EXPERT REVIEWS

DNA vaccines in veterinary use

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DNA vaccines represent a new frontier in vaccine technology. One important application of this technology is in the veterinary arena. DNA vaccines have already gained a foothold in certain fields of veterinary medicine. However, several important questions must be addressed when developing DNA vaccines for animals, including whether or not the vaccine is efficacious and cost effective compared with currently available options. Another important question to consider is how to apply this developing technology in a wide range of different situations, from the domestic pet to individual fish in fisheries with several thousand animals, to wildlife programs for disease control. In some cases, DNA vaccines represent an interesting option for vaccination, while in others, currently available options are sufficient. This review will examine a number of diseases of veterinary importance and the progress being made in DNA vaccine technology relevant to these diseases, and we compare these with the conventional treatment options available.

Keywords: cancer • companion animals • cost • DNA vaccine • economic impact • food animal • infectious disease • veterinary use

Animals play an increasingly important role in society in both the developed and the developing world. In addition, as the global travel of animals, commercial goods and people expands, transmission of disease poses a greater threat than ever before. The demand for animal vaccines must be met in a timely, efficient and economically viable manner. Vaccines for companion animals, which provide for the health and longevity of pets, are generally well established and affordable; the number of companion animals with infectious diseases has decreased significantly since commercial vaccines were made available. Vaccines for livestock are essential for herd health, economic survival of farmers, and the maintenance of trade of meat and other animal products between countries. For livestock, the health of the herd is more important than that of the individual, and the only way to control outbreaks is to cull and mass slaughter herds, resulting in significant economic loss. Vaccination can be an effective preventative measure against infectious diseases; vaccines are therefore required for prophylactic purposes as well as for the control of outbreaks, and need to be affordable on a large scale and easily available. For wildlife, the need is for cheap but effective vaccines that can ideally be consumed orally in the form of bait traps.

DNA vaccines are emerging as a new and important method of vaccination for animals. DNA vaccination involves immunization with a plasmid encoding an antigen of the pathogen. The gene of interest is inserted into a plasmid, along with appropriate genetic elements such as 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 via direct injection, or injection with electroporation or gene gun. The gene of interest then undergoes transcription and translation by host cellular machinery, resulting in the production of an antigenic protein that can induce cellular and humoral immune responses. DNA vaccines have a number of advantages over other vaccination technologies that are of particular interest to veterinary medicine. They have the potential to be less expensive than other commercial vaccines, as they can be produced in large quantities by bacteria and, in the case of certain pathogens, do not require expensive facilities of a high biosafety level. They are temperature stable and safe to transport, which can be important for farms located in remote areas or for wildlife vaccines that need to remain in the open for a prolonged period of time. They are able to drive immunity in the presence of

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maternal antibodies – an issue of great importance, as neonates ingesting colostrum are at a high risk of disease. Different genes can be combined simultaneously, which allows for the development of vaccines against multiple strains of a pathogen and for combination approaches against multiple pathogens. This aspect is especially attractive for livestock, where vaccine cocktails are a useful vaccination option. In addition, because the relevant protein is produced and presented intracellularly, both a cellular and humoral immune response are induced, which gives a more efficient immune response when the animal encounters the natural infection at a later timepoint. DNA vaccines can be administered via a number of routes and techniques, which can alter the immune response and distribution of the protein. A number of DNA vaccines have already been approved for commercial use in both companion and food animals.

A few reviews have been written on the status of DNA vaccines in veterinary medicine, which detail the research and development of DNA vaccines for a number of veterinary diseases [1–5]. This review is unique in that it will examine a number of diseases of veterinary importance, and compare DNA vaccines with currently available vaccines and treatment options. For some diseases, DNA vaccines could be very useful and greatly improve upon current treatment options, whereas for other diseases, ample commercial vaccines are available that have proven efficient and cost effective. This review will consider the impact of the disease and compare current commercial vaccines and treatment options with DNA vaccines being developed, both in terms of cost–effectiveness for those DNA vaccines that are commercially available, ease and frequency of administration, and efficacy of protection.

Companion animals

The number of companion animals in the USA is on the rise. In 2007, there were over 43 million households owning dogs, over 37 million households owning cats and over 2 million households owning horses. Vaccinations comprised the greatest percentage of veterinary expenditures for dogs and the second largest for cats and horses after physical exams (Table 1) [6]. The market for companion animal vaccines is therefore substantial and well established.

Different types of vaccines exist for companion animals. Core vaccines (such as rabies, parvovirus and distemper) are required for all companion animals (dogs and cats), regardless of geographic location or risk of exposure. Noncore vaccines (such as leptospirosis and *Bordetella*) are not required and are used in situations where only some animals truly need to be protected due to higher exposure or risk. Conventional core vaccines have been well established and are mostly effective. For diseases such as rabies where bait vaccines are used for wildlife, a DNA vaccine could be useful; however, further progress needs to be made in developing this mode of administration. This is further discussed in the review. A number of the core vaccines for dogs and cats are summarized in Tables 2 & 3.

Opportunities for DNA vaccines in infectious diseases in companion animals

Infectious diseases represent a continuous threat to companion animals in many places, especially those with limited access to veterinary care. Viral, bacterial and parasitic diseases plague a large swathe of animals, from pediatric animals from pet stores in developed countries to feral animals roaming the streets in underdeveloped areas. DNA vaccines that have been developed for a number of these diseases are detailed in the following sections.

Feline immunodeficiency virus

Feline immunodeficiency virus (FIV) is a lentivirus that causes a progressive immune deficiency syndrome in cats. FIV is similar to HIV in morphology, genomic organization and pathogenesis, and as such, has been of great interest as a model for studying HIV [7]. However, FIV also has an important veterinarian application, as FIV affects both feral and domestic cats. The worldwide prevalence of FIV infection in domestic cats has been reported to range from 1 to 28% [8] and is close to 2.5% in the USA [9]. FIV, like HIV, poses a number of difficulties for vaccine development: FIV targets its host's immune cells, impairing the innate immune response and weakening the adaptive immune system by depleting $CD4^+$ T cells. Another challenge in vaccine development is the genetic diversity of FIV. Six subtypes of FIV have been identified, with most diversity found in Env (between 2 and 15% within a particular subtype and 17–26% between subtypes) [10]. In addition, an error-prone reverse transcriptase, as well as frequent recombination between feline leukemia virus (FeLV) and endogenous FeLV-related retroviruses in the cat genome can lead to mutated strains [11]. A number of vaccine approaches have been tested, including fixed infected cell, inactivated whole virus, subunit, recombinant and dual-subtype vaccines [7]; however,

Table 1. Pet ownership in the USA and veterinary expenditures.

Parameter	Dogs	Cats	Birds	Horses
Number of households owning	43,021,000	37,460,000	4,453,000	2,087,000
Average number owned per household	1.7	2.2	2.5	3.5
Total number in the USA	72,114,000	81,721,000	11,199,000	7,295,000
Total veterinary expenditure	US$16.1 billion	US$7.1 billion	US$102.8 million	US$718.3 million
Expenditure for vaccines	70.2% (= US$11.2 billion)	63.7% (= US$4.52 billion)	13% (= US$13.36 million)	49.3% (= US$210.46 million)

Data taken from [6].

Table 2. Canine vaccination guidelines of the American Animal Hospital Association.

Pathogen	Pathogen family	Vaccine types	Effectiveness (%) and duration of immunity	Vaccination ages	Ref.
Canine parvovirus (core)	*Parvoviridae*	MLV, killed, recombinant	>99 DOI: >7 years	Puppies receive three doses between the ages of 6 and 16 weeks, at 3–4 week intervals. Booster vaccination 1 year later. Revaccination every 3 years	[85]
Canine adenovirus (core)	*Adenoviridae*	MLV and killed, MLV parenteral, killed or MLV topical	>99 DOI: >7 years	Puppies receive three doses between the ages of 6 and 16 weeks, at 3–4 week intervals. Booster vaccination 1 year later. Revaccination every 3 years	[85]
Rabies (core)	*Rhabdoviridae*	Killed	>99 DOI: >3 years	One dose at 3 months. State, provincial and local statutes govern frequency of subsequent administration	[85]
Canine distemper (core)		MLV and recombinant vectored virus	>99 DOI: 3–7 years	Puppies receive three doses between the ages of 6 and 16 weeks, with the final dose administered at 14–16 weeks	[85]
Parainfluenza (noncore)	*Paramyxovirus*	MLV parenteral	Unknown DOI: >3 years	Puppies receive three doses between the ages of 6 and 16 weeks, at 3–4 week intervals. Booster vaccination 1 year later. Revaccination every 3 years	[86]
Bordetella bronchisepta (noncore)	Coccobacilli of the phylum *Proteobacteria*	MLV or killed parenteral	20–24 DOI: <12 months	One dose at 6–8 weeks, one dose at 10–12 weeks. Booster 1 year later. Revaccination every 3 years	[86,87]
Leptospira interrogans (noncore)	*Spirochaete* bacteria	Killed	<50	One dose at 12 weeks, one dose at 14–16 weeks. Annual revaccination	[85]
Borrelia burgdorferi/ Lyme borreliosis (noncore)	*Spirochaete* bacteria	Recombinant outer surface protein A	Unknown	Initial dose at 9–12 weeks, second dose 2–4 weeks later. Annual revaccination	

DOI: Duration of immunity; MLV: Modified live virus.
Data taken from [213].

these have mostly been unable to offer effective protection. A commercial vaccine (Fel-O-Vax® by Fort Dodge, KS, USA) is available against subtype B of FIV, and effectively protects against subtype B and slightly against subtype A, the two most prevalent subtypes in the USA. Several DNA vaccines against FIV, consisting of mutated whole genomes or individually mutated genes, have also been attempted, and these offer limited protection. A representative sample of these vaccines is provided in Table 4.

Cats diagnosed with FIV require extensive supportive care, therefore pet cats that are at risk of infection would benefit from being vaccinated. The available commercial vaccines are likely sufficient to offer protection, as the costs are not prohibitive. Feral cats represent more of a problem. Shelters that practice trap-neuter-release (TNR) programs for feral cats usually test all cats for FIV, vaccinate seronegative adults and euthanize seropositive adults and kittens. TNR programs are expensive and controversial in their effectiveness. If DNA vaccines could be produced more cheaply than current commercial vaccines, they would be a better choice for feral cats. DNA vaccines should also be studied for this disease in this species, as they represent an interesting model for HIV.

Feline leukemia virus

Feline leukemia virus, like FIV, is a retrovirus that can cause fatal disease in cats, including leukemia, lymphoma, anemia and immunodeficiency. There are three subtypes of FeLV – A, B and C, with A being the most prevalent and contagious but the least pathogenic. Oronasal exposure to the virus can lead to a temporary infection with transient viremia (65% of cases) or persistent viremia (30–40%), which causes more severe illnesses, especially in kittens [12]. The prevalence of FeLV in a healthy cat population is less than 0.5% [13] but can range from 0.35% in a free-roaming population to 32.6% in a high-risk multicat household [14]. The virus is shed in saliva, nasal secretions, urine, feces and milk. Cats with the greatest risk of infection are those living with infected cats, cats that venture or live outdoors and may be bitten by an infected cat, and kittens born to infected mothers.

Both FeLV-specific cytotoxic T-lymphocyte activity and virus-neutralizing antibodies contribute to limiting the infection within 4–8 weeks of infection [15]. Vaccines against type A are considered effective against all subtypes [16]. A number of commercial vaccines are available with varying efficacies. Treatment options are mostly palliative and usually require weekly to daily doses,

and are therefore expensive. In most veterinary clinics, the FeLV vaccine is not considered a core vaccine, but is highly recommended. In 2003, sales of vaccines for one of the commercial vaccines (Leucogen, Virbac, France) amounted to €13 million (US$20 million) [17]. A DNA vaccine against FeLV was developed and tested in kittens and adult cats by Hanlon *et al.*, and was found to provide robust protection against persistent viremia (Table 5) [18].

Considering the number of commercial vaccines available for FeLV, the relatively low prevalence of the disease and the relatively low cost of the vaccine, the development of a DNA vaccine against FeLV is not an urgent necessity for strictly clinical purposes. A cheaper vaccine could be useful for feral cat populations, but for cat owners, prophylactic and treatment options are available. FeLV as a model for DNA vaccines could be of interest from a basic scientific point of view, as the virus belongs to the same family as human T-lymphotropic viruses [12].

Rabies

Rabies virus is a lyssavirus of the *Rhabdoviridae* family, which constitutes a serious health threat for domestic animals, humans and wildlife. The virus kills 55,000 people each year and costs the global community over US$583 million [19]. Rabies is transmitted when the virus is introduced into bite wounds, open cuts in skin, or onto mucous membranes from saliva or other potentially infectious material such as neural tissue. The rabies vaccine is required for all domestic animals in the USA and a number of large-scale rabies control vaccination programs for wild animals are in place in a number of states and countries. The most important viral reservoir for the rabies virus is in developing countries, where only 30–50% of the canine population is vaccinated [20]. The difficulty in producing a rabies vaccine lies in inducing enough immunity to fully protect the animal while being in a form that is able to be widely distributed at a relatively inexpensive cost. Vaccines for wild animals present a particular problem in that it is impossible to know if animals obtain the full, necessary dosage of a vaccine and that it is very difficult to revaccinate animals. Vaccination programs for wild animals frequently use vaccines that are not approved for rabies protection in domestic animals, distributed via bait units. The development of a DNA vaccine that would be safe and effective for both wild and domestic animals could be useful, particularly if the duration of immunity can be increased compared with commercial vaccines. Currently available commercial vaccines and DNA vaccines for rabies are listed in Table 6.

There are numerous effective and inexpensive vaccines available for rabies protection for domestic animals. Since rabies vaccines are mandatory, the market has responded with a wide choice of vaccines that offer vaccine protection for similarly aged animals with similar vaccination protocols. DNA vaccines, however, offer the potential advantage of a more rapid induction of neutralizing antibodies [21]. If DNA vaccines can be produced inexpensively, they may also be useful in rabies control programs in developing countries, since vaccination using inactivated live viruses can be expensive.

For the inoculation of wildlife against rabies, recombinant vaccines encoding rabies virus glycoproteins have been shown to provide protection in wildlife such as raccoons, foxes and ferrets in a cost-effective manner [22–24]. DNA vaccines could also be potentially used in a similar manner, as a number of studies have produced orally administered DNA vaccines [25,26]. The hope is for a DNA vaccine against rabies that could be inexpensively produced and effective in both wild and domestic animals.

Table 3. Feline vaccination guidelines of the American Association of Feline Practitioners.

Pathogen	Vaccine types	Effectiveness (%)	Vaccination ages	Ref.
Feline panleukopenia virus (core)	MLV and killed, adjuvanted and nonadjuvanted	>99 DOI: 7 years	Kittens beginning at 6 weeks of age, then every 3–4 weeks until 16 weeks. Booster 1 year later, then every 3 years	[85,86]
FHV and FCV (core)	MLV and killed, adjuvanted and nonadjuvanted	FHV: 52 DOI: 5 years FCV: <75 DOI: 5 years	Kittens beginning at 6 weeks of age, then every 3–4 weeks until 16 weeks. Booster 1 year later, then every 3 years	[85,86,219]
Rabies (core)	Killed, canarypox virus-vectored recombinant	>99 DOI: 3 years	Single dose as early as 8 weeks, 1 year booster. State, provincial and local statutes govern frequency of subsequent administration	[85,86]
Feline leukemia virus (noncore)	Killed, canarypox virus-vectored recombinant	Unknown	Single dose at 8 weeks, second dose 3–4 weeks later. Annual revaccination for cats at risk	
Bordetella bronchisepta (noncore)	Avirulent live	Unknown DOI: 1 year	Single dose at 8 weeks. Annual booster for cats at risk	[86]
Chlamydophila felis (noncore)	Avirulent live, killed	Unknown DOI: 1 year	Single dose at 9 weeks, second dose 3–4 weeks later. Annual booster for cats at risk	[86]

DOI: Duration of Immunity; FCV: Feline calcivirus; FHV: Feline herpes virus; MLV: Modified live virus.
Data taken from [214].

Table 4. Feline immunodeficiency virus vaccines.

Treatment	Vector	Dosage/price	Immune response	Protection	Ref.
Fel-O-Vax (Fort Dodge)	Inactivated whole viruses of subtype A and subtype D with adjuvant	Three initial doses at 2–3-week intervals with annual revaccination; US$599/50 doses (US$11.98/dose)	Antibodies against whole FIV antigen and r-Gag	Protected 82% preventable fraction	
DNA vaccine (Hosie *et al.* 1998)	Whole FIV genome with in-frame deletion in *pol* (FIVΔRT) administered with IFN-γ	100 µg im.	CTL responses to FIV Gag and Env in the absence of a serological response	Lower viral load and protection for 4/10 vaccinated	[122]
DNA vaccine (Gupta *et al.* 2007)	FIV-pPPRΔvif *vif* deletion mutant carrying a 375-bp deletion within the *vif* gene + IFN-γ	600 µg im.	Increased FIV-specific T-cell proliferation, no antiviral antibodies	Similar plasma virus loads in vaccinated and control infected cats	[123]
DNA vaccine (Dunham *et al.* 2002)	FIV with in-frame deletion in either ΔRT or ΔIN genes ± IL-12/18	100 µg im.	Slightly increased CTL responses, no antiviral antibodies	5/18 ΔIN and 2/12 ΔRT protected against challenge with low-virulence FIV; lower viral loads in vaccinated cats infected with more virulent FIV	[124]
Lymphocyte T-cell immune modulator (Imulan)	Glycoprotein that increases leukocyte number and IL-2 production	Weekly injections during first month, every other week during second month, every 4–6 weeks afterwards	Regulator of CD4 lymphocytes and increases IL-2 production	Unknown	

CTL: Cytotoxic T lymphocyte; FIV: Feline immunodeficiency virus; im.: Intramuscular; ΔIN: Integrase; ΔRT: Reverse transcriptase.

West Nile virus

West Nile virus (WNV) disease has emerged as an important disease in horses. The disease is caused by WNV, a flavivirus transmitted to animals by mosquitoes that have acquired the infection by feeding on viremic birds [27]. While the number of cases in the USA has decreased over the past few years, it still represents a significant health threat. During an outbreak in 2002, over 15,000 cases of WNV disease in horses were reported, with only a third of affected horses having recovered. In the states of Colorado and Nebraska alone, for example, 1478 cases of WNV were reported, with a mortality rate of 29%, which cost the states US$600,660 and horse owners US$163,659 [201]. In a study following the outbreak, veterinarians estimated the costs to treat affected horses: US$200 to treat animals with mild disease, US$400 for those with moderate disease and US$250 for equids with severe disease [201]. No effective prophylactic treatments are available for WNV; mosquito control has been the most common method of preventing contraction of the disease, although protective antibodies can control viremia upon infection. Successful vaccination requires the induction of both neutralizing antibodies and cell-mediated immunity [28].

Three vaccines against WNV are currently commercially available, with a DNA vaccine recently developed by Fort Dodge that received US Department of Agriculture (USDA) approval and was released on the market in December 2008. A study by Seino *et al.* in 2007 found that all three commercial vaccines were efficacious in the prevention of WNV-induced encephalitis in horses, with all three resulting in 100% survival against a severe challenge model of encephalomyelitis, whereby all controls exhibited clinical disease (fever, viremia, onset of grave neurological disease and histopathologic lesions in the CNS) and 100% mortality [29]. These vaccines are detailed in Table 7, alongside the DNA vaccine.

Commercial vaccines for WNV are available for varying prices. The largest demand for WNV vaccines is most likely from private horse owners, where efficacy of the vaccine is of the greatest importance. Studies show that the DNA vaccine offers comparable protection and should therefore be an acceptable choice for horse owners. Another key attraction of a DNA vaccine for performance horses is the ability to differentiate vaccinated animals from infected animals [3]. High titers of certain diseases such as WNV can prevent horses from being entered into shows; an animal vaccinated with a DNA vaccine would not face this particular problem. Ease of transport and stability of the vaccine is of further importance, as farms and ranches can require veterinarians to travel long distances with such vaccines. The cost of the DNA vaccine is comparable to the cost of conventional vaccines against WNV. It will be interesting to follow the performance of this first commercial DNA vaccine.

Opportunities for DNA vaccines for cancer in companion animals

In addition to targeting pathogenic agents, DNA vaccines are also being used as cancer vaccines for companion animals. These vaccines incorporate plasmids encoding tumor antigens that induce

the formation of antibodies that are expected to target tumor cells in the vaccinated animals, leading to the regression of tumors. DNA vaccines are, overall, an exciting prospect in the field of companion animal oncology. Many examples of DNA vaccination against tumor antigens in animal models have been developed [30]. Some of these antigens include: a human tyrosinase antigen for canine melanoma (detailed in the following section); the α-folate receptor for ovarian carcinoma [31]; HER-2/neu associated with breast cancer [32]; the paraneoplastic encephalomyelitis antigen Hu D associated with small-cell lung cancer [33]; tyrosine hydroxylase of neuroblastoma [34]; and prostate-specific antigens for prostate cancer [35]. Many of these have resulted in significant tumor protection or the reduction of tumor development and tumor size in mice. Aside from the canine melanoma vaccine, no experiments have yet been conducted in companion animals. However, since many of these cancers do affect dogs and cats, these vaccines represent an interesting possible application of DNA technology.

Canine oral melanoma

One of the DNA vaccines that have been conditionally licensed by the USDA is canine melanoma vaccine (Merial Animal Health Ltd, UK). Canine melanoma is the most common oral tumor in the dog but can also occur in the toes and footpads. The biological behavior of the tumor is closely related to site, size, stage and histologic parameters. Site, for example, influences invasiveness and metastatic properties: tumors in haired skin are not as malignant as tumors in the mucosa or oral cavity. Size of tumors is also prognostic, as is the stage (I, II or III, and IV) [36].

Traditional treatment of canine melanoma involves either surgery (tumor excision), radiation therapy, chemotherapy or immunotherapy, all with fairly poor results. Tumor excision either runs the risk of not removing the entire tumor or is not possible if the tumor has spread to lymph nodes and other areas. Radiation therapy or radiation therapy with chemotherapy can also be used to achieve slightly better results, but treatment is

Table 5. Feline leukemia virus vaccines.

Vaccine	Vector	Dosage	Price	Vaccination ages	Immune response	Protection	Ref.
Leucogen (Virbac, France)	p45 envelope antigen with adjuvant (aluminium hydroxide gel, extract of *Quillaja saponaria*)	Two initial doses at 3–4-week intervals injected im., annual revaccination with single dose	US$99.99/ten doses (US$9.99/dose)	8 weeks	Neutralizing antibodies within 9 weeks	80% preventable fraction against persistent viremia	[88]
Purevax®/ Eurifel (Merial Animal Health Ltd, UK)	Canarypox virus with *env* and *gag* genes and part of the *pol* gene of subgroup A (no adjuvant)	Two initial doses of 0.25 ml 3–4 weeks apart sc., annual revaccination with single dose	US$121.99/25 doses (US$4.88/ dose)	8 weeks	Cytotoxic T-cell response, very limited neutralizing antibody production	78% preventable fraction for persistent viremia	[89]
Leukocell 2 (Pfizer, NY, USA)	Inactivated, adjuvanted, mixed subunits from FeLV strains A, B and C, with sterile adjuvant	Two initial doses of 1 ml 3–4 weeks apart sc., annual revaccination with single dose	US$50.70/ten doses (US$5.07/dose)	9 weeks	Neutralizing antibodies within 7 weeks	Protects more than 70% of artificially immunosuppressed cats against persistent viremia	[90]
Fel-O-Vax LvK (Fort Dodge, KS, USA)	Inactivated whole virus of subgroups A and B with adjuvant	Two initial doses of 1 ml 3–4 weeks apart sc. or im., annual revaccination with single dose	US$74.00/ten doses (US$7.40/dose)	9 weeks	Neutralizing antibodies within 11 weeks	44–100% preventable fraction	
Fevaxyn (Schering-Plough, NJ, USA)	Inactivated whole virus of subgroups A and B with adjuvant	Two initial doses of 1 ml 3–4 weeks apart sc., annual revaccination with single dose	US$119.99/25 doses (US$4.80/ dose)	9 weeks	Unknown	90.4–100% preventable fraction	
DNA vaccine (Hanlon *et al.* 2001)	Two plasmids expressing *gag/pol* and *env* gene with adjuvant plasmids encoding feline IL-12, IL-18 and/or IFN-γ	100 µg of each DNA construct injected im.	Unknown	13–15 weeks	Unknown	100% protection against transient and persistent viremia, 5/6 kittens protected against latent infection	[18]

FeLV: Feline leukemia virus; im.: Intramuscular; sc.: Subcutaneous.

Table 6. Commercial and DNA rabies vaccines.

Vaccine	Vector	Dosage	Price	Vaccination ages	Protection	Ref.
Rabvac™ (Fort Dodge, KS, USA)	Killed virus with adjuvant	One 1-ml dose sc. or im. Revaccinate 1 year later and every 3 years thereafter	US$74.99/50 doses ($1.50/dose)	3 months	Dogs, cats and horses Neutralizing antibodies in dogs within 2 months, 100% against lethal challenge	[20]
Defensor 3® (Pfizer, NY, USA)	Chemically inactivated rabies virus with adjuvant	One 1-ml dose sc. Revaccinate 1 year later and every 3 years thereafter	US$21.95/ten doses (US$2.20/dose)	3 months	Dogs, cats, sheep and cattle, but extends to raccoons and bats	
Prorab® (Intervet, The Netherlands)	Killed virus with adjuvant	One 1-ml dose sc. or im. with annual revaccination	US$13.85/ten doses (US$1.39/dose)	3 months	Dogs, cats and sheep	
Imrab® (Merial Animal Health Ltd, UK)	Inactivated rabies virus	One 1-ml dose sc. or im. Revaccinate 2 year later and every 3 years thereafter	US$102.97/50 doses (US$2.06/dose)	3 months	Cats, dogs, sheep, cattle, horses and ferrets Virus-neutralizing antibodies	
Raboral V-RG® (Merial Animal Health Ltd)	Nonpathogenic virus with small portion of viral RNA encoding G protein of the rabies virus	2 ml of vaccine in fish meal or dog food bait	US$1.30/vaccine-bait unit	For adult wild animals	Protects coyotes and raccoons within 14 days	
DNA vaccine (Tesoro-Cruz *et al.* 2008)	Plasmid encoding glycoprotein of rabies virus	100 µg of DNA im., intranasally and intradermally with booster 30 days later	Unknown	1–2 years	Neutralizing antibodies in cats within 15 days and in mice via passive transfer of cat sera/antibodies. 100% protection in mice. Protection from lethal challenge for cats (100% for intradermal vaccination and 67% for im. route)	[91]
DNA vaccine (Lodmell *et al.* 2006)	Plasmid encoding glycoprotein of rabies virus	100-µg DNA intradermally in ear pinnae	Unknown	12–14 months	Neutralizing antibodies within 2 months in dogs, persisting for 6 months. 100% protection against lethal challenge	[20]

im.: Intramuscular; sc.: Subcutaneous.

expensive and causes significant temporary discomfort to the animal. Nonspecific immunotherapy is also being investigated, with techniques such as allogeneic tumor cell vaccines, dendritic cell vaccines and the use of immune adjuvants (interleukins and granulocyte–macrophage colony-stimulating factor).

DNA vaccination has proven, under certain circumstances, to be safe and effective in combating melanoma. Dogs in stage II or III, where local tumor control has been achieved, respond well to this treatment. The DNA vaccine consists of a plasmid expressing xenogeneic human tyrosinase. Melanoma tumor cells overexpress tyrosine proteins. The human tyrosinase protein is different enough from the canine tyrosinase protein that it will stimulate an immune response, yet similar enough to the canine tyrosinase that the immune response is effective against canine melanoma cells that express tyrosinase. Table 8 details the cost and efficacies of current treatment options against those of the DNA vaccine at a veterinary teaching hospital, as well as the mean survival time for various modalities.

Considering the difficult nature of malignant melanoma and the limited effectiveness of available treatment options, the advent of a DNA vaccine is a welcome development. Unfortunately, the vaccine can only be used in more advanced stages of the cancer, once surgical control of the tumor has been achieved. However, the vaccine is easy to administer and less harsh for the patient than chemotherapy or radiation treatment, with fewer side effects. While administration of the DNA vaccine itself may be inexpensive, additional costs associated with administering it, such as blood tests, radiographs, initial surgical treatment and vaccine administration, add up to a cost similar to that of chemotherapy or radiation therapy. Likewise, administration of the vaccine requires four injections at 2-week intervals, which is a similar time requirement to that of traditional treatment options. Therefore, in terms of cost to the client, the vaccine does not significantly reduce this cost, but can reduce discomfort to the patient. The vaccine has been granted a conditional license. It will be interesting to follow its performance on the market once it achieves full license.

Opportunities for DNA vaccines in livestock

While DNA vaccines represent an interesting possibility for targeting infectious disease and cancer in companion animals, their potential use in livestock is even greater. Livestock production in the USA alone is significant in scale: the USA is the world's largest beef producer, second largest beef export and second largest pork producer. Details of the livestock industry are listed in Table 9.

Most livestock operations incorporate large numbers of animals, where herd immunity is of greater importance than the health of individual animals. Herd immunity involves conferring protection to a large population of animals by vaccinating a significant percentage of the whole group. Herd immunity prevents the rapid spread and decreases the persistence of a disease. This is especially important for diseases with longer incubation periods, where infected animals cannot readily be identified and can transmit disease to other members of a herd. While a large number of commercial vaccines are available for the wide span of livestock diseases, DNA vaccines possess an inherent advantage in that vaccinated animals can be distinguished from infected animals, and since they have the potential to be less expensive to produce, they could be of value in large-scale animal operations. Furthermore, many commercial vaccines come in the form of cocktails, offering protection against several diseases in one vaccine. DNA vaccines are particularly able to provide such an option, as several plasmids encoding different genes can be incorporated into a DNA vaccine. A number of diseases of veterinary importance in livestock are detailed in the following sections.

Bovine respiratory disease complex

The cattle industry is a large and growing industry both in terms of dairy and beef cattle. Growing demand for beef, especially in developing countries, has led to increased and more intensified production. Infectious diseases represent a serious threat, especially in large-scale productions. One of the most important diseases is the undifferentiated fever/bovine respiratory disease complex (BRDC), representing a US$1 billion economic loss to the industry and a US$3 billion cost in preventative and curative treatments [37]. BRDC can cause a large range of clinical symptoms, including subclinical benign infection, fatal mucosal disease, hemorrhagic disease, reproductive failure, congenital abnormalities [38], acute infections with immunosuppression, pyrexia, anorexia, depression, bronchitis, pneumonia and death [39]. BRDC can be caused by a number of pathogens, such as bovine herpes virus, *Mannheimia haemolytica*, *Pasteurella multocida*, parainfluenza type-3 (PI_3), and bovine respiratory synctitial virus (BRSV). The economic cost of the disease is significant: a study by Gunn *et al.* estimated costs of a disease, with losses stemming from the abortion of calves to lost production to heifer death (Table 10). In addition, vaccinated calves sell for a higher price than unvaccinated calves. Therefore, livestock producers should have a vested interest in vaccinating calves efficiently and effectively.

A large number of commercial vaccines are available for each infectious agent. For example, there are over 180 USDA-licensed bovine diarrhea virus (BVDV) vaccines [40], all with varying efficacies and for varying prices. For cattle, herd health is more important than the health of individual cattle. Vaccines will be

Table 7. West Nile virus vaccines.

Vaccine	Vector	Dose	Immunity	Vaccination ages	Price
West-Nile Innovator® (Fort Dodge, KS, USA)	Formalin-inactivated whole virus with MetaStim adjuvant	Two doses at 3–6 week intervals, annual revaccination with a single dose	Within 2 weeks after second dose	Nonvaccinated dam: 3–4 months Vaccinated dam: 5–7 months	US$138.30/ten doses (US$13.83/dose)
Recombitek (Merial Animal Health Ltd, UK)	Lyophilized recombinant canarypox vectored West Nile virus vaccine expressing membrane and envelope proteins, plus a sterile liquid diluent	Two im. doses at 4–6-week intervals, annual revaccination with a single dose	Within 28 days, for 12 months	Unknown	US$169.99/ten doses (US$16.99/dose)
PreveNile™ (Intervet, The Netherlands)	Lyophilized yellow fever West Nile chimera virus vaccine expressing membrane and envelope proteins without adjuvant	Single im. dose	Within 28 days, for 12 months	4 months or older	US$199.99/ten doses (US$19.99/dose)
West-Nile Innovator® DNA (Fort Dodge)	Plasmid encoding PrM and E gene with adjuvant (SP oil, MetaStim)	Two doses 3 weeks apart	Within 28 days	Foals 8–9 months old were found to be protected against viremia	US$199.99/ten doses (US$19.99/dose)

im.: Intramuscular.

Table 8. Treatment options for canine oral melanoma.

Treatment	Dosing/administration	Cost/availability	Results	Ref.
Surgery	Excision surgery and biopsy	~US$3000	MST of 17–18 months for stage I (<2 cm diameter tumor), 5–6 months for stage II (2–4 cm diameter tumor) and 3 months for stage III (>4 cm diameter tumor)	[95]
Radiation therapy	Total dose of 24 Gy over three doses 36 Gy in varying doses	~US$2000	53% complete remission, 30% partial remission, MST of 7 months	[92,93]
Chemotherapy	Carboplatin administered at doses of 300 or 350 mg/m^2 of body surface area	US$3000–4000 (carboplatin is US$1.90–1.95/mg)	28% overall response rate, 24% partial response rate, MST of 165 days	[94]
Merial DNA vaccine (Merial Animal Health Ltd, UK)	Administered after surgical excision of tumor Four 0.4-ml doses administered transdermally biweekly. Booster dose every 6 months thereafter	Available to board-certified veterinary oncologists US$500/dose	MST of 569 days (19 months) for stage II–III dogs	[96]

MST: Mean survival time.

important for disease prevention and disease control in the event of an outbreak. The cost and longevity of vaccines will be as important as their efficiency.

Bovine respiratory synctitial virus

Bovine respiratory synctitial virus causes 60–70% of respiratory disease in cattle and mortality of BRSV can reach 20% in herd outbreaks [39]. Vaccination against BRSV is therefore essential for the industry and further represents a model for the study of human respiratory synctitial virus (RSV). BRSV is an enveloped, single-negative strand RNA virus of the *Pneumovirus* genus and *Paramyxoviridae* family. A number of commercial vaccines are available for BRSV and are routinely administered to calves, including combinations of vaccines for BRSV and other disease agents. However, there are several problems with these vaccines. First, peak incidence of BRSV occurs between 2 and 7 months of age; the immaturity of the young calves' immune system as well as the immunosuppressive effects of maternal antibodies found in the colostrum make vaccination difficult [41]. In addition, these vaccines generally do not induce long-term immunity and have been shown to exacerbate subsequent respiratory disease, probably due to the elicitation of a Th2 response upon vaccination [39]. Since most BRSV vaccines are modified live viruses, they can also trigger abortion in pregnant cows. DNA vaccines represent a promising approach to the vaccination of cattle against BRSV. The Hamers *et al.* DNA vaccine was able to reduce lung lesions and viral shedding [42], while the Taylor *et al.* DNA vaccine stimulated humoral and cell-mediated immunity within 9 weeks of vaccination [41]. Details of current vaccination options are compared with two DNA vaccines in Table 11.

Bovine viral diarrhea

Bovine viral diarrhea virus is a small, enveloped, single-stranded RNA pestivirus of the *Flaviviridae* family. Relative antigenic heterogeneity exists among the different strains, and isolates are divided into two biotypes – cytopathic, which cause changes in host cells, and noncytopathic, which do not cause host cellular changes. Both types can cause disease in cattle, but only the noncytopathic type causes persistently infected animals that continually shed the virus throughout their lives. The virus is also subdivided into two different genotypes – type 1 and type 2. Both cause disease, but type 2 is associated with high mortality and acute, fulminating infections [40]. Despite the genetic heterogeneity, it has been suggested that subgenotypes tend to be herd-specific and geographically clustered, which could potentially reduce the need for expanded crossreactivity in vaccines. Bovine virus diarrhea vaccine (BVDV) not only is a causative agent of BRDC, but is also associated with enteritis in calves and can cause acute hemorrhagic disease [43]. In general, vaccines developed for bovine diarrhea are modified live or inactivated viruses. The former induces longer lasting and broader immune

Table 9. US livestock industry in 2007 according to the US Department of Agriculture.

Industry	Amount produced	Number of animals	Economic value	Ref.
Beef	30 million tons of meat	104.8 million	US$89 billion	[215,216]
Dairy	185.6 million tons of milk	9.25 million	US$35.5 billion	[215]
Poultry	40 million tons of meat	806 million broilers 42.5 million layers	US$35.1 billion	[203,215]
Pork	22 million tons of meat	62 million	US$5.5 billion	[203,215]

Table 10. Costs of bovine respiratory disease complex.

Event	Cost
Immunosuppression of calves	US$6.36/calf/year
Congenital defects, growth retardation, and so on	US$61.5/calf/year
Aborted calf	US$711/cow/year
Delayed breeding of cow due to infection	US$148.5/cow/year
Infected heifer	US$148.5/heifer/year
Death of a cow or heifer	US$1179

responses (including T-cell-mediated responses since replication of the virus amplifies antigens, while the latter induces shorter and sometimes inadequate immunity but is safe to use). Two DNA vaccines, described in Table 12, have been developed against BVDV, including one that induces strong immunity when boosted with the relevant viral proteins [44].

Johne's disease

Bacterial as well as viral diseases represent a serious threat to the livestock industry. One of particular importance is that of Johne's disease. Johne's disease is an intestinal infection of ruminants, affecting cattle, goats and sheep, and is caused by *Mycobacterium avium* subsp. *paratuberculosis*, a bacillus closely related to the causative agent of TB in both cattle and humans (*Mycobacterium tuberculosis*). The USDA estimates that 22% of dairy operations in the USA are infected with M. paratuberculosis and have an infection rate of at least 10% [202]. A study by Ott *et al.* in 1996 estimated that the cost to the dairy industry amounted to approximately US$200–250 million annually (US$22–27 per cow) owing to decreased milk production, higher cow-replacement costs and lower cow-cull revenues [45]. Worldwide, the disease is estimated to cost the industry over US$1.5 billion [46].

Like *M. tuberculosis*, *M. paratuberculosis* is an extremely hardy bacterium, able to survive a wide range of temperatures in water, soil and the air. It is excreted in the feces and milk of infected cattle and generally infects calves at an early age. However, the development of clinical disease, which includes chronic diarrhea, weight loss, intestinal lesions and reduced milk production, usually does not occur until 2 years of age, which makes detection difficult. Further complicating detection efforts is the fact that current detection methods detect less than 50% of infected animals, requiring multiple testing, which can become expensive [47].

Treatment for Johne's disease is expensive, both in terms of cost of drugs and forfeited income, due to drug traces in milk and meat. Treatment also only treats clinical signs, and is therefore not a valid option for herd treatment. Currently, avoiding infection of calves is the most practiced way of controlling disease – calves are birthed in as clean, manure-free environments as possible and removed immediately after birth from their dams. Some cattle practices use milk replacers instead of allowing calves to milk from their dams, and obtain essential colostrums from Johne's-free cows. Culling and isolation of infected animals is also widely practiced.

Vaccination of cattle is controversial, as it is generally not very effective: it does not eliminate or prevent infection of the cow, and only reduces the development of clinical disease and shedding of the virus. It also can induce the formation of granulomatous lesions in the site of vaccination [48]. Two commercial vaccines are available, only one of which is licensed in the USA (Mycopar [Fort Dodge], available in the USA, and Silirum® [Pfizer, NY, USA], available in Australia). An analysis by van Schaik *et al.* in 1996 determined that there was a cost–benefit advantage for vaccinating, since vaccinating a cow cost US$15 and total return of vaccination amounted to US$142 per cow due to reduction of clinical disease (although, interestingly enough, vaccination did not prevent loss of milk production) [49]. However, vaccination is not effective in preventing an outbreak or spread of the disease. Furthermore, vaccinated animals cannot be distinguished from infected animals, which further complicates the detection of a disease that is already difficult to detect. The development of a DNA vaccine that could prevent disease, especially in very young animals, would be of great interest. A number of experimental DNA vaccines have been developed for cattle and sheep and are compared with commercial vaccines in Table 13.

DNA vaccines offer a very possible improvement upon current vaccination and prevention of infection with Johne's disease. Commercial vaccines are at best effective in reducing but not preventing clinical disease, and at worst can cause lesions in the site of vaccination. Current methods of preventing infection include removing calves from their dams at birth, which is stressful to the animals and can lead to the possibility of calves not getting enough immunoglobulin-rich colostrum. Both DNA vaccines cited in Table 13, elicited strong immune responses, and seem to be able to prevent disease in light of the absence of lesions in tissues of certain animals after challenge. Unlike the commercial vaccines, DNA vaccines would allow differentiation between vaccinated and infected animals, therefore limiting the likelihood of an outbreak if the detection of infected animals did not work. DNA vaccines would also prevent the problem of lesions at the site of infection, as well as transmission of bacteria to calves via lactation.

Foot-and-mouth disease

The foot-and-mouth disease (FMD) virus is an apthovirus of the *Picornaviridae* family, which causes FMD, a highly contagious and devastating disease of cloven-hoofed animals, such as cattle, sheep, goats, buffalo and pigs. The disease is characterized by fever, lameness, vesicular lesions of the mouth, tongue and feet, and acute myocarditis in young animals. The virus can be excreted in lesions, saliva, exhaled air, milk, urine, feces, and semen, leading to rapid and easy transmission between animals [203]. FMD represents a serious economic and public-health problem: outbreak of the disease can decimate a nation's livestock industry, such as happened in Taiwan in 1997, where 4 million animals were culled, costing the country US$5 billion. In addition, the presence of FMD, especially

Table 11. Vaccines against bovine respiratory synctitial virus.

Vaccine	Vector	Dosage	Price	Vaccination ages	Immunity	Ref.
Bovi-Shield BRSV (Pfizer, NY, USA)	MLV for BRSV, IBR, PI_3 and BVD	Two 2-ml im. doses 3–4 weeks apart with annual revaccination with single dose	US$25.99/50 doses (US$0.52/dose)	No age restriction, but calves vaccinated before 6 months should be revaccinated after 6 months of age	Serum antibodies within 2 weeks and neutralizing antibodies within 5 weeks	[97,98]
Cattlemaster® Gold™ 5LP5 (Pfizer)	MLV BRSV, chemically altered strains of IBR and PI_3 and inactivated BVD with aluminum hydroxide adjuvant	Two 5-ml sc. doses 3 weeks apart with annual revaccination with single dose	US$59.99/25 doses (US$2.40/dose)	No age restriction, but calves vaccinated before 6 months should be revaccinated after 6 months of age	Serum antibodies in 89% of calves within 2 weeks	[99]
Jencine 4 (Schering-Plough, NJ, USA)	MLV for BRSV, noncytopathic BVD virus	One 2-ml im. or sc. dose with annual revaccination	US$46.96/50 doses (US$0.94/dose)	2 weeks old; calves vaccinated before 6 months of age should be revaccinated after 6 months of age	Unknown	
Pyramid 4 (Fort Dodge, KS, USA)	MLV for BRSV, IBR, PI_3 and BVD with MetaStim adjuvant	One 2-ml dose sc. with annual revaccination	US$77.99/50 doses (US$1.56/dose)	No age restriction, but calves vaccinated before 6 months of age should be revaccinated after 6 months of age	Unknown	
Vira Shield (Novartis, UK)	Inactivated BRSV, IBR, BVD, PI_3 viruses with Xtend® SP adjuvant	Two 5-ml doses sc. 4–5 weeks apart with annual revaccination	US$62.90/50 doses (US$1.26/dose)	No age restriction, but calves vaccinated before 6 months of age should be revaccinated after 6 months of age	Cell-mediated and humoral immunity	
DNA vaccine BRSV (Hamers *et al.* 2007)	Two plasmids expressing the BRSV F and N antigens	2 ml of DNA vaccine im. 4 weeks apart or single dose followed by vaccination with inactivated virus	Unknown	3–6 weeks (plus maternal antibodies)	No antibodies, reduced lung lesions, reduced virus shedding correlated with IFN-γ producing T-cell response	[41]
DNA vaccine BRSV (Taylor *et al.* 2005)	Plasmid expressing F gene of the Snook strain of BRSV	Two doses of 0.25-mg DNA im., intradermally and intratracheally 5 weeks apart	Unknown	2 weeks (no maternal antibodies)	Antibodies within 9 weeks and BRSV-specific lymphocyte proliferative responses within 2–4 weeks No virus found in lungs of 9/11 challenged calves	[41]

BRSV: Bovine respiratory synctitial virus; BVD: Bovine virus diarrhea; IBR: Infectious bovine rhinotracheitis; Im.: Intramuscular; MVL: Modified live virus; PI_3: Parainfluenza type-3; sc.: Subcutaneous.

in developing countries, can greatly affect the export market of cattle to FMD-free countries too. Vaccination campaigns in Western Europe, parts of South America, North America, New Zealand and Australia have rendered these areas FMD-free. However, these campaigns are expensive and certain outbreaks have been linked to the presence of residual live virus in chemically inactivated vaccines [50]. Prophylactic vaccination is now prohibited in the EU and the USA, and a number of other FMD-free countries; however, antigen banks exist that provide different strains of the virus that can be used for outbreak scenarios (Table 14). Prophylactic vaccination is performed in countries where FMD is enzoonotic (especially in Southern Africa and South America); however, there are significant problems associated with vaccine production and development.

No universal prophylactic vaccine currently exists for FMD. Seven very different types of FMDV are believed to exist and there is no cross-protection between serotypes. Another difficulty

Table 12. Vaccines against bovine viral diarrhea disease.

Vaccine	Vector	Dosage	Price	Vaccination ages	Immunity	Contraindication	Ref.
Bovilis (Intervet, The Netherlands)	Inactivated BVD virus	Two 2-ml doses im. 4 weeks apart with annual revaccination with single dose	US$21/25 doses (US$0.84/ dose)	8 months	Reduced viral excretion and neutralizing antibodies	Fetal protection can be achieved if administered 4 weeks before start of gestation Should not be administered within 21 days to slaughter	[102]
Breed-back™ FP 10 (Boehringer-Ingelheim, UK)	BVD Types 1 and 2, MLV, IBR, PI_3 and BRSV, MLV with adjuvant	Two 2-ml doses sc. 14–28 days apart with annual revaccination with single dose	US$9.75/five doses (US$1.95/ dose)	No age restriction, but calves vaccinated before 6 months of age should be revaccinated after 6 months of age	91–100% protection from fetal infection, 14/22 calves free of clinical signs	Should not be vaccinated within 21 days before slaughter Should not use in pregnant cows or in calves nursing pregnant cows	[103]
DNA vaccine (Liang *et al.* 2008)	Plasmid encoding a truncated secreted version of E2 with a tissue plasminogen activator signal sequence, followed by boosting with E2 protein formulated with 10% Emulsigen, a mineral oil in water emulsion, and CpG oligodeoxynucleotide	1 mg of DNA delivered transdermally via needle-free injection, followed by 50 µg of protein	Unknown	8–9 months	Virus-neutralizing antibodies and INF-γ-producing $CD4^+$ T cells, reducing leucopenia, no weight loss or temperature response	Unknown	[100]
DNA vaccine (Nobiron *et al.* 2003)	Plasmid expressing E2 glycoprotein of BVDV	500 µg DNA intradermally	Unknown	3–7 months	Low levels of neutralizing antibodies, elevated T-cell proliferation, reduced febrile responses	Unknown	[101]

BRSV: Bovine respiratory synctitial virus; BVD: Bovine virus diarrhea; BVDV: Bovine virus diarrhea vaccine; IBR: Infectious bovine rhinotracheitis; im.: Intramuscular; MLV: Modified live virus; PI_3: Parainfluenza type-3; sc.: Subcutaneous.

associated with the development of a vaccine for FMD is that the virus is able to persist in cattle and small ruminants, irrespective of vaccination status [51]. Given this and the fact that the virus can persist outside of a host for more than a month and is easily transmitted, vaccination becomes even more difficult.

Vaccine banks generally either hold reserves of fully formulated and tested vaccines that can be used immediately but have a short shelf life, or reserves of antigens, which have longer shelf-lives but need to be formulated into a vaccine before it can be shipped out for use. Characteristics of the three principal vaccine banks are listed in Table 15, as are comparisons of commercial vaccines with DNA vaccines.

DNA vaccination, while still not perfected, represents a potential solution to FMD. Countries that are FMD-free generally do not want to prophylactically vaccinate against FMD, as not only are vaccines costly and induce short-lived protection, but they could also cause an outbreak. A DNA vaccine could address both problems – it would be cheaper than conventional vaccines and would eliminate the need to introduce the virus itself into a disease-free location. In addition, DNA vaccination allows discrimination between vaccinated and infected animals, which would circumvent the problem of the export of meat from countries with FMD. In addition, DNA vaccines elicit both humoral and cellular immunity, which are both essential for control of the disease.

Table 13. Vaccines against Johne's disease.

Vaccine	Vector	Species	Dosage	Vaccination ages	Immunity	Ref.
Mycopar® (Fort Dodge, KS, USA)	Whole-cell bacterin containing inactivated *Mycobacterium paratuberculosis* bacteria suspended in oil	Cattle, sheep	Cattle: 0.5 ml sc. Sheep: 1 ml sc.	Calves: less than 35 days Sheep: 3 months	Cattle: ~90% effective in eliminating clinical disease Sheep: specific humoral and cellular responses elicited	[106,107]
Silirum® (Pfizer, NY, USA)	Killed bacteria in mineral oil adjuvant	Cattle, deer	One dose sc. for cattle and deer	Calves: 14 days Deer: 5 months	Cattle: no antibodies. Reduced colonization of tissues by pathogen Deer: reduced severity of disease	[108,109]
DNA vaccine (Kathaperumal *et al.* 2008)	Four rAgs (85A, 85B, 85C and superoxide dismutase) with two adjuvants (monophosphoryl lipid A and bovine IL-12)	Cattle	100 µg of each antigen and 100 µg of IL-12 im.	5–10 days	Antibodies within 3 weeks; significant IFN-γ production within 11 weeks Significant increases in $CD4^+$ and $CD8^+$ T cells against all four rAgs; rAg-specific expression of IL-2, IL-12 and TNF-α. 4/8 animals did not show bacteria in tissue	[104]
DNA vaccine (Sechi *et al.* 2005)	Three rAgs (*Mycobacterium avium* 85A, BCG 85A and 65K)	Sheep	Three doses of 1 mg of each antigen im. 20 days apart	5 months	Increased IFN-γ and IL-10 expression, increased $CD4^+$ T cells Absence of lesions and bacteria in tissues	[105]

Im.: Intramuscular; rAg: Recombinant antigen; sc.: Subcutaneous.

DNA vaccines effectively adjuvanted – such as with interleukins or complement components [52] – could constitute powerful vaccines. More work needs to be completed on these vaccines, especially in a more diverse range of species; most research has been carried out in swine [53–56]. Attention should also be paid to cattle, goats and sheep, which are also affected by this disease.

Porcine reproductive & respiratory syndrome virus, & swine influenza

The worldwide pork industry is a fast-growing industry, with intensive animal husbandry practices. According to the USDA, the world production of pork in 2008 was forecast to reach 97 million tons of pork [204]. Porcine reproductive and respiratory

Table 14. Vaccine banks for foot-and-mouth disease.

Vaccine bank	Total vaccine stock	Provenance of vaccines	Antigen subtypes	Manufacturing time
International Vaccine Bank (Pirbright, UK)	Antigens equivalent to 3.5 million doses of finished vaccine of each subtype	Manufactured on site	A: 1,500,000 O: 1,000,000 C: 500,000 Asia1: 500,000	Capability of formulating, filling and dispatching up to 500,000 doses of vaccine within 3 days
European Union Vaccine Bank (Pirbright, UK; Lyon, France; di Brescia, Italy)	Antigen equivalent to 5 million doses of vaccine of each subtype	Purchased from European manufacturers	O1 Tur178: 5,000,590 C1 Europe: 2,500,000 Asia1: 2,500,000 O1 BFS: 5,000,692 A24 Cruzeiro: 5,000,874 A22 Iraq: 3,887,124	5 days to release inactivated antigen. Manufacture of vaccines from raw materials takes aproximately 10 weeks
North American Vaccine Bank (Plum Island, USA)	38,417,720 antigens available	Purchased from manufacturers	O: 10,778,718 A : 13,599,002 C : 6,800,000 Asia1: 5,240,000 SAT1: 1,000,000 SAT2: 1,000,000	Can obtain hundreds of thousands of doses of FMD vaccine within days

FMD: Foot-and-mouth disease; SAT: Southern African Territory.
Data taken from [113].

Table 15. Vaccines against foot-and-mouth disease.

Vaccine	Vector	Dosage	Price
Decivac® FMD DOE (Intervet, The Netherlands)	Antigens of chemically inactivated FMDV types O, A, C, Asia1 and SAT1, SAT2, SAT3, with DOE. Combinations of antigens depend on local situation	Cattle, buffalo, pigs: 2 ml Sheep, goats: 1 ml sc. or im.	US$0.05/cow US$0.01/sheep or goat
Aftopor® (Merial Animal Health Ltd, UK)	Purified, inactiaved FMDV types O, A, C, Asia1, SAT1, SAT2, SAT3 in a DOE adjuvant. Combinations of antigens depend on local situation	3–15 µg of antigen/strain for pigs and ruminants Large ruminants and pigs: 2 ml Small ruminants: 1 ml im.	US$2/dose for trivalent vaccine
Bayovac (Bayer, UK)	Trivalent inactivated virus of O, A and C strains in oil emulsion	Cattle and buffalo: 5 ml Sheep and goats: 3 ml im. or sc.. Revaccinate 90 days after and thereafter every 6 months	Unknown
DNA vaccine for swine (Benvenisti *et al.* 2001)	1. Plasmid encoding all viral structural proteins with viral protease of strain O1(G) (pKVI335). 2. Construct with viral P1, 2A and 3CD sequences (pKVI326)	5 µg of DNA with 25 mg gold, by gene gun and sc. Booster inoculation after 3 weeks	Unknown
DNA vaccines for swine (Beard *et al.* 1999)	1. Plasmid encoding entire FMDV genome with mutation at cell-binding site (pWRMHX) 2. Plasmid encoding viral capsid gene P1 and processing proteinase 3C (iP12X3C)	Two injections of 3 µg of DNA bound to 1.5 mg of gold 4 weeks apart, via im., intradermal and gene gun inoculations	Unknown
DNA vaccine for swine (Wong *et al.* 2002)	Plasmid expressing two FMDV VP1 epitopes (pCEIS) coadministered with IL-2 plasmid	Two injections of 200 µg of DNA im. 4 weeks apart	Unknown
DNA vaccine for swine (Cedillo-Barron *et al.* 2001)	1. Plasmid encoding VP1 and a 3D polymerase (pcDNA3.1/3D15) from type O FMDV 2. Plasmid encoding VP1 and NS2B (pcDNA3.1/2B15) from type O FMDV	Three injections of 300 µg of DNA 3–4 weeks apart im. and intradermally	Unknown
DNA vaccine against sheep (Niborski *et al.* 2006)	Plasmid encoding FMDVP1 of O1 strain with ovine GM-CSF with adjuvant PLG microparticles	Three injections of 400 µg of DNA coated on PLG 3 weeks apart intradermally and im.	Unknown

DOE: Double-oil emulsion; FMD: Foot-and-mouth disease; FMDV: Foot-and-mouth disease virus; Im.: Intramuscular; PLG: Poly (D,L-lactide-coglycolide); SAT: Southern African Territory; sc.: Subcutaneous.

syndrome is a disease that has recently emerged as one of the most important pathogens in countries with intensive swine industries. The disease is characterized by reproductive disorders in pregnant sows, perinatal losses and respiratory distress in piglets, causing the industry an estimated US$66.75 million annually in breeding herds and US$493.57 million in growing pig populations [57]. The causative agent of porcine reproductive and respiratory syndrome (PRRS) is a virus of the *Arteriviridae* family of the *Nidovirales* order (porcine reproductive and respiratory syndrome virus [PRRSV]), which shows high sequence variation between European and North American isolates [58].

The disease is particularly difficult to treat and detect. Following infection, the induction of neutralizing antibodies is slow, which enables the virus to persist in the host and propagate to other animals through contaminated mucus, saliva, excrement, semen and possibly via the airborne route [59,60]. Heterogeneity of isolates is a complicating factor for achieving protection via vaccination, as is the fact that in the endemic phase of the disease, many infections are subclinical, thus rendering detection of the disease unreliable [61]. A number of commercial vaccinations are available for PRRSV with varying efficacy, which generally reduce clinical disease rather than preventing infection [62]. A number of DNA vaccines have also been tested that achieve varying results (Table 16). DNA vaccines would be particularly valuable for this disease if a cocktail vaccine against different strains of the virus could be achieved without the possibility of causing an outbreak.

Given the relative inefficacy of commercial vaccines and the contraindications of some of these, there is much room for improvement in finding a solution to PRRSV. DNA vaccine development for PRRSV is still only in the preliminary stages, but offers an interesting alternative to commercial vaccines. In particular, the fact that certain commercial vaccines cannot be used for prophylactic purposes provides a potential DNA vaccine with a distinct advantage, as it would be effective for prophylactic use. Indeed, in Denmark, for example, a national eradication

Table 15. Vaccines against foot-and-mouth disease (cont.).

Vaccination ages	Protection	Ref.
Young animals without maternal antibodies: 2 weeks old, revaccination 4–6 months later Young animals with maternal antibodies: 4 months of age, revaccination 4–6 months later	Adult animals: every 6 months Immunity within 10 days lasting for 6 months in cattle, buffalo, sheep and goats; 4 months in pigs	
Ruminants: starting at 2 weeks of age followed by second vaccination 4 weeks later, with booster every 6 months Pigs: starting at 8 weeks of age followed by second vaccination 4 weeks later, with booster every 4 weeks	Antibodies that reduce clinical signs and mortality following exposure to FMDV in 92% of animals within 16 weeks	[54]
Until 4 months of age	Confers immunity within 21 days, lasting 6 months	
3 weeks old (8.9–13.3 kg)	Vaccination with pKVI326 induced partial protection but no detectable neutralizing antibodies Vaccination with pKVI335 provided no protection	[111]
Unknown	Low levels of neutralizing antibody within 4 weeks Pigs vaccinated with pWRMHX were protected from challenge, those vaccinated with piP12X3C were not	[50]
30–40 kg	10–20-fold T-cell proliferation, low levels of neutralizing antibodies, 100% protection from challenge	[111]
20–25 kg	B-cell, T-cell and antibody response to nonstructural proteins 2B and 3D, but no immunity induced to FMDV15 peptide, no protection against challenge	[112]
6–12 months	Transient T-cell response, some humoral immunity Protection against FMD symptoms, inhibition of viral replication	[113]

DOE: Double-oil emulsion; FMD: Foot-and-mouth disease; FMDV: Foot-and-mouth disease virus; Im.: Intramuscular; PLG: Poly (D,L-lactide-coglycolide); SAT: Southern African Territory; sc.: Subcutaneous.

vaccination program was unsuccessful, as the vaccine virus was found to be transmitted between boars for a period of 8 weeks and was introduced to herds via semen [63]. Similarly, a DNA vaccine would be more advantageous in achieving herd immunity, as it would be able to be administered to all animals, including boars and pregnant sows. Further research must be undertaken to study the immune mechanisms of these DNA vaccines and of the virus itself in order to achieve a viable vaccination option.

The recent human outbreak of H1N1 swine flu, which was first reported in 2009 in human populations in Mexico, has caused great alarm. This hemagglutinin has not been circulating routinely for close to half a century. As such, human immune resistance to this strain is currently low, so the infections of pigs with influenza as a component of translation of infection to human populations has become of increased importance, and suggests that a renewed focus on such vaccination for porcine populations is in order. The variability of H1N1 is particularly amenable for approach by DNA vaccine technology. Recently, studies by Laddy *et al.* [64,65] and Chen *et al.* [66], using consensus-based immunogens, appear particularly promising in the H5 system (see the section on avian influenza later). Furthermore, the use of electroporation to improve DNA delivery in pig populations has already been licensed for clinical application. Such combination DNA vaccine approaches may be attractive for the prevention of swine influenza in commercial farming. As this DNA approach is a non-live, nonspreading, nonreplicating delivery platform, concerns over attenuation and inadvertent spread are avoided. Furthermore, vaccination can be easily distinguished from true infection, thus allowing for protection of herds in case of exposure to this pathogen.

Opportunities for DNA vaccines in birds: avian influenza

Avian zoonoses have only fairly recently emerged as a public health issue, yet avian diseases have been a prevalent and constant threat to birds of all species, from poultry to zoo birds to

Table 16. Vaccines against porcine reproductive and respiratory syndrome.

Vaccine	Vector	Dose	Vaccination ages	Price
Ingelvac RespPRRS/Repro™ (Boehringer-Ingelheim, UK)	Modified live virus	2 ml im.	3 weeks and nonpregnant females	US$57.99/50 doses (US$1.16/dose)
Suvaxyn® PRSS (Fort Dodge, KS, USA)	European-type modified live virus	2 ml im.	4 weeks	Unknown
Progressis (Merial Animal Health Ltd, UK)	Inactivated virus with oil excipient	Two 2-ml doses im. at 3–4-week intervals with revaccination within 60–70 days	2–3 weeks	Unknown
Porcilis® PRRS (Schering-Plough, NJ, USA)	Attenuated live European strain with Diluvac Forte adjuvant	Two 2-ml doses im.	2 weeks	US$16.64/25 doses (US$0.66/dose)
DNA vaccine (Hou *et al.* 2008)	Plasmid coexpressing GP5 gene of PRRSV and swine ubiquitin	Three 500-µg doses im. at 3-week intervals	30 days	Unknown
DNA vaccine (Xue *et al.* 2004)	Plasmid coexpressing PRRSV ORF5, ORF7 genes and porcine IL-2 or IFN-γ	Four 500-µg doses im. at 2-week intervals	30 days	Unknown
DNA vaccine (Jiang *et al.* 2006)	Plasmid coexpressing GP5 and M gene of PRRSV	Two doses of 100 µg im. 4 weeks apart	3 weeks	Unknown

PRRSV: Porcine reproductive and respiratory syndrome virus; im.: Intramuscular; ORF: Open-reading frame.

wild birds. Birds represent a significant part of agriculture and the economy: approximately 850 million poultry birds contribute US$35.1 billion to the US economy annually, while 11 million pet birds constitute the third most popular companion pet after dogs and cats [6]. Poultry in large-scale operations are routinely vaccinated against a number of diseases: day-old chicks are vaccinated against Marek's disease, and can be vaccinated against Newcastle disease and infectious bronchitis. Older birds can be vaccinated against fowl pox, fowl cholera and avian encephalomyelitis. Poultry in smaller scale operations and in developing countries, however, are not vaccinated as often, as vaccines can be expensive and are usually produced in large-dose vials intended for commercial use. In such operations, culling is the most frequent method of disease control. Vaccination of wild birds is, for obvious reasons, impractical and frequently impossible. Currently available avian vaccines are not always effective and some only treat symptoms rather than preventing disease [67,68]. DNA vaccines therefore have a potential application in avian disease. One such disease, which represents a grave threat to avian and human health, is avian influenza.

Avian influenza virus (AIV) is an orthomoxyvirus that has garnered a great deal of attention in the past few years. Its virulence and its potential to spread to humans have generated fears of pandemics among humans and panzootics among birds. Outbreaks of avian influenza, in particular of the H5N1 strain, have occurred in a number of countries, such as in Hong Kong in 1997, 2001 and 2002, resulting in the slaughter of over 3 million chickens, a loss of HK$200 million (~US$25 million) and six human deaths [69]. Several subtypes of AIV exist, classified by the antigenicity of the surface proteins hemagglutinin (H1–16) and neuraminidase (N1–9). Certain subtypes, when introduced into a host, can become highly pathogenic, thus switching from low-pathogenic avian influenza (LPAI) to highly pathogenic avian influenza (HPAI). Wild waterfowl are considered carriers of LPAI viruses. HPAI viruses not only cause up to 100% mortality in a wide range of avian species, but can also infect mammalian species [70]. Symptoms of HPAI in poultry range from cessation of egg laying, to loss of appetite and depression, to sudden death. The virus is shed in saliva, nasal secretions and feces.

The vaccination of poultry and zoo birds has been undertaken in a number of countries, subject to strict risk assessment and surveillance requirements by national authorities. The EU, for example, which initially had a nonvaccination policy, subsequently passed Avian Influenza Directive 2005/94/EC, which allowed for both the emergency and preventative vaccination of zoo birds and poultry. In Hong Kong, the USDA developed an AI Hong Kong H5N1 Response Plan that allowed vaccination to be used in eradication program for HPAI [71]. China has recently announced plans to vaccinate all 4 billion of its chickens [72]. Concomitantly, a number of differentiating infected from vaccinated animal (DIVA) techniques have been introduced, with varying advantages and disadvantages [73].

Vaccination has several advantages, including reducing the risk of birds becoming infected, reducing mortality and reducing shedding of the virus in the event of an outbreak, which can help prevent spread of the disease. Several commercial vaccines

Table 16. Vaccines against porcine reproductive and respiratory syndrome (cont.).

Immunity	Contraindication	Ref.
Vaccination of infected animals: reduced duration of viral shedding, but no reduction of viral load in tissues no change in proportion of persistently infected pigs	Boars should not be vaccinated. Not recommended for use in naive herds. Duration of protection is ~4 months	[116]
Reduced preweaning mortality of piglets but no prevention of clinical signs or viremia	None	[117]
Induction of PRRSV-specific antibodies Reduction of the reproductive disorders and of the number of early farrowings still-births	None	
Reduced morbidity in fattening pigs, improvement of reproductive performance and reduced transplacental virus transmission in breeding pigs	Pregnant sows should only be vaccinatedafter previous exposure to European PRRSV	
Enhanced T-cell proliferation No neutralizing antibodies Lower viral replication and distribution in tissues Lack of lesions in 4/6 vaccinated pigs.	Unknown	[59]
Reduced viral replication Absence of lesions in 2/3 pigs vaccinated with ORF5/IFN-γ, 1/3 vaccinated with ORF5/IL-2 and 1/3 vaccinated with ORF7/IL-2	Unknown	[115]
Neutralizing antibodies within 10 weeks. Enhanced splenocyte proliferation activity	Unknown	[116]

PRRSV: Porcine reproductive and respiratory syndrome virus; im.: Intramuscular; ORF: Open-reading frame.

are available, including inactivated vaccines, recombinant fowl pox H5 vector, combination vaccines of different AI strains and AI with Newcastle disease [74]. A number of DNA vaccines are also being developed for both poultry and humans. Table 17 examines both commercial and experimental DNA vaccines for use in poultry.

Vaccination of fowl for AIV, while permitted in many countries, is still not always encouraged. The Avian Influenza Directive 2005/94/EC, for example, limits preventative vaccination programs to programs authorized only for specific birds in specified regions, and are subjected to rigorous surveillance and control requirements. For example, in 2006 France vaccinated 900,000 ducks and geese, while Germany vaccinated three commercial poultry holdings of ducks, geese and layer chickens. However, the standard practices of keeping poultry separated from wild birds and, in the event of an outbreak, the culling of birds, are much preferred. Concerns about outbreaks due to vaccines are not unfounded, and DIVA is not always highly effective, which can have an impact on the import/export of poultry markets. Vaccination can also mask the occurrence of disease in a farm, delaying its detection. Furthermore, the virus has a tendency to mutate, which may render commercial vaccines ineffective. Vaccination is also difficult and expensive, as commercial vaccines must generally be administered one-to-three times. Vaccination trials of 400–600 commercial farms in Italy cost €2–4 million a year, with the vaccine costing €0.10–0.15 per bird [205]. DNA vaccines could most likely provide a less expensive option, and, in the case of vaccines such as that of Jiang *et al.* [75], would require fewer injections. DNA vaccines would also have a 'built-in' DIVA, which would require fewer steps in the vaccine program. The safety of DNA vaccines would prevent the possibility of an outbreak resulting from vaccination, and perhaps most promising of all is the fact that DNA vaccines can elicit both humoral and cellular immune responses. Protective antibodies must be matched to the strain of AI, whereas cellular immunity can achieve greater cross-protection – a particularly important prospect given the number of strains of virus and the ability of the virus to mutate [76]. The DNA vaccines by Laddy *et al.* [64] or Chen *et al.* [66], for example, are consensus-based vaccines that provide protection against multiple strains of AI. Furthermore, the use of microelectrodes and electorporation as shown by Laddy *et al.* to induce strong HAI and broad micro-neutralization titers in several species of animals, including non-human primates [76], using consensus DNA immunogens appear to be a highly attractive option for avian influenza, as well as perhaps for swine flu as previously discussed.

Opportunities for DNA vaccines in fish: infectious hematopoietic necrosis

Another target for DNA vaccines today is fish. As the oceans become depleted of fish due to overfishing and pollution, intensive farming of fish is becoming more widespread and economically important. The scale of these fisheries is expanding: for example, in the 1970s, there were 70,000 acres of catfish farm ponds in the USA, with approximately 20,000 fish per acre. In 1996, US catfisheries occupied over 90,000 acres, producing a total of 270,000 tons of fish annually [206]. The Alaskan salmon market

Table 17. Commercial and DNA vaccines for avian influenza.

Vaccine	Species	Vaccine vector	Dosage	Vaccination age	Protection	Ref.
Poulvac FluFend (Fort Dodge, KS, USA; product label)	Chickens, ducks and turkeys	Inactivated AIV, H5N9 strain A/CK/Italy/22A/H5N9/1998 with oil emulsion adjuvant	Chickens: two doses of 0.5 ml im. within 14 days Ducks: two doses of 0.2 ml sc. within 3 weeks Turkeys: three doses of 0.5 ml sc. at 21-day intervals	Chickens: from 2 weeks Ducks: from 1 day Turkeys: from 8 days	Protection against clinical signs and mortality and reduced viral excretion within 3 weeks Significant antibody titers persist in chickens for at least 22 weeks	
Nobilis Influenza H5 (Intervet; product brochure, summary of product trials)	Poultry and ducks	Inactivated AIV for various subtypes with oil emulsion adjuvant	Chicken: two doses of 0.5 ml within 4–6 weeks im. or sc. Ducks: 1-ml dose sc.	Chickens: from 1 day	Complete protection in chickens and ducks against virus transmission from infected birds and clinical disease	[67]
Trovac AI H5 (Merial; product label and Bublot *et al.* 2006)	Chickens, potentially mammals (immunogenic in cats)	Live recombinant fowlpox recombinant expressing the HA gene of AI H5 subtype isolate	Chickens: 0.2 ml sc.	Chickens: from 1 day	Protection within 1 week, lasting 20 weeks, significantly decreased virus shedding	[68]
DNA vaccine (Laddy *et al.* 2008)	Mice, ferrets and macaques	Set of synthetic consensus DNA constructs encoding AIV A antigens H5HA, H1NA, NP	Mice: three doses of 25 µg 2 weeks apart im. with electroporation Ferrets: three doses of 200 µg 1 month apart im. with electroporation Macaque: two doses of 1 mg 1 month apart im. with electroporation	Mice: 6–8 weeks Ferrets: 4–6 months	Macaques: unknown cellular and humoral immune responses, protection from morbidity and mortality, and reduction of viral shedding in mice and ferrets	[64]
DNA vaccine (Kodihalli *et al.* 2000)	Chickens	Plasmids encoding HA and NP genes transfixed to gold particles, delivered by gene gun	Two 10-µg doses 3 weeks apart im.	3 weeks	HA plasmids: protection of 86% of chickens NP plasmids: protection of 42% of chickens	[119]
DNA vaccine (Jiang *et al.* 2007)	Chickens	Plasmids expressing optiHA and wild-type HA genes	One 100-µg dose im.	3 weeks	Antibodies within 1 week, complete protection against challenge	[75]
DNA vaccine (Le Gall-Reculé *et al.* 2007)	Chickens	Prime–boost: plasmid encoding H7 and M1 viral proteins from an Italian H7N1 LPAI virus	Two 100-µg doses 3 weeks apart	4 weeks	Significant decrease in cloacal and tracheal shedding of virus	[120]

AIV: Avian influenza virus; HA: Hemagglutinin; im.: Intramuscular; LPAI: Low pathogenic avian influenza; NP: Nuclear protein; sc.: Subcutaneous.

produces close to US$900 million worth of fish annually [207]. Fish are raised for food and sport, and many fisheries are also involved in conservation efforts.

Owing to intensive fishing practices, fish are particularly vulnerable to a number of bacterial and viral diseases. Previous methods of disease control included the introduction of bacterins and vaccines in feed over prolonged periods of time, with varying degrees of success. Attenuated or modified viral and bacterial organisms have also been introduced directly into the water, but this method runs the risk of viruses reverting to virulent forms and contaminating rivers, lakes, other hatcheries and wildlife. Recently, fisheries have had to resort to individually injecting fish with vaccines. A number of vaccines for fish are available today, such as for Koi herpes virus, pasteurellosis, vibriosis, enteric septicemia of catfish, piscirickettsiae, infectious salmon anemia, *Moratella viscosis*, and a number of streptococci and mycobacteria [208].

One disease that has been a scourge of the industry has been infectious hematopoietic necrosis (IHN). This disease, caused by the IHN virus of the *Rhabdoviridae* family, results in symptoms characterized by extensive necrosis of hematopoietic tissues (spleen, kidney and liver). Transmission occurs horizontally between fish, as the virus is shed in feces, urine, sexual fluids and external mucus. The virus can also be transmitted from farmed populations to wild populations. The severity of outbreaks depends on a number of factors, including species and age of fish, rearing conditions and water temperature [209]. Previously, the only method of preventing disease was to avoid exposure to the virus through hygienic rearing practices, such as disinfection of fertilized eggs, incubating eggs, and raising fry and young fish in isolated sites.

The Canadian salmon industry, which produced 98,441 tons of fish valued at US$543,634,000 in 2006 [210], was particularly vulnerable to IHN. From 2001 to 2003, the virus caused losses in the order of US$200 million Canadian dollars (~US$198 million) [208]. A DNA vaccine against IHN virus for Atlantic farmed salmon was developed by an affiliate of Novartis (Aqua Health Ltd, PE, USA), with permission from appropriate Canadian regulatory authorities, and granted a commercial license. The vaccine has been shown to provide strong protection against the virus and will hopefully be able to be used in other countries soon (Table 18).

A number of other DNA vaccines for fish have been tested (Table 18) including for viral hemorrhagic septicemia virus, infectious pancreatic necrosis virus (*Birnaviridae* family), infectious salmon anemia virus and *Mycobacterium marinum*. While these vaccines have achieved varying degrees of success, DNA vaccines for fish remain an attractive option for several reasons. Given the size of hatcheries and the number of fish in them, a cheap vaccination option is necessary; inactivated viruses would be prohibitively expensive and attenuated viruses can cause disease. In addition, the introduction of viral pathogens in water could lead to contamination of other bodies of water and the fish in them. DNA vaccines are safer for fish as they are not formulated with an oil adjuvant that can cause peritonitis [77]. They are also safe for the consumer, as the fish are consumed months or even years after vaccination, and the quantity of DNA used is very small. At this point, immune mechanisms of protection achieved by DNA vaccines are poorly known; however, the effectiveness of these vaccines is fairly well established.

Future directions for DNA vaccines

DNA vaccines are currently mainly being developed to vaccinate against diseases. However, alternate uses of DNA inoculation are also being considered. One that has enjoyed success in terms of efficacy is the inoculation of pigs with plasmids encoding the gene for porcine somatotropin. Vaccination of gilts or pregnant

Table 18. DNA vaccines for fish.

Pathogen	Species	Vaccine vector	Dosage	Vaccination age	Protection	Conventional treatment	Ref.
IHNV (Apex-IHN by Novartis, UK)	Atlantic salmon	G-protein gene	Two 20-µg doses im.	30 g or larger	73% protection	Avoiding exposure to the virus	[208]
IPNV (Mikalsen *et al.* 2004)	Atlantic salmon	*VP2* gene	25 µg of DNA im.	Postmolts of 20 g or larger	RPS* of 84%	Commercial vaccines: inactivated virus or structural virus proteins expressed in *Escherichia coli*	[124]
VHSV (Lorenzen *et al.* 2001)	Rainbow trout	G-protein gene	1 µg of DNA im.	Fry 0.5 g (3 months after hatching)	RPS of 98%		[125]
Mycobacterium marinum (Pasnik and Smith 2004)	Striped bass	*AG85A* gene (secreted fibronectin-binding protein)	25 or 50 µg doses with booster 14 days later im.	40–50 g (~5 months)	Low levels of AG85A-specific antibodies, lymphoproliferative responses within 42 days, 80% RPS for 25-µg dose, 90% for 50-µg dose	No chemotherapeutic agents; avoiding exposure to bacteria	[126]

*Relative percent survival (= one cumulative mortality vaccinated group/cumulative mortality control group).
im.: Intramuscular; RPS: Relative percent survival.

sows results in increased piglet viability, size at birth, growth and performance, via the upregulation and increased activity of IGF [78,79].

A commercial growth hormone-releasing hormone vaccine for pigs has been developed and approved for use in Australia by VGX Pharmaceuticals (LifeTide SW5; PA, USA). A single vaccination, delivered via electroporation to pigs, resulted in increased weights and increased growth rate of piglets, reduced mortality in piglets and dams, and an increased number of offspring in sows [80]. Neither the USDA nor the US FDA have yet approved the use of such vaccines, nor of any form of growth hormone treatment for the swine industry. However, such DNA vaccines have been approved in other countries and may be of great value in countries such as China, which is the world's largest consumer and producer of pork. Indeed, according to a report by the USDA, the Chinese consumed 51 million metric tons of pork in 2006, roughly half of the world's total pig consumption (versus 8.6 million metric tons in the USA) [211]. This technology could also be useful in developing countries where animal owners are predominantly resource-poor, small-scale operators with little land and few animals, who must operate on few resources and capital. Similar to the use of bovine somatotropin in dairy cows to increase milk production, the use of porcine somatotropin has many advantages. Pigs are not only healthier at birth and beyond, but also leaner, which translates into healthier meat for consumption. Somatotropin is species specific and studies have shown that porcine somatotropin is active only in swine, not cattle or humans, thereby guaranteeing its safety for human consumption [212]. Furthermore, if more meat can be derived from a smaller number of animals, then fewer resources will be required for pork production, which will make pork production less environmentally detrimental.

Conclusion

Ultimately, the factors that will make a DNA vaccine attractive for a certain disease will include its reduced cost, its ease of transport and administration, its ability to act in the face of maternal antibodies, the ability to differentiate diseased animals from vaccinated animals, and the reduced likelihood of the vaccine to cause an outbreak. In addition, DNA vaccines have the ability to induce both cellular and humoral protection since antigens can be delivered and processed intracellularly, which can improve upon certain traditional vaccinations. DNA vaccines are also powerful in that they have extended boosting capability for continued immunotherapy, since the vaccine can be administered repeatedly without inducing neutralizing antibodies to the plasmid. However, all of these factors must be weighed against the ability of the vaccine to offer protection to the host. If the number of currently commercially available DNA vaccines is low, it is because prior DNA vaccines did not induce a high enough degree of protection in larger animals and humans. The improvement of DNA vaccine immune potency must be achieved, through promising technologies as improved formulations or simple electroporation, or alternate vaccination strategies should be considered, such as prime–boost approaches, cytokine gene adjuvants or other adjuvant formulations.

In terms of large food-animal production, vaccines are essential not only for individual animal health, but also for herd health and, by extension, human health. The large number of commercial vaccines available for a number of large animal diseases allows breeders and producers ample choice for vaccination strategies. In addition, an advantage of many commercial vaccines is that they provide a cocktail of immunizations for a relatively low price. If DNA vaccines are to compete with commercially available vaccines, the price of production will need to be minimal. The advantage of a DNA vaccine, especially in large herds or production establishments, is its ease of transport and storage compared with commercial vaccines. In addition, a DNA vaccine specific to a certain disease could be more useful in an outbreak scenario where control of the spread of the disease is desired. An additional significant advantage is the potential ability of DNA vaccines to act in the face of maternal antibodies [3]. For neonates ingesting colostrums and maternal antibodies, commercial vaccines are generally inefficacious, yet young calves are very susceptible to a number of infectious diseases. The current method of disease prevention for calves is immediate postnatal separation of the calf from its dam; therefore, the ability to induce immunity in young animals in the face of maternal antibodies could be very valuable.

Expert commentary & five-year view

DNA vaccines should be developed for diseases where traditional vaccination is not very effective (such as PRRSV or in diseases where various regional serotypes exist) or only treats clinical signs and does not prevent disease (such as FMD). DNA vaccines should also be looked into as an option if there is concern about traditional vaccination causing an outbreak in a herd or causing adverse effects on the animal (such as a vaccine-induced sarcoma in cats or lesions at the site of vaccination in pigs). However, improvements must be made in the degree of protection induced by DNA vaccines, as well as in the delivery method. Several methods of delivery have been developed, including naked DNA delivery by injection, gene gun delivery, lipid-based and polylactide-coglycolide (PLG) microparticles (which allow for oral delivery but can damage delivered DNA), mucosal delivery (suppository or oral delivery), intramuscular or intradermal injection followed by electroporation, or via a transdermal device, a spring-powered device used to inject the vaccine intramuscularly without a needle. The naked DNA delivery has a low rate of uptake; gene-gun delivery limits the amount of DNA that can be incorporated in the vaccine and is expensive due to the use of gold beads, the encapsulation process of PLG microparticles is harsh and can potentially damage DNA, mucosal delivery is able to effectively prime but not induce a satisfactory immune response [81]. Electroporation is a preferred method for administering DNA vaccines and has shown to be effective for both intradermal and intramuscular injections [83,84], and newer and simpler electroporation systems could become very practical in terms of vaccinating large numbers of animals, and in lowering the level of discomfort to the vaccinated animal.

DNA vaccines are starting to gain a foothold in the veterinary commercial market. A number of properties discussed in the review make DNA vaccines a safe, efficient and attractive option

for veterinary use, making the future use of these vaccines highly likely. Further development of DNA vaccines will also improve production methods, which will decrease the costs of these vaccines. As a result, we expect that the use of DNA vaccines will increase within the next 5 years. However, improvements need to be made in terms of boosting immunogenicity, improving delivery methods and enhancing formulations. Electroporation, for example, has significantly improved efficacy of DNA vaccines, while other methods of delivery, such as the use of lentiviral vectors, show promise too. Diverse methods of purifying DNA and producing vaccines have also been developed, including fermentation methods, gene recombination processes and PCR scale-ups, which will allow for improved production capability. New methods of vaccination, such as priming with a DNA vaccine and boosting with a protein vaccine, have been shown to be very effective and might be an avenue of further exploration. A large number of trials for both animal and human DNA vaccines are ongoing for a wide range of infectious diseases and cancers. For now, DNA vaccines have made more headway in their use in animals than in humans; however, it is likely that they will prove possible and efficacious for both continued animal and human use.

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Key issues

- Although tremendous strides have been made in terms of fighting infectious disease using vaccines in both companion and production animals, infectious disease still poses a considerable threat to the health, wellbeing, and economic value of animals and production systems.
- There are a number of important animal diseases for which conventional vaccination is not possible, not permitted or ineffective.
- DNA vaccines have a number of advantages for veterinary use, including safety and ease of transport, the ability to drive immunity in the presence of maternal antibodies, the ability to differentiate vaccinated from infected animals, and the potential to be cost effective to produce.
- DNA vaccines have been shown to induce a potent immune response for a number of diseases of veterinary importance, and four DNA vaccine-based products have been made commercially available for equids, swine, canines and fish.
- However, improvements in immunogenicity, plasmid delivery and production methods are important to allow DNA vaccines to become more widely applicable.
- DNA vaccines have the potential to expand beyond the realm of infectious disease to the fields of cancer or the enhanced production capacity of food animals.

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