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REVIEW ARTICLE

# The development of veterinary vaccines: a review of traditional methods and modern biotechnology approaches

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**Abstract** The immunization of animals has been carried out for centuries and is generally accepted as the most cost-effective and sustainable method of controlling infectious veterinary diseases. Up to twenty years ago, most veterinary vaccines were either inactivated organisms that were formulated with an oil-based adjuvant or live attenuated vaccines. In many cases, these formulations were not very effective. The discovery of antigen/gene delivery systems has facilitated the development of novel prophylactic and therapeutic veterinary vaccines. To identify vaccine candidates in genomic sequences, a revolutionary approach was established that stems from the assumption that antibodies are more readily able to access surface and secreted than cytoplasm proteins; as such, they represent ideal vaccine candidates. The approach, which is known as reverse vaccinology, uses several bioinformatics algorithms to predict antigen localization and it has been successfully applied to immunize against many veterinary diseases. This review examines some of the main topics that have emerged in the veterinary vaccine field with the use of modern biotechnology techniques.


## Introduction

Vaccinations are an effective method of preventing a wide range of animal diseases. The field of vaccinology has yielded several effective vaccines that have significantly reduced the impact of some important diseases in both companion animals and livestock. Today, the vast majority of licensed veterinary vaccines are in the form of live attenuated, killed/inactivated microorganisms, cell membrane compounds or toxoids (McVey & Shi, 2010; Unnikrishnan, Rappuoli, & Serruto, 2012). Live attenuated vaccines can be very effective because they induce both cellular and humoral immune responses (da Costa, Walker, & Bonavia, 2015; Rizzi et al., 2012). However, a major concern that is associated with vaccines of this nature is the potential risk of reversion of the microorganism for a virulent phenotype (Shimoji et al., 2002; Unnikrishnan et al., 2012). Killed/inactivated vaccines are typically safer; however, they may be less effective than attenuated vaccines. The commercial vaccines based on toxoids (inactivated toxins) have some drawbacks since they require complex components in culture medium. The limitations of the three existing vaccine types in combination with the fact that

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several diseases have yet to be successfully treated with an efficient vaccine entails there is a need for better and safer vaccines that can prevent, control or eradicate animal diseases (Dunham, 2002; Redding & Weiner, 2009).

Recombinant vaccines represent an attractive strategy by which the limitations of conventional vaccines can be overcome, and a number of rationally designed and subunit vaccines have already reached the veterinary market. Efforts to develop more effective vaccines against a large number of diseases using recombinant DNA technology are in progress around the world. Recombinant vaccines are developed based on rationally designed recombinant highly purified antigens through structure-based design, epitopes focusing or genomic-based screening (Correia et al., 2014; Dellagostin et al., 2011). In addition to enhancing understanding of the genes responsible for virulence and facilitating the identification of the determinants of protective immune responses, these molecular approaches have provided new methods of developing novel vaccines against infectious, parasitic or metabolic diseases.

However, the inherent immunogenicity of recombinant antigens is often low in comparison to the more traditional vaccines, and there is a need for potent and safe vaccine adjuvants to ensure that recombinant vaccines can succeed. The low immunogenicity frequently observed in recombinant antigens occurs due to a lack of exogenous immune activating components. Recombinant antigens can be offered in different adjuvants, and the immunomodulatory effects are dependent upon the particular adjuvant used in conjunction with specific antigens.

In this review, we summarize the conventional and recombinant vaccines used in veterinary medicine and the molecular approaches that have led to the development of new vaccines in recent years. We have focused on vaccines that target infectious diseases.

## Conventional veterinary vaccines

Historically, the development of veterinary vaccines was based on empirical trial-and-error approaches that were designed to mimic, by vaccination, the immunity induced by natural infection (Doolan, Apte, & Proietti, 2014). The conventional ''isolate, inactivate or kill and inject'' approach can induce protection against a wide range of bacterial and viral pathogens. The majority of the licensed veterinary vaccines that are currently in use are inactivated (killed), live-attenuated vaccines or toxoids. In fact, the widespread use of these vaccines has contributed considerably to the improvement of animal and public health. However, conventional vaccines are generally expensive to produce, and need to be administered multiple times to induce optimal immunity (Delany, Rappuoli, & Gregorio, 2014; Meeusen, Walker, Peters, Pastoret, & Jungersen, 2007).

Additionally, the whole-organism approach to vaccination is almost exclusively restricted to pathogens that can be cultured *in vitro*. Although this process has been successful for a number of ''simple'' pathogens with relatively low antigen variability, it has not been effectively applied to vaccinate against pathogens that have high antigenic diversity or/and are capable of evading or misdirecting the host immune response (Doolan et al., 2014). Also, traditional vaccine design is based on a strategy that involves mimicking the immunity induced by natural exposure; however, in the case of many pathogens, this is suboptimal and robust sustained protection may require inducing an immunity that exceeds the natural biological immunity while also ensuring the adverse effects associated with stimulating the inflammatory response are minimized (Zepp, 2010). This is especially true for chronic infections, in which the pathogen is able to co-exist with the host for an indefinite period of time despite the presence of immune responses induced by the host and targeted against the pathogen (Doolan et al., 2014).

Live-attenuated modified vaccines are capable of inducing both humoral and cell-mediated immune responses. In contrast, inactivated vaccines offer improved safety profiles but cannot provide effective long-term protection. They may also cause adverse side effects due to undesirable components. Toxoids induce reliable humoral immunity, but little or no cell-mediated immunity (Moreira et al., 2016). The types and key features of conventional and next-generation approaches to the development of veterinary vaccines are presented in Table 1.

## Live-attenuated veterinary vaccines

Live attenuated vaccines are created by passage of viruses or bacteria in an unnatural host or cell. After multiple passages of the virus or bacterial strain in various media, the strain is administered to the natural host in the hope that random mutation has delivered a non-virulent and replicative infectious agent (Meeusen et al., 2007). However, the strains that are present in most of the existing live attenuated bacterial vaccines are not highly protective. In addition, they have many drawbacks. For example, they cause local inflammation and other unwanted reactions and they can revert to virulence. Additional issues include the inability to effectively culture the bacteria or virus, the possibility of inducing an autoimmune response, and the need for refrigerated storage (Babiuk, Pontarollo, Babiuk, Loehr, & Van Drunen Littel-van den Hurk, 2003; Meeusen et al., 2007). As the live attenuated organism can still infect target cells, these vaccines can replicate and induce both cellular and humoral immunity and, generally, do not require an adjuvant to be effective.

The process of producing virus vaccines is very complex because it uses living cells; as such, it is difficult to achieve standardization. Live-attenuated vaccines are also challenging to formulate because of the macromolecular complexity of viruses and bacteria; viruses can be enveloped or non-enveloped. In comparison to inactivated vaccines, live-attenuated viruses are easier to produce, do not require the use of adjuvants in the formulation, and only require minimal downstream processing (van Gelder & Makoschey, 2012). While naturally occurring attenuated viruses or viruses obtained after passage in different animal species or cell cultures were used as vaccine strains in the early vaccines, today, targeted mutagenesis can be applied to generate vaccine virus strains.

The reverse vaccinology approach to vaccine design can create recombinant vaccines that are generally safer and more immunologically defined than the traditional live-

**Table 1** Characteristics of vaccines currently available for veterinary use.

| Type of vaccines | Characteristics |
|---|---|
| Live-attenuated | Live strains are not highly protective;<br>Reversion to virulence to a more virulent phenotype can occurs;<br>Need for refrigerated storage;<br>Induce both cellular and humoral immunity. |
| Inactivated (killed) | Inactivated vaccines offer good safety profiles;<br>Cannot provide effective long-term protection due to the destruction of the pathogen replication;<br>Many inactivated vaccines are unable to cope with the prevailing strains in the field;<br>Frequently, new vaccines have to be generated from field strains with new outbreaks. |
| Toxoids | The amount of toxin produced *in vitro* is unpredictable;<br>High levels of biosafety are required. |
| Recombinant subunit | Well-defined composition;<br>No risk for pathogenicity;<br>Can be produced in a variety of protein expression systems;<br>Possibility for cost-efficient production and purification;<br>Primarily humoral immune response;<br>Need of adjuvant. |
| RNA/DNA-based | Humoral and cellular immune responses (antigen presentation by both MHC class I and II molecules);<br>Challenges in adequate cellular uptake and expression;<br>Long-term persistence of immunogen;<br>Risk of integration into host genome not completely excluded;<br>Unstable and quite expensive production (for RNA vaccines). |
| Vectored-based | Induce both cellular and humoral immune responses;<br>*In vivo* amplification systems available;<br>Some vaccines are commercially available with a well-known safety record;<br>Viral vectors allow for efficient infection of target cells. |

attenuated vaccines (Delany et al., 2014). When molecular approaches are employed, the obtained deletions and mutation can be identified. The targets for these deletions are the genes that are responsible for important metabolic processes, but that allow the development of immune response. Therefore, this approach represents a viable strategy by which some of the drawbacks associated with live-attenuated vaccines can be overcome.

## Inactivated veterinary vaccines

Inactivated vaccines currently consist of bacterins of one or more bacterial species or serotypes, or killed viral strains formulated most often in an oil or aluminum hydroxide adjuvant (Meeusen et al., 2007). Inactivated vaccines are stable in field conditions and less expensive to produce than live vaccines. The vaccine virus is usually grown in cell culture, either in roller bottles or bioreactors. The inactivation of the vaccine virus for the production of killed vaccines is achieved by physical or chemical treatments that cause denaturation of the proteins or damage to the nucleic acids. The inactivated antigen may be further purified and mixed with an adjuvant (van Gelder & Makoschey, 2012).

Inactivated vaccines offer improved safety profiles but cannot provide effective long-term protection due to the destruction of the pathogen replication (Cho, Howard, & Lee, 2002). A large number of viral infections are caused by viruses that have multiple serotypes (*e.g.*, bluetongue virus and influenza viruses). As a consequence, many of the existing viral vaccines are often unable to cope with the prevailing strains in the field, and new vaccines have to be generated from field strains in response to new outbreaks (Meeusen et al., 2007).

## Toxoids

Vaccination is the best preventive measure available to control the diseases caused by bacterial toxins. The vaccines that are currently commercially produced consist of inactivated native toxins (toxoids) combined with conventional adjuvants, which, although efficient, present some production limitations. For example, the amount of toxin produced *in vitro* is unpredictable, and some of the toxins are potent biological toxins that require high levels of biosafety (Arimitsu et al., 2004).

The use of recombinant vaccines can overcome these limitations, since they can be produced efficiently in large amounts and usually present low reactogenicity and toxicity. As such, they represent promising alternatives to the current commercial vaccines. For example, the production of recombinant *Escherichia coli* toxins takes only 2–3 days using simple growth media and formaldehyde for inactivation. This production method does not require many biosafety precautions because the toxic domain of the protein can be removed (Moreira et al., 2016).

## Conventional subunit vaccines

Subunit vaccines usually contain part of the target pathogen and provoke an immune response against that component only. Polysaccharide vaccines are a type of subunit vaccine that is composed of long chains of carbohydrate molecules that make up the surface capsule of the bacteria. The absence of additional antigenic components that are capable of stimulating T cells means that purified polysaccharides are incapable of recruiting sufficient T-helper activity

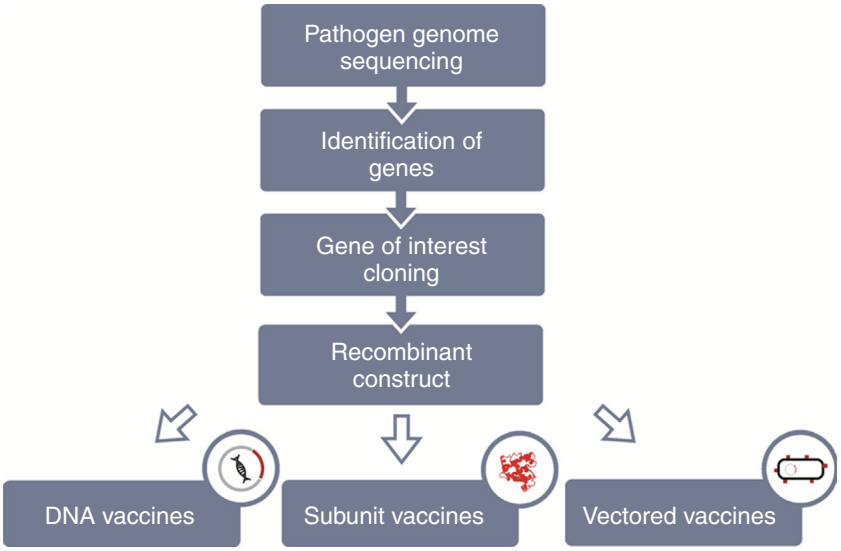


**Figure 1** Biotechnological approaches to vaccine development using recombinant DNA techniques. The gene encoding the antigen is isolated and either expressed and purified from a protein-production system, or is expressed directly by the vaccine recipient following injection of an engineered plasmid or a live vector. Prime-boost strategies combine different antigen delivery systems to broaden the immune response.

to mount a protective immune response. This problem has been overcome through polysaccharide–protein-conjugate technology *via* which the polysaccharide antigen is covalently linked to a carrier protein, typically an inactivated toxin (toxoid), like the tetanus or diphtheria toxoids. By using a conjugate vaccine, the immune responses to the polysaccharides are dramatically improved (Dintzis, 1992).

VLP vaccines are virus-like particles that do not contain replicative genetic material but permit presentation of antigens in a repetitive, ordered array similar to the virion structure, which is thought to increase immunogenicity (Jennings & Bachmann, 2008). Their close resemblance to native viruses in terms of the molecular scaffolds and absence of genomes entail that VLPs can effectively elicit both humoral and cell-mediated immune responses without requiring an adjuvant. However, these approaches have yet to be employed in a commercial vaccine (Liu et al., 2012).

## Biotechnology applied to next generation vaccine development

Genomic analyses of pathogens and enhanced understanding of the mechanisms of pathogenesis has resulted in new antigen discovery and the development of recombinant veterinary vaccines. A large amount of draft and whole-genome sequencing of viruses, prokaryotes, and eukaryotes pathogens has been performed (Kremer et al., 2016; Pizza et al., 2000; Tettelin et al., 2000; Vasconcelos et al., 2005). These advancements have also improved antigen discovery and the characterization of variability between viral pathogens, which typically contain fewer than ten genes, and eukaryotic pathogens, which often encode >10 000 genes (Aurrecoechea et al., 2007; Cho et al., 2002). The genome sequencing technologies and the approaches used to screen the genome and proteome of a pathogen have greatly improved the efficiency of antigen discovery (Seib, Zhao, & Rappuoli, 2012) because relevant antigenic structures can identify and produce recombinant vaccines that contain only the antigen necessary to elicit protective immunity.

Genomic databases generally contain whole genome sequences and the complete repertoire of encoded proteins from which vaccine screening is possible (Bagnoli et al., 2011). Surface-exposed antigens, secreted proteins, and toxins are commonly viable vaccine candidates against bacterial infections (Ravipaty & Reilly, 2010). However, further *in vivo* investigation of antigens is still necessary and desirable. Comparative genomic analysis software can be used to perform gene comparative analysis by basic sequence similarity searches. Sequence similarity algorithms facilitate the comparison of predicted coding sequences (ORFs) with known genes/proteins in public databases, and are commonly used to predict the degree of gene conservation among a bacterial population.

*In silico* analysis may also result in enhanced protein antigen qualities such as expression and solubility. As native gene sequences retain their own specific codon usage that reflects the composition of their respective genomic tRNA pools, gene sequences may be optimized for higher expression levels in any heterologous system (Bagnoli et al., 2011). One drawback of reverse vaccinology is that it cannot be used to predict polysaccharides or lipids, which are often included in vaccines as active compounds. Fig. 1 has shown a scheme of recombinant vaccine development strategies.

Thus, the advances in genomics and other ''omics'' have given rise to a ''third generation'' of vaccines that are developed through the use of novel technologies such as reverse vaccinology (Dellagostin et al., 2011; Rappuoli, Pizza, Giudice, & Gregorio, 2014). This approach allows identification of a broader spectrum of vaccines candidates, including proteins that had not been identified and/or no abundant. In addition, enable the identification of potential targets without the need to grow pathogens in the laboratory. The reverse vaccinology has been resulted in veterinary

vaccines that protect against an increased range of vaccine-preventable diseases. These next-generation vaccines can be multivalent, are highly purified, deliver an improved safety profile, and offer a viable alternative to the more reactogenic whole cell vaccines (Oliveira et al., 2015; Rappuoli, 2001).

### Recombinant subunit

Subunit vaccines contain short, specific proteins of a pathogen that are noninfectious because they lack the ability to replicate in the host. Protective antigens allow recombinant vaccines to be administered as safe, non-replicating vaccines. There is currently a large amount of scientific interest in the identification of immunogenic and protective antigens for animal pathogens.

Cloning the gene coding for the antigen is often necessary to better characterize and produce the identified antigen. *E. coli* has been used extensively as a host for heterologous protein expression; however, this approach has some limitations relating to the yield, folding, and posttranslational modifications of the recombinant protein (Heinson, Woelk, & Newell, 2015; Simionatto et al., 2010). An alternative host to *E. coli* is the methylotrophic yeast, *Pichia pastoris*. This yeast strain has emerged as a powerful and inexpensive expression system for the heterologous production of recombinant proteins that facilitates genetic modifications, allows the secretion of expressed proteins, permits post-translational modifications, and produces a high yield (Ghosh & Nagar, 2014; Hartwig et al., 2010).

The expression of antigens in heterologous systems enhances the safety of both the manufacturer and the user by eliminating the need for the use of a virulent or partially virulent microbe to induce immunity. The additional benefits of subunit vaccines are that they incorporate proteins in their most native form, thereby facilitating correct protein folding and the reconstitution of conformational epitopes (Eshghi, Cullen, Cowen, Zuerner, & Cameron, 2009). By incorporating more than one protein into a subunit vaccine, it is possible to invoke immunity to more than one strain or serotype of a bacteria or virus pathogen (Dellagostin et al., 2011). The potential drawbacks of subunit vaccines are they offer only a moderate level of immunogenicity and require adjuvants to generate robust immune responses.

### Vectored vaccines

The use of antigen/gene delivery systems has facilitated the development of novel prophylactic and therapeutic vaccine candidates. Vector vaccine technology uses a vector to deliver protective protein(s) to the immune system of the vaccinated host. These vectors are usually immunogenic and can display multiple antigens. Recombinant vector vaccines are classified as live vector vaccines and naked DNA vaccines. Plant vaccines are also vector vaccines that have significant potential in veterinary medicine.

Classical live vectors are attenuated bacteria or viruses that, in addition to inducing their own natural immunity, can also be used as carriers to express the immunogenic antigens of other pathogens. Poxviruses, which include the vaccinia, fowlpox, and canarypox viruses, have been successfully used as vectors for exogenous genes. Poxviruses can accommodate large amounts of foreign genes and can infect mammalian cells, resulting in the expression of large quantities of encoded protein. Currently, the canarypox virus vector system has been used as a platform for a range of veterinary vaccines including those against WNV, canine distemper virus, feline leukemia virus, rabies virus, and equine influenza virus. The bacterial attenuated vector BCG has been studied for several years. Recombinant BCG offers significant potential to express a large number of heterologous antigens and can induce solid immunity (Rizzi et al., 2012).

The use of plants to produce and deliver immunogenic antigens *via* food sources is highly beneficial. The use of transgenic plants represents an innovative development that has opened new avenues in the vaccine industries. In veterinary vaccinology, transgenic plants can produce and deliver immunogenic antigens *via* animal feed (Shams, 2005).

### DNA and RNA

DNA vaccines induce antigen production in the host itself. DNA (or RNA) vaccine can be defined as a plasmid that contains a viral, bacterial, or parasite gene that can be expressed in mammalian cells or a gene encoding a mammalian protein (noninfectious diseases). The gene of interest is inserted into a plasmid along with appropriate genetic elements such as strong eukaryotic promoters for transcriptional control, a polyadenylation signal sequence for stable and effective translation, and a bacterial origin of replication. The plasmid is transfected into host cells and transcribed into mRNA, which is subsequently translated, resulting in the host cellular machinery producing an antigenic protein. The host immune system recognizes the expressed proteins as foreign, and this can lead to the development of a cellular and humoral immune response.

Immunization of animals with naked DNA encoding protective viral antigens would, in many ways, represent an ideal procedure for viral vaccines because it not only overcomes the safety concerns associated with live vaccines and vector immunity but also promotes the induction of cytotoxic T cells after intracellular expression of the antigens (Meeusen et al., 2007).

## Adjuvants for recombinant veterinary vaccines

The low immunogenicity frequently observed in pure recombinant antigens occurs due to a lack of exogenous immune-activating components such as nucleic acids, lipids, lipopolysaccharides (LPS), proteins, cell membrane components. Recombinant antigens can be offered in different adjuvants, and there is frequently a need to enhance the immunogenicity (except DNA vaccines). The addition of adjuvants to vaccine antigens delivers several advantages, such as dose sparing, increased efficacy in the elderly, and broadening of the cell or/and humoral immune response.

Subunit recombinants are typically better tolerated than inactivated or live attenuated pathogens; however, they are generally less immunogenic and require the addition of an adjuvant to achieve protective immune responses (Soema,

Kompier, Amorij, & Kersten, 2015). The immunomodulatory effects are dependent upon the particular adjuvant used in conjunction with specific antigens.

Several adjuvants have been evaluated for use in veterinary vaccines, such as mineral salts (aluminum) (Li, Aldavel, & Cui, 2014); emulsions (Montanide) (Miles et al., 2005; Peter, Men, Pantaleo, Gander, & Corradin, 2001); biodegradable polymeric microparticles, and nanoparticles. In addition, an alternative range of adjuvants has been described as ''immune potentiators'' because they exert direct effects on immune cells, thereby leading to their activation (Ott, Radhakrishnan, Fang, & Hora, 2000). Examples of these include Toll-like receptor (TLR) agonists such as monophosphoryl lipid A (MPL) (Garçon, Wettendorff, & Van Mechelen, 2011); saponins, and bacterial exotoxins (Marchioro et al., 2013).

Some adjuvants act by sequestering antigens in physically restricted areas, known as depots, to provide an extended time period of antigenic stimulation. Thus, several veterinary vaccines are in the form of emulsions in oil. This relatively old-fashioned technology is, nonetheless, a powerful approach that achieves a strong inflammatory response and slow antigen liberation, exactly what recombinant subunit vaccines lack. In contrast to the strongly immune-activating emulsion-type adjuvants, aluminum salt adjuvants are not capable of inducing Th1 or cell-mediated immune activation to any significant degree; however, they are efficient Th2 inducers, giving rise to high antibody titers in the vaccinated individual.

Several groups have independently proposed the use of nano or microparticles to develop controlled-release vaccines. Depending on their size, particles are internalized by either phagocytosis or endocytosis. The antigens are either adsorbed on the surface of the nanoparticles or encapsulated inside the nanoparticle matrix (Slütter et al., 2009). Currently, polymeric microparticles have not yet been successfully developed as a vaccine product. Microparticles generally enhance the induction of Th2-type, humoral immunity, while nanoparticles promote Th1-based, cell-mediated immune responses (Li, Aldayel, & Cui, 2014).

**Table 2** Recombinant veterinary vaccines available in 2017.

| Animal species | Pathogens | Vaccine type |
|---|---|---|
| Cats | Feline leukemia virus | Vectored |
| Cats | Rabies virus | Vectored |
| Cattle | *Ripcephalus (Boophilus) microplus* | Subunit |
| Cattle | *Ripcephalus (Boophilus) microplus* | Subunit |
| Dogs | Canine distemper virus | Vectored |
| Ferrets | Canine distemper virus | Vectored |
| Fish | Infectious Hematopoietic Necrosis Virus | DNA |
| Horses | Influenza virus and Tetanus toxin | Vectored |
| Horses | Influenza virus | Vectored |
| Horses | West Nile virus | Vectored |
| Horses | West Nile virus | DNA |
| Poultry | Infectious Laryngotracheitis virus | Vectored |
| Poultry | Avian influenza virus | Vectored |
| Poultry | Marek's disease virus | Vectored |
| Poultry | Newcastle disease virus | Vectored |
| Poultry | *Mycoplasma gallisepticum* | Vectored |
| Raccoons/coyotes | Rabies virus | Vectored |
| Sheep/goats | *Echinococcus granulosus* | Subunit |
| Swine | Classical swine fever virus | Vectored |
| Swine | Porcine circovirus | Subunit |
| Swine | *Actinobacillus pleuropneumoniae* | Subunit |
| Swine | Classical swine fever virus | Vectored |
| Swine | Porcinecircovirus | Subunit |
| Swine | Porcinecircovirus | Vectored |

## Perspectives of the reverse vaccinology in animal health

The development of veterinary vaccines is a challenging task; however, reverse vaccinology is highly promising as a mechanism of veterinary vaccine development. Significant progress has been made in the field of vaccinology during the era of genomics, and next-generation vaccines are set to have an increasing impact on animal health. We can expect many more advances in vaccinology and the development of new effective veterinary vaccines that not only protect against infectious diseases but also against other diseases or chronic disorders. In fact, reverse vaccinology is now being applied to many bacterial, viral, and eukaryotic pathogens and, in all cases, has been successful in providing novel antigens for the design of new vaccines (Bagnoli et al., 2011; Buonaguro & Pulendran, 2011). Moreover, the ability of rational design to improve candidate antigens can provide increased protection against antigenically variable pathogens (Seib et al., 2012). Table 2 shows the recombinant veterinary vaccines that are commercially available in 2017.

Genomics has catalyzed a shift in vaccine development toward sequence-based approaches, which use high-throughput *in silico* screening of the entire genome of a pathogen to identify genes that encode proteins with the attributes of immunogenic vaccine targets (Seib et al., 2012). The genes are expressed using foreign protein expression systems, including *E. coli*, yeast, and insect or mammalian cells, and are then purified and injected into a host to elicit immunity. In addition, the expression of recombinant proteins in plants could be a viable alternative to conventional expression systems and, therefore, they represent a versatile tool for the production of edible vaccines.

The low immunogenicity frequently observed can be overcome by the use of a specific adjuvant. Discovery and development of new adjuvants for recombinant targets is essential because purified protein antigens do not always induce the desired protective and sustained immune response against different target pathogens. For instance, there is an acute need to develop effective and safe vaccines

against important veterinary diseases. As novel genome-based technologies and new adjuvants continue to emerge, it is expected that new veterinary vaccines for important diseases will be within reach.

Novel effective veterinary vaccines are in high demand as a means of controlling new and re-emerging pathogens. A wide range of vaccine technologies has been applied to develop veterinary vaccines. Each approach has its inherent advantages and challenges. Almost all of the existing veterinary vaccines were developed using traditional vaccinology methods, which relied on screening a few candidates at a time based on the known features of the pathogen. However, over the last decade, there has been a significant acceleration in the advancement of biotechnological techniques and the ability to sequence a pathogen's genome has provided vaccinologists with access to its entire antigenic repertoire. Such advances provide a great opportunity to create vaccines that are less dangerous but more effectively immunogenic than those developed by traditional methods.

Reverse vaccinology represents a promising approach to the discovery of recombinant vaccines against infectious, parasitic, and ever metabolic diseases. There is a distinct need to develop more potent, safer, better-characterized vaccines, in which different antigens can be combined, allowing for the development of vaccines against multiple strains of a pathogen. Recombinant vaccines fulfill this criterion and, as such, they are especially attractive for use as animal vaccines, for which vaccine cocktails are a useful vaccination option. The application of a biotechnological approach to the development of new effective veterinary vaccine candidates is fundamental and should be explored in depth.

## Conflicts of interest

The authors declare no conflicts of interest.

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