CHAPTER 11

Pathogen evolution in a vaccinated world

Andrew F. Read and Margaret J. Mackinnon

Introduction

The evolution of drug resistance undermines the effectiveness of chemotherapy. In contrast, vaccines do not fail with the same depressing regularity in the face of pathogen evolution. Indeed, vaccination has eradicated one human disease, provides robust control in the developed world of another eight, and protects individuals against over a dozen more. Rightly, vaccination is viewed as a medical triumph. Yet it is argued that the long-term control of acute childhood diseases like smallpox, polio, and measles does not mean vaccines are evolution-proof. The pathogens now being targeted are quite different from the organisms responsible for those diseases, and some of the vast evolutionary experiments currently being conducted with vaccines are generating pathogen evolution. As shall be seen, a variety of evolutionary responses to vaccination are possible, including the evolution of more virulent pathogens. In general, little is known of what will happen and how evolution can be directed for human betterment. For much of the past century, the development and use of new drugs took place with little consideration of the evolutionary consequences (cf. Chapter 10). It is our contention that we should not repeat that complacency with vaccines: their evolutionary consequences need to be understood so that the benefits of this most successful of disease control measures can continue to be reaped.

We structure our discussion around the following superficially attractive statements. As shall be seen, each is at least partly wrong.

- Vaccine-induced immunity simply replaces natural immunity, and so vaccination has no consequences for pathogen evolution.
- Vaccination has worked effectively for more than a 100 years. If vaccine-driven evolution were going to cause problems, it would be obvious by now.
- Even if vaccine-resistant mutants do evolve, they will do us less harm than wild type pathogens.

Vaccines have consequences for pathogen evolution

Host immunity imposes massive selection on pathogen populations: host–parasite interactions are one of the richest of evolutionary battlefields. But unlike drugs, which impose totally novel selection pressures, vaccines work by eliciting immune mechanisms generated by natural infection in any case. Unfortunately, as shown by the examples of the following eight diseases, this does not make vaccines evolution-proof.

Hepatitis B

Hepatitis B virus (HBV) is a globally significant cause of hepatitis, liver cirrhosis, and liver cancer. An effective vaccine has been used as part of national childhood vaccination programs since the early 1980s. These campaigns have dramatically reduced the prevalence of the virus and the associated disease (FitzSimons *et al*. 2005). The vaccine contains recombinant hepatitis B surface antigen (HBsAg). The so-called *a* determinant of HBsAg is the major target for the neutralizing antibodies

produced during natural infection or following vaccination. In 1990, the first single amino-acid substitution in the S gene coding for the *a* determinant was reported; since then more mutants have been discovered. These mutants exist at low frequencies in unvaccinated individuals and at least some are transmissible. A key feature is that these mutants can coexist with vaccine-induced anti-HBsAg antibodies; indeed, they are often responsible for vaccine breakthrough. Importantly, there is good evidence that these mutants are more often found in vaccinated individuals, and that they are increasing in frequency in vaccinated populations (Fig. 11.1) (François *et al*. 2001; Hsu *et al*. 2004; FitzSimons *et al*. 2005). Thus, HBV populations are evolving in response to widespread vaccination. Proposals to incorporate other HBV antigens into the vaccine are being considered (François *et al*.

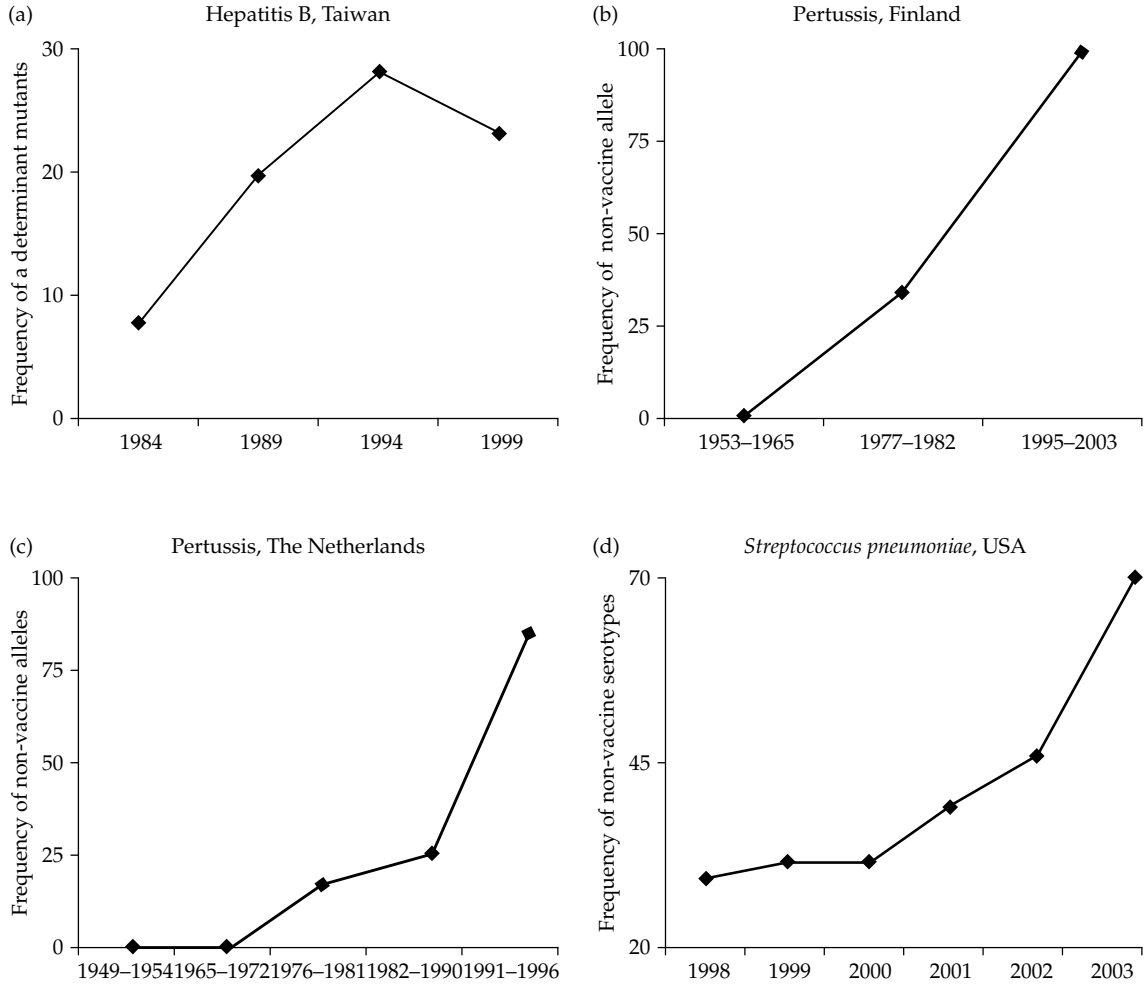

Figure 11.1 Examples of the evolution of vaccine-adapted mutants following the introduction of widespread vaccination. (a) The spread of mutant forms of the *a* determinant of the surface antigen of hepatitis B virus in Taiwan after universal vaccine began in 1984 (data from Hsu *et al*. 2004). (b) The spread of non-vaccine alleles of pertactin in *Bordetella pertussis* populations in Finland (data from Elomaa *et al*. 2005) and (c) The Netherlands (data from van Loo *et al*. 1999). Widespread pertussis vaccination was introduced into those countries in 1952 and 1953, respectively. (d) Increasing frequency of non-vaccine serotypes of *Streptococcus pneumoniae* after the introduction in the United States in 2000 of a pneumococcal conjugate vaccine containing 7 of 90 possible serotypes (data from Flannery *et al*. 2006). Note that in all cases, vaccination substantially reduced disease incidence.

2001; FitzSimons *et al*. 2005; Kimman *et al*. 2006), but it is not obvious that these will obviate the problem, for variants at these other sites occur, presumably because they also provide at least some escape from naturally acquired neutralizing antibodies.

Pertussis

Also known as whooping cough, pertussis is a respiratory disease caused by the bacterium *Bordetella pertussis*. The introduction of whole-cell vaccines in the middle of the last century resulted in dramatic decreases in disease incidence. The virulence factors of *B. pertussis* can be divided into adhesins, such as pertactin (*prn*), and toxins, such as pertussis toxin (*ptx*). Adhesins facilitate attachment to the host, and toxins are involved in immune evasion and possibly resource extraction. Many of these virulence factors are polymorphic, and major changes in allele frequencies have been recorded worldwide (van Loo *et al*. 1999; Hallander *et al*. 2005; Hardwick *et al*. 2007), some of which are associated with vaccination. For instance, in Finland and Holland, the frequency of the vaccine-type pertactin allele (*prn1*) went from essentially 100% in the pathogen population to less than 5% after the introduction of nationwide vaccination (Figs. 11.1b,c) (van Loo *et al*. 1999; Elomaa *et al*. 2005). Similarly, in several countries, the *ptxA1* allele, not present in the vaccine strains, has replaced the vaccine alleles (Elomaa *et al*. 2005; van Amersfoorth *et al*. 2005). Non-vaccine alleles tend to be more frequent in vaccinated individuals than in unvaccinated individuals (Mooi *et al*. 1998; Mastrantonio *et al*. 1999).

Pneumococcal disease

Acute infections with the bacterium *Streptococcus pneumoniae* cause pneumococcal disease, which can present as meningitis, septicemia, and pneumonia, and which is an important cause of death among infants and the aged. *Streptococcus pneumoniae* has about 90 known serotypes (types of capsular polysaccharides); they vary in prevalence and virulence. In the 1990s, clinical trials with vaccines containing 7–11 of these polysaccharides showed decreases in these targets and increases in non-vaccine strains, a phenomenon known as strain (serotype) replacement (Lipsitch 1999). Following the widespread use of such vaccines in childhood immunization programs, strain replacement is now visible at a population level. For instance, in the United States, widespread use of a 7-valent conjugate vaccine began in 2000. Disease incidence has declined dramatically, but non-vaccine serotypes are now increasing in frequency both among disease cases (Fig. 11.1d) and in the asymptomatic carriage population (McEllistrem *et al*. 2003; Huang *et al*. 2005; Flannery *et al*. 2006). The increase in non-vaccine serotypes can fully compensate for the decline in the vaccine serotypes, resulting in no net change in *S. pneumoniae* prevalence in the community (Huang *et al*. 2005). Some of the evolution may be a consequence of a simple rise in the frequency of strains with non-vaccine capsular types filling the niche vacated by the strains targeted by the vaccine, although it is also possible that existing strains are acquiring non-vaccine capsular polysaccharides (Porat *et al*. 2004).

Diphtheria

Infections with toxin-producing strains of the bacteria *Corynebacterium diphtheriae* can cause respiratory disease characterized by lesions in the upper respiratory tract, particularly the tonsils, pharynx, larynx, and nose. The disease lesions are due to a specific phage-encoded cytotoxin; widespread immunization with the detoxified toxin ('toxoid' vaccine) has reduced the disease from a major child killer to one rarely seen at all, at least in rich countries. Various authors have attributed this to reductions in the frequency of the toxin-encoding phage in the bacterial population (e.g. Pappenheimer 1984; Ewald 1994, 1996, 2002; Soubeyrand and Plotkin 2002). So far as we are aware, the only data showing this evolution come from Romania during the third quarter of last century (Fig. 11.2). Here, the frequency of *C. diphtheria* strains that were toxigenic declined from over 80% to less than 5% after the introduction of the vaccine (Pappenheimer 1982).

Malaria

There is currently no malaria vaccine, but many candidate vaccines are in various stages of trial.

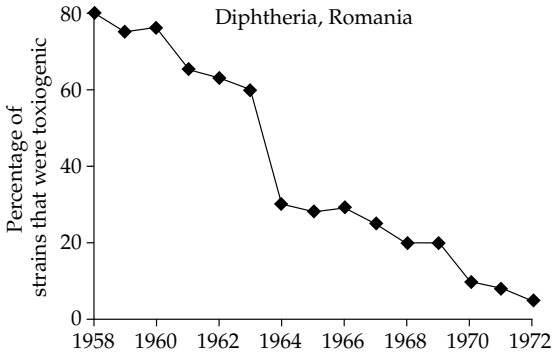

Figure 11.2 Decreasing frequency of toxin-producing strains of *Corynebacterium diphtheriae* in Romania after the introduction of widespread immunization with the diphtheria toxoid vaccine in 1958 (data from Pappenheimer 1982).

One of these trials demonstrated that vaccine-driven evolution is going to be an important consideration. The vaccine, known as Combination B, contained several antigens encoded by different *Plasmodium falciparum* genes. Antigenic loci in malaria are notoriously polymorphic, and Combination B contained a single antigen from each of three polymorphic loci. One of these loci, *msp2*, is dimorphic in nature, where a single parasite has one of two allelic forms. The vaccine contained just one of these forms. In a Phase II vaccine trial in Papua New Guinea, the vaccine did not protect against disease, but the non-vaccine allele rose in frequency in the vaccinated people (Genton *et al*. 2002). This sort of evolution—selection against vaccine strain alleles—is a major concern for malaria vaccine developers (e.g. Mahantry *et al*. 2003; Matuschewski 2006).

Avian influenza

In 1995, the Mexican government was one of the first to use vaccination to try to control H5N2 avian influenza in poultry with very widespread immunization of commercial chickens. The antigenic variants that existed prior to vaccination were well controlled by vaccine-induced immunity, but new lineages arose after vaccination that replaced the originals. These new viral lineages are antigenically distinct and less successfully controlled by the vaccine (Lee *et al*. 2004). Similar evolution has been seen in H9N2 influenza viruses following widespread vaccination of poultry in China (Li *et al*. 2005). This may mean that poultry influenza vaccines need continual modification to track viral evolution in response to vaccine-induced immunity (Lee *et al*. 2004), just as human influenza vaccines need to track viral evolution in response to natural immunity.

Marek's disease

Marek's disease virus (MDV) is a cancer-causing herpes virus that costs the global poultry industry more than US$1 billion annually. The virus became economically important with the intensification of the chicken industry after WWII. In the United States, vaccination of chickens with live virus from a related non-oncogenic strain was used from the late 1960s. This first generation vaccine initially provided good control, but within a decade it was not providing adequate protection against virulent viral strains that appeared in the 1970s. In the 1980s, a second generation vaccine consisting of two non-oncogenic strains was introduced, but this too began to fail as more virulent strains subsequently evolved. In the 1990s a third generation vaccine consisting of an attenuated form of an oncogenic strain was introduced. Losses have once again subsided, but there is great concern in the poultry industry that the third generation vaccine may eventually be undermined by the evolution of even more pathogenic strains. Importantly, the two generations of vaccine that failed were undermined by strains antigenically identical to the oncogenic strains of the pre-vaccine era. Changes in viral aggression and immunosuppressive capacity, not antigenic type, caused the vaccine failure (Fig. 11.3) (Witter 2001; Davison and Nair 2004).

Infectious bursal disease (IBD)

IBD is an immunosuppressive disease of chickens caused by a birnavirus and is responsible for many cases of respiratory and enteric disease. From the mid 1980s, vaccination failures began to be described in poultry operations around the world. In the United States, vaccine breakthrough was due to the evolution of antigenically novel strains

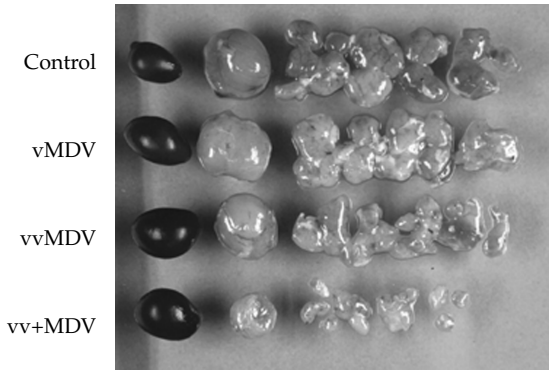

Figure 11.3 Lymphoid organs of chickens: normal chicken (control) and those infected with vaccine-breakthrough strains of MDV. Virulent MDV (vMDV) strains were responsible for the downfall of first generation of anti-MDV vaccines, and successively more virulent strains (vv and vv+) for the downfall of second generation vaccines. Photo by B. W. Calnek, with permission.

against which classical IBDV vaccines were not sufficiently protective. In contrast, vaccine-breakthrough strains in Europe belong to the ancestral IBDV serotype but were instead more aggressive. Like the newly evolved MDV strains, these very virulent European strains cause more severe disease in unvaccinated birds, with mortality rates of up to 60% (van den Berg 2000; Rautenschlein *et al*. 2005; Le Nouën *et al*. 2006).

Thus, vaccines are not evolution-proof

These examples show that vaccines can provoke and even be overcome by pathogen evolution. Most obviously, vaccines that target a subset of strains can give a competitive edge to those not present in the vaccine. But even in the absence of strain-specific effects, widespread vaccination can alter the immune pressures pathogens experience. By reducing the number of fully susceptible hosts in a population, vaccine programs alter the likelihood that a pathogen will encounter and evolve in non-immune hosts. Vaccination can also create a new niche of weakly immune hosts. Where the relative fitness of competing pathogen strains depends on the immune status of their host, changing immune profiles of a population will prompt pathogen evolution.

In extreme cases, vaccines can even be the main or only source of pre-existing immune selection. In many intensified farming situations, such as the poultry and pig industries, large numbers of fully susceptible individuals come together for relatively short periods. Here, naturally acquired immunity will often have little impact on pathogen evolution, partly because major efforts are made to prevent natural infections in the first place, but mostly because animals are slaughtered before natural immunity has time to build up in the host population (Lee *et al*. 2004; de Jong *et al*. 2007). When epidemics do occur, vaccine-induced immunity will be a major source of immune selection on the pathogen.

Why has vaccination worked despite evolution?

If vaccine adaptation is possible, why have pathogens subject to vaccination for many decades not evolved and caused the failure of immunization programs? Vaccination successfully eradicated smallpox and is close to eradicating polio. It has provided sustainable control for several decades against several other diseases, including measles, pertussis, diphtheria, mumps, and rubella. In this section, we argue that one should not draw from this history the lesson that evolution is unimportant. The success stories to date concern a peculiar subset of infectious diseases. The lesson of Marek's disease is salutary: vaccines can fail in the face of pathogen evolution. It is simply too early to be confident that MDV will be the exception rather than the rule.

Not all infectious diseases are alike

The vaccine success stories involve acute childhood infections. A striking feature of the natural history of these diseases is that first infections invoke immunity that is sterilizing, strain-transcending, and usually life-long. Such acute infections either kill their host or are rapidly cleared. The pathogens persist by exploiting susceptible individuals, typically non-immune children. Why natural selection failed to find these organisms a way to penetrate previously exposed hosts is unclear, but it is evident that it did not. There must have been intense selection on all of them to break though natural

immunity in the pre-vaccine era. An evolutionary solution would have been no easier in the vaccine era. Acute childhood diseases were easy targets for vaccination: natural immunity was already evolution-proof; all that was needed was for vaccines to induce something similar.

In the case of smallpox, eradication did not even require vaccines to elicit the evolution-proof level of immunity produced by natural infections. The proportion of people that need to be vaccinated to eradicate a disease is determined by R_0, one measure of pathogen fitness. R_0 for smallpox is one of the lowest for any human disease, and any mutants able to escape vaccine-induced immunity would have had an even lower R_0 (or else they would have evolved anyway). Escape mutants would thus be even easier to eradicate than wild type. Only if the smallpox vaccine had been very weakly cross protective could any epitope variants have escaped eradication and saved the species (McLean 1995).

The diseases that are the focus of much of today's vaccine development differ notably from acute childhood infections. The populations of pathogens causing diseases like flu, malaria, and pneumococcal disease frequently consist of a rich diversity of strains able to successfully infect previously infected individuals. Individual infections of diseases like HIV, sleeping sickness, tuberculosis, and malaria are often chronic, with infections persisting in partially immune hosts due to immunosuppression and antigenic variation. These 'hard diseases' are a great challenge for vaccine developers, arguably because for these pathogens, natural selection has found them an evolutionary solution to natural immunity: antigenic flexibility.

Moreover, in contrast to vaccines against the successfully controlled diseases that induce sterilizing immunity, many vaccines against other diseases leak, allowing wild type pathogens to transmit though vaccinated hosts. The absence of sterilizing immunity makes it possible for natural selection to probe vaccine-induced immunity for weaknesses. Notably, with Marek's disease, where two generations of vaccines had to be abandoned in the face of viral evolution, immunization is extremely leaky (Islam *et al*. 2006).

Is it too soon to be confident?

Even for some of the acute childhood infections, it may be too early to say what the evolutionary outcome will be. A standard result in population genetics (and the theory of drug resistance) is that advantageous mutations even under strong selection can be spreading in a population for some time—even decades—before they become detectable. Mathematical models of vaccine-driven evolution show the same thing: it might take decades to tip the balance in favor of vaccine-resistant strains (McLean 1995; Wilson *et al*. 1999; Gandon *et al*. 2001). This is particularly so when vaccine coverage is low to begin with (e.g., Hepatitis B) and when the favored mutant is initially rare in the population.

Moreover, vaccines of the future might also impose novel immune selection. Whereas current vaccines work by mimicking natural immunity, many vaccine developers are deliberately attempting to stimulate immunity targeted at pathogen epitopes not seen by natural immunity, sometimes with effector mechanisms not deployed naturally. Frequently a motivation behind such attempts is to avoid the antigenic polymorphism of loci seen by natural immunity (e.g., Alonso *et al*. 2005; Matuschewski 2006). Such technical breakthroughs have the potential to impose completely novel selection pressures, just as drugs do.

Pathogen adaptation in vaccinated populations

In this section, we survey the sorts of pathogen phenotypes that vaccine-driven evolution might produce. In the next, we use this framework to address the consequences of these different evolutionary outcomes for public and animal health.

To study the evolutionary responses of pathogens to vaccine-imposed selection, it helps to consider the fate of pathogen variants that differ in some way from wild type pathogens. Wild type pathogens are those favored by natural selection in unvaccinated populations and, following Gandon and Day (2007), we refer to variants that rise in frequency after the introduction of widespread immunization as vaccine-favored or vaccine-adapted variants. These could also be called 'vaccine-escape'

or 'vaccine-resistant' mutants. We are tempted to use those terms because they capture the essential concept, but in much of the literature 'escape' and 'resistance' are equated with mutations at protective epitopes. For instance, François *et al*. (2001: 3803) explicitly equate vaccine escape in HBV with mutations in the envelope genes that result 'in nonrecognition by neutralizing antibodies induced by vaccination'. As we will argue below, epitope alterations are only one possible mechanism of vaccine adaptation.

To simplify matters, we hereafter refer to vaccine-favored variants as mutants, although they might be existing strains, genotypes or serotypes, or de novo mutants. All that matters is that their phenotypes be heritable. To rise in frequency, these vaccine-adapted mutants must be selectively favored in vaccinated hosts. This may mean that they are better able to invade/infect/penetrate the defenses of a vaccinated host, or that once inside the host, they have a higher per day transmission rate, or that they are cleared less quickly by the immune system than are wild type strains.

In principle, parasite adaptation to immunized populations could involve a variety of different pathogen phenotypes. We argue that only a subset of this variety has been so far looked for—and therefore seen—and that broader thinking on this is required. Two pathogen traits have been the focus of previous considerations: epitope evolution (*sensu* François *et al*. 2001) and virulence evolution. Biomedical scientists have been concerned with the former; the latter has recently received some attention from evolutionary biologists. The distinction is somewhat artificial (epitope variants can differ in virulence), but we begin with these traits for historical and heuristic reasons.

Epitope evolution

Vaccine-adapted mutants can arise by alterations in the genes encoding the pathogen epitopes that are recognized by vaccine-induced immunity. Some of these changes can make the structure of the protein they encode differ so much from wild type that the mutants are not recognized by vaccine-primed host responses. Epitope variants are well known in many diseases and are in part responsible for the chronic nature of many viral and protozoal diseases and the slow acquisition of protective natural immunity in diseases such as malaria. Epitope variants are a major cause of vaccine breakthrough in individual patients in several diseases including hepatitis B, whooping cough, and pneumococcal disease, where epitope differences among the capsular polysaccharides and surface antigens are responsible for the evolution seen in populations immunized against a subset of circulating strains (Fig. 11.1).

Virulence adaptation

A different class of possible outcomes is what might be called 'virulence adaptation.' Here, widespread immunization imposes direct selection on pathogen virulence determinants, so that the subsequent evolution involves the adjustment of intrinsic pathogen virulence, the virulence observed when the pathogen infects an immunologically naïve host. We define virulence as the harm done to hosts following infection.

- *Decreased virulence*—The intrinsic virulence of diphtheria went down in Romania after the introduction of a toxoid vaccine (Fig. 11.2). The standard explanation for this evolutionary outcome is as follows (Pappenheimer 1984; Ewald 1994, 1996, 2002; Soubeyrand and Plotkin 2002). Virulence is due to the production by the bacteria of a toxin that enhances pathogen fitness by allowing the bacteria to obtain nutrients when resources in the immediate vicinity are scarce. This gives toxin-producing strains (tox+) a fitness advantage over toxin-less (tox–) strains in unvaccinated hosts. But the toxoid vaccine induces anti-toxin immunity, reducing the benefits of producing the toxin. This immunity does not target the pathogen directly, just its products. The toxin constitutes as much as 5% of the total protein synthesized by the bacterium. Consequently, tox– strains are better adapted to an immunized population because they avoid the metabolic costs of producing toxin for little gain. Hence intrinsic virulence evolves downwards.
- *Increased virulence*—More recently, we proposed that vaccines could drive virulence in the opposite direction (Gandon *et al*. 2001, 2003; Read *et al*. 2004; Mackinnon *et al*. 2007). Our argument

is a logical consequence of the best-studied theory of why evolution does not always produce benign parasites. The virulence trade-off hypothesis posits that there are fitness benefits associated with virulence, as well as costs. The benefits (why selection favors virulent strains at the expense of the avirulent) are assumed to be the production of more transmission forms per unit time and/or longer time before immune clearance. The cost of virulence (why selection penalizes excessively virulent strains) is the truncation of the infectious period by host death. Natural selection favors those pathogen strains able to optimally balance these costs and benefits to maximize overall fitness. Further details of the trade-off model and other theories for the evolution of virulence are given by Ebert and Bull (Chapter 12).

For vertebrate diseases, the strongest evidence supporting the trade-off model comes from myxomatosis and our own experimental work with rodent malaria in laboratory mice (Mackinnon and Read 2004a; Mackinnon *et al*. 2007). In *Plasmodium chabaudi*, we have documented genetic variation in virulence on which selection can act. Host death shortens infectious periods, but more virulent strains transmit more successfully, are less rapidly cleared by the host, and have an advantage in competition with less virulent strains. Importantly for what follows, these advantages of virulence also accrue in immunized hosts (Fig. 11.4).

Now, consider what might happen if a new vaccination program is used to attack a pathogen that is optimally balancing the costs and benefits of virulence. Presumably a vaccine is used because it protects the host against death. This means the fitness cost of virulence—the force selecting against virulence—is relaxed by vaccination. Since there are still fitness benefits of virulence, more aggressive strains will spread in vaccinated populations because they are now less likely to kill the host. Even if vaccination reduces pathogen titers and transmission rates, virulent strains will still produce more transmission stages than less virulent strains. In fact, in an immunized host, they may produce disproportionately more transmission stages if immunity is more effective against less aggressive strains. Consequently, vaccinated individuals create the conditions that favor the spread of intrinsically more virulent parasites.

This verbal argument is supported by rather general mathematical models (e.g., Gandon *et al*. 2001, 2003; Porco *et al*. 2005; André and Gandon 2006; Ganusov and Antia 2006; Massad *et al*. 2006; Miller *et al*. 2006). More specific population-level models, parameterized for endemic high-transmission malaria, confirm the argument and show that the evolution of virulence can take place on time scales of a few decades, comparable to the time taken for resistance to drugs like chloroquine to become clinically relevant (Gandon *et al*. 2001).

An important corollary to this argument is that the mode of action of the vaccine matters: the vaccines must leak (allow at least some transmission of wild type pathogens) and reduce disease (risk of death). Vaccines that stop hosts from becoming infectious do not alter the relative costs and benefits of virulence: they thus do not directly drive virulence evolution. Indeed, transmission reduction alone can even impose minor downward selection on virulence indirectly via epidemiological processes. Less transmission means fewer multiply infected hosts (Gandon *et al*. 2001), and in theory (e.g., Frank 1996) and for malaria in practice (de Roode *et al*. 2005; Bell *et al*. 2006) within host competition can favor virulence.

Strikingly, outbreaks of virulent strains in vaccinated populations have been seen in two poultry diseases, Marek's disease and infectious bursal diseases (see above), as well as in feline calcivirus disease in domestic cats (Hurley *et al*. 2004; Coyne *et al*. 2006; Radford *et al*. 2006). In all three cases, virulence evolution has eroded vaccine efficacy, and hosts infected with these vaccine-breakthough strains are at greater risk of death. While it is difficult to know whether these strains have arisen in response to vaccination, in all three cases, vaccination provides less protection against the virulent strains than it does against progenitor strains. Similarly, experimental evolution of rodent malaria parasites showed that immunization promoted the evolution of virulence (Mackinnon and Read 2004b). Because from the pathogen's perspective on virulence evolution, genetic resistance is vaccination by another means, we also mention that the evolution of more resistant rabbits in Australia

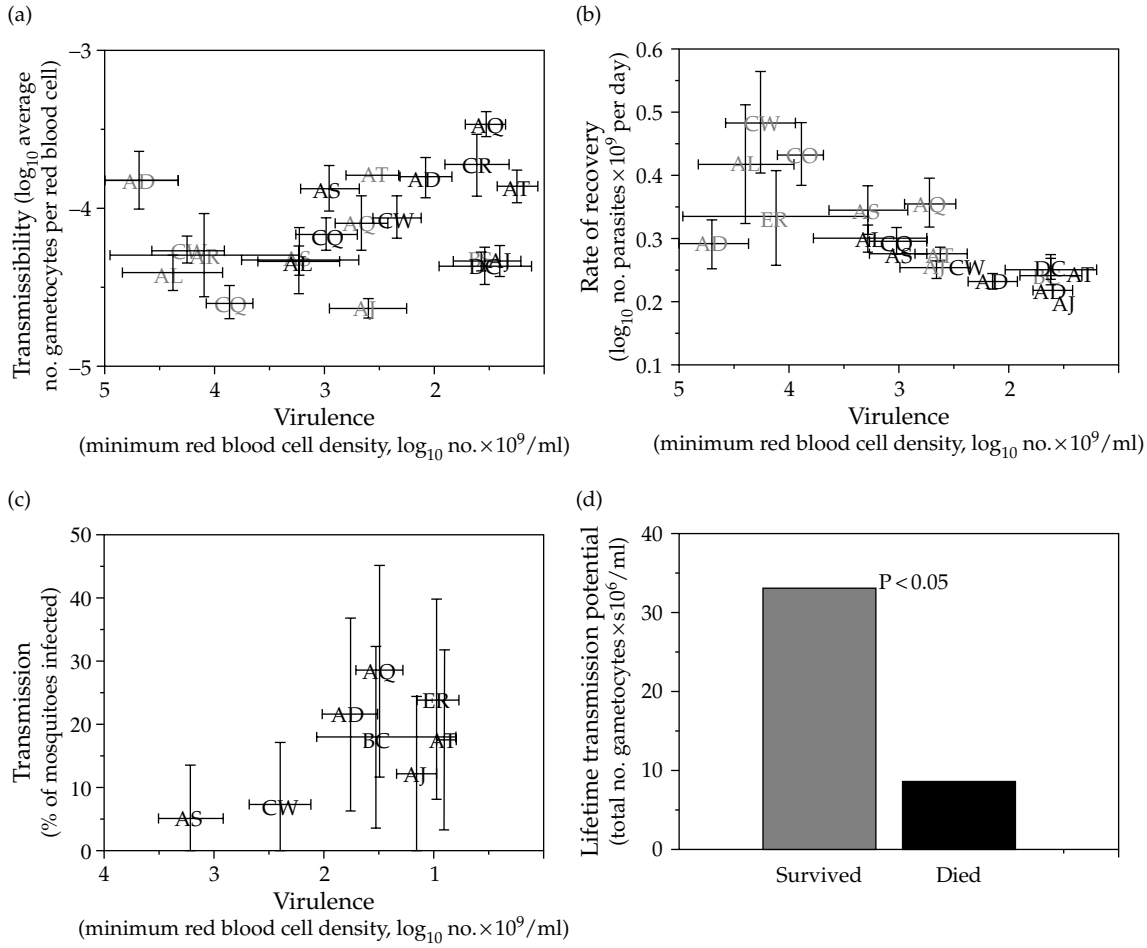

Figure 11.4 Benefits and costs of virulence in rodent malaria. Groups of laboratory mice (C57/Bl, female, $n = 7-17$) were infected with one of 10 strains (clones) of wild-caught rodent malaria parasite *Plasmodium chabaudi*. Parasite clones that were more virulent were also more transmissible (a, c) and more persistent (b). They were also more productive in semi-immune hosts: thus virulence was of positive benefit to parasite fitness. However, mice that died had lower transmission potential than mice that survived, causing a fitness cost of virulence (d). Thus these data support the assumptions of the virulence trade-off model. Virulence was measured as the minimum red blood cell density experienced by the mouse during its infection; transmissibility was measured by the average number of transmission stages (gametocytes) produced during the infection or as infectivity to mosquitoes over a 4-day period; and persistence was measured as the rate of decline in parasite density post-peak parasitemia. Each lettered symbol represents the mean for the parasite clone and the bars represent standard errors of the means. Black symbols indicate that the mouse was naïve to infection when inoculated; lighter symbols indicate that the mouse was made semi-immune prior to infection. Data from Mackinnon and Read (1999; 2003) and Mackinnon *et al.* (2002).

led to the evolution of more virulent myxoma virus (Fenner and Fantini 1999).

Other possible vaccine-adapted phenotypes

Epitope changes and virulence adaptation are the main phenotypes that have been considered in the context of vaccine-driven evolution. But it seems likely that other phenotypes might also be favored in vaccinated populations, including these:

- enhanced immunosuppression, for instance by enhanced production of immunomodulatory substances;

• the evolution or enhanced production of smokescreen molecules, immunogenic molecules on pathogen surfaces, whose only function is to distract the immune system from functionally important molecules;
• changes in patterns of antigenic variation, including antigenic repertoires and rates of change;
• changes in tissue tropism into immunologically privileged sites and/or increased sequestration; and
• activation of alternate host cell invasion pathways.

There are undoubtedly other strategies yet to be discovered.

The health consequences of vaccine-adapted pathogens

What will be the health outcomes of vaccine-driven pathogen evolution? There are several possibilities.

First, vaccine-adapted mutants might spread but with little health impact. Small differences in relative fitness can easily lead to large changes in gene frequencies with little impact on overall pathogenicity or infectiousness. In pertussis, several very large changes in allele frequencies at genes encoding toxins and adhesins occurred during the whole cell vaccine era with apparently little public health impact (Denoël *et al*. 2005).

Second, vaccine efficacy could be eroded, with the spread of vaccine-adapted mutants leading to reduced individual protection. This would be equivalent to classical drug resistance, and could, in extreme cases, lead to the abandonment of the vaccine, as happened with the early generation Marek's disease vaccines. There are calls to include new epitopes in some existing human and veterinary vaccines to pre-empt this (e.g., François *et al*. 2001; Radford *et al*. 2006). The existence of considerable standing variation among strains is one of the reasons vaccines do not yet exist for diseases such as malaria and HIV.

Third, intrinsic virulence could evolve. Clearly, this will enhance the population-wide health benefits of vaccination if virulence goes down: individuals will be exposed to less pathogenic strains. The continued circulation of unproblematic strains may also help boost or maintain vaccine-induced immunity (Ewald 1996). In contrast, if *more* virulent strains evolve, unvaccinated individuals who get infected will suffer more severe disease, as will vaccinees if vaccine efficacy declines. But what of the population-wide health burden? Vaccines reduce disease severity, and often transmission. Does a lower force of infection, and hence reduction in the number of hosts at risk of becoming diseased, more than compensate for deaths due to increased intrinsic virulence? We modeled the widespread use of a leaky blood-stage malaria vaccine in a human population subject to year-round high-intensity malaria transmission. Parameter values were chosen to mimic a situation like that in Tanzania. As predicted, intrinsic virulence increased, and the public health benefits were eroded through time, with vaccine-induced reductions in population-wide mortality rate receding from that achieved immediately after vaccination was introduced. At intermediate levels of vaccine coverage, total mortality actually exceeded that in the pre-vaccine era (Gandon *et al*. 2001). We are cautious about drawing strong policy conclusions from these models, because complex epidemiological situations are hard to capture. Nonetheless, the exercise does show that in principle, vaccine-driven evolution can worsen the public health burden. If such evolution did occur, withdrawal of the vaccine would expose even more people to the full intrinsic virulence of the vaccine-adapted strains.

Clearly, a key issue is whether the intrinsic virulence of vaccine-adapted strains is greater or less than that of the wild type. We think there is a general feeling in the vaccine community that because escape mutants will be less fit than wild type (otherwise, they would have evolved anyway), vaccine-adapted mutants will be less damaging than wild type pathogens. Indeed, experimental insertion of immune-evading epitopes into wild type influenza A and HBV reduces viral replication rates relative to lines in which wild type epitopes have been inserted (Kalinina *et al*. 2003; Berkhoff *et al*. 2006). Apparently, the conformational changes required for immune evasion led to less efficient viral replication. Several other types of vaccine-adaptation might similarly reduce replication efficiency. It is well known in the context of drug resistance (Chapter 10) that such fitness costs may

in due course be reduced by compensatory mutations elsewhere in the genome (so far as we know, not yet studied for epitope mutants). Even so, initially penalized vaccine-adapted variants should never acquire more than wild type replication efficiency by compensatory mutation.

But vaccine-adapted mutants need not have lower replication abilities than wild type. For instance, pathogens allocating more resources to smokescreen molecules might grow less aggressively than wild type pathogens in unvaccinated individuals, but they might also grow better in vaccinated hosts precisely because of the smokescreen. Overexpression of immunomodulatory substances might be favored in vaccinated individuals, while overexpression in unvaccinated hosts might kill them. Similarly, higher replication rates may enable more transmission and longer persistence in the face of vaccine-induced immunity. Indeed, as we have mentioned above, intrinsically more virulent vaccine-breakthrough strains have been seen in feline calicivirus, Marek's, and infectious bursal diseases. Even epitope mutants need not be less virulent. Many epitopes are themselves often virulence factors. A vaccine-adapted strain might have immune-evading epitope conformations that also make it highly efficient at binding host cells and hence too pathogenic to be favored by selection in an unvaccinated world. Thus there is no *a priori* reason to assume that vaccine-adapted variants will necessarily be less virulent. They may not have evolved in an unvaccinated world precisely *because* of their excessive virulence.

Predicting evolution

Predicting the direction vaccine adaptation will take is extremely challenging, because a lot of quantitative biological data are required. First, data are needed on fitness costs and benefits associated with putative vaccine-favored mutants in vaccinated and non-vaccinated hosts. Whether a particular mutant spreads depends on the relative magnitudes of these costs and benefits. Costs and benefits vary widely with host, pathogen, and epidemiological circumstances and are unlikely to map easily onto categorization of the cellular and molecular mechanisms involved in vaccine adaptation. Our argument that malaria vaccines could favor the spread of more virulent malaria parasites is based on costs and benefits of virulence that we measured in laborious experimental work with rodent malaria (Fig. 11.4). While we expect the logic will apply to other species of malaria and to other diseases where virulence is intimately linked with transmissibility, different natural histories can generate different predictions (e.g., Ganusov and Antia 2006).

Even very good knowledge of the fitness costs and benefits of vaccine adaptation is insufficient to predict evolutionary outcomes: a whole host of epidemiological factors are also important (Restif and Grenfell 2007). For instance, what is the level of natural immunity, and how will vaccination change this? What effect will vaccination have on levels of herd immunity? What fraction of the pathogen population lives in non-immune versus immunized hosts? What subset of the pathogen population does the vaccine cover/target? How does vaccine coverage alter the force of infection of the disease? For pathogen populations in which a rich strain structure interacts with virulence evolution, predictions become very problematic, particularly because complex population dynamics typically follow a perturbation of the epidemiological system. Evolutionary prediction requires disease-specific models parameterized by a very good knowledge of relevant details. We doubt that simple generalities will emerge.

For instance, it has been argued that it is highly desirable to have vaccines that selectively remove virulent strains, leaving mild strains to circulate and induce supplementary protection (the so-called 'virulence antigen strategy': Ewald 1994, 1996; Soubeyrand and Plotkin 2002; Ebert and Bull 2003). This sort of generality makes us nervous. Vaccines targeted at virulence determinants will not always lead to reduced virulence. Toxoid vaccination, for example, selectively targets parasite toxins. Anti-toxin immunity might indeed select against toxin production, but the production of *more* toxin may be a way for the pathogen to retain the resource-acquisition or immunosuppressive benefits of the toxin in the face of anti-toxin antibodies. Toxin epitopes could also evolve. The optimism behind the virulence antigen strategy may

be particularly misplaced when virulent strains are targeted at antigens unconnected with the virulence. For example, if the capsular polysaccharides of *Streptococcus pneumoniae* and other pathogenic bacteria such as *Neisseria meningitidis* and *Haemophila influenza* are not the only cause of strain variation in virulence, vaccines directed at the currently more virulent serotypes could simply prompt capsular switching. In that case, virulent strains would take on non-vaccine capsular types (Maiden and Spratt 1999).

The classic case invoked in support of the virulence antigen strategy is diphtheria. In Romania, this evolved to be less virulent after the introduction of the toxoid vaccines because toxigenic strains decreased in frequency (Fig. 11.2). As we described earlier, the standard explanation for this is that anti-toxin vaccination greatly reduced the resource acquisition benefits of the toxin, making the metabolic costs of producing it not a price worth paying (Soubeyrand and Plotkin 2002). This argument may be correct. But an alternative expectation is that toxin production (and hence virulence) increases (that is, that *tox*++ strains should evolve) because the benefits of increased resource acquisition via toxins can be had with less risk of death because the host is now protected by the vaccine (Gandon *et al*. 2001). The contradictory predictions arise because the two arguments differ in what is considered the cost of virulence: host death or the metabolic costs of toxin production. In fact, increased or decreased virulence can evolve depending on the relative magnitude of the two costs (Gandon *et al*. 2002; Read *et al*. 2004). Thus, one needs to know a lot to predict what will happen.

In the case of diphtheria, one can of course ask the empirical question of what actually happened. Without doubt, the incidence of diphtherial disease has declined in the face of vaccination, but what we want to know is what evolution has occurred. This is a question about the frequency of the toxigenic strains in the bacterial population. So far as we know, the only relevant data are those reproduced in Fig. 11.2. Frustratingly, the study that generated those data has apparently never been published in the primary literature, and so far as we know, there are no other such data published. However, we note that diphtheria case fatality rates have remained unchanged in the United States despite 60 years of widespread vaccination (Mortimer and Wharton 1999), and they have actually *increased* in Delhi (Singh *et al*. 1999). It is quite possible that geographically variable evolutionary outcomes are occurring in diphtheria. In the veterinary context, different evolutionary trajectories have been seen even within the same host–pathogen combination. Epitope variants of infectious bursal disease virus were responsible for vaccine breakthrough in the U.S. poultry industry; in Europe, hyperpathogenic variants were responsible (van den Berg 2000; Rautenschlein *et al*. 2005; Le Nouën *et al*. 2006).

We reiterate: very system-specific analyses are needed to predict evolutionary trajectories. We doubt that simple generalities will emerge.

Watching evolution

The difficulties of prediction make it even more important that we should watch and learn from the experiments already underway. One of the most obvious ways of doing this—taking samples from clinical cases of vaccine failure—is often done. However, to make sense, such analyses need to be set into context: samples of pathogens from vaccinated and unvaccinated people, and from disease cases and asymptomatic infections, are required. But even these are not sufficient to predict the direction of pathogen evolution because there are population level influences, such as herd immunity, at play: thus one needs to measure change at the population level. To detect or study evolutionary change, random samples of a pathogen population are needed from each of those four groups before widespread vaccination is introduced, and then with successive samples over subsequent decades. The existence of such series data for pertussis (van Loo *et al*. 1999; Hallander *et al*. 2005; Hardwick *et al*. 2007) is one of the reasons it has played such an important part in discussions of vaccine-driven evolution. There are encouraging signs that such collections are being made for at least some other human diseases (e.g., Pebody *et al*. 2006). We believe this should be routine for all human diseases and for many veterinary diseases when new vaccines are being introduced.

Genetic change is easiest to study where relevant genes are known: molecular epidemiology on epitope variants and known virulence determinants

is relatively straightforward. However, it is much harder when relevant genetic variation is unknown, as is the case for many virulence determinants. In these cases, what is needed is some phenotypic marker of virulence (e.g., determination of the frequency of toxigenic strains of diphtheria), but in many cases, virulence can only be assayed by comparing lines inoculated into a laboratory standard host. The ability to do these assays is what enabled the evolution of myxomatosis to be so successfully studied (Fenner and Fantini 1999). It will be extremely challenging to detect virulence changes for human diseases. Against the background of falling disease incidence that should follow from the introduction of effective vaccination, it may be rather late in the day when increases in virulent breakthrough strains are noticed. There is also usually no ethically acceptable experimental host in which the virulence of strains can be compared. Comparisons of case fatality rates over time are confounded by changes in clinical medicine and other environmental changes, including changes in disease cofactors. It is substantially easier to track virulence changes in the veterinary context where experimental infection of relevant hosts is possible. It may be no accident that this is where increasingly virulent strains in vaccinated populations have been detected (MDV, IBDV, and feline calicivirus (FCV)).

Based on our discussion above, we think surveillance and archiving of pathogen samples is especially warranted for infectious diseases where existing or new vaccines:

- fail to prevent transmission, or where immunity wanes and vaccinated hosts become leaky;
- target a subset of strains in a population;
- target virulence determinants;
- target epitopes not normally seen by natural immunity;
- involve novel technologies that, for instance, induce better than natural immunity or induce immune effectors not normally evoked;
- could be overcome by increased production of virulence factors, such as toxins and immunosuppressive substances; or
- provide prophylactic vaccination in normally naïve populations and thus constitute a major new source of immune selection on pathogens.

Assessment of the potential of vaccination to prompt virulence evolution should begin by considering what selection pressure prevents more virulent strains from dominating current populations, and then asking (1) Will vaccination relax that? and (2) Do virulent strains have a fitness advantage in vaccinated hosts? For diseases where both answers are yes, non-sterilizing vaccination could generate more virulent pathogens.

Coda

Society has developed very robust procedures for testing the safety and efficacy of vaccines for individuals. It is much harder to assess the long-term consequences of vaccine use: which vaccines will be undermined by evolution; which will create more or less virulent pathogens. By definition, evolutionary experiments take some time, and replication at the whole population level is problematic. Mathematical models clarify thinking but cannot yield the certainty of a clinical trial. Nonetheless, the complexity of the issue does not make the problem go away. In our view, vaccine evaluation processes should involve evolutionary assessments at all points in the vaccine pipeline, from design to rollout and beyond. In particular, during early trial stages, data can be gathered on the effects of vaccination on transmission of wild type pathogens, and whether more virulent strains are likely to benefit from a vaccinated world. Such data are a necessary first step to evaluating evolutionary risk and deciding among competing vaccine strategies.

Research on vaccine-driven evolution is in its infancy, and there are many open issues. Some of these involve vaccine design. Can we prevent the evolution of antigenic targets by focusing on naturally invariant epitopes? Do broad spectrum multitarget vaccines make epitope evolution less likely—and if so, virulence evolution more likely? Other questions involve the evolutionary experiments currently underway: will leaky vaccination of poultry against avian influenza lead to the evolution of strains more virulent to humans (so called 'monster' strains; Anon 2004)? Will the efficacy of vaccines against bacterial capsular polysaccharides decline because of serotype replacement and capsular switching? Do changing farm practices lead to pathogen evolution that undermines current

vaccines? What will be the evolutionary consequences of vaccines targeted at specific disease syndromes such as cerebral malaria or pregnancy-associated malaria?

Vaccines have been and continue to be the most successful infectious disease control measure after hygiene. Yet it seems likely that at least some national immunization programs are going to have to be adjusted in response to pathogen evolution (Kimman *et al*. 2006; Radford *et al*. 2006), and agricultural vaccines have already been rendered useless by pathogen evolution. If the repeated evolution of drug resistance has taught us anything, it is that it is better to think about evolution in advance.

Summary

1. The evolution of vaccine-resistant pathogens is not obviously as big a problem as the evolution of drug resistance. We argue that it is nonetheless a problem that is likely to grow. There is no reason for complacency.

2. Vaccine-driven pathogen evolution has been seen in several infectious diseases.

3. Vaccine-induced immunity does not simply substitute for the selective pressures imposed by infection-induced immunity. Vaccines can alter the immune landscape experienced by pathogens, and hence their evolution, by targeting subsets of strains in a population, reducing the number of fully susceptible individuals in a population, and creating or expanding classes of semi-immune hosts.

4. Vaccines of the future are likely to evoke novel immune pressures. These novel vaccine technologies will likely impose completely novel selection for resistance, as do drugs.

5. Vaccination against the acute childhood diseases such as smallpox, polio, and diphtheria has occurred for decades without being undermined by pathogen evolution. However, these diseases were easy targets: natural immunity was evolution-proof; all vaccination needed to do was to induce something very similar.

6. Infectious diseases now under assault by vaccination are different: natural infections induce leaky, often strain-specific immunity that usually wanes. Vaccines against these diseases will likely induce immunity to which natural selection has already found solutions.

7. Some agricultural vaccines have already failed in the face of pathogen evolution (e.g., Marek's disease) and the jury is still out on others, including some against human diseases (e.g., pertussis). It may take decades for vaccine resistance to become apparent.

8. A wide variety of pathogen phenotypes can be favored by natural selection in vaccinated populations. Most biomedical research has concentrated on epitope variation, but the evolution of increased virulence may also occur when vaccination relaxes the natural selection against virulence.

9. It seems likely that vaccines could provoke the evolution of enhanced immunosuppression and changes in patterns of antigenic variation, tissue tropism, and invasion pathways. There has been little analysis of these possibilities.

10. Predicting evolutionary consequences of vaccination in advance is extremely difficult. There may be no population-wide health implications of vaccine-driven pathogen evolution, or it could improve or worsen disease burdens. Anything is possible.

11. Evolutionary analysis is particularly warranted where vaccines are leaky, target subset of strains or virulence determinants, involve novel technologies, or relax selection against virulence.

12. Vaccination is one of the most cost-effective methods of public and animal health improvement. Continuing past successes and realizing the full potential of vaccination requires evolutionary considerations at all stages of vaccine design and implementation.

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