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# Recent Advances in Vaccine Technologies



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## KEYWORDS

- Vaccines • Inactivated • Attenuated • Subunit • Peptide • Vector • DIVA
- Nucleic acid

## KEY POINTS

- Traditional vaccine technologies are based on killed/inactivated and live/attenuated approaches.
- Novel killed/inactivated vaccination strategies include antigen subunit, protein, and peptide vaccines.
- Novel live/attenuated vaccination strategies include modified live, marker/differentiating infected from vaccinated animals, vector, and nucleic acid vaccines.
- New vaccine technologies often find their first commercial application within veterinary medicine.

## INTRODUCTION

Most vaccines that are available today rely on either inactivated (killed) or live attenuated (weakened) technologies. Such approaches have been successfully used to address many of the important veterinary and human diseases. However, both techniques have their limitations and associated potential problems.

Inactivated vaccines must be totally innocuous and noninfective. Problems with field outbreaks in the past have occasionally been attributed to incomplete inactivation. Such problems should not, and would not, exist if more reliable inactivants, inactivation procedures, and innocuity testing were used within the manufacturing process. Furthermore, because the manufacture of such vaccines involves the culture of large amounts of the infectious agent, there is a potential hazard to the personnel involved and the environment. Vaccines grown in eggs, tissue culture, or simply culture medium may contain unwanted “foreign” proteins, which could affect immunogenicity or be potentially allergenic/reactogenic. Finally, inactivated vaccines have certain limitations on their mode of presentation and as a consequence the nature of the immune response they can elicit. The response to vaccination may be limited

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The author has nothing to disclose.

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Vet Clin Small Anim 48 (2018) 231–241  
<https://doi.org/10.1016/j.cvs.2017.10.002>

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and of short duration with adjuvants or immunostimulants required to enhance their overall immunogenicity/efficacy.

Attenuated vaccines must be precisely controlled and characterized in order to provide the required level of protective immunity without causing significant disease symptoms within the host animal. There is also a low risk that the attenuated antigen may revert to full virulence, and careful reversion to virulence safety studies must be carried out. Furthermore, in culturing the vaccine antigen, it is possible that other infectious agents may be introduced that could themselves lead to undesired side effects when the vaccine is used in the field.

Because of these and other reasons, including protective efficacy, economy of manufacture, and whether the infectious agent can be produced in vitro, scientists have turned their attention more and more to the new vaccine technologies. These vaccine technologies include split-product, subunit, isolated protein, peptide, marker vaccine, live vector, and nucleic acid approaches.

## **KILLED VACCINE STRATEGIES**

### ***Natural Split-Product and Subunit Vaccines***

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By identifying suitable subunit, protein, or peptide antigens as vaccine candidates, natural split-product and subunit vaccines must be delivered to the target animals in order to elicit the desired protective immune response. The simplest and most basic form of subunit vaccine is one in which the infectious agent has simply been disassembled or broken up into its component parts. Some current influenza vaccines, known as split-product vaccines, consist of formalin inactivated virus that has been treated in order to lyse the viral envelope and release both the external envelope proteins and the internal nuclear and matrix proteins. A further refinement has been to use the purified envelope glycoproteins hemagglutinin and neuraminidase alone in a subunit vaccine in order to reduce the risk of any toxic side effects. Unfortunately, split-product and subunit vaccines for influenza have tended to have reduced immunogenicity when compared with whole virus products. Attempts to improve this situation have concentrated on modifying antigen presentation by delivering the viral glycoproteins within lipid vesicles, which can be composed of either virus-derived lipids (virosomes) or added nonviral lipids (liposomes).<sup>1</sup> In this way, artificial "empty" viruses can be created that can display improved immunogenicity. Polymeric preparations of isolated proteins in the form of micelles are also more immunogenic than the protein monomer.<sup>2</sup> In recent times, such multimeric presentation systems are often collectively referred to as virus-like particles or VLPs.<sup>3</sup> A development that offers both polymeric presentation and built-in adjuvant activity, for further enhancing immunogenicity, is the immunostimulating complex or ISCOM.<sup>4</sup> The first successful commercial veterinary application of this technology was for equine influenza,<sup>5</sup> and these vaccines have been studied for mucosal delivery.<sup>6</sup> Split product and cell culture subunit vaccines are also currently marketed for feline leukemia virus (FeLV) disease. Although each has been shown to be immunogenic, their overall degree of efficacy particularly in the face of an oronasal challenge has been inconsistent. However, once again by presenting the surface glycoprotein gp70/85 of FeLV in an ISCOM, neutralizing antibodies were elicited in all vaccinated cats, and complete protection was demonstrated against a subsequent oronasal challenge.<sup>7</sup>

As well as these new generations of veterinary viral subunit vaccines, many current bacterial vaccines are based on toxin or pilus subunits. Although antitoxin antibodies will neutralize the harmful effects of the bacterial infection, antipilus antibodies will block colonization by preventing attachment. Good examples are the F4 (K88),

F5 (K99), F6 (987P), F7 (F41), and F18 fimbrial adhesion antigens of enterotoxigenic *Escherichia coli* (ETEC), which in current vaccines are used to prevent neonatal diarrhea in calves and pigs. Indeed *E coli* strains engineered to overproduce these antigens were probably the first examples of the use of recombinant DNA technology to develop improved commercial vaccines.<sup>8</sup>

### **Recombinant Subunit and Protein Vaccines**

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Vaccines produced using overexpressed proteins recovered from genetically modified *E coli* provide a link between natural subunit vaccines and those derived using recombinant DNA technology. Although subunit vaccines produced from the natural infectious agent still fulfill an important role, the cost of producing and purifying immunogen can be prohibitive. Indeed, once the immunogenic proteins have been identified, it becomes the goal of many researchers to produce large quantities of those proteins in a sufficiently pure form to generate safe and effective vaccines. The emergence of recombinant DNA technology meant that foreign genes could be inserted into expression vectors and then introduced into cells that act as “production factories” for the foreign proteins encoded for by those genes. In many cases, this provides a relatively inexhaustible and cheap source of protein from the infectious agent for vaccination studies.

1. Bacterial expression: The first recombinant expression systems were established using *E coli* bacteria. This was the natural choice because it had been used to develop the early concepts and understanding of molecular biology. This expression system can provide relatively large quantities of defined proteins and was thus heralded as the answer for many subunit vaccines. However, because of the fact that prokaryotic cells have different mechanisms for processing and trafficking, expressed proteins are often incorrectly folded. In addition, signal sequences, glycosylation sites, and disulfide bonds, which occur in many candidate vaccine proteins, can either result in toxicity, insolubility, or rapid degradation within the bacterium. Nevertheless, one of the first recombinant veterinary vaccines to be successfully produced was based on the gp70 surface glycoprotein of FeLV expressed in *E coli*, known as the p45 protein.<sup>9</sup>
2. Yeast expression: The widespread use of *Saccharomyces cerevisiae*, baker's yeast, as an industrial microorganism has made it a natural choice for an alternative antigenic protein expression system. It has the added advantage, over prokaryotic systems, that posttranslational modification of proteins is carried out in a manner similar to that used by higher eukaryotic cells, and therefore, recombinant proteins are more likely to be correctly folded. Yeast-expressed proteins will also be glycosylated, although this glycosylation will be distinct from that carried out by mammalian cells. Further developments in yeast expression have concentrated on exploring the potential of another strain of yeast (*Pichia pastoris*), which has been used to express human hepatitis B vaccines based on the virus surface antigen (HBsAg) at levels as high as 400 mg/L. These expression levels are 10-fold higher than reported levels for this protein in *S cerevisiae*.<sup>10</sup>
3. Insect cell expression: A more recent and highly novel expression system has been developed using insect ovarian cells from *Spodoptera frugiperda* infected with a baculovirus vector, *Autographa californica* nuclear polyhedrosis virus. These viruses possess a strong promoter that controls the production of a 29-kDa polyhedron protein, which accumulates eventually to constitute up to 50% of total infected cell protein. Therefore, by replacing the polyhedrin gene with a selected foreign gene, high levels of recombinant protein may be produced. These proteins

will also undergo posttranslational modification, including glycosylation, phosphorylation, and signal peptide cleavage. However, once again the glycosylation pattern is known to be different from that seen on mammalian cell-derived proteins. Expression levels as high as 1 g per liter could be expected, although actual levels can vary considerably from 1 to 600 mg depending on the antigen. Insect cell expression has been successfully used in veterinary vaccines against porcine circovirus type 2<sup>11</sup> and classical swine fever (CSF).<sup>12</sup>

4. Mammalian cell expression: Because many veterinary pathogens will infect and replicate in cultured mammalian cells, they would appear to be the natural choice for an expression system if one desires authentically processed proteins for a sub-unit vaccine. However, they do present several technical problems, and expression levels can be somewhat lower than those achieved using the alternative expression systems described above. Nevertheless, several systems are available for the expression of proteins in mammalian cells and have been successfully used to express candidate vaccine proteins for bovine viral diarrhea (BVD),<sup>13</sup> CSF,<sup>14</sup> and VLPs for Japanese encephalitis virus.<sup>15</sup>
5. Plant cell expression: An additional emerging expression system that warrants mention is the use of plant cells. Although in the past plant geneticists have largely concentrated on crop improvement, some recent studies have shown that plants may provide a useful expression system for mammalian proteins. To express foreign genes in plants, it is necessary to splice a plant promoter, terminator, and, generally, a regulatory sequence onto cloned complementary DNA. Selectable markers may also be incorporated to facilitate identification of recombinants, and the expression hosts can be plant either cell cultures or whole plants. The first licensed vaccine to use this expression system was against Newcastle disease virus (NDV) infection in poultry,<sup>16</sup> and it is being investigated for many other vaccine applications, including infectious bronchitis virus, infectious bursal disease virus, ETEC, BVD, and bovine herpes virus.<sup>17,18</sup>

### **Peptide Vaccines**

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By identifying and sequencing important immunogenic sites on infectious agents, these can in many cases be mimicked using short chains of amino acid (peptides). The first indication that such peptides had vaccine potential was demonstrated in 1963 using a plant virus, tobacco mosaic virus. In this study, a chemically isolated hexapeptide fragment from the virus coat protein was coupled to bovine serum albumin and used to elicit rabbit antibodies that would neutralize the infectious virus. Two years later, a synthetic form of the same peptide was used to confirm this observation. However, it was more than 10 years before the next example of a peptide that elicited antiviral antibody appeared following work by Sela and colleagues on a virus that infects bacteria, MS2 bacteriophage.<sup>19</sup> The emergence of more accessible techniques for sequencing proteins in 1977, coupled with the ability readily to synthesize peptides developed by Merrifield in 1963,<sup>20</sup> led to an upsurge in experimental peptide vaccine research in the 1980s.<sup>21</sup> The first demonstration that peptides could elicit protective immunity *in vivo* in addition to neutralizing activity *in vitro* was obtained in 1982 using an animal virus, foot-and-mouth disease virus (FMDV).<sup>22</sup> A detailed study of both enzymically and chemically cleaved fragments of viral protein 1 (VP1) from the virus of FMDV serotype 0 had identified 2 regions between amino acids 138 to 154 and 200 to 213, which were found on the surface of the virus, and fragments containing these regions were able to induce neutralizing antibodies against the homologous virus. Studies using chemically synthesized peptides corresponding to several regions of VP1 led to the identification of similar sites on the molecule (141–160 and 200–213), which when coupled to a protein carrier, keyhole limpet hemocyanin (KLH), and inoculated into

guinea pigs would raise neutralizing antibodies that could protect against experimental infection. Although in these early studies the peptide had an immunogenic activity that was only 1% or less of that seen with the inactivated virus particle on an equal weight basis, the levels of neutralizing antibody produced were several orders of magnitude greater than that obtained with the whole VP1 molecule. This observation in laboratory animal models has subsequently been supported by the demonstration of protective immunity to peptide vaccination in both cattle<sup>23</sup> and pigs.<sup>24</sup>

Once a candidate peptide is identified or predicted, then it must be delivered to the immune system in a suitable manner in order to elicit not just a high titer antipeptide response but also antipeptide antibodies that will recognize and neutralize the infectious agent. Indeed, there has been a widely held view that, due to their relatively small molecular size, peptides are poor immunogens and thus require carrier-coupling to enhance their immunogenicity. Because of this, there are many examples of elegantly defined peptides, which, having been coupled in an uncontrolled manner to large undefined carrier proteins, produced antipeptide antibodies that totally failed to recognize the native protein.

Defined peptides, which, having been coupled in an uncontrolled manner to large undefined carrier proteins, produced antipeptide antibodies that totally failed to recognize the native protein.<sup>25</sup> The concept that peptides behave like haptens is in many cases misguided. Experiments using the 141 to 160 peptide from FMDV have demonstrated that the role of KLH as a carrier in priming for a peptide response is fundamentally different from its role in hapten priming because an uncoupled peptide or peptide coupled to a different carrier (tetanus toxoid) could boost a response in peptide-KLH primed animals. This observation has led to the demonstration of helper T-cell and B-cell determinants on this relatively small peptide.<sup>26</sup> Indeed, it is now clear that uncoupled peptides can be immunogenic provided they contain appropriate antibody recognition sites (B-cell epitopes) as well as sites capable of eliciting T-cell help for antibody production (Th-cell epitopes). These Th-cell epitopes must interact with class II major histocompatibility complex (MHC) molecules on the surface of antigen-presenting cells and B cells and subsequently bind to a T-cell receptor in the form of a trimolecular complex. The Th cells will provide signals in the form of chemical messengers (lymphokines) to specific B cells, which result in differentiation, proliferation, and antibody production. With this knowledge, synthetic peptides can be constructed with appropriate sites for antibody production plus additional T-cell epitopes.<sup>27,28</sup> This approach has been further exploited using a peptide containing B-cell epitopes within a consensus sequence based on residues 129 to 169 of Asian type O viruses linked to a promiscuous artificial Th-cell site from measles virus, which has now been commercially licensed for use as an FMDV peptide vaccine in swine.<sup>29</sup>

The requirement for multiple copy peptide presentation has been investigated using recombinant DNA technology by fusing small peptide sequences to the genes coding for larger proteins in order to produce several novel constructs. The use of peptide sequences fused to bacterial proteins as immunogens has the potential advantage of a completely uniform and defined structure compared with the uncharacterized and variable nature of peptide/carrier conjugates prepared by chemical cross-linking. This approach has been used to express FMDV peptides fused to the N-terminus of B-galactosidase in *E coli* cells. B-galactosidase was chosen because it had been shown that antibodies can be produced to foreign proteins located at the N-terminus, and it was known to contain several helper T-cell sites. Preliminary experiments with B-galactosidase and TrpLE fusion proteins indicated that multiple copies of the inserted peptide sequence may be beneficial. Subsequently, the immunogenicity of 1, 2, or 4 copies of FMDV VP1 peptide 137 to 162 fused to the N-terminus of

B-galactosidase was studied in both laboratory animals and target species. The protein containing one copy of the viral determinant elicited only low levels of neutralizing antibody, whereas protective levels were elicited by proteins containing 2 or 4 copies of the determinant.<sup>30</sup> Furthermore, single inoculations of the 2-copy and 4-copy proteins containing as little as 2 µg or 0.8 µg of peptide, respectively, were sufficient to protect all laboratory animals against challenge infection. The equivalent of 40 µg of peptide in the 4-copy protein also protected pigs against challenge infection after one inoculation. Thus, the immunogenicity of the multiple copy peptide/B-galactosidase fusion proteins is similar to that obtained using a synthetic multiple antigen peptide system.<sup>31</sup>

A further development of the fusion protein concept for multiple peptide presentation has led to the production of particulate structures with epitopes repeated over their entire surface, similar to VLPs. The earliest examples of these are based on HBsAg, hepatitis B core antigen (HBcAg), and yeast Ty proteins, which spontaneously self-assemble into 22-, 27-, and 60-nm particles, respectively. It has been shown using HBcAg fusion particles (CFPs) that the immunogenicity of FMDV peptide can approach that of the inactivated virus. Indeed, as little as 0.2 µg of FMDV VP1 142 to 160 peptide corresponding to 10% of the fusion protein, presented on the surface of CFPs, gave full protection to guinea pigs.<sup>32</sup> In subsequent experiments, N-terminal CFPs were shown to be 100-fold more immunogenic than free disulfide dimer synthetic peptides containing B- and T-cell determinants and 10-fold more immunogenic than carrier-linked peptide. This activity appears to be dependent both on the provision of T-cell help from the HBcAg and on particle formation. CFPs are also immunogenic with or without conventional vaccine adjuvants in a wide range of species. Furthermore, systemic responses can be elicited by oral or nasal administration and in a T-cell-independent manner. This last property of the CFPs offers the possibility of developing vaccine-based therapies for immunocompromised individuals infected with immunodeficiency viruses.<sup>33</sup>

Although only a limited number of peptide-based vaccines have been licensed to date, they offer the opportunity of moving vaccines from relatively undefined biological entities to more defined pharmaceutical-like products, and they have now been used to elicit immune responses against a wide variety of veterinary viruses, including rabies virus, FeLV, bovine rotavirus, bovine enterovirus, canine parvovirus, respiratory syncytial virus, equine herpes virus, and bovine leukemia virus.<sup>21</sup>

## LIVE VACCINE STRATEGIES

### *Modified Live Marker/Differentiating Infected from Vaccinated Animals Vaccines*

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New technology vaccines can also be used as a valuable tool in disease control and eradication programs by enabling the user to differentiate infected from vaccinated animals. These marker or DIVA (differentiating infected from vaccinated animals) vaccines can be recombinant deletion mutants of wild-type pathogens or subunit/peptide vaccines. They will require an accompanying diagnostic test for screening, and they can make it possible for vaccines to be used more readily in nonendemic situations. Early examples of such rationally attenuated glycoprotein deletion mutants have been used for the control of pseudorabies and CSF in pigs and infectious bovine rhinotracheitis in cattle.<sup>34</sup>

### *Live Vectored Vaccines*

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Live attenuated vaccines offer several distinct advantages over conventional inactivated and subunit vaccines. By replicating in the host, they more accurately

mimic natural infection, and they are often easy to administer, provide long-lived immunity, and stimulate a more “comprehensive” immune response, including humoral antibodies, secretory antibodies, and cytotoxic T cells. For these reasons, scientists have investigated ways of delivering subunit or peptide vaccines using live vectors.

1. **Virus vectors:** Most virus vector studies have concentrated on relatively large DNA viruses, in particular, poxviruses, herpesviruses, and adenoviruses. The most common virus vector to be applied experimentally is the orthopoxvirus vaccinia, successfully used in the vaccination campaign to eradicate smallpox.<sup>35</sup> The observation that a 9000-base-pair segment of the vaccinia virus genome could be deleted without affecting either its infectivity or its ability to replicate led to the development of recombinant vaccinia viruses with inserted foreign genes. Indeed, it has been shown that up to 25,000 base pairs of foreign DNA can be inserted into the virus, which offers the potential for inserting several genes into a single vector to produce a multicomponent vaccine. Despite these numerous positive observations, there are several potential problems associated with the use of vaccinia. In producing the initial recombinant, it is possible that cell tropism and pathogenicity may be affected. Because of its broad host range, there may also be problems with virus dissemination and recombination with other poxviruses under field conditions. However, the biggest question is undoubtedly that of safety. Despite its excellent track record in the smallpox eradication campaign, vaccinia has been known on very rare occasions to cause serious adverse reactions. In spite of these reservations, vaccinia recombinants have been used under strict supervision in the field in an attempt to control the spread of rabies in wildlife in Europe and North America. In view of its promising properties, much attention has been given to further rational attenuation of the vaccinia virus. For example, insertion into the TK gene has been shown to produce a marked reduction in pathogenicity, and further deletions or insertions have been investigated to produce a safer vector for general vaccination purposes. One such vector is known as modified vaccinia virus Ankora, and this has been recently used to develop a vaccine against Middle East respiratory syndrome coronavirus infections in camels.<sup>36</sup> An alternative approach that is being actively pursued for veterinary purposes is to use poxviruses, which have a more restricted host range. Much of this work has concentrated on the use of avipoxviruses, in particular, fowlpox and canarypox, as vectors for various veterinary species. These have been successfully exploited for several diseases, including Newcastle disease, avian influenza, equine influenza, rabies, FeLV, and canine distemper.<sup>37,38</sup> Other poxviruses that have been studied as veterinary vaccine vectors include capripox virus for rinderpest, racoonpox for rabies, parapox for pseudorabies, suipox for swine influenza, and myxoma virus for rabbit hemorrhagic disease.<sup>35,39</sup> Veterinary herpes viruses (eg, infectious bovine rhinotracheitis virus, feline herpes virus, and pseudorabies virus) and adenoviruses (eg, canine, equine, avian, and chimpanzee adenovirus) are also being developed as vectors. One particularly notable example is the herpesvirus of turkeys, which has been particularly successfully applied as a vector within the poultry industry for bivalent vaccines against Marek disease and IBR, IBD, or NDV.<sup>40</sup> In addition, a commercial trivalent vector vaccine has recently been developed against Marek, NDV, and IBD.<sup>41</sup>
2. **Bacterial vectors:** Recent studies on the rational attenuation of bacteria in order to produce suitable safe oral vaccines have introduced the possibility of using the vaccine strains generated as live vectors for foreign proteins. Majority of the

work in this area has concentrated on producing invasive strains of salmonella that are sufficiently attenuated so as not to cause any pathogenic disease symptoms when delivered orally to the host. Initial studies looked at generating auxotrophic mutants by removing or modifying important genes involved in the aromatic (aro) or purine (pur) synthesis pathways. Auxotrophic attenuation relies on the absence of the required nutrient in the host tissue, for example in the case of aro mutants the critical compounds are probably p-aminobenzoic acid and 2,4-dihydroxybenzoate. Both double aro and combination aro, pur mutants have been generated. Vaccination results are somewhat mixed; however, induction of local and systemic antibody and cell-mediated responses following oral immunization highlights the potential of this approach, and successful attenuated vaccines against salmonella in poultry have been produced.<sup>42</sup> Salmonella has also been used experimentally to vector several antigens, including *E coli*, *Shigella dysenteriae*, *Helicobacter pylori*, and transmissible gastroenteritis virus.<sup>43</sup> A further development in this field has come from studies into the use of Bacille Calmette-Guerin, a live attenuated bovine tubercle bacillus currently used to immunize humans against tuberculosis, as a vector. This mycobacterium is known to be safe and immunogenic. Furthermore, it can be given as a single oral dose; it is fairly heat stable, and it is inexpensive to produce. As a result, it has been engineered for overexpression homologous Ag85b as well as heterologous *E coli* and enterovirus 71 proteins. Other potential bacterial vectors include *Vibrio cholera*, *Yersinia enterocolitica*, *Listeria monocytogenes*, *Lactobacillus casei*, and *Streptococcus gordonii*.<sup>44</sup>

3. Protozoal vectors: One further and highly novel vector technology is based on the use of a live protozoan parasite (*Eimeria*) that has been genetically modified to deliver homologous or heterologous antigens to poultry. Such vaccines would use the currently licensed commercial attenuated strains that have been developed to vaccinate chickens against coccidiosis. Foreign genes would be expressed within the attenuated vectors using enzyme-mediated integration. The resultant transgenic strains could then be delivered in order to provide broader protection against coccidiosis infections or dual protection against coccidiosis and another infectious disease of chickens. The proof of concept for this approach has recently been reported by engineering a modified strain of *Eimeria tenella* to deliver the CjA protein of *Campylobacter*. This recombinant vaccine has been shown to provide between 86% and 91% immune protection against *Campylobacter jejuni* challenge when compared with unvaccinated and wild-type *E tenella* vaccinated controls ( $P < .001$ ).<sup>45</sup>

### **Nucleic Acid Vaccines**

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A relatively new vaccine technology that falls between live and killed approaches is the nucleic acid vaccine. These vaccines are based on DNA cloned into a delivery plasmid or the direct injection of messenger RNA. They can be produced cost-effectively, and the endogenous protein synthesis mimics a natural infection. Thus, the antigens are presented in their native form and will elicit both MHC class I and class II T-cell responses as well as an antibody response. In addition, there is no risk of infection, and these vaccines can be used to bypass passive immunity.<sup>46</sup> The first licensed applications of this technology in 2005 were for the control of infectious hematopoietic necrosis virus disease in Canadian Atlantic salmon<sup>47</sup> and for the control of West Nile virus in horses.<sup>48</sup> DNA vaccines have also been licensed in Europe for salmon pancreas disease.<sup>49</sup>

## SUMMARY

The field of veterinary vaccination has seen many significant advances in technologies over the past 25 years, with the introduction of several vaccines based on novel recombinant DNA technology. Such vaccines are designed to offer the farmer, owner, and clinician safer and more efficacious alternatives to existing vaccine technologies. In addition, they can have the added advantage of ease of administration and improved stability. Indeed, many new vaccine technologies often find their first commercial application within veterinary medicine, and, with the current interest in One Health approaches to humans, animals, and the environment, veterinary vaccines have an important role to play in the development of novel approaches. This article has covered some of the many new inactivated/killed and attenuated/live vaccination strategies that are now available to the veterinary research worker. A great deal more still needs to be understood about the nature of the responses required to elicit full protective immunity to several diseases. This knowledge should enable the development and construction of new generations of vaccines with more defined properties. It is already apparent that veterinary medicine will play a key role in such developments, and it is clear that this very active research area offers a great deal of potential for the development of further vaccine technologies in the future.

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