Vaccination: a way to address questions in behavioral and population ecology?

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Host–parasite interactions have become an important topic in behavioral and population ecology. Among other fitness-related traits, the response to parasitism has implications for individuals, but also for the dynamics of host–parasite interactions. In this context, active immunization (vaccination) is increasingly used as an immunological tool to investigate the costs and associated trade-offs of the host immune response to parasitism. Active immunization experiments also provide information on the relationships between parasite resistance and sexual selection, and on implications of the variability of immune responses within natural populations.

A parasite can be defined as an organism living in or on another living organism (a host), which obtains part or all of its organic nutrients from the host, and causes a degree of damage to its host [1]. This broad, ecological definition includes pathogens such as fungi, bacteria and viruses, which feed in or on living organisms. These parasites have been termed microparasites, as opposed to the more classical term macroparasites.

Because parasites are such a significant part of the biotic environment of most species and vice versa, there has been much recent interest in the evolution and ecology of host–parasite interactions. Attention has focused on: (i) determining the role of parasitism as a driving force behind the behaviour of individuals [1]; and (ii) understanding the evolution and dynamics of natural populations [2–4]. This work has led to several questions such as how costly is the response to parasites and how does this cost relate to investments in other life history traits? Could parasitism affect sexual selection processes? Why does a parasite cause asymptomatic infection in one host, but can harm another? The evolutionary ecology approach stresses the importance of genotypic and phenotypic variability in natural populations: the host genotype affects susceptibility to a parasite [5] and vice versa; furthermore, it is the phenotypes of the hosts and parasites that are involved in the interactions [6], and thus environmental effects on phenotype development also have to be considered.

As a result of the intimate and often long-term nature of host–parasite interactions, natural selection has driven host–parasite coevolution [7]. There are two important features of host and parasite reciprocal responses: (i) the responses are more or less specific; and (ii) the responses can be more or less inducible [7]. Immunity is one of the host responses to parasites, which can either be innate (i.e. present regardless of the biotic environment of the individuals) or acquired (i.e. activated only in response to challenge) [8]. In this context, the acquired immune response (AIR) is of particular interest to evolutionary ecologists [9]. It is a good example of how an inducible defense can track complex changes in the biotic environment [10]. Moreover, the variability of the AIR among individuals can be related to various evolutionary processes, and its ecological implications are potentially wide [3,11,12]. The AIR is, for instance, of interest to biologists concerned with the evolution of adaptive phenotypic plasticity because of its inducibility [10], specificity and, in some cases, its ability to be transferred maternally from mother to offspring [13–15]. In addition, the mechanisms behind an AIR are complex, but well known as a result of detailed molecular and medical studies [16].

Vaccination (active immunization) is used, in most cases, to protect individuals against an infectious agent to which they could be exposed. Active immunization often relies on the humoral immune response (with the production of antibodies) elicited by exposure to an antigen, which can either be a killed or an attenuated live microorganism, or some other product with antigenic properties (e.g. sheep red blood cells). Such immune challenges are not always applied to protect individuals, but can be used to study characteristics associated with their serological response. Vaccination represents a potentially powerful tool to address fundamental questions on the evolutionary ecology of host–parasite interactions. By experimentally exposing an organism to an antigen and by controlling the context of this exposure (statistically and/or experimentally), it is possible to answer several questions related to the organism's ability to respond to parasites. This approach can be used within natural populations to address many aspects of the evolutionary ecology of parasitic diseases [2].

Here, we focus on how active immunization has been used as a tool to tackle ecological questions at different levels, and conclude by underlining the benefits that should be gained from further links between the evolutionary ecology of host–parasite interactions and

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Box 1. Potential collateral effects of vaccines

The term vaccination conveys an idea of harmlessness, but the potential collateral effects of vaccination should be considered for ethical and scientific reasons, as follows.

Hypersensitivity and/or immediate allergic reactions

Hypersensitivity and/or immediate allergic reactions are relatively rare (e.g. one febrile reaction per 19 000 people and 26 per 76 000 people suffer from a local reaction to the diphtheria–tetanus vaccine [64]). However, in ecological studies, the vaccines used have often been designed for other species, which might lead to an increase in the frequency of such hypersensitivity and/or allergic problems.

Interactions with other components of the immune system

For example, the Newcastle disease virus (NDV) vaccine has antitumoral properties (Box 1) which could result from cytokine release [e.g. production of interferon-β (IFN-β)] [65]. This could lead to false conclusions, especially when both humoral and cell-mediated immunocompetence are tested in the same animal: thus, NDV vaccination could influence the cell-mediated immune response.

Toxicity of adjuvants

Vaccine adjuvants [66] are necessary to produce strong and sustained immune responses, but they can influence much more than the strength and duration of an immune response (e.g. behavioral and physiological side effects). For instance, a reduced investment in reproduction following an experimental immune challenge could be due to effects from the adjuvant and not to the mounting of the immune response *per se*.

Stress effects

Most of the vaccination experiments involve the capture of individual subjects. The effect of the potential stress associated with the capture and manipulation of individuals is often believed to be controlled for by comparing the results with those of a group of individuals injected with a saline solution. However, acute stress can suppress certain components of immunity and increase others [67], which should be taken into account when interpreting the results of experiments.

Cross protection

Many studies induce immunization against antigens that are nonpathogenic or to which animals are not usually exposed. However, some vaccines can induce nonspecific innate immunity [68]: hence, animals from the vaccinated group could be protected against some pathogens, although animals from the control group are not. This could reduce the ability to detect the cost of an immune challenge.

immunology. In doing so, the need for careful interpretations of results is stressed and some potentially fruitful lines of investigations are identified. We then consider vaccination more for its main medical and veterinary purpose (i.e. to protect individuals against infectious diseases [17]) because it provides the opportunity to discuss the evolutionary and ecological implications of immunization in natural populations.

Trade-offs and costs

The life history theory predicts that variability among individuals in the expression of a trait, here an immune response, should be a function of allocation strategies among traits and constraints [18]. According to several studies, immune response to parasites is costly and the existence of detectable trade-offs is expected [11]. Resources used to mount an immune response could be diverted from costly activities such as reproduction [19–21]. An individual facing an increase in a costly activity, such as the rearing of an experimentally increased number of offspring, could also have fewer resources to invest in its immune response [22,23]. In both cases, vaccination has been used to mimic exposure to a pathogen that would elicit a specific immune response [12] (Table 1). Individuals from different species have, for instance, been exposed to novel nonpathogenic antigens or true vaccines, and evidence of such trade-offs has been reported, although not in all cases (Table 1). Much of this recent interest in the life history implications of immunology has involved the avian immune system, and the protocols used have been inspired by immunocompetence studies in poultry (e.g. Ref. [24]). Recent studies using individuals from wild bird populations have assessed the cost of antibody production by measuring changes in their metabolic rate following exposure to an antigen [25,26]. Immune responses under different levels of cold stress have also been compared [27]. Overall, these studies suggest that, at least in certain environmental conditions, there are costs and trade-offs associated with mounting a specific immune response. In this context, vaccination appears to be a powerful tool, provided that the results are carefully interpreted. Individuals vary in their response to different antigens and in how they respond to identical antigens. Moreover, the production of antibodies after first exposure to an antigen is only one type of response among many that are involved in parasite immunity.

Vaccination reflects the complexity of natural responses to parasites, both redundant and overlapping, which depends on many factors. For instance, vaccination involves memory cells and sometimes both cell-mediated and humoral immune responses, and potential trade-offs between these responses can hinder results [3] (Box 1). Experimental design, with individuals randomly attributed to treatment and control groups, and careful choice of vaccination agent and protocol could help disentangle some of the processes at work. To date, few studies have investigated the potential interactions between the treatment and vaccination protocols used. In addition, the choice of antigen, the timing and mode of antigen administration, and sampling design (Table 2) could be important, which relates to the difficulty of attributing a level of immunocompetence to individuals and stresses that trade-offs could exist at different levels. Possessing a functional immune system does not have the same cost as responding to a particular challenge. Klasing's article [28] highlights the need to distinguish between the low energetic cost of antibody production and the actual cost of an acute immune response involving inflammation and other physiological components of such a response. Finally, heterogeneity among individuals in the overall ability to invest in different traits will affect the patterns observed [21,29].

Immunocompetence and sexual selection

Heterogeneity among individuals will also affect mate choice. Theoretical and empirical studies have indicated

Table 1. Ecological studies involving exposure to a novel antigen and quantification of antibody production[a]

Subject	Antigen used	Species (Latin name)	Outcome	Refs
Life history and immune function trade-offs				
Immune challenge				
	Diphtheria–tetanus vaccine	Pied flycatcher (*Ficedula hypoleuca*)	Activation of immune defense results in reduced breeding success.	[20]
	SRBC	European starling (*Sturnus vulgaris*)	Activation of immune defense does not reduce reproductive output.	[19]
	SRBC	Tree swallow (*Tachycineta bicolor*)	Reproductive effort reduces long-term immune function in breeding tree swallows.	[47]
Manipulation of life history traits, or correlational study between a trait and immunocompetence				
	KLH	Tree swallow	Humoral immunocompetence correlates with date of egg laying, and reflects workload.	[48]
	NDV vaccine	Collared flycatcher (*Ficedula albicollis*)	Reproductive effort reduces specific immune response and parasite resistance.	[23]
	SRBC	Zebra finch (*Taeniopygia guttata*)	Reproductive effort decreases specific immune response.	[22]
Nutritional status				
	SRBC	Serin (*Serinus serinus*)	Immune capacity correlates with food availability.	[49]
	Diphtheria–tetanus vaccine	Pheasant (*Phasianus colchicus*)	Sexual ornaments reflect nutritional conditions during early stages of growth.	[50]
	Several antigens	Poultry	Nutrition affects resistance to infectious diseases.	[28]
Energetic costs of mounting an immune response				
	Diphtheria–tetanus vaccine	Blue tit (*Parus caeruleus*)	Cold-stressed induced immunosuppression.	[25]
	SRBC	Great tit (*Parus major*)	Immune challenge increases basal metabolic activity.	[26]
Immune response and sexual selection				
Immunocompetence reflected by secondary sexual traits				
	KLH	European starling (*Sturnus vulgaris*)	Song-bout length reflects immunocompetence.	[33]
	SRBC	Barn owl (*Tyto alba*)	Spottiness of plumage reflects immunocompetence.	[51]
Use of sexual hormones				
	SRBC	Fairy wren (*Malurus cyaneus*)	Testosterone treatment decreases humoral immunocompetence, but free-living males with high testosterone are more immunocompetent.	[52]
	KLH	European starling	Testosterone treatment decreases humoral immunocompetence.	[53]
	SRBC	House sparrow (*Passer domesticus*)	Testosterone treatment enhances expression of a sexual trait, but interacts with corticosterone to affect humoral immunocompetence.	[54]
Immune challenge affects secondary sexual traits				
	SRBC	Blackbird (*Turdus merula*)	Immune challenge decreases intensity of bill color.	[55]
Maternal immunological effects				
	NDV vaccine	Barn swallow (*Hirundo rustica*)	Females mating with more attractive males (sexual ornamentation manipulated) invest more antibodies in their eggs.	[40]
	SRBC	Tree swallow	No evidence of maternal transfer of antibodies.	[56]

[a] Abbreviations: KLH, keyhole limpet haemocyanin; NDV, Newcastle disease virus; SRBC, sheep red blood cells.

that male secondary sexual traits can convey reliable information on their ability to resist pathogens and parasites in a wide range of dimorphic species [30,31]. The degree of resistance could be reflected in secondary sexual traits, such as bright colors, which could be used as cues in mate choice. This is suggested to be the result of a trade-off between sexual trait expression and immune function [10,32]. To explain the link between a signal expressed through secondary sexual traits and the quality of individuals, Folstad and Karter [32] proposed the immunocompetence handicap hypothesis – a factor (e.g. testosterone) that enhances the development of secondary sexual traits also suppresses immunity. Several studies that tested predictions linking mate choice and immune function used vaccination protocols to assess the immunocompetence of individuals (Table 1). Duffy *et al.* [33] observed a positive relationship between song-bout length, a trait that is involved in mate choice in male European starlings *Sturnus vulgaris*, and humoral immunocompetence, as assessed by exposure to a novel antigen. Antigen exposure experiments assess the ability of an individual to fight disease, rather than measuring natural parasite loads, as was performed in several earlier studies [34]. Natural parasite loads reflect past exposure to parasites and thus not only the individual's ability to fight parasites.

Evidence of a potential immunosuppressive effect of testosterone was reported in some studies (Table 1), but the relationship between the neuroendocrine system and the immune system is complex. These systems interact via cytokines and hormonal and neuronal pathways, and constitute a finely tuned regulatory framework, distinct from single one-way action–reaction schemes [35,36]. This should be kept in mind when addressing sexual selection or stress-related topics (Box 1). Vaccination can nevertheless help define which components of the immune system are stimulated (humoral or cellular; Table 2) and at what time scale (chronic or short term).

Table 2. Sources of variability in host response to vaccination[a]

Variability	Ecological implications	Refs
Caused by antigens		
Pathway activated		
Inactivated virus vaccines, subunits or toxins generally induce the exogenous pathway [characterized by high levels of serum antibodies, and a lack of CMI and mucosal immunity (e.g. lack of IgA)]	Used to investigate the effects of different parasites (e.g. intracellular or extracellular) and to assess different costs on host response.	[17]
Live vaccines (natural or attenuated) or action of adjuvants induce the endogenous pathway (e.g. CD4$^+$, Th1 or CD8$^+$ produced)		
Interference with other antigens injected	Used to examine the effect of simultaneous exposure to different parasites (superparasitism).	[57]
Caused by adjuvants		
Titer and duration of antibody response	Used to determine the level of immune challenge in hosts.	[58]
Antibody avidity, specificity, isotype or subclass distribution	Used to study the dynamics of host immune response.	
Stimulation of innate immunity	See Box 1 for implications.	[58]
Induction of CMI	Used to test costs associated with CMI.	[17]
Caused by ecological factors or individual characteristics		
Environment and/or ability to use the environment	The focus of most ecological studies. Some of these factors, when not considered as co-variates, could become confounding variables in correlative studies.	
Food availability (quantity of different components such as carotenoids)		[59,50]
Level of stress (e.g. social interactions, thermic stress, quality of habitat)		[36,60]
Individual characteristics		
Age		[61]
Sex		[17]
Immune status of host and previous infections		
Individual genetics: MHC genes		[62]
Individual genetics: non-MHC genes		[63]
Immunosuppressed or not (e.g. YOPI)		[17]
Caused by vaccination protocol and/or vaccination history		
Re-exposure to antigen	Used to test predictions related to the dynamics of immunity in a host.	[17,47]
Number of and interval between two injections	Used to investigate the practical implications of study design.	[17]
Interactions between factors	Used to investigate interactions between ecological and/or immunological processes.	[17]

[a] Abbreviations: CMI, cell-mediated immunity; Ig, immunoglobulin; MHC, major histocompatibility complex; Th1, T helper cell type 1; YOPI, young, old, pregnant or immunodepressed.

Vaccination can also be used to address the importance of the genetic basis of the processes studied. Lines of domestic chickens, *Gallus domesticus*, selected for antibody response to sheep red blood cells showed, as predicted, different secondary sexual traits (i.e. ornament sizes and testosterone levels), suggesting the existence of a trade-off between immunocompetence and ornamentation [37]. Vaccination can also address basic assumptions and predictions of hypotheses linking parasite resistance and sexual selection, such as the existence of a heritable susceptibility to parasitism in natural populations [5].

Ecology and evolution of the dynamics of immunity

An important characteristic of the humoral response, associated with its inducibility and specificity, is its temporal dynamics. The level and components of the humoral immune response can vary over time and among individuals. Can the kinetics of the response to different parasites be explained in adaptive terms? Do individuals in natural populations manipulate their risk of exposure to parasites to become immunized in order to avoid the deleterious effects of potential later re-exposures? (See, for example, Ref. [38].) The costs and benefits of such strategies can be difficult to assess. One example where such strategies could be interesting to investigate is the maternal transfer of antibodies from mother to offspring [14,39]. How does earlier exposure of the mother to the antigens affects the amount of antibodies transferred [14]? Saino *et al.* [40] have shown that female barn swallows (*Hirundo rustica*), mated to males with elongated tails (a secondary sexual trait), will transfer more antibodies to their eggs against an antigen to which they have been experimentally exposed by vaccination than those by females mated to non-elongated or short-tailed males. The existence of such maternal effects can also be important for epidemiological studies: the maternal transfer of antibodies against rabies can affect the interpretation of temporal variability of sero-prevalence in field surveys conducted in red fox *Vulpes vulpes* populations [41].

The dynamics of the immunity of individuals will have ecological consequences at population levels. Most of these implications are well known because they have been incorporated in population models for a long time [e.g. susceptible, exposed, infected, resistant (SEIR) types of models] [42]. The study of their effects on the dynamics of host–parasite interactions has benefited greatly from large-scale vaccination programmes. Comparisons of the effect of vaccination programmes on the dynamics (changes in numbers of new cases over time) of measles and whooping cough in UK cities have indicated strong spatial and non-linear effects which can be

Box 2. Vaccination and the evolution of virulence

The possibility that parasites might evolve in response to the selection pressure imposed by a vaccination programme has been explored [69]. Early studies have focused on the spread of escape mutants, which display epitopes that are not recognized by the immune system of vaccinated individuals [69,70]. Recent theoretical developments have considered alternative counter-adaptations to vaccination involving pathogen life history traits, namely virulence (induced host mortality) and transmission rate [44]. These models have incorporated evolutionary theory for virulence evolution into an epidemiological framework. This theory predicts that parasite life history traits will evolve to maximize parasite fitness. In particular, competition among parasite strains could lead to the evolution of higher virulence as a result of trade-offs between transmission and virulence. For instance, the more virulent strain in a host might have an increased probability of outcompeting other strains, and thus of being transmitted. With regard to the effect of vaccination programmes, mathematical models have been used to investigate the potential effects of vaccines that vary in their type and level of efficiency [44]. The exact outcome of the modelling will depend on the biology of each particular host–parasite interaction and on modelling assumptions [44,71–72], but vaccines designed to reduce parasite growth rate and/or toxicity are susceptible to diminish selection against virulent parasites. This could subsequently lead to the evolution of higher levels of intrinsic virulence and thus to more severe disease in nonvaccinated individuals. Conversely, vaccines designed to block infection would induce no such effects and can even select for lower virulence. This is because a parasite following a strategy that would generate optimal virulence in a resistant host will induce a different-than-optimal virulence in a susceptible host, depending on the conditions provided for virulence evolution. Vaccines that reduce the within-host growth rate of the parasite or act against toxins could select for higher virulence, if they reduce the risk of host death at no cost to the parasite, and hence reduce selection against more-virulent mutants.

This approach stresses the need to understand how different vaccines work, and the evolutionary and epidemiological implications. It also underlines the need for good knowledge of the biology of the systems considered, and for monitoring the indicators of virulence evolution as part of large-scale vaccination programmes. The theory of the evolution of virulence is likely to be applied further to different scenarios involving vaccines.

interpreted as disease-specific responses to dynamical noise: in the case of measles, the dynamics became irregular and spatially uncorrelated in the vaccination era (i.e. not correlated to nearby localities), whereas the reverse pattern was observed with whooping cough dynamics [43].

The evolutionary consequences of vaccination have been considered, but mostly in theoretical terms [44]. The effects could be dramatic. Some vaccines (e.g. against hepatitis B, poliomyelitis) do not provide full protection against disease. Such partially effective (imperfect) vaccines used on a large scale might favor the selection of more-virulent parasites, increase infection severity and favor non-immune selection throughout a population [44] (Box 2).

Conclusion

Modified vaccination protocols have been proven useful in addressing various questions in behavioral and population ecology. The humoral response is complex and is only one of the possible responses of hosts to parasites, which should be taken into account when designing field experiments and interpreting their results. Conversely, the application of an evolutionary ecology approach to address crucial immunological and epidemiological questions is also important. Using vaccination in ecological studies, apart from its healthcare purpose, should help to fill the gap between evolutionary ecology and epidemiology [45,46].

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