θ because the parasites are more constrained. As pointed out by André et al. (2003), a large increase of their replication rate reduces the ratio between transmissibility and virulence, $b/u(1 + r)^\theta$.

at stage T, the case mortality of a host of type i (n or v) infected by a parasite of type j (n or v, for naive-adapted or vaccine-adapted strain) is:

$$\chi_t^i = 1 - \sigma_i(T_t^i) - \bar{D}_t^i, \tag{10}$$

where $\bar{D}_t^i$ is the expected duration of the infection (all causes of stop confounded) and $\sigma_i(T_t^i)$ is the probability of host survival until clearance at time T_t^i (see Appendix 2 available online). As virulence, the case mortality induced by a vaccine-adapted parasite infecting a naive host (χ_n^v) is very high, while the infection of a vaccinated host by a naive-adapted parasite yields a very low case mortality (χ_v^n). However, in contrast with virulence, the case mortality of a vaccinated host infected by a vaccine-adapted strain is always lower than the case mortality of a naive host infected by a naive-adapted

strain ($\chi_v^v < \chi_n^n$). This results from the fact that vaccination and its effect on the replication rate of immune cells decreases the length of infection T. This reduced infection length increases the probability to survive. This yields:

$$\chi_n^v > \chi_n^n > \chi_v^v > \chi_v^n. \tag{11}$$

The overall consequence on the population of infected hosts can be evaluated with the average case mortality, $\bar{\chi}$, which is the expected probability of ultimate parasite induced host death of an infected host chosen randomly among all the newly infected ones. The overall effect of vaccination on average case mortality is presented on Figure 7b. In contrast with its effect on average virulence (Fig. 7a), vaccination (whatever the coverage) always yielded lower average case mortality than in the absence of vaccination. In other words, even when vaccination selects for more virulent strains, the probability of dying once infected is always reduced. As for average virulence, however, the average case mortality is minimized for intermediate values of vaccination coverage.

The cost of parasitism for the host population

In addition to these contrasting measures of mortality that focus on infected hosts, it may be important to weight these costs by the actual prevalence of the disease. This average host mortality is a function of both the virulence and the prevalence of the parasite in the different types of hosts:

$$\overline{\overline{m}} = \delta + \bar{\alpha}_n^n \frac{I_n^n}{N} + \bar{\alpha}_n^v \frac{I_n^v}{N} + \bar{\alpha}_v^n \frac{I_v^n}{N} + \bar{\alpha}_v^v \frac{I_v^v}{N}, \tag{12}$$

where $\overline{\overline{m}}$ is the expected mortality of a randomly chosen host (susceptible or infected, naive or vaccinated) in the population and $N = \Sigma_{i=n,v} S_i + \Sigma_{i,j=n,v} I_i^j$ is the total host population size. At demographic equilibrium, $\overline{\overline{m}}$ is also given by the inverse of the average expected life span of a newborn host, as calculated in the Appendix 5 (available online only at http://dx.doi.org/10.1554/05-220.1.s1). Therefore, $\overline{\overline{m}}$ is a synthetic measure of the parasite load on the entire host population, taking into account the effects of virulence levels, clearance rates, and transmission rates of each of the four types of infections as well as their respective prevalences.

Higher vaccination coverage may have a nonmonotonic effect on average mortality (Fig. 7a, solid line). First, increased coverage lowers average mortality because the prevalence of r_n is reduced. Second, host mortality may increase with higher coverage because of the increase of the prevalence of r_v. Finally, when r_v is fixed, higher coverage decreases mortality because of the increased protection of susceptible hosts. Note that, in Figure 7a, above a threshold value p_m of vaccination coverage ($p_1 < p_m < p_2$), the average mortality becomes higher than without any vaccine. However, if the parasite prevalence is low (e.g., when transmission ability, b, is low), the rise in virulence is compensated by the large prevalence reduction due to vaccination (see Fig. 5a, when b is low the average mortality cannot be increased by vaccination). In contrast with the average virulence, which is always increased at 100% coverage, the average host mortality may thus be reduced by the vaccine, even after parasite counter-evolution.

Discussion

The evolutionary analysis presented above explores the effect of different vaccination strategies (different coverages and different vaccines) on the long-term evolutionary outcome of the parasite population. In contrast with previous studies, the functional relationship between virulence and transmission (both within and between different hosts) is not imposed but derives from a mechanistic description of within-host dynamics. These functional relationships define the matrix of constraints that will shape the evolution of the parasite in this heterogeneous host population (Gandon 2004). The within-host dynamics (and the emerging matrix of constraints among traits) that we use in our model are similar to what has been observed for *Myxoma* virus (Best and Kerr 2000) and rodent malaria (Mackinnon and Read 2003): more virulent parasites have higher transmission rates and are cleared less rapidly by the immune system. Even more interesting is the observation that, in both these systems, immunity could select for more virulent parasites (Fenner and Fantini 1999; Mackinnon and Read 2004), as predicted by our models. The mechanistic approach developed in the present paper contrasts with the models of Gandon et al. (2001, 2003), who also studied the effects of vaccination but used artificially imposed functional relationships between virulence and transmission in naive and immunized (vaccinated) hosts. The present approach is also based on some artificially imposed assumptions (all models are), but they act at a lower level of the host-parasite interaction. Our approach yields very similar results but it also reveals new evolutionary and epidemiological outcomes. We first note these similarities and then discuss the differences.

The model analyzed by Gandon et al. (2001, 2003) showed that antigrowth vaccines may select for larger parasite growth rates and, consequently, for larger virulence (when measured on naive hosts). This is also what we found in this model, where the antigrowth effect is explicitly modeled. As in Gandon et al. (2001, 2003), we also found that such virulence evolution may erode the benefits carried by the vaccines or even yield higher overall mortality in the host population at intermediate coverage (when naive hosts are still present and get infected by vaccine-adapted parasites). Gandon et al. (2003) also pointed out how certain shapes of the trade-off between transmission and virulence may result in evolutionary bistability, where two different virulence strategies may be locally evolutionary stable. The mechanistic model used here shows that such trade-off shapes emerge easily from within-host dynamics when the vaccine efficacy is relatively high and thus leads to evolutionary bistability (compare Fig. 3 in this paper with fig. 3g in Gandon et al. 2003). We also explored the robustness of this result with alternative assumptions on the relationships between parasitemia, transmission, virulence, and recovery (e.g., using, the assumptions of Antia et al. 1994), but we recovered evolutionary bistability under these different assumptions (not shown). Such bistability thus appears to be a rather robust property of acute infections. Increasing replication rate above a resident parasite (adapted to naive hosts) is a costly strategy in naive hosts and carries hardly any benefit in vaccinated hosts unless replication rate is much higher (Fig. 1). As a consequence,

intermediate strategies are maladapted on both types of hosts and this yields evolutionary bistability.

In contrast with Gandon et al. (2001, 2003), our model showed that different virulence strategies could coexist. This is possible because in our model infected hosts can recover (i.e., be recruited back into the susceptible population). As a consequence, parasites indirectly affect the composition of the susceptible population, which yields negative frequency-dependent selection. In other words, the rarest parasite strain is favored because the type of host it is adapted to is the most frequent among susceptibles. This does not occur if clearance is not considered (e.g., Gandon et al. 2001, 2003) because the susceptible class is then only made of newborns and/or immigrants and is hence unaffected by local parasites.

A second difference from Gandon et al. (2001, 2003) is the asymmetry of the reproductive values in different hosts that emerge from the within-host dynamics. The naive-adapted parasite has a high reproductive value in naive hosts but a very small one in vaccinated hosts (Fig. 1). In contrast, the vaccine-adapted parasite, replicating faster, is able to infect both vaccinated and naive hosts with quite similar reproductive values (Fig. 1). This is qualitatively true with all the trade-off models tested (not shown). In acute infections the naive-adapted parasite is a specialist and the vaccine-adapted a generalist. Replicating too slowly is very costly for parasites in the race with immune system, whereas replicating too fast is only a matter of reducing slightly the reproductive output of the infection. A faster replication rate is therefore a way for parasites to enlarge their range of accessible hosts. But this fast-replicating generalist strategy carries some costs (otherwise it would displace the specialist). Indeed the maximal reproductive value on vaccinated hosts is lower than the maximal reproductive value on naive hosts. Why is adaptation to vaccinated hosts unable to restore the reproductive value of wild-type parasites in naive hosts? This effect of vaccination emerges from the properties of acute infections: with an exponentially growing immune system, the length of infection is strongly restricted, because the density of immune cells soon becomes incompatible with parasite replication. As a consequence, the only way for parasites to exploit vaccinated hosts is by generating very intense and yet short infections (see the high transmissibility in Fig. 2a). However, this strategy is costly (the cost of harming its host) and does not allow retrieving the original reproductive success of wild-types, because the efficiency of parasite transmission relative to virulence is necessarily reduced in intense infections (see also André et al. 2003). Indeed, in this model, the ratio between transmission and virulence is $b/[u(1 + r)^{\alpha}]$ and is thus a decreasing function of parasite growth rate.

Concluding Remarks

As noted in the introduction, an alternative evolutionary response of the parasite to immunity (naturally or artificially induced) or any kind of drug treatment is to hide from the action of these different resistance mechanisms and to become invisible. The emergence of escape mutants has been documented against antibiotics (e.g., Swartz 1994; Heinemann 1999) and vaccines (e.g., Carman et al 1990; Earn et al. 2002; Goulder and Watkins 2004). Our model does not allow the emergence of these mutants, but there are interesting similarities between the evolution of escape and virulence strategies. In both cases the evolutionary process can be reduced to the competition of a wild-type strategy against a treatment-adapted strategy (r_n and r_v in our model). In addition, the generalist ability of the vaccine-adapted strain, r_v, resembles the ability of the escape mutant to hide from host resistance. Furthermore, the cost often carried by escape mutants (e.g., Andersson and Levin [1999] in the case of antibiotic resistance and Smith [2004] for immune escape) is analogous here with the fact that, when measured on naive hosts, the virulent mutant has a lower reproductive value than the wild-type, $B_n(r_v) < B_n(r_n)$. For drug resistance, it has been shown that the cost carried by escape mutants can be compensated through the evolution along other phenotypic dimensions (e.g., Levin et al. 2000). In the same way here, although parasite evolution is considered only along one dimension (replication rate), various other parasite traits could evolve in response to vaccination and yield a reduction of the cost of adaptation to vaccinated hosts. Therefore, even if virulence and escape strategies may be viewed as very different evolutionary responses to host resistance, they are very similar and can be analyzed within a common framework. Such a framework may yield an integrated view over the multidimensional evolutionary response of parasites to medical interventions. Another important aspect that should be developed within this framework is the possibility to track simultaneously the short-term epidemiological and evolutionary dynamics after vaccination (Day and Proulx 2004; Day and Gandon 2005). When virulence is expected to increase (e.g., see Fig. 3), this would allow us to determine the speed of this evolution.

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