Chapter 1

Challenges in Veterinary Vaccine Development and Immunization

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Abbreviations

AI(V)	Avian influenza (virus)
BVD	Bovine viral diarrhea, caused by the Pestivirus, BVDV
FMD	Foot-and-mouth disease, caused by the Picornavirus, FMDV
IB	Avian infectious bronchitis caused by the Coronavirus, IBV
IBD	Infectious bursal disease caused by the Birnavirus, IBDV
ILT	Infectious laryngotracheitis, caused by the Herpesvirus, Gallid herpesvirus 1 (GaHV-1/ILTV)
MDV	Marek's disease, caused by the Herpesvirus, Gallid herpesvirus 2 (GaHV-2/MDV)
ND	Newcastle disease, caused by the Paramyxovirus, NDV
PRRS	Porcine reproductive and respiratory syndrome, caused by the Arterivirus, PRRSV
PRV	Pseudorabies virus (Suid herpesvirus 1), the causative agent of Aujeszky's disease

1 Introduction

Infectious diseases of livestock have a direct major financial impact globally through production losses arising from morbidity and mortality. Such losses can include poor weight gain or productivity, condemnation of product, lower commercial return, and inability to trade nationally and internationally. A number of infectious diseases of mammals and birds are of additional global concern due to their zoonotic potential, their ability to be carried across geographical boundaries, their ability to jump species, and to evade or subvert host immune defenses and to throw-off more virulent variants. Examples include influenza viruses, *Salmonella*, and *Leishmania*. The direct and indirect social and economic costs associated with infection are hard to assess [1], but can be dramatic. For example, the H1N1 influenza pandemic in Mexico in 2009 directly affected tourism, the service sector, retail trade, transport, entertainment,

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the agricultural industry (particularly pig farmers) and depressed international investment. The outbreak is estimated to have reduced economic activity by 0.3–0.5 % of gross domestic product (i.e., between US$ 2.7 and 4.5 billion) [2]. The 2001 foot and mouth disease (FMD) outbreak in the UK took 7 months to eradicate, resulted in the slaughter of more than six million animals and was estimated to cost £8 billion to the public and private sectors [3], as well as having considerable environmental costs [4].

Vaccines can be used to prevent, manage, or eradicate disease and are set to become increasingly important as front-line control tools, especially as bacteria progressively emerge with wide resistance to available antibiotics and the burden of parasites resistant to antiparasitics increases. The demand for alternative means of controlling disease and enhancing livestock health is driven by increasing concern of consumers over the potential for drug and antibiotic residues in meat [5] and greater awareness of the burden of antibiotic resistance in the environment [6]. However, vaccines are not a "silver bullet." To be most effective they invariably need to be deployed within comprehensive control strategies that include detailed understanding of the disease epidemiology, biosecurity, quarantine, surveillance, diagnosis, education, and control of the disease vector or reservoir species. It was this combination of measures that resulted in the eradication of Rinderpest through vaccination [7]. Indeed, veterinary vaccines can be remarkably effective. As well as enabling Rinderpest to be eradicated, the development of safe, affordable rabies vaccines efficacious in a variety of species has resulted in dramatic reductions in the burden of this devastating disease in some continents [8] and vaccination against the parasitic protozoa *Eimeria* has been a major success in the fight against avian coccidiosis, arguably one of the most economically important livestock diseases in the world [9]. The recent deployment of the first genetically modified live bacterial vaccine for avian pathogenic *E. coli* has opened the market for a new range of vaccines [10].

The focus of this review is on vaccination against infectious disease. Other applications of vaccination include those designed to provide protection against noninfectious diseases such as allergies and cancers, and those designed to control fertility and production. For consideration of vaccination for these applications in veterinary species the reader is directed to the excellent review of Meeusen et al. [11]. The reader may also wish to read the recent review by Knight-Jones et al. that describes aspects of the evaluation of veterinary vaccines and how this compares and contrasts with human vaccine evaluation [12].

In the following figure (Fig. 1) we present a framework that describes the different elements that may be considered when developing veterinary vaccines. This review focuses more on the scientific elements at the center of the figure, but the cost of development, practicality of use, challenges to licensing, and the even-

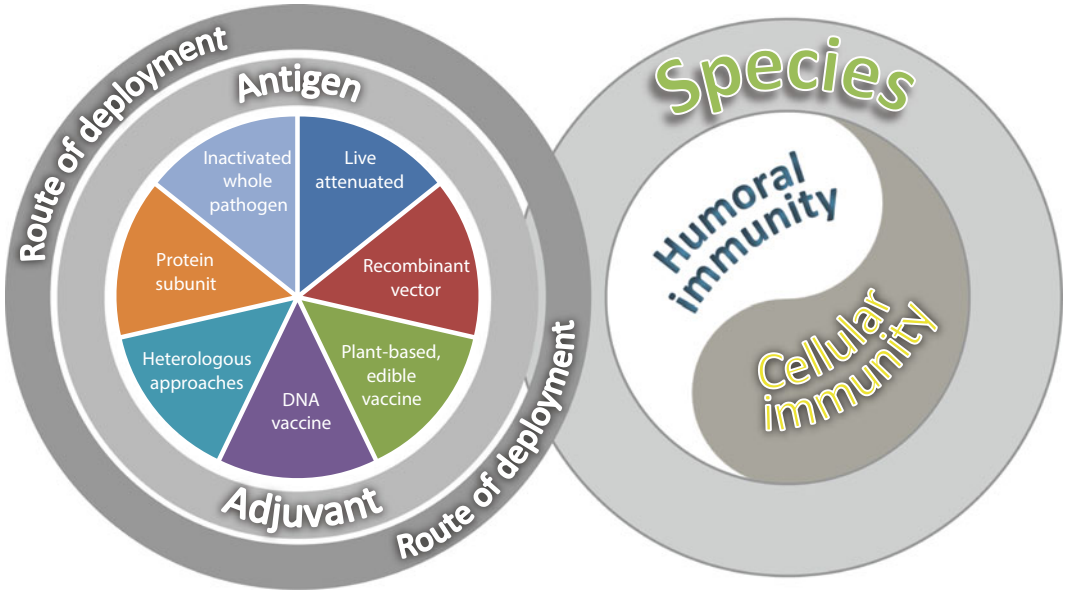

Fig. 1 Different elements that have to be considered when developing veterinary vaccines

tual market value of the vaccine are all crucial considerations that may ultimately dictate whether a veterinary vaccine proceeds to market. Readers are directed to the excellent online resource, Vetvac (http://www.vetvac.org/index.php), a free searchable global database of commercially available livestock vaccines. One can search by vaccine name, pathogen, manufacturer, host species, and country of interest, and combine search terms. For access to research data for commercial vaccines and vaccines in clinical trials or in early stages of research, readers are directed to the Vaccine Investigation and Online Information Network (VIOLIN) database (http://www.violinet.org). For researchers in the UK, the Veterinary Vaccinology Network (www.vetvaccnet.org) is a multidisciplinary network with the aims of facilitating knowledge exchange and discussion, fostering development and uptake of novel tools and technologies, and addressing unmet needs in protective immunity in the field of veterinary vaccinology [13].

2 Choice of Target Species

The target host species for vaccine development is often dictated by the economic impact of the disease or the risk the species represents for onward transmission of a pathogen, although it may also be a candidate for vaccination if it is valuable to protect in its own right, e.g., companion animals, rare species or zoological collections. Typically the species of concern is targeted directly for

vaccination. However, it may be that the vaccine is targeted to a reservoir species that presents a risk. For example, European badgers (*Meles meles*) may be vaccinated against bovine tuberculosis (TB) in England and Wales with BCG (Bacillus Calmette–Guérin) (BadgerBCG, Animal and Plant Health Agency, UK) in an effort to break the transmission of *Mycobacterium bovis* infection between badgers and cattle. There are also experimental vaccines against *Toxoplasma gondii* infection of domestic cats that could be used to reduce excretion of oocytes into the environment, thereby protecting sheep from infection with the parasite resulting in abortion [14]. Another important application of vaccination of veterinary species is to protect humans from zoonoses. Examples of this include vaccination of domestic dogs and sylvatic carnivore species to protect against rabies in humans and domestic and companion animals; vaccination of poultry and pigs against zoonotic serovars of *Salmonella spp.*; vaccination of cattle against enterohemorrhagic *Escherichia coli* O157:H7 [15]; and the proposed vaccination of dogs against *Leishmania spp.* to protect humans against visceral leishmaniasis [16].

Where there are multiple host species for the same pathogen, there may be a lack of information on the efficacy of a vaccine in all affected species. The efficacy of a vaccine may vary between species, making extrapolation from one to another difficult. For example, because of their commercial value, chickens and turkeys are the focus of avian influenza (AI) vaccination and the only bird species for which there are licensed vaccines. Whilst ducks and geese may be significant reservoirs of AI viruses, including highly pathogenic variants, the performance of vaccines in these species is largely unknown.

3 Choice of Vaccine Approach

3.1 Inactivated Whole-Pathogen

There are many examples of the use of inactivated whole-pathogens as successful veterinary vaccines spanning several decades. These include inactivated viruses, e.g., for swine and avian influenza and bovine viral diarrhea (BVD), parasites, e.g., for leishmaniasis and spontaneous abortion in cattle caused by *Neospora caninum*, and bacteria, e.g., immunization of dogs against *Borrelia* spp. Inactivation is usually brought about by heat or chemical treatment or irradiation.

The advantage of vaccines based on the whole-pathogen is that they are generally stable and retain a high proportion of the antigens of the live pathogen. However, by definition they are unable to infect or replicate in the host or express antigens associated with active metabolism, replication, or other life-cycle stages. As a consequence, inactivated whole-pathogen vaccines often require booster immunizations and the inclusion of adjuvants to achieve adequate protection.

One novel approach involves the creation of bacterial "ghosts." Bacterial ghosts are nonliving gram-negative bacterial cell envelopes devoid of cytoplasmic contents while maintaining their cellular morphology and native surface antigenic structures. They are produced by bacteriophage protein-mediated lysis of the bacteria. As well as containing intrinsic adjuvant properties, bacterial ghost preparations can be made containing additional antigens that are expressed in the envelope complex of the bacteria before they are lysed. The advantages of bacterial ghosts include the simplicity of the production method, safety, independence from the cold chain, and versatility to express multiple antigens as a combination vaccine. We are not aware of any commercial vaccines based on bacterial ghost preparations. Recent experimental evaluation of ghosts prepared from *Salmonella enterica* serovar Enteritidis carrying the *E. coli* heat-labile enterotoxin B subunit as an adjuvant gave very encouraging protection to chickens against challenge with a virulent Salmonella Enteritidis strain [17].

Inactivated whole-virus vaccines may not induce cross-protection from one viral geno/sero-type to another, e.g., for FMDV, possibly due to inactivated whole-pathogen vaccines working via the induction of antibody-mediated immunity and not via the induction of cell-mediated immune responses which may be more broadly cross-reactive, although this limitation may be overcome by including multiple inactivated types in the same vaccine preparation.

3.2 Live Attenuated Pathogens

Live attenuated vaccines are reduced virulence versions of the target pathogen that retain the ability to undergo limited replication within the host, thereby inducing cellular and/or humoral immune responses that are relevant to conferring protection against the fully virulent organism. As a consequence, live attenuated vaccines rarely require an adjuvant to be effective and can be administered in a way that mimics the natural route of infection. They can be highly effective vaccines capable of providing lifelong immunity. For example, the eradication of Rinderpest virus, only the second pathogen after smallpox virus to have been eliminated via human intervention, was the result of the targeted use of an efficacious live attenuated vaccine [7]. Vaccination against *Trichophyton verrucosum* with an attenuated strain of *T. verrucosum* (Bovilis Ringvac LTF-130, Merck Animal Health) has all but eradicated bovine ringworm from the national herd in Norway [18].

A significant advantage of live vaccines is that they express a wider range of relevant pathogen antigens, including those that require active metabolism. This is particularly important for vaccines against protozoan or helminth parasites since antigens may be differentially expressed between life cycle stages. The commercial protozoal vaccine Toxovax (MSD Animal Health) protects ewes against infection with *Toxoplasma gondii*. The attenuated vaccine strain of *T. gondii* (S48) cannot form cysts and is therefore unable

to persist. The commercial helminthic vaccine Bovilis® Huskvac (MSD Animal Health) protects cattle against the lungworm *Dictyocaulus viviparus*. The vaccine contains 1000–2000 viable *D. viviparus* infective third stage larvae that are irradiated to prevent their development into the mature adult stage.

Pathogen attenuation is often induced by serial passage through in vitro culture or infection of alternative hosts with reliance on random mutations to result in reduced virulence in the target host. The paradigm for such a vaccine was the development of BCG vaccine against TB. Starting with a virulent bovine strain of *M. bovis,* Albert Calmette and Camille Guérin cultured the bacteria on a medium composed of ox bile, glycerine and potato and then subcultured the bacteria at roughly 3 weekly intervals. After 11 years or approximately 230 subcultures the bacteria failed to produce progressive TB when injected into a variety of mammalian species, including cattle. Since that time, BCG remains the only TB vaccine licensed for use in humans and has been the subject of numerous trials in cattle to test its ability to protect against bovine TB. As has been observed for humans, BCG's ability to confer protection to bovine TB is highly variable. However, its main limitation is that it can sensitize cattle to produce a positive tuberculin skin-test reaction, the mainstay of surveillance and control for bovine TB. Defining the genetic lesions in BCG responsible for attenuation became possible with the advent of whole-genome sequencing. The availability of the complete genome sequence data for many pathogens now permits selective deletion or disruption of genes to result in targeted attenuation. A good example of this is the recently launched avian pathogenic *E. coli* vaccine, Poulvac® *E. coli* (Zoetis) [10].

Despite their success for some diseases, there are a number of problems with many inactivated whole-pathogen or live attenuated vaccines including that the immune responses they induce are often indistinguishable from those elicited by natural infection. Thus, they do not readily allow for differentiation between infected versus vaccinated animals (DIVA), which makes them less suitable for use in disease eradication efforts. Some notable examples include foot-and-mouth disease (FMD), leptospirosis, brucellosis and bovine TB. Vaccines may interference with surveillance methods in two different ways: either it is not possible to differentiate the wild-type pathogen from its vaccine strain in a diagnostic sample, e.g., for infectious bursal disease (IBD), Newcastle disease (ND), and FMD; or a vaccine generates false positivity in an immunodiagnostic test. For example, seroconversion following vaccination against IBD or sensitization of vaccinated livestock to the single bovine intradermal tuberculin test as a consequence of vaccination with the paratuberculosis/Johne's disease vaccine, Silirum® (CZ Veterinaria) [19] or BCG. In the latter case, considerable effort has been invested in the characterization and validation of DIVA diagnostic reagents that

might permit the use of BCG in cattle. In some cases, the gene product disrupted for attenuation may encode an immunodominant, unprotective, nonessential antigen and this can be used as the basis of a DIVA test to discriminate vaccination from infection with wild-type pathogen.

Attenuated virus vaccines are generally considered more efficacious than inactivated whole-virus vaccines since they induce stronger T cell responses, high titers of virus-neutralizing antibodies and provide a longer duration of protection from clinical disease. However, there is a risk that the vaccine virus can revert to a virulent form or recombine with field viruses and cause disease. This was seen with attenuated vaccines for both BVD and porcine reproductive and respiratory syndrome (PRRS). In the case of attenuated vaccines against AI, there is an inherent risk of gene reassortment with wild-type viruses and the emergence of pathogenic variants. Infectious laryngotracheitis (ILT) is a particular problem for the intensive poultry industry. Attenuated vaccines for ILT, particularly those derived by passage in chicken embryos, have been associated with a number of side effects, including residual virulence, transmission to naïve birds, latent infection with subsequent reactivation and shedding of virus, and reversion to virulence after passage in vivo. Most recently, recombination between attenuated ILT vaccines in the field has been shown to be responsible for the emergence of new virulent viruses that have caused widespread disease.

In pregnant animals, live vaccines present a risk of vertical transmission of the attenuated pathogen that can result in fetal complications or persistent infection [20]. As a result, some live attenuated viral vaccines are not licensed in a number of countries. Attenuated bacterial vaccines may also retain a degree of virulence that provides impetus to developing safer vaccines of equal efficacy. For example, the most widely used live attenuated vaccines for *Brucella abortus* and *B. melitensis* can induce abortion in the host and brucellosis in people.

3.3 Protein Subunit

The major advantage of subunit vaccines is their safety. However, their production as recombinant protein relies on knowledge of the protective antigen. In many cases this is either unknown or protection is mediated through a variety of antigens. The latter may not necessarily be an issue, as exemplified by commercial vaccines available for porcine contagious pleuropneumonia where four or five recombinant proteins from the causative organism *Actinobacillus pleuropneumoniae* are combined to provide protection against all known *A. pleuropneumoniae* serotypes. A further limitation is that the recombinant form of the antigen may not induce the same type or extent of immune response as the native antigen because it doesn't preserve native conformation. This is a particular issue for vaccines against parasites and viruses where the target for vaccination is often a glycoprotein.

As for inactivated whole-pathogen vaccines, subunit protein vaccines are often poorly immunogenic and require booster immunizations and inclusion of adjuvants to achieve adequate protection. When added to their relatively high cost of production, this makes them less attractive commercially. Nonetheless, there are some commercial subunit vaccines based on recombinant protein, e.g., the Porcilis PCV vaccine (MSD Animal Health) is based on the baculovirus-vectored expression of recombinant ORF2 protein of porcine circovirus type 2 (PCV2), the causative virus of porcine circoviral disease including the post-weaning multi-systemic wasting syndrome of pigs.

More rarely, the subunits may be native proteins often isolated from the supernatants of pathogen cultures. An example of this is the soluble parasite antigens released by culture of *Babesia canis*. When combined with adjuvant, these antigens form effective vaccines against canine babesiosis.

3.4 Recombinant Vector

Exogenous vaccine genes can be presented and expressed in the context of a vector organism. Frequently the vector is a virus, such as a herpesvirus, adenovirus, or poxvirus, but bacterial vectors are also used, including BCG and *Salmonella*, as well as bacterial endospores [21]. Some recombinant vector vaccines are licensed for use, such as a vaccine for H5 clade AI based on a recombinant fowlpox virus vector (Trovac-AIV H5, Merial), a vaccine for equine influenza based on canarypox virus (Proteq-Flu/Recombitek, Merial) and rabies vaccine based on recombinant vaccinia virus. Recombinant poxviruses are particularly attractive vaccine vehicles as they are environmentally robust, genetically stable, safe, produce long-lasting immunity and can accommodate a large amount of foreign DNA. The vaccinia virus vectored rabies vaccine has been particularly successful as an oral vaccine vector against rabies in wild carnivores, resulting in substantial control of the disease throughout Western Europe and the USA. Virally vectored recombinant vaccines have been developed against ILT in an effort to address the numerous side-effects seen with attenuated viral vaccines (reviewed in Ref. [22]). Some of these have been licensed recently for use in some areas of North and South America, such as Vectormune® (FP-LT, Ceva Animal Health), based on a recombinant fowlpox vector.

An attractive approach is to make an attenuated form of a target pathogen as the vector organism with the aim of generating a bivalent vaccine eliciting protective immunity to both the vector and the heterologous antigen(s) it expresses. No such vaccines have yet been licensed using a bacterial or parasite vector but have been for viral vectors. Simultaneous protection against Marek's disease virus (MDV) and either IBDV (Vaxxitek HVT + IBD, Merial) or ILTV (Innovax®–ILT, Intervet International B.V; Vectormune® HVT-LT, Ceva Biomune) has been possible using turkey herpesvirus as the vector to express IBDV or ILTV anti-

gens. Turkey herpesvirus is nonpathogenic for chickens but confers cross-protection to MDV. Encouraging results have also been seen with live *Salmonella* vectors expressing peptide epitopes from *Campylobacter* proteins [23]. Recent progress in the genetic manipulation of *Eimeria* species presents the exciting opportunity for the creation of transgenic parasite lines as host-specific vaccine delivery vectors expressing one or more foreign proteins to provide simultaneous protection against coccidiosis and other veterinary or zoonotic pathogens [24]. However, it is also worth noting that preexisting anti-vector immunity can neutralize these vaccines and significantly diminish their immunogenicity.

3.5 DNA Vaccine

DNA vaccines are based on the ability of injected plasmids to express vaccine antigens, under the control of an appropriate eukaryotic promoter, in host tissue, in particular muscle cells and skin epithelia. Recombinant plasmid DNA is both relatively cheap to produce and sufficiently stable to avoid the necessity for a cold-chain in many cases. However, the level of protective immunity induced by DNA vaccination is often low unless relatively large quantities of DNA are injected, so as for recombinant protein vaccines, their cost is often prohibitive. One application where they have been found to be particularly successful is in protecting fish against viral diseases, such as infectious hematopoietic necrosis in Atlantic salmon (Apex-IHN, Novartis). At present, fish must be injected with the DNA vaccine intramuscularly, a process that is surprisingly efficient (see videos at http://www.norvacc.com/video-7.html).

3.6 Plant-Based/ Edible Vaccines

The expression of recombinant vaccine antigen(s) in plants that could be fed to target species in order to generate and maintain protective immunity is an attractive option that has been explored for two decades; recently in the EU FP7 project PLAPROVA (project reference: 227056). This 3 year project completed in 2012 (http://cordis.europa.eu/project/rcn/89887_en.html) and focussed on AIV, blue tongue virus and PRRSV. There have also been encouraging results using recombinant antibodies against *E. coli* O157:H7 produced in plants [25, 26]. A challenge is overcoming the propensity for oral vaccines to induce immune tolerance. The first plant-based vaccine (for ND) was licensed in 2005. As well as protecting against viruses of domestic species, the approach also shows promise for the delivery of parasite antigens to the gut associated lymphoid tissues (e.g., for fasciolosis, schistosomiasis, poultry coccidiosis, porcine cysticercosis and ascariosis) or passive immunization through the delivery of plant-expressed antibodies. The reader with an interest in progress in plant-based, edible vaccines is directed to recent reviews of the subject [27–29]. Despite the promise of plant-based vaccines there are concerns with public acceptance of GM foodstuffs for livestock and the risk they pose to contamination of the human food chain or the environment [30].

3.7 Heterologous Approaches

Some approaches to vaccination exploit a synergy where two different vaccines to the same pathogen are combined to augment protective immunity. We break these down into two broad approaches. The first has been termed, heterologous prime-boost. The second approach exploits what we refer to as combination vaccines.

3.7.1 Heterologous Prime-Boost

In this scenario, the host is first primed with one type of vaccine, such as a live viral vector expressing antigen(s), followed by boosting with another vaccine, such as a live attenuated vaccine that expresses the same antigen(s) present in the priming vaccine. The objective is to boost or enhance immunity to the antigen(s) in a way that is more effective than using the same vaccine for priming and boosting. Comprehensive proof of principle for this approach has been demonstrated for vaccination against *M. bovis,* the cause of bovine TB. A number of vaccination strategies have been evaluated for their protective effect in a bovine challenge model (reviewed in Ref. [31]). Currently the most effective vaccination strategy against bovine TB is based on priming the immune system with the live attenuated BCG vaccine followed by boosting with a subunit vaccine containing protective antigens that are present in BCG. A number of these heterologous prime-boost regimes have conferred greater relative protection to cattle than immunization with BCG alone. The most promising combinations combine a prime with BCG followed by boosting with either modified vaccinia virus Ankara strain (MVA) or attenuated adenoviruses expressing the mycobacterial antigen Ag85A [32].

Another example is the comprehensive evaluation of heterologous prime-boost vaccination regimes against pseudorabies virus (PRV) infection causing Aujeszky's disease in pigs [33]. In this study the efficacy of a conventional modified live vaccine was compared with the efficacy of different prime-boost regimes. These consisted of homologous prime-boost regimes (DNA–DNA vaccination or parapox virus–virus vaccination) or heterologous prime-boost regimes (DNA–virus or virus–DNA), all expressing glycoprotein D of PRV. The different prime-boost regimes resulted in variable levels of immunogenicity and protection against challenge infection. Most effective was the regime of priming with DNA followed by boosting with the parapoxvirus vector. This regime resulted in strong antibody responses comparable to the responses obtained after prime-boost vaccination with the modified live vaccine and a level of protection to challenge better than the other prime-boost regimes. From a practical perspective, heterologous prime-boost approaches can suffer from the disadvantage that two vaccines must be produced/administered in the place of one. Furthermore, there is added practical complexity that the two vaccines must be administered often in the correct sequence to achieve the required protection.

3.7.2 Combination Vaccines

In this scenario two different vaccines to the same pathogen are administered simultaneously, with the objective of enhancing protective immunity. There are numerous successful examples of this approach. Typically the combination is against different strains of the same pathogen using the same vaccine form. An example of this is Poulvac IB Primer (Zoetis), a lyophilized vaccine containing two attenuated strains of avian infectious bronchitis virus (IBV): Massachusetts serotype H120 and Dutch variant strains D207/D274. Alternatively, the combination may be based on different vaccine types. For example, the simultaneous administration of live and inactivated vaccines against NDV provides better protection and has been used successfully in control programs in areas of intense poultry production. In some cases the licensed vaccine contains multiple vaccines against different pathogens, e.g., the RECOMBITEK® C4 (Merial) vaccine comprises a modified live virus and a canarypox vector to confer protection against canine distemper, Adenovirus Type 2, Parainfluenza, and Parvovirus, and the RECOMBITEK® C6 (Merial) vaccine adds a liquid suspension of inactivated cultures of *Leptospira canicola* and *L. icterohaemorrhagiae* to confer additional protection against Leptospirosis.

4 Choice of Antigen

Many of the points relating to the choice of vaccine antigen have been alluded to already. An essential consideration is whether sufficient protective immunity can be produced using a single antigen or whether multiple antigens are required. Indeed, it may not even be known what the protective antigens are or the mechanisms of protective immunity, which may guide an antigen identification or evaluation strategy. Even if the protective antigen is known there are still important considerations and constraints that often dictate the type of vaccine that is developed; for example, the extent to which the antigen varies naturally and whether it is necessary to retain native antigen conformation to establish protective immunity with the vaccine. Single-stranded RNA viruses, such as influenza, lentiviruses including feline immunodeficiency virus (FIV) and nidoviruses such as IBV and PRRSV evolve rapidly by antigenic drift and shift meaning a vaccine developed to one variant may provide limited cross-protection to heterologous variants, presenting a major obstacle for vaccine development. In some cases, vaccination with two genetically divergent vaccines to broaden the protection against heterologous types can be effective, as in the case of the Poulvac IB Primer (Zoetis) vaccine to avian IBV, described above.

A novel experimental vaccine for leishmaniasis extends consideration of the vaccine antigen to targets beyond the pathogen itself. In this study, vaccination was to the bite of the sand fly

vector. Immunity generated in a hamster model to a fly salivary protein resulted in protection against *Leishmania infantum*, suggesting a new approach to vaccination against infections transmitted by ectoparasites [34].

5 Choice of Immune Response To Be Targeted

This is frequently an aspect of vaccination that is poorly defined for the pathogen and/or the target species. This is exacerbated if the pathogen is difficult to work with experimentally or relatively little is known about the immune response of the target species and suitable reagents for its study are lacking. Good examples for this are the development of equine vaccines (reviewed in Ref. [35]) and vaccines against avian influenza (AI) in Anseriformes, such as ducks and geese [36, 37]. Only since 2004 has the full complement of horse immunoglobulin heavy chain constant region genes been described. The horse is atypical in that it expresses seven IgG subclasses. To achieve maximal protection to infections mediated by Fc receptor or complement-mediated elimination mechanisms, it appears vaccines should elicit IgG antibodies of particular IgG subclasses; other subclasses offering less effective protection [38]. Importantly, as the authors of this work point out, since IgG plays key roles in both serum and mucosal compartments in the horse, these considerations are applicable to both systemic and mucosal vaccination strategies. Vaccination of Anseriformes with existing AI vaccines requires a higher dose of antigen compared with chickens or the addition of a strong stimulator for the immune response to be effective. Differing immunoglobulin genetics is considered to be a significant contributing factor to this [36].

This said it is debatable whether it is necessary to have a clear understanding of the protective immune mechanisms before vaccine development can proceed. However, a good understanding of immunological correlates/surrogates of protection can reduce the need for expensive challenge experiments as part of the vaccine development process. Commercial vaccines, such as BCG for TB and Fel-O-Vax FIV for FIV are widely used vaccines yet the precise basis for their protection is unclear. This means we do not know why they fail to protect certain individuals. Poor understanding of the basis for protective immunity makes it hard to develop improved vaccines on a rational basis.

Even when a significant amount is known about the nature of protective immunity, the challenge may be that effective protection requires stimulation of different elements of immunity at different stages and in different anatomical locations. For example, antibodies only protect at the initial site of influenza infection whereas cellular responses, especially cytotoxic T-lymphocytes (CTL) are needed once initial infection has occurred. These considerations

dictate how the antigen is presented, e.g., vaccine-derived antigenic peptides must be processed and presented by MHC class I cytosolic or cross-presentation pathways for CTL responses to be generated.

In addition, the immune response required to protect against one pathogen may be antagonistic to the response required to another type of pathogen. This is best exemplified by the difference in protective immunity required against helminthic pathogens, that is characterized by the type 2 immune response, compared to the response required for intracellular pathogens, that is characterized by type 1 immune responses. This of course is a generalization but it highlights how antagonism between the two broad arms of immunity can be a hurdle to vaccination; underlying concomitant infections may skew the immune response making redirection of the immune response by vaccination a challenge.

As innate immunity is considered to be evolutionary primitive compared to acquired immunity, many elements of the innate response are common amongst veterinary species, such as the universal existence of pattern recognition receptors (PRRs) able to respond to pathogen-associated molecular patterns (reviewed in Ref. [39]). Increasing our understanding of the innate immune response to pathogens should result in the development of molecular adjuvants to enhance and/or refine the host response to vaccination.

6 Adjuvants

An adjuvant enhances the magnitude or duration of immunity, can accelerate the onset of immunity, direct its nature, prolong immunological memory, reduce the dose of antigen required to establish immunity, or a combination of these actions. They do this by either sequestering the antigen or targeting it to an antigen-presenting cell (APC), by activating the APC, or modifying the behavior of T-cells. Some vaccines contain inherent adjuvanticity due to their ability to stimulate the innate immune system via engagement of PRRs. Inactivated whole-virus or subunit vaccines invariably need an adjuvant to boost delayed or weak protective immunity, e.g., for swine influenza virus or PRRSV, especially where the pathogen downregulates host immunity, e.g., PRRSV and to overcome the effects of maternal antibodies on young animals (a form of vaccine interference—*see* Subheading 9).

The choice of adjuvants is considerable. One advantage faced by those developing vaccines for veterinary species compared to human is that the use of adjuvants is currently less restricted. There have been numerous reviews of adjuvants for use in humans and animals over the last 20 years and we would refer readers to those listed below in particular. In the following table (Table 1) we present

Table 1
Summary of adjuvants available for veterinary vaccine development by type

Type of adjuvant	Examples (incl. brand name where appropriate) Those underlined are in use in licensed vaccines	Notes
Oil emulsion	Freund's Complete and Incomplete Adjuvants, Montanide®, Titermax®, Ribi®, SAF®, MF59	May be W/O (water in oil) or O/W (oil in water), or further combinations, e.g., W/O/W
Microparticle	Aluminum hydroxide, potassium aluminum sulfate (alum), aluminum phosphate (alhydrogel), calcium phosphate, immune stimulating complexes of Quillaja saponins (ISCOMs), poly(lactide-co-glycolide) (PLG), alginate, liposomes, non-ionic block copolymers, virosomes, cochleates, poloxamers, virus-like particles (VLPs)	
(Immuno)-active compounds	Saponin (Quil A or QS-21), DDA, Monophosphoryl lipid A (MPL A), cytokines (IL-1, -2, -6, -8, -12, TNF-α, GM-CSF, MIP-2, type I interferons), chitosan	Cytokines have been evaluated particularly in ruminants, pigs, and birds
Microbial derived	Heat-labile enterotoxin and cholera toxin (LT, CT) and mutants thereof (LTK63, LTR72), (lipo)polysaccharides, CpG oligonucleotides, lipopeptides, flagellin and other Toll-like receptor agonists	
Synthetic polymers	Polyanhydrides, polyesters, polyester amides, dextran	

Information in this table was partly taken from data presented in the following reviews to which the interested reader is directed: [40–44]

a synthesis of information described in these reviews and gleaned from other published studies. It is almost certainly not exhaustive but serves to describe the wide range and nature of adjuvants available or under development. Some adjuvants could be described under more than one type but these, e.g., saponin and CpG oligonucleotides, are listed only once for simplicity. Many veterinary adjuvant-vaccine formulations are proprietary and their compositions have not been disclosed. The reviews provide more detail for the different adjuvants regarding their composition, structure, mode of action, type of immune response they stimulate (where known), target host species, and pathogen for which they have been evaluated.

The use of adjuvants in veterinary species has not been without notable side effects. For example, the occurrence of vaccine-associated malignant sarcomas in cats is attributed to the use of aluminum salt

adjuvanted vaccines [45]. The hemorrhagic disorder; bovine neonatal pancytopenia ("bleeding calf syndrome") that emerged in 2007 in several European countries was reported to be linked to the use of the BVDV vaccine PregSure®BVD. Moreover, this association was attributed by some to the presence of significant amounts of bioprocess impurities within the vaccine combined with a powerful adjuvant system [46]. This apparent association led to the withdrawal of the product from the market in 2011.

7 Route of Vaccination/Efficacy of Delivery

Considerations over the most appropriate route of delivery for the vaccine may be driven by practicality, concerns over local reactogenicity, or attempts to enhance or direct the immune response in a desired way. Since the route of entry for many pathogens is at mucosal surfaces, the induction of immunity at mucosal surfaces is critical to prevent infection. Therefore numerous attempts have been made to deliver vaccines to mucosal surfaces (oral, ocular, nasal). It is often generalized that a common mucosal immune system exists whereby antigenic stimulation of immunity at one mucosal site results in the secretion of IgA at a distant mucosal site. However, in many cases this has shown not to be the case. Instead there is functional compartmentalization and limited reciprocity between sites. Basic understanding of the extent to which the target species shares a common mucosal immune system is an essential consideration in determining the most appropriate route of immunization. For instance, whilst oral immunization may confer protection in the respiratory tract, the converse may not be true.

The oral route is likely to be the favored route for targeting populations or larger groups of animals, especially wildlife species and poultry. However, in the case of vaccine delivery for wildlife it is dependent on presentation in bait and the most suitable bait and baiting strategy may differ between species and contexts, as exemplified by rabies vaccination [47]. Automated *in ovo* vaccination is an emerging technology for poultry, e.g., using the Inovoject® System (an Embrex® BioDevice from Zoetis) to deliver Inovocox® vaccine against coccidiosis. The manufacturers claim advantages for the system over oral or parenteral vaccination of chicks such as consistent and uniform vaccine delivery, reduced chick stress, earlier immune response and protection, and significant labor savings. DNA vaccination may be improved through attempts to improve transfection efficiency, such as transcutaneous injection, biolistic particle delivery, or electroporation (reviewed in Ref. [48]), but these methods are not yet in routine use with livestock. For fish, the route of vaccine delivery is an important factor in influencing efficacy. The most efficient delivery route at present is intramuscular (IM) injection [49], but suitable delivery strategies

for mass vaccination of small juvenile fish have yet to be developed. Other methods evaluated for vaccination of fish include scarification of the skin, intraperitoneal injection, intrabuccal administration, cutaneous particle bombardment using a gene gun, or immersion [50, 51]. The ideal approach would be oral or immersion delivery of vaccine, but so far gene gun mediated delivery appears the most promising alternative to IM injection although it remains at the research stage.

8 Illustrative Examples

The challenges and the diversity of approaches taken to veterinary vaccine development are well illustrated by a few examples for which the authors have particular experience.

8.1 Porcine Reproductive and Respiratory Syndrome (PRRS)

PRRS is arguably the most important disease impacting the swine industry worldwide. Improving the efficacy of vaccination against PRRS is a major challenge particularly since the PRRS virus (PRRSV) is rapidly evolving and diversifying. Progress is hampered by uncertainty over the viral targets of protective immunity and significant knowledge gaps in the understanding of the mechanisms of host protective immunity to PRRSV infection. The lack of reliable correlates of immunity that mean novel vaccines need to be tested empirically and the genetic diversity of PRRSV means extrapolation of results between isolates is risky.

According to www.vetvac.org, there are currently 25 commercially available PRRSV vaccines; 15 live attenuated and ten inactivated vaccines, which are derived from both the North American and European PRRSV genotypes. Modified live vaccines (MLVs) were rapidly developed following the almost simultaneous emergence of the two PRRSV genotypes in North America and Western Europe some 25 years ago. The market leading MLV (Ingelvac PRRS MLV, Boehringer Ingelheim) was based on a North American genotype isolate and it has now been attributed as being responsible for the introduction of North American PRRSV to over eight countries outside of this continent [52]. This sharply illustrates the capacity of attenuated PRRSV to revert to virulence, a property facilitated by a high-mutation rate during PRRSV replication. In addition, there are numerous reports of PRRS disease outbreaks being caused by "vaccine-like" isolates [53–58]. Despite these safety issues, MLVs continue to be widely used, which is undoubtedly driven by the limited efficacy of inactivated vaccines particularly against heterologous strains. Inactivated PRRSV vaccines are therefore best suited as autogenous or "farm-specific" vaccines as proposed by Geldhof et al. [59, 60].

PRRSV-specific antibody responses can be observed from 7 to 10 days post-infection [61], however, these antibodies often do

not neutralize PRRSV infectivity [62]. Neutralizing antibodies (nAbs) may not be observed until at least 4 weeks post-infection, and titers, when measurable, are lower than those elicited by other viral infections [61, 63, 64]. Passive transfer experiments have shown that nAbs can provide a dose-dependent protection against PRRSV [65–67] and whilst data on protection against heterologous strains by passive transfer is limited, these studies suggest that vaccination strategies inducing high-titer nAbs may be efficacious. Consequently, the majority of approaches to develop the next generation of PRRSV vaccines have focussed on targeting the nAb response. During PRRSV infection antibodies are directed against a broad range of viral antigens and nAb responses have been mapped to GP2, GP3, GP4, GP5, and M proteins [68–76]. The early identification of highly conserved linear epitopes in the ectodomain of the major glycoprotein GP5 [73, 74, 77, 78] focussed vaccine development efforts on this antigen. However, recombinant GP5 protein was poorly immunogenic, failed to provide protection and could exacerbate disease upon challenge [79–82]. Expression of GP5 by plasmid DNA or viral vectors, alone or in conjunction with other PRRSV structural proteins, showed better immunogenicity, but typically failed to induce high titer nAbs and at best conferred only a degree of protection [83–92]. Other studies have shed doubt on whether GP5 represents the prime vaccine candidate, including: the observation that glycosylation sites on GP5, proposed to mask antibody epitopes, are highly variable amongst strains [93]; studies with chimeric viruses have shown that GP5 is nonessential for infection of macrophages [94]; pigs engineered to lack the GP5 receptor sialoadhesin show an unaltered course of PRRSV infection [95]; and affinity purified GP5-specific Ab fail to neutralize PRRSV infectivity in vitro [76, 96]. There is consequently an increased focus on the minor envelope proteins, GP2, GP3, and GP4, which form a glycosylated complex essential for infectivity [97–99]. The evaluation of the neutralization of PRRSV strains by hyperimmune sera revealed significant differences in the sensitivity to neutralization that did not associate with the sequences of previously described linear nAb epitopes nor to N-linked glycosylation sites [100]. Interestingly, a proportion of sera exhibited significant neutralizing activity against all isolates suggesting that these sera contain nAb specific for conserved epitopes that may be poorly exposed and consequently immunogenic in most PRRSV strains. This study highlights our limited understanding of the nAb response to PRRSV but suggests that the identification of the structures recognized by these broadly cross-neutralizing Ab should be a priority for the PRRS research community.

Since the resolution of viremia typically precedes the appearance of nAbs, it is likely that T cell responses are more important to the control and clearance of the virus. Upon PRRSV infection,

virus specific IFN-γ secreting T cells are typically detected in blood after 7–14 days and continue to increase with time long after the resolution of viremia [101], which may reflect the persistence and delayed clearance of antigen in the lungs or lymphoid tissues. Few studies have attempted to characterize the PRRSV-specific T cell response in any detail. CD4 T cells are necessary to drive PRRSV-specific proliferative responses in vitro [102], CD8 T cells are the predominant population expanded by PRRSV stimulation in vitro [103] and both CD4 and CD8 T cells contribute to PRRSV-specific IFN-γ responses [104]. While IFN-γ is known to inhibit PRRSV replication at least in vitro [105, 106], cytotoxic killing of infected cells by CD8 T cell may represent a more effective protective effector mechanism [107], although this has yet to be shown convincingly for PRRSV [103]. CD8 T cells are the dominant population infiltrating the lungs during PRRSV infection [108] and during resolution of infection they are the major source of PRRSV-specific IFN-γ (Graham et al. unpublished data). Investigation into the PRRSV antigen-specificity of T cells is limited and often the phenotype of responding T cells was not discerned. T cell reactivity against both structural and non-structural proteins has been described [104, 109–112]. However more research is required to better define PRRSV T cell antigens and to test whether they may be used to induce protective immune responses.

8.2 Bovine Viral Diarrhea (BVD)

BVD is an economically important infectious disease of cattle caused by infection with the pestivirus BVD virus (BVDV). BVD is characterized by leucopoenia, fever, depression, diarrhea, dehydration, anorexia, salivation, nasal discharge, gastrointestinal erosions, and tissue hemorrhages. However, clinical presentation is dependent on a number of factors including virus strain, immune, reproductive, and age status of the host, as well as the presence of co-infections. The majority of BVDV strains cause a transient acute infection in healthy animals that is cleared within 10–14 days. Transient immunosuppression, thought to be a consequence of immune cell death within lymph nodes and gut-associated lymphoid tissue and reduced numbers of circulating leukocytes, increases susceptibility to secondary infection resulting in respiratory and enteric disease [113]. BVDV infection has a major impact on the reproductive success of the host and may result in abortions or the birth of persistently infected calves that play a key role in the epidemiology of BVD [114].

Reflecting its commercial impact BVD neatly illustrates the range of approaches available for vaccine development. There are around 140 registered BVD vaccine products currently in use around the world (www.vetvac.org). These are culture attenuated modified live virus (MLV) or inactivated/killed virus vaccines, formulated as either monovalent BVDV preparations or multiva-

lent vaccines including other pathogens implicated in the bovine respiratory disease complex [115]. Whilst good cross protection is observed against BVDV type 1 strains, the failure of existing BVDV-1 based vaccines to protect against some emerging BVDV type 2 strains has resulted in inclusion of the latter in new vaccine preparations [116]. MLV vaccines are generally thought to be more efficacious since they evoke stronger virus-specific T cell responses, induce high titers of virus neutralizing antibodies and provide a longer duration of protection from clinical disease than inactivated vaccines. However, there are safety concerns over the potential for MLVs to revert to virulence or recombine with field viruses and cause disease. In addition, MLV-vaccinated animals may develop transient viremia and shed vaccine virus [117, 118] and in the case of pregnant animals, MLVs pose the risk of vertical transmission of the vaccine strain that can result in fetal complications or persistent infection [20]. Consequently, MLVs are not licensed in a number of countries including the UK. Neither MLV nor inactivated vaccines allow for differentiation between infected versus vaccinated animals (DIVA), which limits their utility in efforts to eradicate BVDV [119].

The development of next-generation BVD vaccines have primarily focussed on the delivery of the E2 glycoprotein since it represents the major target of the neutralizing antibody response. A variety of approaches have been experimentally evaluated in cattle. These include DNA plasmids [120–122], eukaryotically expressed recombinant protein to preserve conformational epitopes [123–125], or combined heterologous DNA prime-protein boost regimes [126, 127] or via live viral vectors [128–131]. Whilst many of these studies have shown encouraging results, to date none of these vaccines has been licensed.

8.3 *Salmonella*

Salmonella are an economically important cause of diarrhea and systemic infections in animals. Furthermore, they are a zoonotic pathogens and a major cause of diarrhea and systemic disease in humans world-wide, most commonly as a result of consumption of contaminated foodstuffs of animal origin. In the European Union (EU), over 100,000 human cases are reported each year. The European Food Safety Authority (EFSA) has estimated that the overall economic burden of human salmonellosis could be as high as EUR 3 billion a year. Poultry meat, eggs, and egg products are frequently associated with *Salmonella* outbreaks as is pork and contact with infected animals.

Salmonella Enteritidis, Typhimurium, Virchow, Hadar, and Infantis are the most commonly implicated serotypes in human disease in Europe. They are also the most commonly isolated serotypes from poultry. Moreover, *Salmonella* Enteritidis (SE) and to a lesser extent, *Salmonella* Typhimurium (ST) are commonly associated with egg related outbreaks [132]. More recently the

emergence of monophasic strains has complicated diagnosis and indeed vaccination programs [133]. Despite these challenges the use of *Salmonella* vaccines in laying flocks has contributed to a significant reduction in human cases of salmonellosis in the UK. It is widely accepted that vaccination of laying hens confers protection against *Salmonella* infection and results in decreased level of on farm contamination [134] and has contributed to the decline of the *Salmonella* Enteritidis epidemic [135]. Interestingly, in some European countries (Austria, Belgium, The Czech Republic, Germany, and Hungary) vaccination of laying flocks is compulsory. In other countries it is permitted and often recommended (Bulgaria, Belgium, Cyprus, Estonia, France, Greece, Italy, Latvia, Lithuania, The Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, and the UK). Conversely, in a few countries vaccination is prohibited (Denmark, Finland, Sweden and Ireland) [136]. In the UK, the majority of commercial scale egg producers subscribe to the British Egg Industry Council (BEIC) Quality Assurance Scheme that provides a code of practice (Lion Code) on farm hygiene and welfare standards, including *Salmonella* vaccination. Vaccination against *Salmonella* began in laying flocks in the UK in 1998 for farms that subscribe to the BEIC Lion Code Scheme [137–139].

At present, both live and inactivated vaccines are commercially available to vaccinate laying flocks [140]. Live vaccines generally confer better protection than the inactivated ones, as they are able to induce both cell mediated and humoral immune responses [136, 141]. However, they may persist in the environment and can present issues for the clinical diagnostic microbiology laboratory. As SE and ST are considered to be the most important serovars for public health in Europe, existing commercially available live and inactivated *Salmonella* vaccines for poultry are generally targeted against one or both of these serovars. In the UK, three live vaccines and two inactivated vaccines are currently available [141–143]. These vaccines are used singularly or combined. To maximize protection, vaccination programs that combine live and inactivated vaccines are sometimes used [144]. Within these vaccination programs, oral vaccines are administered in two or three doses during the rearing period of the pullets and are complemented by one or two injections of killed vaccine (normally close to point of lay) [140]. Currently used vaccination programs are licensed for use against biphasic variants of ST, that is expressing two different flagellar antigenic specificities. Their efficacy against monophasic Salmonella Typhimurium (mST), which only express a single flagellar antigenic specificity, has not yet been fully investigated [133, 144]. It is likely that ST vaccines have a similar protective effect for mST as for biphasic ST. However, there are no data available concerning the efficacy of current vaccination programs [145].

A long term goal is to develop vaccines for broiler chickens and also to investigate the use of vectored vaccines that could be used to protect layers, broilers and breeders against a number of patho-

gens, including *Campylobacter*, *E. coli*, *Salmonella*, *Brachyspira*, and *Clostridia* through the use of a single economically viable commercial vaccine.

8.4 Bovine Tuberculosis (*Mycobacterium bovis*)

Bovine tuberculosis (bTB) is a major challenge for livestock globally, a zoonosis, and a significant threat to the cattle industry of England and Wales. Efforts to eradicate the disease from the bovine population are hampered where there is a wildlife reservoir of infection. In England and Wales, the primary wildlife reservoir is the European badger (*Meles meles*), a species protected under national law. In these countries it will take a combination of measures targeting both cattle and wildlife to eradicate bTB. One of the disease control measures being pursued is vaccination, both of badgers and cattle.

At present, the developed vaccine agent for tackling bTB in both cattle and badgers is the live attenuated BCG strain of *M. bovis*. It has been administered to humans since 1927 and is one of the most widely used of all current human vaccines. BCG was licensed for intramuscular vaccination of badgers against bTB by the UK Competent Authority (Veterinary Medicines Directorate) in 2010, following 10 years of studies carried out by the Animal and Plant Health Agency (APHA; formerly the Animal Health and Veterinary Laboratories Agency, AHVLA and the National Wildlife Management Centre of the Food and Environment Research Agency, FERA, now also part of APHA). The licensed vaccine "BadgerBCG" (APHA) has a Limited Marketing Authorization and is currently available for use in the UK by vets and trained lay vaccinators under prescription from a veterinary surgeon.

Use of BadgerBCG over large geographical areas is restricted by the need to trap badgers and inject them, an approach that is relatively expensive and labor intensive. More practical would be an oral form of BCG that could be delivered to badgers in baits. The efficacy of BCG given orally has been demonstrated for cattle, brushtail possums (*Trichosurus vulpecula*) [146], wild boar (*Sus scrofa*) [147], and white-tailed deer (*Odocoileus virginianus*) [148], as well as badgers [149]; each following experimental *M. bovis* infection of captive animals, but also against natural infection in wild possums [150]. However the dose for effective oral administration of BCG is higher than that given parenterally because BCG is killed and degraded in the gut and uptake is relatively inefficient [151]. Experimental studies in possums have suggested that in order to generate immunity it is necessary for oral BCG to retain viability to the point of delivery to the intestine [152]. This has been facilitated through formulation of BCG in a lipid matrix that provides a stable storage and delivery vehicle that protects the live attenuated bacillus during passage through the stomach [146]. Recent success using heat-inactivated *M. bovis* to experimentally vaccinate wild boar orally has increased the number of candidate

oral vaccines for bTB [153, 154]. The Governments of England and Wales have funded research into the development of an oral vaccine for badgers since 2005. Candidate vaccine baits for badgers have been identified and are being evaluated for palatability and efficacy (degree of protection afforded to badgers that consume a vaccine bait), but the formulation of the vaccine itself is only one element. Linked to this is the need for a practical deployment strategy which will maximize uptake among the target badger population and, as far as possible, minimize consumption by other wildlife species or cattle [155].

Regarding cattle, BCG was first demonstrated to be an efficacious vaccine against bTB in 1911 (reviewed in Ref. [156]). Extensive work has been carried out since to optimize the dose and route of administration of BCG vaccine to cattle. Whilst no single vaccine currently offers equal or superior performance to BCG, when used in combination with BCG several offer enhanced protection, e.g., recombinant human adenovirus-vectored mycobacterial antigens [157, 158]. Further assessment of this adenovirus-based strategy as well as development of other approaches should result in vaccine protocols that impart better protection than with BCG alone, and in particular could prolong the duration of immunity. For the foreseeable future, vaccine strategies for bTB in cattle will need to include BCG. The problem with this is that vaccination with BCG sensitizes cattle to tuberculin-based diagnostic tests, including the single intradermal comparative cervical skin test (SICCT). This sensitization is the reason a diagnostic test is needed that will allow accurate detection of infected cattle amongst the vaccinated animals (a so-called DIVA test) and so allow use of a BCG-based vaccine for bTB control alongside a test and slaughter program [159]. A longer-term research goal is the development of vaccines that do not sensitize cattle to tuberculin-based diagnostic tests. This would allow the SICCT to be used alongside vaccination. Close communication and collaboration with research groups working to develop novel human TB vaccines means there is a route to evaluate promising bTB candidates in cattle (embracing a "One Health" approach to vaccine development).

9 Conclusions, Issues, and Needs

Vaccination of veterinary species has a long and successful history and remains an extremely active area of research. Review of PubMed.gov shows that since 2004 there have been an average of over 500 publications each year on veterinary vaccination, reaching their peak over the last 3 years. In writing this overview we have only been able to dip our toe into this vast sea of literature. However, we identified a number of particular issues and cross-cutting needs

that require further attention by the research community, companies, government, and regulators. We summarize these here.

9.1 Vaccine Interference

Vaccine interference is an aspect of veterinary vaccination that requires further evaluation and discussion. The term itself is confusing and is variably interpreted as either referring to the situation where vaccination against one pathogen may compromise the protective immunity induced by vaccination to another, or where the presence of maternally derived antibodies interfere with vaccination in newborn animals. The reader is referred to a helpful review of this subject [160]. The review focuses on experience from human vaccine development and considers vaccine interference in the contexts of the nature and dose of the individual vaccine components, the presence of preexisting immunity, the stage of immunological maturation, genetic and environmental background, vaccine schedule, and mode of vaccine delivery.

The presence of interfering maternal antibodies is a significant consideration in a variety of veterinary vaccine settings. They cause problems for the vaccination of young piglets against influenza, they are the most important obstacle in the establishment of control programs against IBD, they are the primary cause of failure of canine parvovirus type 2 vaccination, and interference by high titers of maternal antibodies prevents the development of an antibody response following vaccination with either a killed or attenuated BVDV vaccine. In countries where control of FMD relies predominantly on vaccination, newborn animals ingest specific anti-FMDV antibodies in the colostrum. This maternally derived antibody provides immediate protection against infection with FMDV but also interferes with the development of active immunity following vaccination leaving young animals susceptible to FMDV infection when maternal antibodies wane. Currently available vaccines for FMD cannot overcome this effect.

9.2 Incomplete Protection and Vaccine Escape Variants

Vaccines rarely produce sterilizing immunity and in some cases exert a powerful selective pressure on pathogens, resulting in the emergence of variants for which the vaccine no longer provides adequate protection. This does not have to arise from the emergence of a new variant but could simply arise from the use of a vaccine that does not provide sufficient cross-protection from one pathogen geno/sero-type to another resulting in the dominance of one type already in circulation. This may be part of the explanation of the failure to control canine distemper virus (CDV) infection in Korea, where at least two different CDV genotypes are in circulation that differ significantly from the genotypes present in vaccine strains [161]. Ensuring a vaccine is effective against a range of circulating strains or variants can be secured by including multiple types in the same vaccine preparation but there is a significant cost to such a strategy. Alternatively autogenous vaccines can be used.

Autogenous vaccines are derived directly from the variant(s) responsible for the disease outbreak, e.g., for *Mycoplasma bovis*. However, this approach cannot prevent the emergence of new variants that escape vaccine-induced immunity through mutation. This is particularly the case for viral pathogens where a high infectious load combined with a low fidelity of genome replication provide an environment for the selection of new variants. There are some good examples of this. First is the TJ strain of PRV, which is a variant of PRV that appears to be emerging along with others in China's pig population in the face of vaccination with the live attenuated vaccine strain, Bartha-K61, which until now has played a critical role in the control of Aujeszky's disease in China [162]. Sequence analysis indicates that these emerging PRV variants cluster to a relatively independent clade in the phylogenetic tree and that protection against these variants with the Bartha-K61 vaccine is incomplete [163]. Second is IB in poultry. IB is caused by an RNA virus that readily undergoes mutation and recombination so that important antigenic variants appear which evade existing vaccine protection. While conventional vaccines work well against homologous types, new strategies are needed to counter this instability. The simple use of two genetically different vaccines to protect against a wide range of heterologous types is now a widespread practice that has been very effective thus far (reviewed in Ref. [164]).

9.3 Mass Application of Vaccines

Mass application of vaccines can be an important consideration in reducing the cost of vaccination by avoiding the need to vaccinate individual animals manually and as a tool in combating disease outbreaks. Mass vaccination of poultry is already performed regularly against a variety of respiratory and gastric pathogens using application by aerosol/spray or in drinking water. Mucosal vaccination has the advantage of inducing both local and systemic immune responses. *In ovo* vaccination offers the advantage of reduced labor costs, mass administration and the induction of an earlier immune response, as described in Subheading 7. For rapid intervention with vaccine during a disease outbreak such as AI, mass application of vaccine is desirable in order to achieve rapid coverage of susceptible birds. An AI vaccine that could be applied by spray or aerosol would be ideal, but aerosol vaccination using live virus is not desirable because of its zoonotic potential and because of the risk for virus reassortment. The next generation of AI vaccines based on recombinant vectors holds out hope for safe and efficacious mass vaccination of susceptible birds as an alternative to preemptive culling in an outbreak [165].

The success of rabies vaccination in the European continent was undoubtedly the result of a safe, effective, and cost-effective vaccine combined with the ease of mass distribution of millions of

edible vaccine baits over large geographical areas. However, there can be a naïve assumption that successful disease eradication is simply a matter of vaccinating enough susceptible animals. The successful eradication of Rinderpest required detailed consideration of the principle of herd immunity and careful application of the vaccine based on detailed epidemiological information. Readers are directed to the excellent review of Roeder and Taylor that sets out the principle of herd immunity and some of the factors which militate against mass vaccination achieving effective levels of herd immunity [166].

9.4 Economics and Incentives

Before embarking on the lengthy and costly road towards a licensed vaccine, initial impact assessment is necessary in order to assess the relative merits of different disease intervention options, including vaccination. This is most likely to be meaningful when it is done in partnership between policy-makers, vaccine manufacturers, funders, and stakeholders. Even when a compelling benefit–cost ratio is found it does not mean a vaccine will necessarily follow [167]. Disease control programs that utilize vaccination but rely on its voluntary uptake are at risk of failure if willingness to vaccinate is too low to reach satisfactory vaccination coverage to stop the spread of the disease. There have been a number of interesting studies exploring the willingness of stakeholders (typically livestock farmers) to vaccinate and the factors that influence this decision. These include studies on Bluetongue in the Netherlands [168], poultry vaccination in developing countries [169] and farmers' confidence in vaccinating badgers as a means to controlling bTB in cattle in the UK [170]. Important lessons emerge from these studies, such as the importance of financial incentives and when they should be applied during a disease control program, the characteristics of the disease, farmers' perceptions of disease risk, the efficacy of the vaccine and other available control options, the availability of resources, and the existence and effectiveness of the veterinary infrastructure, and the wider social and political context. Where there is little incentive to use a vaccine, the best endeavors can fail. An excellent example of this is the vaccination of cattle against *E. coli* O157:H7, reviewed recently by Matthews et al. [171]. These authors point out that in Canada, where the first *E. coli* O157:H7 vaccine was developed and fully licensed, uptake of the vaccine is currently less than 5 % of the market. The authors suggest that this is a likely consequence of the fact that the infection causes no clinical disease in cattle. Therefore, there is little economic incentive for the farmer who bears the cost of vaccination, but receives no direct perceived benefit. For a wider consideration of the economics of veterinary vaccination, the reader is also referred to the review of McLeod and Rushton [172].

9.5 Harmonization

The separation of licensing bodies for human and veterinary medicines has been cited as a reason for delays in the licensing of veterinary vaccines [171]. Whilst the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and its veterinary counterpart, VICH, have been pivotal over the last two decades in harmonizing technical requirements for human and veterinary product registration respectively across Europe, Japan, and the USA there needs to be greater join up between the human and veterinary sectors, not least regarding how the cost might be shared across stakeholders if the conceptual benefits of a "One Health" approach are to become a reality [173]. Progress is being made. For example, STAR-IDAZ (http://www.star-idaz.net/) is a recently established network of 24 partners in 18 countries brought together with funding from the European Commission for the purpose of sharing information, improving collaboration on research activities and working towards common research agendas and coordinated research funding on major animal diseases affecting livestock production and/or human health.

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