



## Evidences of parasite evolution after vaccination

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### ARTICLE INFO

#### Article history:

#### Keywords:

Virulence  
Vaccine  
Escape mutant  
Evolutionary medicine  
Infectious disease

### ABSTRACT

We present a brief review of some of the empirical evidence of parasite evolution in response to vaccination. The available data shows that very different pathogen strategies can be selectively favored as a result of vaccination. However, this data often lacks a qualitative and/or quantitative assessment of the benefits and the costs associated with these alternative strategies. Without this type of information to calibrate theoretical models it will be difficult to predict the potential risks associated with vaccine-induced evolution. Our purpose here is therefore to stimulate future research into quantifying these effects.

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### Introduction

Vaccination is one of the most efficient health policies for protecting human and animal populations against infectious diseases [1]. The immediate benefit associated with vaccination, however, may be rapidly eroded if vaccine-adapted variants of pathogens arise and spread in the population. But how likely is vaccine-driven evolution? Here we discuss six different, well-studied examples demonstrating the evolutionary potential of parasites in response to vaccination.

Before getting into the details of the different case studies it is necessary to lay out a clear and unambiguous language for discussing and comparing the various examples. We refer to the predominant strain (or strains) present prior to vaccination as the 'wild-type' strain, and to strains that are selectively favored in vaccinated hosts as 'vaccine-favored variants' (Fig. 1). The selective advantage of a vaccine-favored variant in a vaccinated host is typically believed to arise from differences from the wild type in one or more of three critical epidemiological parameters: its transmission rate between hosts, the recovery rate of the host infected with such a variant, and the mortality rate that the variant induces in the host. It is also commonly believed that vaccine-favored variants suffer some fitness cost in naïve hosts (Fig. 1), since otherwise one would expect them to reach appreciable frequencies, even in the absence of vaccination. As with the benefit enjoyed by a vaccine-favored variant, it is thought that this cost in naïve hosts also arises from differences from the wild type in one or more of the three epidemi-

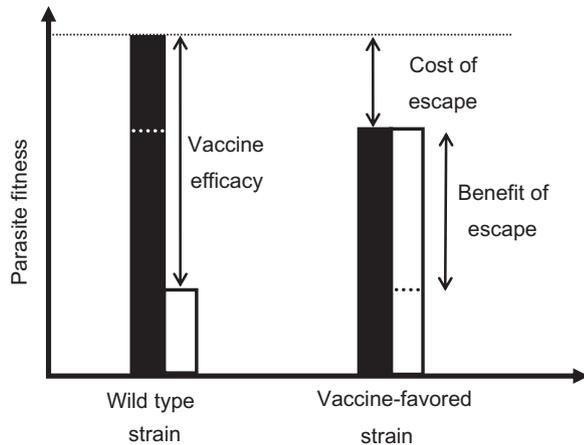
ological parameters mentioned above. Not surprisingly, these costs and benefits also play a central role in making predictions about the evolutionary consequences of vaccination [2], and therefore we focus predominately on them below.

For each of the six examples presented, we provide a brief background and then discuss the available evidence for how the costs and benefits of vaccine-favored variants are realized. We note at the outset, however, that aside from a very small number of experimental studies, most of the available evidence of vaccine-induced evolution comes from uncontrolled, observational studies. As such, it should be borne in mind that factors in addition to (or instead of) vaccination might well have played a role in the evolution of some of the parasites considered. Indeed, one of our main purposes with this article is to stimulate future research aimed at quantifying these costs and benefits.

### Hepatitis B virus

There are six main genotypes (A–F) of human hepatitis B virus (HBV), and they share a common immunodominant region on the outer protein coat (surface antigen), termed the *a* determinant. The neutralizing (protective) antibodies induced by vaccination target epitopes located within the *a* determinant. The use of HBV vaccines started in the 1980s in developed countries and dramatically reduced the incidence of the disease. However, there is now growing evidence that mutations occurring within the surface antigen allow replication of HBV in vaccinated people. In 1990 a mutation (a single amino acid substitution) in the S gene coding for the *a* determinant of the surface antigen was described [3]. Since then, several other HBV variants have been found in many countries. Interestingly these mutants have also been identified in unvac-

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**Figure 1.** Schematic representation of the comparison between a wild-type strain and a vaccine-favored variant. The lifetime reproductive success (a relevant measure of fitness at endemic equilibrium) is plotted in naïve hosts (in black) and in vaccinated hosts (in white) for both strains. The efficacy of the vaccine can be evaluated by the reduced performance of wild-type strain in vaccinated hosts. The cost of adaptation to vaccination is evaluated in naïve hosts while its benefit is evaluated in vaccinated hosts (in both cases, relative to the performance of the wild-type strain).

inated hosts [4], though in lower frequency than in vaccinated individuals. This suggests that, although there is likely a cost associated with these variants (in terms of their fitness in naïve hosts; Fig. 1), it might be relatively small. The epidemiological models of Wilson et al. [5,6] assume that the vaccine-favored variant remains infectious to naïve hosts but that its transmission rate is lower than the wild type. Consistent with this hypothesis is the fact that some escape variants have been shown to remain infectious and pathogenic in unvaccinated chimpanzees [40]. Unfortunately there is no experimental evidence on the nature and the magnitude of these costs. Currently not much is known regarding the epidemiological parameters through which the vaccine-favored mutants gain their advantage in vaccinated hosts, but it seems plausible that they enjoy both an increased duration of infection (i.e., reduced recovery) as well as an increased rate of transmission.

### Pertussis

Pertussis (or whooping cough) is a highly transmissible bacterial infection of the respiratory tract, and was a major cause of childhood morbidity and mortality in the prevaccination period. In the 1950s several countries started mass vaccination programs against *Bordetella pertussis*, with a whole cell vaccine made out of different strains. This effectively reduced the incidence of pertussis, but in many countries pertussis remains an endemic disease. Worse still, in some highly vaccinated populations the incidence of pertussis has started to increase again since 1990s. It has been suggested that this reemergence could be due to the spread of vaccine-favored variants. For example, Mooi et al. [7,9] showed that new mutations in two surface proteins, pertactin and pertussis toxin, have appeared after the start of vaccination, replacing the variants which are found in the pertussis vaccines. Both proteins are virulence factors and induce protective immunity in humans and other animals. These mutations may thus be involved in evading the immune response induced by vaccination. In fact these new variants were observed more frequently among vaccinated individuals than in unvaccinated individuals [7]. This suggests a potential selective advantage in vaccinated hosts and/or a cost in naïve ones. Experimental results with vaccinated and unvaccinated mice are partially consistent with this hypothesis. The more recent variants perform relatively better (i.e., reach higher densities) in vaccinated

mice than the wild type [8,9]. Furthermore, in naïve mice the variants were found to have an overall lower performance than the wild type (Ref. [9]; Mooi, personal communication). However, no information is available yet regarding the impact of these mutations on the epidemiological parameters of transmission, recovery rate or virulence.

### *Streptococcus pneumoniae*

*S. pneumoniae* consists of 90 known serotypes (types of capsular polysaccharides) with variable prevalence and virulence [10]. In contrast with HBV vaccines, pneumococcal vaccines have been designed to target a subset of the circulating serotypes. The vaccines used in three clinical trials include only between 7 and 11 serotypes. Several studies reported a decrease in the incidence of the disease but also showed evidence of serotype replacements (reviewed in Ref. [11]). The serotypes that were not included in the vaccines reached higher frequencies in vaccinated individuals in three independent clinical trials of pneumococcal conjugate vaccines [12–14]. In this situation, the vaccine-favored variants were already present at appreciable frequencies before vaccination, however, suggesting that the costs associated with these variants are relatively low. As with the case of HBV vaccines, virtually nothing is known about the epidemiological parameters through which the vaccine-favored mutants gain their advantage, but again it is plausible that this comes about through both an increased duration of infection (i.e., reduced recovery) and an increased transmission rate.

### Marek's disease

Marek's Disease Virus (MDV) is an avian herpes virus that causes substantial losses in the poultry industry. Vaccination started in the 1950s but new virus strains rapidly emerged. These strains have the ability to infect and exploit vaccinated birds. Witter [15] showed experimentally that these emerging strains are more able to cause disease than ancestral strains in both naïve and vaccinated hosts. However, in contrast with the previous examples, there is evidence that these mutants are also much more virulent (i.e., they induce more extreme symptoms) in both naïve and vaccinated individuals [15–17]. Interestingly, it appears as though subsequent generations of vaccines have then been followed by even further increases in virulence. This increased virulence may be viewed as one component of the cost (induced death may limit transmission) associated with a better performance on vaccinated hosts. This particular disease seems like a very promising case in which experimental quantification of the costs and benefits of vaccine-favored variants would be readily possible.

### Malaria

Several types of vaccines are currently developed against human malaria [18–20]. Although there is not yet any conclusive indication of how these vaccines affect malaria evolution, at least one study in humans has demonstrated that vaccination exerts selective pressure in favor of strains not included in vaccines [21]. Furthermore, there is experimental evidence from rodent malaria that vaccination can affect virulence evolution. Mackinnon and Read [22] performed an experimental evolution study with the rodent malaria *Plasmodium chabaudi* in laboratory mice. They evolved multiple lines of *P. chabaudi* in immunized and naïve mice by repeated serial passage of blood-stage parasites. After 20 passages they observed that the lines that evolved in immunized mice became more virulent in both naïve and immunized mice. This suggests

that these lines adapted to immunized mice by increasing their level of host exploitation. In support of this hypothesis Mackinnon and Read [23–25] showed that the more virulent strains persist at higher densities and for longer. Thus, as for MDV, the more virulent strains could be viewed as vaccine-favored variants where the cost associated with this adaptation would be the increased virulence in naïve mice. The benefit of this increased virulence would be a consequence of the covariances among the different life-history traits [23–25] leading to increased transmissibility and decreased recovery rate in immunized hosts.

## Diphtheria

The bacteria *Corynebacterium diphtheriae* causes human respiratory infections, and the disease that it induces is caused by toxin production. Although we failed to find any experimental evidence, it is generally assumed that toxin production (governed by a *tox* gene carried by a bacteriophage) confers a competitive advantage to the bacteria [26,27]. The synthesis of the toxin, however, carries metabolic costs. Toxin production may represent as much as 5% of the total bacterial protein synthesized [28]. Diphtheria vaccines started to be used in the 1920s. The peculiarity of these vaccines is that they are not directed towards the organisms producing the toxin but towards the toxin itself. Such toxoid vaccines are made by treating the toxin with heat or chemicals to destroy its ability to cause illness while retaining the capacity to stimulate protective immunity. From the parasite point of view, this antitoxin immunity removes the benefit of producing the toxin and, since this toxin is costly, could select for variants that do not produce the toxin (i.e., *tox*– strains). These strains can be viewed as vaccine-favored variants because, without paying the cost of toxin production, they can exploit vaccinated hosts more efficiently than the wild type (i.e., *tox*+ strains). Pappenheimer [26] reports that this type of evolution may have occurred in Romania where an intensive vaccination program was carried out between 1958 and 1972. During this period the fraction of immune individuals rose from 60 to 97%, while the morbidity fell from 600 per 100 000 to only one per 100 000 individuals. Interestingly, a survey of the characteristics of the *C. diphtheriae* carried by the population during the same period of time revealed that the proportion of the *tox*+ strains dropped from 87 to only 4%. In contrast, in other countries, diphtheria remains endemic in spite of vaccination [29,30]. It should also be noted, however, that the potential impact of the vaccine on toxin production remains controversial. Given that the diphtheria toxoid vaccine is imperfect [31] the production of *more* toxin may be an alternative way to overcome the effect of the toxoid. Depending on the magnitude of the cost and the benefit associated with the toxin, vaccination may either select for or against toxin production [17,32]. Unfortunately there does not yet appear to be any quantitative estimates of these parameters.

## Discussion

The above empirical examples show that vaccine-favored variants do occur and, in some cases, may even be implicated in the reemergence of disease. Nevertheless, it is clear that there is a lack of information regarding the nature and the magnitude of the costs incurred by these mutants in naïve hosts, as well as the benefits that they gain in vaccinated hosts (see Fig. 1). These factors are crucially important since they strongly influence the evolutionary consequences of vaccination [2]. For example, previous theoretical studies have demonstrated that if the costs paid by vaccine-favored variants in naïve hosts are expressed through increased virulence

(as seems to be the case in Marek's disease and rodent malaria), then we would expect vaccination to induce the evolution of higher levels of virulence (when measured in naïve hosts) because it drives such variants to higher frequency (Refs. [33–36], see other papers in this issue). On the other hand, if the costs are paid through other epidemiological parameters such as reduced transmissibility, then this is not typically the case [37–39,5]. Therefore one critical aspect of future empirical work in this area will be to determine the nature of these costs and benefits for different parasite strains. We hope that this article, in some way, stimulates such research and motivates collaborative research projects between scientists studying vaccines and evolutionary biologists.

## Acknowledgements

We would like to thank Margaret Mackinnon, Frits Mooi and Andrew Read for discussions and comments on an earlier draft of this paper. S.G. was supported by the Centre National de la Recherche Scientifique (France) and by an ANR grant “jeunes chercheurs”. T.D. was supported by a grant from the Natural Sciences and Engineering Research Council of Canada and the Canada Research Chairs program (Canada).

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