Introduction to Veterinary Vaccines

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Abstract

Vaccination of animals has been carried out for centuries, and it is the most cost-effective and sustainable method of controlling infectious diseases. Veterinary vaccines not only are important to animal health but also play a vital role in reducing transmission of zoonotic diseases to humans and in securing food supply for humans. Conventional inactivated (killed) or live-attenuated vaccines constitute the majority of licensed veterinary vaccines that are currently in use. The widespread use of these vaccines not only substantially contributed to animal welfare and public health but also led to a successful global eradication of rinderpest, one of the animal diseases with major economic consequences in many parts of the world. Despite these successes, there are some limitations associated with conventional vaccines, and there are still several diseases that have yet to be successfully treated, demonstrating the need for better and safer vaccines. Recombinant vaccines represent an attractive strategy by which some of the limitations of conventional vaccines can be overcome. In the recent past, the veterinary field has witnessed the most successful applications of recombinant vaccines where more than a dozen viral-vectored vaccines, subunit, DNA, and virus-like particles-based vaccines were licensed for veterinary use, and many more are under development. There is a wave of rationally designed vaccine innovations ahead of us to benefit animals, animal owners, and ultimately humans.

Keywords

Antigens · Conventional vaccines · DNA vaccines · Rational design · Recombinant vaccines · Subunit vaccines · Vector vaccines · Veterinary vaccines · VLP-based vaccines

Learning Objectives

After reading this chapter, you should be able to:

- Describe how veterinary vaccines were developed starting with conventional vaccines to genetically engineered ones with the emphasis on vaccines for viral and bacterial diseases
- Explain the progress made from traditional to technology-based modern vaccines using examples of licensed vaccines
- Discuss remaining challenges and future research directions in developing improved veterinary vaccines, where appropriate

Note: Veterinary vaccines for parasitic and noninfectious diseases (allergy, cancer, fertility, etc.) are not covered here, and the reader is advised to refer to recent excellent reviews on these subjects [1–3].

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1 Introduction

Vaccination aims to mimic the development of naturally acquired immunity. The terms "vaccine" and "vaccination" are derived from Variolae vaccinae, first coined by Edward Jenner in 1796 to describe the inoculation of materials collected from a lesion on a milkmaid suffering from cowpox to confer protection against the related human smallpox virus [4, 5]. Louis Pasteur is another pioneer who discovered how to make vaccines from attenuated microbes in the mid-nineteenth century. He developed the earliest vaccines against fowl cholera, anthrax, and rabies [6, 7]. Subsequent breakthroughs in vaccine development came out in the 1950s by adaption of in vitro preparation using chicken embryos and tissue culture cells for large-scale production [8, 9].

Vaccination is the most widely used tool in veterinary medicine to prevent and control animal diseases, and it represents the most cost-effective and sustainable intervention. Protection of livestock from infectious diseases contributes to better welfare, helps to enhance their productivity and profitability for livestock producers, and also ensures the provision of healthy and nutritious food such as eggs, milk, and meat products for consumers. Further benefit of immunization of livestock animals is the reduction in antibiotic use and a subsequent reduction in their residue in animal products contributing to public safety. On the other hand, the purpose of vaccination in companion animals is primarily aimed at their welfare by preventing particular infectious diseases. The close association between people and their pets would not be as carefree without vaccination. Regardless of the animal types, vaccination serves as a primary defense to prevent diseases in animals and also further transmission from animals to humans. Rabies is an excellent example of vaccine-preventable viral zoonotic disease which occurs in more than 150 countries and territories. Dogs are the main source of human rabies deaths, contributing up to 99% of all rabies transmissions to humans [10]. Through mandatory dog vaccination, dog-mediated rabies has been eliminated from Western Europe, Canada, the United States, and Japan, demonstrating that rabies elimination is feasible through vaccination of dogs and prevention of dog bites. In the developed world, the fight against rabies is brought to another level by introducing vaccines that can be administered orally into wildlife to control rabies in wild animals and also prevent the spillover to domestic animals. An outstanding example of such a vaccine is RABORAL V-RG®, the first licensed vaccinia virus-based vector vaccine expressing the glycoprotein of rabies virus. RABORAL V-RG® allowed oral vaccination on a large scale using baits containing vaccine [11]. The introductory chapter is divided into conventional and genetically engineered veterinary vaccines sections, where a broader coverage is devoted to genetically engineered veterinary vaccines in general, and viral vector vaccines in particular, as it is the main subject of this textbook.

2 Conventional Live and Inactivated Veterinary Vaccines

The majority of the licensed veterinary vaccines that are currently in use are inactivated (killed) or live-attenuated/modified live vaccines (MLV). Vaccine strains for inactivated vaccine preparation can be pathogenic wild-type isolates that are subsequently killed to avoid risk of causing disease. Thus, inactivated or killed vaccines preparations are generally safe and do not pose a risk of reversion to virulence. However, they are unable to infect host cells and activate cytotoxic T cells, and consequently, they are less protective and generally require strong adjuvants to induce the required level of immunity. Because of a higher production cost and the need for adjuvants, inactivated vaccine preparations are relatively more expensive and generally require multiple administrations by parenteral route to induce optimal immunity [1, 12]. Recent research toward improving the effectiveness and duration of immunity of inactivated vaccines has focused on the development of improved adjuvants [13].

Strains for MLV preparations, in contrast, need to be sufficiently attenuated by serial passaging of viruses or bacteria in an unnatural host or cell with the hope that random mutations result in a non-virulent, but replicative competent infectious agent [1]. Such vaccine preparations can replicate in target cells and induce both cellular and humoral immunity and generally do not require an adjuvant to be effective. As opposed to inactivated vaccine preparations, live vaccines can be administered by mass administration routes such as spray, drinking water, in ovo, etc. The major drawback of live vaccine preparations is the potential risk of reversion to pathogenic wild type as well as being a potential source of environmental contamination. Despite such disadvantage, MLV products have played a major role in successful disease control and eradication. An excellent example is the eradication of rinderpest from the globe that was declared on 25 May 2011 [14]. This achievement is critically dependent on the use of the "Plowright" vaccine [15], which is an attenuated vaccine produced from the Kabete O strain passaged 90 times in tissue culture [16]. In summary, conventional live and inactivated vaccines for a wide range of infectious diseases have been available for several decades and are still being developed for some emerging diseases. They are widely used in veterinary medicine and contribute considerably to the improvement of animal and public health [1, 2, 17].

3 Genetically Engineered Veterinary Vaccines

The availability of rich information on viral and bacterial genomes along with the advancement of available genetic tools allowed specific modifications or deletions to be introduced into the genome, with the aim of producing well-defined and stably attenuated live or inactivated viral or bacterial vaccines. Because of their relatively smaller size, viral genomes were used to be more amenable to genetic manipulations than bacterial genomes, and as a result, most of the genetically engineered licensed vaccines are based on viral vaccines. However, recent advances in genome editing opened up new avenues for multiple applications by allowing genetic material to be added, removed, or altered at particular locations in the genome of organisms much larger than viruses [18, 19].

Despite the method used, the ability to identify and selectively delete genes from a pathogen has allowed the development of "DIVA vaccines" which, combined with suitable diagnostic assays, allow differentiating infected from vaccinated animals (DIVA). The DIVA principle not only extends to vaccines with deleted genes but also includes subunit and other types of vaccines that induce an antibody response that is different from the antibody response produced by the wild-type organism. An accompanying diagnostic method, tailored for optimal sensitivity and specificity toward the epitopes distinguishing the vaccine from the wild-type, accounts for the DIVA diagnostics test. The first DIVA vaccine was used to differentiate between infected and vaccinated pigs for pseudorabies [20]. Such DIVA vaccines and their companion diagnostic tests are now available for several diseases including infectious bovine rhinotracheitis, classical swine fever, foot-and-mouth disease, etc. [17].

4 Subunit and Virus Like Particles (VLP)-Based Vaccines

Subunit-based vaccines contain only part of the virus or bacteria that is capable of inducing protective immune response against that component only. Thus, subunit vaccines can be compatible with the DIVA principle as long as the pathogen consists of another protein capable of consistently inducing antibodies in wild-type infected animals. The protective antigen or multiple of antigens of pathogens can be generated as recombinant proteins in various expression systems such as E. coli, yeast, baculovirus-insect cell, etc. to be used as subunit vaccines. A large quantity of recombinant proteins can be expressed, purified, and formulated in most of the cases with a potent adjuvant to be used as a safe and nonreplicating subunit or VLP vaccines. A

typical subunit veterinary vaccine composed of a single protein is a recombinant OspA vaccine for the purpose of preventing Lyme disease in dogs caused by the spirochete, *Borrelia burgdorferi*. Interestingly, even a non-adjuvanted recombinant OspA (Recombitek® Lyme) was demonstrated to completely prevent *Borrelia burgdorferi* infection in vaccinated dogs [21]. Diagnostic assessment of Lyme "positive" or "negative" is made using a popular test kit (SNAP®), which is a patient-side, lateral flow ELISA technology that can detect antibody or antigen to a variety of vector-borne parasites, including C6 antibody to *Borrelia burgdorferi* [22]. An example of a more complex subunit vaccine was also developed against porcine contagious pleuropneumonia, a severe disease of pigs with hemorrhagic necrotizing pneumonia and high mortality in the acute form. The disease is caused by Actinobacillus pleuropneumoniae, and prevention by vaccination has been severely restricted by the prevalence of 15 different serotypes. The two available subunit vaccines are composed of either acellular A. pleuropneumoniae four extracted proteins (Porcilis® APP) or five recombinant proteins (Pleurostar APP™), which confer some degree of cross-protection against all tested serotypes [1]. There is currently a large amount of scientific interest in the identification of immunogenic and protective antigens for animal pathogens and expressing them in a safe heterologous expression system; thereby, handling of virulent or partially virulent microbes can be eliminated by the manufacturer as well as the end user.

As shown above, the end product of a subunit vaccine is recombinant protein(s), whereas in VLP-based vaccines, the protein(s) further spontaneously assemble into supramolecular structures resembling infectious viruses or, in some cases, subviral particles. Thus, VLPs are structurally similar to infectious viruses and thus are highly immunogenic but, because they lack viral nucleic acid, are noninfectious and safe [23]. In addition, VLPs do not induce antibody responses to internal or nonstructural viral proteins, thereby allowing distinction between vaccinated and infected animals [24]. These advantages have made VLPs attractive vaccine candidates for many viral diseases [25–29]. In the veterinary field, two baculovirus-expressed VLP-based vaccines were approved and widely used (Ingelvac® CircoFLEX and Porcilis PCV®) to provide protection against disease caused by porcine circovirus type 2 (PCV2) [30, 31]. PCV2, a member of the Circoviridae family, is associated with postweaning multisystemic wasting syndrome, a swine disease characterized by wasting, weight loss, respiratory distress, and diarrhea, that has a severe economic impact on production [32]. The immunogens in both vaccines are VLPs formed by the assembly of the single capsid protein encoded by the open reading frame 2 of PCV2. The assembly of these VLPs from single proteins allows a cost-effective vaccine production at large scale in the baculovirus-insect cells expression system. Although VLPs have been produced for a wide range of viruses under experimental condition, clearly not all are equally suitable for the development of commercial vaccines, due to several challenges ranging from scalability and cost-effectiveness to the need of co-expressing multiple proteins, including viral envelope proteins [30, 33, 34].

5 DNA Vaccines

Similar to subunit or VLP-based vaccines, DNA vaccines are encoding only part(s) of the pathogen, but antigen is produced intracellularly mimicking antigen expression by replication of live pathogen, thereby leading to the development of both humoral and cellular immune response. In addition, innate immune responses are also stimulated as plasmids contain molecular elements such as unmethylated CpG motifs that are not prevalent in mammal, avian, and fish cells [35, 36]. DNA vaccines are safe as production of plasmids does not involve manipulation of infective antigens. As immune responses are developed only against those coded antigens in the plasmid, DNA vaccines are also compatible with the DIVA principle. The DNA vaccines licensed so far are veterinary vaccines that include APEX-IHN® to prevent infectious hematopoietic necrosis (IHN) in farm-raised salmon and West

Nile-Innovator® DNA to prevent horses from West Nile disease. The first USDA-licensed therapeutic vaccine, ONCEPT® Canine Melanoma, is also a DNA vaccine that is proven to extend the lives of dogs treated for oral melanoma [37]. One of the main drawbacks of DNA vaccination in large animals and poultry has been their relative low efficacy and relatively high cost of goods mainly due to the large amounts of DNA needed to be injected in order to achieve a strong response. Thus, several strategies are under development in order to achieve better responses with less amounts of DNA.

6 Viral Vector-Based Vaccines

Viruses have evolved sophisticated structures and mechanisms to infect cells, and hence, they serve as efficient delivery vectors of various antigens. The concept of viral vector was introduced in 1972 when Jackson et al. created recombinant DNA from the SV40 virus by genetic engineering [38]. A decade later, the use of vaccinia virus as a transient gene expression vector was described [39, 40]. Thereafter, nonpathogenic or host-restricted viruses carrying foreign genes have been used as delivery vehicles that can be administered into a host, creating protective immunity to the inserted proteins. Although host-restricted vector viruses will not be productively replicating within the tissues of the vaccinated animals, they are able to express the foreign gene [41]. Viral vector-based vaccines are mostly used without an adjuvant, and like subunit and DNA vaccines, they allow for differentiating infected from vaccinated animals (DIVA). The first licensed viral vector-based vaccine (RABORAL V-RG®) is an oral rabies vaccine bait that contains an attenuated ("modified-live") recombinant vaccinia virus vector expressing the rabies virus glycoprotein gene of Evelyn-Rokitnicki-Abelseth rabies virus [42, 43]. RABORAL V-RG® has been in continuous use since 1987 when it was first field-tested in foxes in Belgium [44]. Thereafter, approximately 250 million doses have been distributed globally [11]. Before its introduction, rabies control in wildlife relied mostly on depopulation and the vaccination of individual animals. RABORAL V-RG® allowed oral vaccination on a large scale using vaccine-containing baits, and several countries have used RABORAL V-RG® safely without any adverse effects and have achieved complete rabies control [45, 46].

Subsequent poxvirus-based licensed products include TROVAC™-AIV H5, which is a bivalent recombinant fowlpox virus expressing the H5 antigen of avian influenza virus. This product has had a conditional license for emergency use in the United States since 1998 and has been widely used in Central America, with over four billion doses administered [47]. As the vaccinated birds will not develop antibodies against matrix protein/nucleoprotein, this vaccine can also be used with a DIVA approach. Additional fowlpox-based bivalent vaccines include Vectormune® FP-LT and Vectormune® FP-MG, which are indicated as aids in the prevention of fowlpox and infectious laryngotracheitis and fowlpox and Mycoplasma gallisepticum, respectively [48].

The most extensively used poxvirus vector platform in animal health is the ALVAC platform based on canarypox virus. Canarypox virus has the advantage of being more host-restricted than vaccinia virus. Because of the host restriction, canarypox virus recombinants produce abortive infections in mammalian cells, but they still effectively express inserted foreign genes. Currently, the canarypox virus or ALVAC vector platform has been used as a vaccine vector for a range of veterinary diseases of companion animals, including canine distemper, feline leukemia, rabies, West Nile, and equine influenza [49–54]. With the exception of equine influenza vaccine (PROTEQFLU®), all ALVAC-based vaccines are non-adjuvanted. Interestingly, the PROTEQFLU® contains a polymer adjuvant, and through the induction of both cell-mediated and humoral immunity, it is claimed to produce sterile immunity 2 weeks after the second of two doses [55].

Outside of poxviruses, the most successful and widely used vaccine vector in veterinary medicine is herpesvirus of turkeys (HVT). First and

foremost, HVT has been widely accepted as a safe and effective vaccine against Marek's disease in chickens for almost 50 years [56, 57], providing an excellent vector backbone for the induction of protection against two or more poultry diseases. HVT has also unique features including the ability to cause persistent infection. As a result, a single dose of vaccine delivered in ovo to 18-day-old embryo or subcutaneously to 1-day-old chicks at the hatchery [58] induces a lifelong immunity. Like poxviruses, herpesvirus genomes can accommodate long fragments of foreign DNA without compromising their ability for normal replication [59]. These promising properties of HVT as a vector have led to the development and commercialization of HVT-based recombinant vaccines against important poultry diseases, including IBD, ND, and ILT [VAXXITEK® HVT + IBD, Innovax®-ILT, VECTORMUNE® ND, etc.]. One of the limitations of this extremely effective vector platform is the interference when more than one recombinant HVT vaccines are simultaneously administered. Hence, most of the recent research in this area has focused on either expressing more than one foreign genes or developing compatible viral vectors that could be combined with HVT in inducing protection against multiple avian diseases in multivalent vaccines [60].

7 Future Perspectives and Research Directions

Combination Vaccines Prevention rather than treatment is the most effective means of controlling infectious diseases in animal health. With the number of vaccines growing, combination vaccines are becoming more important. Protection against several diseases with fewer injections while maintaining the efficacy and safety of single-component vaccines helps not only to reduce costs but also to simplify overloaded immunization schedules [61]. In using combination vaccines, each component of the vaccine must be assessed individually and in combination to avoid an inappropriate immune response as a result of unwanted interaction of the various components with each other [62]. In general, one observes higher interference in live multivalent vaccines than in killed multivalent vaccines. Thus, the challenge in this respect is to be able to combine multiple antigens in one vaccine without substantially reducing the efficacy and safety of the individual components.

The enormous potential of expressing multiple protective antigens from two or more pathogens in a single vector also opens the door for a new generation of multivalent viral vector vaccines. This is especially true of larger viral vectors, like herpesviruses and poxviruses, where there are few restrictions imposed by gene packaging limits. However, as we attempt to push the biological boundary by inserting multiple genes, issues like genetic instability of the vector and/or insert or less efficient replication of the vector backbone becomes more of a problem. Genetic instability is one of the major challenges in making viral vector-based vaccine candidates as some vectors are simply incompatible with certain sequences, length, or configuration of the insert. Consequently, the virus acting as the vector may introduce mutations or deletions into the inserted gene as well as into part of the vector backbone to abrogate proper expression of the transgene(s). It is, therefore, necessary to analyze the stability of these vectors during the early stages of vaccine research by serial rounds of replication in cell culture as well as in target animal species as required by the regulatory agencies (reversion to virulence or back passage studies). Obviously, the efficacy of a multiantigen vector should be satisfactory to meet the regulatory requirements as well as customer's need under field conditions.

Overcoming Maternal/Preexisting Immunity Maternal immunity, a form of passive immunity, plays a vital role in protecting young animals against severe disease upon infection with virulent field virus. However, maternal immunity is not without its negative effects. It often interferes with active immunization and is the most common cause of vaccine failure in animals. As

vaccinations to most animals are preferably given at a young age, overcoming the interference by maternal immunity after vaccination is one of the greatest challenges in animal health. Although maternally derived antibody (MDA) interference is complex as many factors can influence the outcome after vaccination, one possibility that may be pursued to achieve this goal is to implement a heterologous prime-boost strategy. An excellent example that demonstrated the usefulness of this strategy in circumventing MDA interference used a vectored fowlpox virus expressing the hemagglutinin gene of avian influenza H5. The fowlpox-AI recombinant was administered at 1 day of age for early priming of chickens with MDA against fowlpox or avian influenza followed by a booster vaccination with whole virus-inactivated vaccine [63].

More and Improved Bacterial Vaccines Apart from improving animal health and productivity, veterinary vaccines have a significant impact on public health through reductions in the nontherapeutic use of antibiotics and hormones and their residues in the human food chain. The emergence of antibiotic-resistant bacteria in food-producing animals as well as in humans and the increasing restriction of the use of nontherapeutic antibiotic in livestock evidently promote the use of vaccine rather than antibiotics. Unfortunately, for a number of bacterial pathogens, vaccines are either still missing or some of the existing vaccines are not completely protective. One of the reasons for the poor protection is the existence of many different serotypes for a given disease and the poor level of cross-protection between serotypes. The growing power of combining sequencing, structural, and computational approaches may help the design of novel cross-protective immunogens suitable for future improved bacterial vaccine development.

Rational Antigen Design Prospects for new vaccines stem primarily from advances in genetic engineering and the ability to define the antigens responsible for inducing protective immunity. Recent advances in genomics, structural, and computational biology are opening the door to new technologies such as reverse vaccinology that allow designing novel vaccines against diseases unamenable to traditional vaccine development strategies [64, 65]. These approaches allow identification of a broader spectrum of vaccine candidates, including proteins that had not been identified and/or not abundant. The ability to rationally design candidate antigens can provide more cross-protective antigen candidates against antigenically variable pathogens [66]. In this regard, the parallel discovery and development of new and improved adjuvants for recombinant targets is essential to obtain the desired level and duration of protective immune response against different target pathogens. To speed up the implementation of these new technologies in animal health, closer interaction between research groups developing human and veterinary vaccines should be encouraged as common technologies can be applied in each areas. Consequently, animal vaccine research scientists can lean on medical research for some breakthrough technologies, but may also contribute to human vaccine development, as they are able to bridge the gap in translating results obtained in small-rodent models to large animal (human) application.

8 Summary

Much progress has been made in expanding the range of existing veterinary vaccines and introducing new technology-based vaccines with increased efficacy and reduced side effects. Due to the ability to directly test new vaccine candidates in target animal species, veterinary vaccines have a relatively quicker route to the market and thus are at the forefront of testing and commercializing innovative technologies, as exemplified by the successful licensing of vectored, subunit, DNA, and VLP-based veterinary vaccines. However, there are still many problems that remain to be addressed, and there is ample scope to incorporate new knowledge and technologies such as mRNA, replicon, and particle-scaffold-based platforms into vaccine design to fill the gaps. A better understanding of

the molecular and immunological disease processes as well as the interaction between pathogens and the host immune system is likely to be required to improve the effectiveness of new and improved veterinary vaccines. Moreover, the interchange between scientists working on animal and human disease will remain essential to be prepared for the ever-present threat of new and reemerging diseases. The increased recognition of the "One Health" concept undoubtedly helps to foster this research collaboration. Based on the past significant advances in vaccinology and future opportunities for new innovations, it is beyond doubt that vaccines will continue to play a crucial role in maintaining and improving the health of animals and ultimately humans.

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