

Animal vaccination and the evolution of viral pathogens

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Summary

Despite reducing disease, vaccination rarely protects against infection and many pathogens persist within vaccinated animal populations. Circulation of viral pathogens within vaccinated populations may favour the development of vaccine resistance with implications for the evolution of virus pathogenicity and the emergence of variant viruses. The high rate of mutations during replication of ribonucleic acid (RNA) viruses is conducive to the development of escape mutants. In vaccinated cattle, unusual mutations have been found in the major antigenic site of foot and mouth disease virus, which is also involved in receptor recognition. Likewise, atypical changes have been detected in the immunodominant region of bovine respiratory syncytial virus. Large deoxyribonucleic acid (DNA) viruses are able to recombine, generating new genotypes, as shown by the potential of glycoprotein E-negative vaccine strains of bovine herpesvirus-1 to recombine with wild-type strains. Marek's disease virus is often quoted as an example of vaccine-induced change in pathogenicity. The reasons for this increase in virulence have not been elucidated and possible explanations are discussed.

Keywords

Evolution – Marek's disease – Pathogenicity – Pathotype – Receptor – Tropism – Vaccine – Virulence – Virus.

Introduction

The production of animal proteins for human consumption depends on the reduction or elimination of diseases, which can cause losses directly through morbidity and mortality, and indirectly through increased condemnations at processing plants, decreased growth rates and/or increased susceptibility to other pathogens. The tendency towards increases in size of production units, often associated with the presence of multi-age groups on a farm, complicates the control of pathogens. The developments in the poultry industry provide an excellent

example of the increase in size of production units, with some farms having over one million layers producing eggs for consumption. Although the introduction of many pathogens can be reduced by applying strict biosecurity measures, including the use of filtered-air positive pressure housing, the associated costs often make this approach impractical. The development of veterinary vaccinology has been essential in providing cost-effective approaches to prevent and control infectious diseases in animals. Besides improving the animal health sector, animal vaccination has also substantially enhanced public health by preventing the occurrence of several zoonotic diseases, and reducing the use of veterinary drugs and hence drug residues in the

food chain (38). Recent advances in vaccinology have defined new directions for vaccine development strategies, and new generation vaccines are rapidly gaining scientific acceptance (22, 49, 56). Nevertheless, the rapid development of new veterinary vaccines, and their widespread use to protect large populations of animals against a growing number of infectious diseases, have raised questions about the potential consequences of mass vaccination strategies for the evolution of pathogens, in particular rapidly evolving viruses.

Because of high population numbers, rapid replication cycles and elevated mutation rates, viral pathogens exhibit an important capacity for variation and adaptation. This is particularly true for ribonucleic acid (RNA) viruses, which lack proofreading capability and replicate at maximum viable mutation rates. Mutation rates during RNA replication have been estimated to be in the range of 10^{-3} to 10^{-5} errors per nucleotide copied (Fig. 1). As a consequence of such limited replication fidelity, individual RNA genomes have only a fleeting existence, and RNA viruses evolve as heterogeneous populations of variant genomes collectively termed viral quasispecies (12, 13, 15). Viral genomes within quasispecies are subjected to a continuous process of variation and competition. Genome

subpopulations best adapted to replicate in a given environment will dominate the population, while unfit mutants are kept at low levels. Unfit mutant populations in one environment may nevertheless be fit in a different environment, and modulation of frequencies of genome subpopulations is the key to adaptability of RNA viruses. The biological and medical relevance of the quasispecies dynamic of RNA viruses has been extensively documented in several recent publications (12, 13, 15). Although several small deoxyribonucleic acid (DNA) viruses can reach evolution rates similar to those of RNA viruses, elevated mutation rates would be incompatible with the maintenance of the genetic information contained in large DNA viruses with complex genomes over 100 kilobases (kb) in size. Large DNA viruses are thus less prone to error than RNA viruses (Fig. 1). Acquisition of new genetic information proceeds mainly by gene duplication, lateral gene transfer by recombination between related viral genomes, and host gene capture (47).

Besides important differences in their genetic organisation, viruses can also produce a wide range of clinical manifestations upon replication in their hosts. Viral infections may be inapparent or they may cause acute or chronic diseases either directly, as a result of viral replication in infected tissues, or indirectly by triggering immunopathological responses (33). Many viral and host functions, as well as environmental factors, are presumed to influence the outcome of an infection. It is therefore often difficult to determine the nature of virus–host interactions responsible for these disparate effects. A number of recent studies involving several important animal pathogens, such as bovine respiratory syncytial virus (BRSV), foot and mouth disease virus (FMDV), bovine herpesvirus-1 (BoHV-1) and Marek's disease virus (MDV), have provided evidence that evolution of viral pathogens within vaccinated populations has not only important implications for the development of vaccine resistance, but may also promote the emergence of variant viruses with altered pathogenicity or host tropism.

In this review we will discuss some of the factors that may drive the development of pathogens with modified pathogenicity in vaccinated populations. The development of vaccine resistance to MDV will be examined in detail because MDV vaccines provide the best example of changes in field strains in association with vaccination. Marek's disease (MD) vaccines are probably the most intensively used vaccines against a DNA virus in any species. In the United States of America (USA) alone approximately 10 billion chickens are vaccinated against MD every year and at least an equal number of chickens are vaccinated elsewhere in the world. Moreover, the emergence of MDV strains with increased pathogenicity is often quoted as an example of vaccine-driven evolution of a DNA pathogen (18, 19, 39).

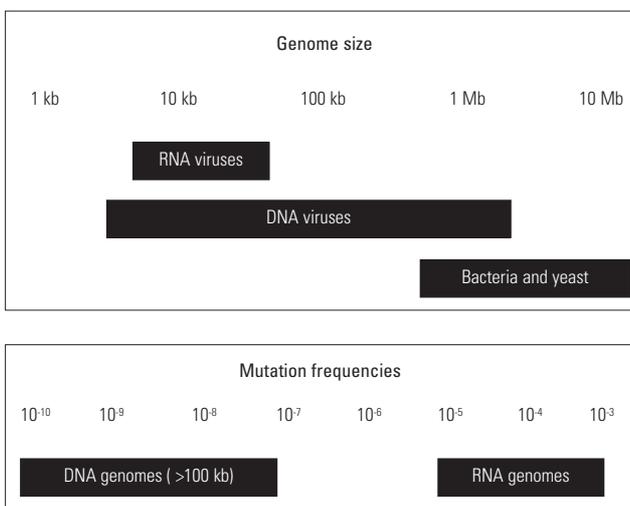


Fig. 1
Genome sizes and mutation frequencies for DNA and RNA microorganisms

The tolerance of DNA and RNA microorganisms to accept mutations decreases with genome size. RNA viruses are confined to one log variation in genome size and display average mutation frequencies of 10^{-4} errors per nucleotide copied (14). DNA viral genomes span more than 2.5 logs in size, overlapping the smallest bacterial genomes such as *Mycoplasma genitalium* (580 kb). The evolution rate for DNA viruses with small genomes varies from 10^{-4} (parvovirus) to 10^{-8} (papovavirus) substitutions per nucleotide per year (14), while the rate is estimated at 3.5×10^{-8} for large DNA viruses such as human herpesvirus-1 (152 kb) (42)

Imperfect vaccines

Although vaccination allows control of the clinical manifestations of a disease, vaccine-induced immunity generally does not protect against viral infections, and limited virus replication and shedding can still be observed in vaccinated animals. Optimal protection of each individual within large populations is also generally not achievable by mass vaccination strategies, because the level of protection conferred by vaccination can be influenced by multiple factors, the most important being:

- damage to vaccine
- improper administration
- immaturity of the host immune system
- inhibition by maternal antibodies
- immunosuppressed state of the host
- enhanced susceptibility of the host
- insufficient time between vaccination and exposure
- antigenic differences between circulating viruses and vaccine strains
- development of vaccine resistance.

Thus, in the absence of sterilising immunity, or as a consequence of sub-optimal protection, many pathogens still persist within vaccinated populations.

The selective pressure that vaccine-induced immunity may exert on evolving pathogens is largely unknown. A recent analysis of the genetic diversity of BRSV strains, using a large collection of field isolates, revealed a continuous evolution of BRSV, especially in countries where vaccination is widely used. Remarkably, unusual mutations mapping within the conserved central hydrophobic part of the G attachment protein of BRSV were observed in some recent French isolates (55). The biological significance of these mutations in the immunodominant region of BRSV G protein is not known, but they may contribute to the lack of cross-protection between vaccine and field isolates (55). Another example of interaction between vaccine-induced immunity and the genetic diversity of RNA viral populations replicating in the animal host is provided by a large-scale challenge experiment with FMDV in peptide-vaccinated cattle (52). Synthetic peptides conferred only partial protection, and some immunised animals developed lesions upon challenge with virulent virus. FMDV mutants escaping neutralisation in peptide-vaccinated animals were characterised by unusual single amino acid substitutions affecting both virus antigenic structure and receptor-binding specificity (Fig. 2) (4, 6, 52, 53).

The risk of recombination between attenuated or vectored viral vaccines and their wild-type counterparts in co-

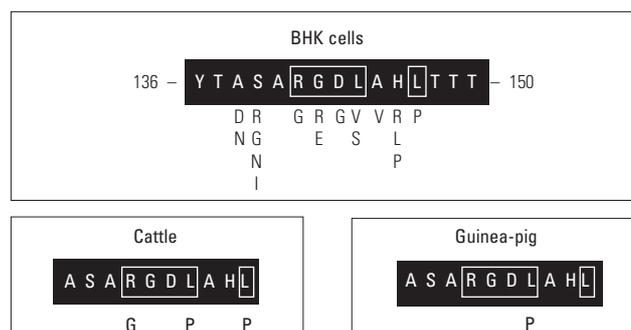


Fig. 2
Exploration of new antigenic/receptor binding structures by foot and mouth disease virus

The overlap between antibody and receptor-binding sites at the surface G-H loop of capsid protein VP1 of foot and mouth disease virus (FMDV) favours the co-evolution between antigenicity and cell tropism in this important animal pathogen. The emergence of unusual FMDV variants displaying both altered antigenicity and modified host cell tropism has been reported in different biological situations including the selection of monoclonal antibody resistant mutants in baby hamster kidney (BHK) cells (31, 41), challenge experiments in peptide-immunised cattle (52, 53), and virus adaptation to the guinea pig (5, 37). The amino acid sequence of the capsid protein VP1 G-H loop region corresponding to antigenic site A of FMDV C-S8c1 is indicated in black boxes. The Arg-Gly-Asp (RGD) integrin-binding motif and flanking amino acid residues promoting FMDV serotype C binding to BHK cells are designated by white-framed boxes (32). Single amino acid replacements found in antigenic site A of FMDV variants are listed for each position

infected animals is generally considered to be a major safety concern for the development and use of live vaccines. The consequences of co-infections were recently assessed in a series of studies involving the alphaherpesvirus BoHV-1. These studies revealed that BoHV-1 exhibits a high potential for genetic diversification in co-infections of the animal host and that recombination is an important mechanism in alphaherpesvirus evolution (46). Several European countries have initiated control programmes based on the use of marker vaccines in which the gE gene of BoHV-1 has been deleted. These marker vaccines, combined with serological detection of gE-specific antibodies, allow differentiation between naturally infected and vaccinated animals. Remarkably, virulent BoHV-1 recombinants carrying the vaccine gE-negative phenotype can be generated *in vitro* by co-infection of attenuated gE-negative with virulent gE-positive BoHV-1 strains (35, 36). As live attenuated marker vaccines can be administered intranasally, at the natural portal of entry of BoHV-1, co-infections between marker vaccine strains and wild-type BoHV-1 can be encountered under natural conditions. This suggests the potential for emergence of recombinant BoHV-1 with the virulence of the wild-type strain and the phenotype of the vaccine strain (54).

Recent epidemiological evidence supports the hypothesis that oncogenic strains of MDV evolve towards pathotypes

with increased virulence, and this evolution is probably driven, at least in part, by MD vaccination (59, 60, 61). However, there are many factors involved in optimal MD vaccination, which will be discussed in detail in the section 'Marek's disease: a case study on evolution of virulence'.

Although it is often difficult to predict the consequences at the population level of mechanisms evidenced at the animal level, these different studies suggest that circulation of pathogens within vaccinated populations may have a number of implications for the development of vaccine resistance, the evolution of virus pathogenicity, and even for the emergence of mutant viruses with altered tissue or host tropism.

Antigenic variation and shifts in receptor usage

Despite early evidence that antigenic changes in influenza virus haemagglutinin could be linked to modifications in sialic acid recognition (50), it is commonly assumed that antibody-binding sites and receptor recognition motifs on virus particles are physically separated. The main argument is that amino acid residues involved in receptor recognition should be invariant, as variation would be lethal, while antigenic sites are inherently variable to allow the virus to cope with the host antibody response. In contrast to this conventional wisdom, structural studies have shown that there is some overlap between these two regions in a number of viral systems, including FMDV (5, 6, 57).

In FMDV, amino acids which are critical for the recognition of integrin receptor molecules, in particular the highly conserved Arg-Gly-Asp (RGD) motif located at the protruding G-H loop of capsid protein VP1, are also involved in the interaction with multiple neutralising antibodies (14, 17). Although integrins are probably the major class of receptor molecules used by FMDV in the animal host, virus evolution in cell culture can render RGD-dependent interactions dispensable for infectivity and develop alternative pathways to recognise and enter cells (17, 24). Because the RGD motif located at the G-H loop of capsid protein VP1 is also a key part of several epitopes recognised by neutralising antibodies, the capacity of FMDV to develop and use RGD-independent mechanisms of cell recognition has considerable implications for the evolution of virus antigenicity. When FMDV entry into cells is restricted to RGD-dependent interactions, the amino acid residues which are critically involved in integrin recognition remain invariant while mutations in flanking residues allow the virus to escape immune surveillance. This situation changes dramatically

upon FMDV acquisition of RGD-independent mechanisms of cell recognition. The relaxation of the constraints imposed by integrin recognition allows FMDV to explore new antigenic structures at the G-H loop of VP1, and variant viruses with highly unusual substitutions, such as Arg-Gly-Gly (RGG) or even Gly-Gly-Gly (GGG) sequences instead of the RGD, can be selected (31, 41). This provides FMDV with a new repertoire of antigenic variants and an improved capacity to escape neutralisation (Fig. 2). Absence of integrin recognition by FMDV harbouring altered RGD motifs has confirmed that changes in FMDV antigenic structures can be linked to modifications in receptor usage, and suggests that viruses which use the same surface site for receptor recognition and antibody binding harbour the potential for co-evolution of antigenicity and receptor usage (5, 6, 53).

The concept of a receptor binding site accessible to antibodies may also have important implications for adaptation of FMDV in the field. The genomic changes that can endow FMDV with the capacity to use alternative mechanisms of cell recognition are minimal (5, 6), and antigenic variants with altered receptor-binding specificities are likely to be present in the mutant spectrum of FMDV replicating in the animal host. A recent study analysing the genetic changes selected during adaptation of FMDV to guinea pigs documented the progressive dominance of an unusual amino acid replacement affecting both the antigenic structure of the G-H loop of VP1 and its interaction with the integrin molecules expressed in various cell lines commonly used to propagate FMDV (Fig. 2) (4, 37). Remarkably, the antigenic alteration found in the guinea pig-adapted virus was also identified in several FMDV mutants escaping neutralisation by anti-FMDV antibodies in peptide-vaccinated cattle (Fig. 2) (52, 53). These results with FMDV mutants generated *in vivo* illustrate the high potential for RNA virus adaptation in the face of an immune response.

Marek's disease: a case study in evolution of virulence

Virulence, the capacity of a pathogen to cause disease, is a phenotypic trait which is subjected to variation and natural selection. MDV provides an excellent example of variation in its potential to cause different disease syndromes and natural selection towards increased virulence. Since the first description of polyneuritis in four roosters by József Marek in 1907 (29), MDV has shown a propensity to cause a number of different disease syndromes including lymphoproliferative syndromes, commonly referred to as MD, lymphodegenerative disease, central nervous system disease syndromes, and atherosclerosis (65). Selection for

increased pathogenicity was already noticed before vaccines were introduced in the early 1970s. The changes in the broiler industry in the 1950s had two important consequences. First of all, the housing system changed, with a drastic increase in the density of chickens per square metre. The birds were also subjected to intensive genetic selection to improve production parameters. During the same period a dramatic increase in MD incidence occurred (8) and the term 'acute MD' was introduced. It is impossible to prove if this change in pathogenicity was caused by a change in the virus, a change in the genetics of the host, a change in the environment of the host or a combination of these factors. The incidence of MD continued to increase in broilers, with condemnations reaching approximately 3% in Delmarva (a peninsula in the USA encompassing Delaware, Maryland and Virginia) and Georgia, until the herpesvirus of turkeys (HVT) vaccine became available around 1970. Since the introduction of MD vaccination in the USA, there have been two periods with increased MD condemnations, which were first noted in Delmarva. The first one, in the early 1980s, led to the introduction of the bivalent HVT+SB-1 vaccine. The second occurred in the mid 1990s and led to the introduction in the USA of the CVI988 vaccine (40), also known as 'Rispen'. The increase in condemnations in the early 1980s was significantly lower than the level of condemnations before the introduction of vaccines, and the second spike was again lower than the first one. Notwithstanding these spikes in condemnations, MDV vaccines are still highly effective in preventing the disease (9, 62). In 2002, MD condemnations were in general less than 0.001% in the USA, although condemnations may be higher in some regions, e.g. Delmarva, which had a condemnation rate of 0.011% in 2002 (34). Worldwide there are few problems reported at this time (20, 34). Spikes in condemnations are sometimes seen in various parts of the world, but these may be caused by many factors and do not necessarily indicate the presence of more virulent strains.

Economic considerations

Before discussing some of the biological factors of MDV–host interactions it is important to address man-made factors influencing these interactions. The poultry industry provides the best example to illustrate some of the problems preventing optimal vaccine-induced protection in production animals. Primary chicken lines are under continuous selection for improved production characteristics, in which selection for disease resistance constitutes only one of the many components. The consequence is that the host is continually changing, which may impact virus–host interactions. In the USA profits per broiler are minimal and highly variable from year to year. Over the last five to six years profits for

broilers have ranged from approximately \$0.10 to actual losses of approximately \$0.01 per pound (0.45 kg) (J. Smith, personal communication). As a consequence the pressure to reduce costs is high. The down-time between production cycles is approximately 14 days in the USA (J. Smith, personal communication) and litter is frequently re-used for a number of cycles (34), thus providing an immediate challenge to newly placed birds before adequate acquired immune responses have developed. MD vaccines are some of the more expensive used in broilers, with average prices in the USA ranging from \$2.25/1,000 doses for HVT to \$10.75/1,000 doses for HVT/CVI988. To reduce costs these MD vaccines are routinely used at half or one quarter of the recommended dose in broilers, although these vaccines are used at the recommended levels in layers and broiler breeders. Although there are differences between the USA and other major poultry production areas of the world, the general trend to reduce costs leads to improper vaccination techniques and inadequate biosecurity, thus increasing the risk of selection for strains with increased pathogenicity.

Nomenclature of Marek's disease virus strains

Marek's disease virus is generally referred to as serotype 1 MDV (MDV-1), and all MDV-1 strains have oncogenic potential unless the strains are attenuated in cell culture (65). Serotype 2 MDV (MDV-2) strains are non-oncogenic chicken herpesviruses, while serotype 3 consists of HVT. These three serotypes belong to the recently established *Mardivirus* genus within the *Alphaherpesvirinae* subfamily of the *Herpesviridae* (<http://www.ncbi.nlm.nih.gov/ICTVdb/Ictv/index.htm> [accessed on 20 September 2006]). Witter *et al.* (58, 63) have developed a system to determine the pathotype of MDV-1 strains isolated from vaccination breakdowns. Briefly, genetically defined chickens (line 15 × 7), positive for maternal antibodies for the three serotypes and vaccinated with HVT or HVT+SB-1 (a MDV-2 strain), are challenged with virus strains with known pathogenicity and with the new strains. Depending on the development of MDV in the groups vaccinated with HVT or the bivalent vaccine, new isolates are classified as virulent (v), very virulent (vv) or very virulent plus (vv+) MDV. European strains are sometimes classified as hypervirulent, which is probably comparable to the vv+ classification in the USA.

Pathogenesis of Marek's disease

The pathogenesis of MD has been extensively reviewed and further references can be found in these reviews (2, 10). Infection occurs by inhalation of cell-free virus and is probably transferred to B lymphocytes by phagocytic cells. Until recently, macrophages were generally considered to be resistant to MDV infection and replication, but Barrow

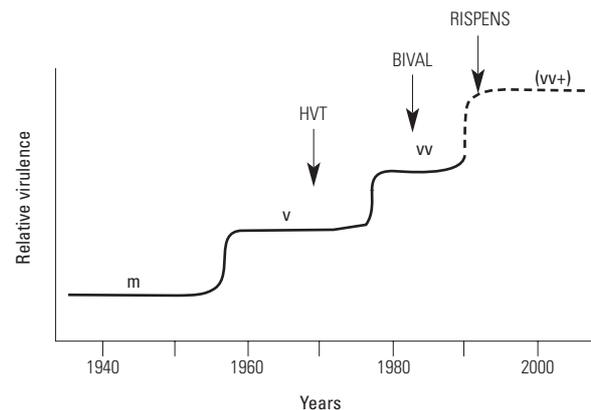
et al. (7) provided support for the role of macrophages by demonstrating the presence of MDV transcripts in the cytoplasm and nucleus of splenic macrophages after infection with a hypervirulent strain of MDV. However, virus particles could not be demonstrated in the nucleus and it is not known if the presence of transcripts represents an abortive or productive infection. The first lytic replication phase mainly occurs in B cells, although some T cells can also be involved. The lytic phase can normally be demonstrated as early as three to four days post infection (PI). Subsequently, activated T cells expressing major histocompatibility complex (MHC) class II and CD4 become infected with MDV, while resting T cells appear to be refractory to infection. Around seven days PI, latent infections are established in the activated T cells. Depending on factors such as genetic resistance of the host, virulence of the MDV strain, vaccination status and presence of immunosuppressive viruses, tumours may develop mostly in CD4⁺, MHC class II⁺ T cells.

Infectious virus is produced in the feather follicle epithelium (FFE), and shedding of MDV starts around 14 days PI, which is before the onset of MD mortality, although experimentally infected birds may die within this period with the early mortality syndrome (65). Quantitative polymerase chain reaction (qPCR) assays have been developed to measure MDV genome copies in the FFE, and initial data suggest that peak shedding occurs between two and five weeks PI (1, 3, 23), although viral DNA has been detected as early as seven days PI (3).

Marek's disease vaccines, applications and protective mechanisms

Since the introduction of HVT in the USA in the early 1970s, 'new' vaccines or new combinations have been introduced twice in the USA. In 1983 the bivalent combination HVT+SB-1 became available, and CVI988 was licensed in 1996 to protect against field viruses with increased pathogenicity (Fig. 3). However, CVI988, which is currently considered the 'gold standard' for MD vaccines (9), has been used in the Netherlands since 1971 and afterwards in other European countries (see 34 for details). Thus, since the introduction of MDV-2 strains (SB-1 and 301B) no new MDV vaccines have been developed that are better than CVI988. Witter and Kreager (64) found that new vaccine strains could be obtained that were comparable to, but not better than, CVI988. They actually questioned if the efficacy of MD vaccines is limited by some, unspecified, type of biological threshold.

Vaccine-induced immunity protects against viral replication, resulting in a significant reduction of the lytic replication phase of the challenge virus (43). However, vaccination does not prevent the establishment of infection in the lymphoid organs nor, and more importantly, in the



m: mild
v: virulent
vv: very virulent
vv+: very virulent plus (hypervirulent)
HVT: herpesvirus of turkeys vaccine
BIVAL: bivalent HVT+SB-1 vaccine
RISPENS: CVI988 vaccine

Fig. 3

Evolution of Marek's disease virus isolates.

Stepwise evolution of virulence of Marek's disease virus isolates: past history and future predictions. There seems to be a relationship between introduction of new vaccines and the development of more virulent pathotypes

From (59), with permission of Taylor and Francis Ltd (<http://www.tandf.co.uk/journals>)

FFE. Interestingly, qPCR studies suggest that vaccination with HVT does not reduce shedding of MDV-1 at 56 days PI (23). Additional studies are needed to determine if different pathotypes differ quantitatively in virus shedding and if this is modified by vaccination.

Are virulence- and transmission-related traits linked to pathogen fitness in Marek's disease virus?

Although it has been clearly shown that more virulent MDV pathotypes have evolved since the introduction of vaccines, the molecular basis for the evolution of MDV has not been elucidated (45). Early virus replication in nonvaccinated birds is prolonged for more virulent pathotypes compared with less pathogenic strains (66) and is higher in nonvaccinated than in vaccinated birds independent of the pathotype (43), suggesting that the evolution of MDV is related to the early virus–host interaction, perhaps by interference with immune responses. However, genes important for viral replication, e.g. viral interleukin (vIL)-8, pp38 (11, 21, 26) and several glycoproteins, are highly conserved across pathotypes (25, 48), which is of interest because these genes, with the

exception of vIL-8, are recognised by cell-mediated and/or humoral immune responses (30, 44). Mutations in the Meq gene correlating with virulence were described by Shamblin *et al.* (48). These authors argued that Meq is not a likely target for vaccine-induced selection because the Meq gene is not encoded by HVT or SB-1 and Meq is mostly expressed during latency and in transformed cells. Moreover, deletion mutants for Meq can cause cytolytic infections comparable to infection with wild-type virus, but do not cause tumours (28).

Gandon and colleagues (18) have suggested that vaccines reducing the growth rate of a pathogen, e.g. MD vaccines, would lead to evolution of the pathogen towards increased virulence, while vaccines blocking infection would not induce such effects and may even lead to decreased virulence. They also suggest that virulence and transmission are related traits intimately linked to pathogen fitness (39), although this hypothesis is not generally accepted (16). If this hypothesis is correct, it would suggest that the more virulent MDV pathotypes should be more efficient in virus shedding from the FFE than less virulent pathotypes. As was mentioned before, there is a lack of quantitative data on MDV shedding from the FFE. Baigent *et al.* (3) quantitated the number of CVI988 genome copies in FFE from different commercial chicken breeds and found differences between the breeds, but comparative studies with other pathotypes have not yet been reported. However, there may not be an intimate link between virulence and transmission in MD, based on studies using deletion mutants. Very virulent MDV or vv+MDV mutants lacking the pp38 gene (21), vIL-8 gene (11) or the first intron of vIL-8 (26) show a significant reduction in lytic virus replication and a subsequent reduction in tumour incidence, probably as a consequence of reduced virus load. However, these viruses do produce cell-free virus in the FFE (11, 21). It will be important to conduct quantitative studies to determine if the amount of virus in the FFE is directly related to replication of mutant viruses in the lymphoid organs and if there are quantitative differences between wild-type and mutant viruses in the amount of virus produced in the FFE.

The future of Marek's disease protection

Based on past experience it is expected that more virulent pathotypes than vv+ strains of MDV will emerge. Based on the work by Witter and Kreager (64) it is less certain that improved vaccines will be available in the near future. Recombinant fowlpox virus (rFPV)-based vaccines expressing MD glycoproteins have shown some promise under experimental conditions (27). Based on experiences with rFPV vaccines for avian influenza (51) it is not clear if these vaccines will give sufficient protection if chicks are positive for FPV maternal antibodies. In addition, in order

to be used by the industry any new vaccine must be cost-effective, reducing MD contamination at a cost comparable to current vaccine prices.

Future strategies for protection against MD may have to depend on a combination of vaccination, increased genetic selection, and perhaps transgenic approaches. All current commercial and experimental vaccines are based on the reduction of virus replication rather than prevention of infection. It is not known if vaccines preventing MDV infection can be developed. To be successful these vaccines need to block entry of virus into phagocytic cells, which may be complicated by the nature of cell-free virus produced in the FFE. Most, if not all, cell-free virus particles are encased in keratin, and vaccines designed to block virus–cellular receptor interactions may therefore not work. Mechanisms to block virus replication after entry into susceptible cells (using macrophages, B cells, activated T cells, epithelial cells) may be possible using RNA interference (RNAi) approaches. Transgenic approaches, which may not be acceptable to consumers, will be needed to express the RNAi sequences in all potential target cells.

Conclusion

In a global economy with open markets and frequent exchanges of animals and livestock products, the control of infectious diseases has become a major concern for the farming industry. Vaccination, when available, is probably the most effective method of protecting animal populations against economically relevant pathogens, and mass vaccination strategies have largely contributed to the control of infectious diseases in endemic areas.

Despite reducing the clinical manifestations of the disease, vaccination rarely protects against viral infection, and many pathogens still persist within vaccinated populations. Epidemiological studies have documented a rapid and continuous diversification of different viral pathogens in large populations of animals protected by vaccination, suggesting that the evolution of these pathogens was probably driven, at least in part, by vaccination. The biological implications of vaccine-driven evolution of viral pathogens remain largely unknown, and recent evidence suggests that the development of vaccine resistance may also be associated with modifications in pathogenicity or host tropism, as illustrated by the emergence of MDV pathotypes of enhanced virulence and the selection of FMDV variants with modified receptor-binding specificities. Recombination between attenuated marker vaccines and field strains of BoHV-1, with the potential emergence of virulent BoHV-1 expressing the phenotypic traits of the marker vaccines, provides another

remarkable example of virus adaptability upon evolution in vaccinated populations. This has implications for control and eradication strategies based on serological differentiation between vaccinated and infected animals.

The evolution of viral pathogens circulating in vaccinated-populations may represent a new challenge for the animal health sector. There is a need to better understand the dynamics of viral pathogens replicating in the field, in particular those evolving in animal populations protected by vaccination. ■

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La vaccination des animaux et l'évolution des virus

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Résumé

Si la vaccination parvient à réduire l'incidence des maladies, il est rare qu'elle confère une protection contre le processus infectieux, de sorte que nombre d'agents pathogènes continuent à circuler au sein des populations d'animaux vaccinés. Cette circulation virale risque de favoriser l'apparition d'une résistance aux vaccins, avec des conséquences sur l'évolution de la pathogénicité des virus et l'émergence de virus variants. Les taux élevés de mutation accompagnant la multiplication des virus à acide ribonucléique (ARN) favorisent l'apparition de mutants d'échappement. Chez des bovins vaccinés, des mutations inhabituelles ont été observées au niveau du site antigénique majeur du virus de la fièvre aphteuse qui est également impliqué dans la reconnaissance de récepteurs. De même, des modifications atypiques ont été détectées dans la région immunodominante du virus respiratoire syncytial bovin. Les grands virus à acide désoxyribonucléique (ADN) ont la capacité de se recombiner et de créer de nouveaux génotypes, comme c'est le cas des souches vaccinales de l'herpèsvirus bovin de type 1 porteuses d'une délétion dans le gène codant pour la glycoprotéine gE, qui présentent un risque de recombinaison avec les souches sauvages. Le virus de la maladie de Marek est souvent cité comme exemple de la modification du pouvoir pathogène induite par la vaccination. Les causes exactes de cette virulence accrue restent à élucider ; les auteurs examinent quelques explications possibles de ce phénomène.

Mots-clés

Évolution – Maladie de Marek – Pathogénicité – Pathotype – Récepteur – Tropisme – Vaccin – Virulence – Virus. ■

Vacunación de animales y evolución de los patógenos virales

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Resumen

Pese a que reduce la incidencia de la enfermedad, la vacunación pocas veces protege contra la infección y muchos agentes patógenos siguen presentes en poblaciones animales vacunadas. La circulación de agentes patógenos virales en esas poblaciones puede favorecer el desarrollo de resistencia a las vacunas, con implicaciones para la evolución de los virus y la emergencia de variantes virales. La elevada tasa de mutaciones durante la replicación de los virus ácido ribonucleico (ARN) favorece la emergencia de mutantes de escape. En ganados vacunados se han observado mutaciones inusuales en el sitio antigénico principal del virus de la fiebre aftosa, implicado también en el reconocimiento de receptores. Del mismo modo, se han detectado cambios atípicos en la región inmunodominante del virus respiratorio sincitial bovino. Los grandes virus ácido desoxiribonucleico (ADN) pueden recombinarse, dando lugar a nuevos genotipos, como lo muestra el potencial de las cepas vacunales del herpesvirus bovino de tipo 1 con delección en el gen de la glicoproteína E para recombinarse con cepas salvajes. El virus de la enfermedad de Marek suele citarse como un ejemplo de cambio de patogenicidad provocado por la vacunación. Aún no se han elucidado los motivos por los que aumenta la virulencia y los autores contemplan las posibles explicaciones.

Palabras clave

Evolución – Enfermedad de Marek – Patogenicidad – Patotipo – Receptor – Tropismo – Vacuna – Virulencia – Virus.



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