Review

Developing vaccines against foot-and-mouth disease and some other exotic viral diseases of livestock

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Vaccines remain the main tool for the control of livestock viral diseases that pose a serious threat to animal and occasionally human health, reduce food security, distort trade in animals and their products, and undermine agricultural development in poor countries. Globalization and climate change increase the likelihood for new patterns of emergence and spread of livestock viruses. Conventionally attenuated and killed virus products have had spectacular success, and recent examples include the global eradication of rinderpest and the control of bluetongue in the UK and northern Europe. However, in many cases, livestock vaccines could benefit from improvement in some properties (e.g. stability, speed of onset and duration of immunity, and breadth of cross-protection to different serotypes or strains) and in some cases are not available at all. Compared with human vaccines, uptake of livestock products is highly cost-sensitive and their use may also need to be compatible with post-vaccination screening methods to determine whether or not animals continue to be infected. Requirements and prospects for new or improved vaccines are described for some priority viral diseases with potential for transboundary spread, particularly for foot-and-mouth disease.

Keywords: livestock viral vaccines; foot-and-mouth disease; African swine fever; peste des petits ruminants; bluetongue; Rift valley fever

1. THREATS POSED BY EXOTIC LIVESTOCK VIRUSES

Livestock viral diseases are an important cause of animal suffering and lost productivity. Livestock producers in the UK and northern Europe have benefited from the fact that many of the most severe of these diseases have either been always absent or have been eradicated. Examples include rinderpest (RP), foot-and-mouth disease (FMD), peste des petits ruminants (PPR), African swine fever (ASF) and bluetongue (BT). However, these ‘exotic’ viral diseases cause serious direct and indirect losses in other parts of the world, often depressing agricultural development in some of the poorest countries, distorting trade patterns and compromising food security [1,2]. Moreover, factors such as globalization and climate change increase the risk of these diseases being introduced or re-introduced [3,4] especially for those that can be spread indirectly in food, by fomites or by insect vectors. Long-term control of these diseases requires measures to be applied at source mainly in the poor countries which are the reservoirs of infection. In the meantime, wealthy countries with highly industrialized systems of densely stocked and fully susceptible livestock populations need contingency plans to deal with possible incursions.

Vaccination is often a critical element in both situations, and reliance on slaughtering animals to halt epidemics is considered increasingly unacceptable on both economic and ethical grounds [5,6].

2. VETERINARY VACCINE REQUIREMENTS

Livestock keeping is mainly a commercial activity and therefore vaccine uptake is cost-sensitive, regardless of whether or not vaccination programmes are funded by livestock keepers or governments. In developing countries where resources are often lacking, as well as having a low unit cost, the ideal vaccine for livestock disease control should be stable at ambient temperature and induce a long period of protection. In practice, locally produced vaccines used in developing countries may be of poor quality and efficacy and may even give rise to outbreaks of disease owing to inadequate inactivation of the vaccine virus [7]. On the other hand, vaccines produced in industrialized countries may be too expensive for use in developing countries, leading to inadequate vaccine uptake for effective disease control. In contrast, vaccines designed for use in an emergency situation, following an incursion of an exotic viral disease into a normally disease-free region may command a high price, but without contingency provision to stimulate advance development and production, a suitable vaccine may not be available. For emergency vaccines, a rapid onset of protection is more important than the duration of protection.
In addition, after the use of emergency vaccination, there is often a need to be able to demonstrate the establishment or re-establishment of disease freedom for trade purposes. Particularly for countries with significant livestock-related export industries, this factor can have a decisive effect on the selection of control options. Internationally agreed approaches to substantiating disease freedom are developed by the World Organization for Animal Health [8]. As vaccination reduces the incidence of disease, clinical observation may no longer be an acceptable method of surveillance and post-vaccination serology may be required to show an absence of infection. This necessitates the development of the so-called ‘negative marker vaccines’, which fail to induce an antibody response to the full range of epitopes that elicit antibodies following infection. This allows tests for antibodies to infection-specific epitopes to be used to identify infection among vaccinated animals—so-called ‘DIVA’ tests that Differentiate Infection in Vaccinated Animals [9].

3. VACCINE USE AND AVAILABILITY
This year will mark the end of the global RP eradication campaign and worldwide elimination of one of the most deadly scourges of cattle. Vaccination using a live, cell culture-attenuated RP virus (RPV) vaccine was the cornerstone of this campaign and success was aided by the fact that the virus existed only as a single serotype and that one dose of vaccine could provide lifelong protection [10]. The related morbillivirus that causes PPR, a disease of sheep and goats with a case fatality rate of up to 80 per cent in naive populations, is still at large however, and has increased in range over the past 10 years despite the availability of several live attenuated vaccine strains [11]. Recent incursions have brought the disease into close proximity with Europe, following outbreaks in Thrace in 2007 and in Morocco in 2008. Vaccination was widely used in both regions to bring the disease under control, and in Morocco over 21 million sheep and goats were vaccinated. Another recent example where the use of a conventional, cell culture grown, virus vaccine (in this case killed) has been highly effective is the control of BT in Northern Europe, including its complete elimination from the UK following voluntary, mass vaccination of sheep and cattle in 2008 [12]. Killed vaccines have also been used for many years to control FMD. These vaccines were instrumental in controlling and then eradicating the disease from most of mainland Europe [13,14] and their large-scale use over many years is leading to progressive eradication of the disease in South America [15]. Very large quantities of the vaccine are also being used in parts of Asia, such as India and China, and global production probably approaches 2 billion doses annually [16]. As well as being manufactured for use in endemically infected countries, FMD vaccines are also stored as frozen antigen reserves or vaccine banks, ready for rapid formulation and emergency use in FMD-free countries [17,18]. The European vaccine bank holds approximately 40 million doses of vaccine divided between different serotypes and strains.

ASF is an example of an important livestock viral disease for which no vaccine is available. The disease is caused by a large DNA virus that is adapted to infect warthogs and soft ticks without causing disease. However, infection of domestic pigs is associated with a fatal haemorrhagic disease that precludes pig-keeping in much of Africa and has on several occasions spread beyond the continent, most recently into central Europe [19].

4. DEVELOPMENT OF IMPROVED VACCINES
In contrast with human vaccines, novel vaccines for livestock can be evaluated in the target species at an early stage of the vaccine development process. However, most vaccine development has taken place in the developed world where there are restrictions in handling dangerous pathogens and experimental infection with exotic viruses of large animals such as cattle can take place only in specialized high containment facilities that are expensive and in limited supply. Use of small animal models has not always proved a reliable surrogate for the natural host, an example being the case of synthetic peptide vaccines that were effective in protecting guinea pigs against FMD but were unreliable in cattle [20]. Rapid progress in recombinant technology and different delivery systems have created an explosion in opportunity to develop new vaccines. In order to evaluate these possibilities without live viral challenge of vaccinated animals, a high priority is to develop a better understanding of the correlates of immune protection for the diseases in question. A difficulty here is that the immunological toolbox of reagents needed to dissect livestock immune responses is less developed than for humans and mice.

5. FOOT-AND-MOUTH DISEASE VACCINES
The different vaccine qualities that are of importance for FMD control in endemically affected countries compared with their use in an emergency situation have been reviewed [21].

Despite many years of research, reviewed by Grubmann [22], the basic immunogen within FMD vaccines has changed little in 40 years, although oil adjuvants have often replaced aluminium-based alhydrogel. Subunit and DNA vaccines have so far proved less potent than whole, inactivated viral capsids, and differences in host species susceptibility have hampered attempts to produce an optimally attenuated live vaccine. Adenovirus-vector vaccines delivering interferons or FMDV capsid proteins, co-expressed with the viral protease required for their processing, have been shown to provide rapid-onset protection against FMD in pigs and cattle [22], but very large doses are needed for the latter species. Baculovirus-derived virus-like particles (VLPs) are also highly immunogenic [23] and offer advantages of safe production and freedom from non-structural viral proteins (NSPs) making them compatible with a DIVA test (see below).

Improvement in techniques for purifying viral capsids and removing NSPs from conventional inactivated virus preparations has given rise to a negatively marked product. Animals that become infected despite vaccination can then, in principle, be detected serologically based on the presence of antibodies against NSP [9]. However,
because of the difficulty in completely eliminating NSP from vaccines, non-specific serological reactions to these proteins can still be induced, especially after multiple vaccinations. Consequently, it is difficult to prove the absence of infection by serological testing alone, which must rather be thought of as one of a range of measures to substantiate this [24]. In practice, stamping out continues to be selected for emergency FMD control by industrialized countries that were previously disease-free. This was the case in the UK in 2001 and although vaccination was used in the Netherlands in 2001 and also in the later stages of a more recent incursion in Japan in 2010, the vaccinated animals were in both cases slaughtered as quickly as possible so that vaccination was used to help buy time to slaughter animals without disease spread rather than as an alternative to culling. The reasons for this are complex and not entirely scientific, but may be attributed partly to uncertainty over the efficacy of the vaccine available [25], a lack of preparation for NSP-based surveillance (at least in 2001) and a desire to avoid the need to carry out DIVA testing owing to possible delay that this would entail for the recovery of official FMD-free status for trade as well as uncertainty over its acceptability to trade partners. The latter problem might be ameliorated by clarification of the precise requirements for the detection and elimination of FMDV carrier animals [26]. Preparedness for the use of vaccines could also be improved by the development of better vaccine selection techniques [27].

Antibodies directed at the FMDV capsid appear rapidly after infection or vaccination and are protective, and there is a strong correlation between antibody levels induced by vaccination and protection [28]. Although there is also a cell-mediated response to vaccination that correlates with protection [29], CD4 T-cell depletion of cattle infected with FMDV showed that the antibody response is partly T-cell independent and that lack of CD4+ T cells did not affect recovery from infection [30]. Infection with FMDV leads to a long-lasting protective immunity and is associated with virus retention in light zone germinal centres of lymph nodes [31]. In contrast, immunity induced by vaccination with inactivated virus vaccine is short-lived. Induction of stronger T-cell responses and more efficient sequestration of antigen may improve the memory responses after vaccination and prolong the duration of protection. Stabilization of recombinant viral capsids might therefore have advantages in terms of generating a more potent and long-lasting immunity as well as for an improved thermal stability and shelf life prior to administration. Considering that the usual portal of entry of FMDV is through the oropharynx, there is also scope for further investigation of mucosally active vaccines to deliver protection at this site.

Another challenge for FMD vaccines is the existence of seven serotypes of FMDV that are not cross-protective, with the possible exception of animals sequentially infected with multiple serotypes [6]. In addition, antigenic differences within some serotypes are also sufficient to impact upon cross-protection [32]. A better understanding of the viral determinants of cross-reactivity and their immunodominance or its lack, is needed to improve vaccine selection as well as the development of more cross-protective vaccines.

One approach to the problem of responding rapidly to changing vaccine strain requirements is the development of infectious copy-based master seed vaccine strains into which novel capsid genes could be rapidly inserted [33]. Prospects for the development of a more cross-protective vaccine have been increased by the observation that a DNA prime/heterologous boost regimen induces broadly cross-reactive neutralizing antibodies. Thus, vaccination of pigs with DNA-encoding FMDV serotype O capsids followed by boosting with inactivated FMDV serotype O antigen stimulated the production of antibodies that neutralized not only FMDV serotype O, but also serotypes A, C and Asia 1 [34]. This response is similar to that seen in animals primed with plasmid DNA encoding H1N1 influenza haemagglutinin (HA) and boosted with seasonal flu vaccine or replication-defective adenovirus (AdV) 5 encoding the homologous HA, which developed broadly neutralizing influenza virus antibodies [35]. The generation of more cross-reactive antibodies by a gene-based heterologous prime-boost strategy may be related to the ability of DNA vaccines to increase the diversity of the CD4+ T-cell response [36]. DNA prime/heterologous boost vaccination may also provide more durable immune protection, and this approach may be of value in FMD endemic countries.

6. PESTE DES PETITS RUMINANTS’ VACCINES

Several different live-attenuated PPRV vaccine strains have been used in Africa and Asia, and historically RPV vaccine was also used as it is fully cross-protective. A prototype DIVA vaccine was developed by substituting the N gene of PPRV into an infectious copy of the RPV vaccine strain [37]. Additional genes have since been substituted and the ensuing vaccine was shown to protect goats [38]. However, use of vaccines containing RPV genes may be considered unwise now that RP is eradicated. Although it has not yet been possible to rescue PPRV from full-length cDNA using reverse genetics, the ability to manipulate the genome of PPRV would provide the opportunity to develop a live, attenuated marker vaccine. However, effective deployment of a live-attenuated PPRV vaccine depends upon a cold-chain and this, together with the cost of vaccine administration and the nature of sheep and goat farming, makes vaccination campaigns against PPRV difficult.

Alternative vaccines with DIVA potential have been developed using viral vectors such as sheep and goat pox to express PPRV H or F surface glycoproteins and these can protect against experimental PPRV infection [39,40]. Advantages of using sheep and goat pox virus vectors are that they can also provide protection against this important pox virus, which has a similar range of occurrence to PPRV. Furthermore, the pox vector is highly thermostable. However, there are conflicting reports on the ability of sheep and goat pox vaccines to protect against each other [41] and the effect of pre-existing sheep or goat pox immunity on vaccine efficacy is not known, although the PPRV-neutralizing antibody response in goats vaccinated on two occasions with a recombinant capripox-expressing PPRV H (rCPV/H) can be boosted six months later by a third vaccination with rCPV/H [40].
7. AFRICAN SWINE FEVER VACCINES

Currently, there is no effective vaccine available for ASF in domestic pigs. However, complete protection can be achieved by vaccination with low-virulence isolates of ASFV [42]. This opens up the possibility of developing rationally attenuated ASFV vaccines, although not all pigs vaccinated with low-virulence isolates are protected against challenge. For example, inbred pigs of the cc haplotype infected with a low-virulence ASFV isolate, OURT88/3, were not always protected against challenge with the closely related virulent isolate, OURT88/1 [43]. In contrast, complete protection could be induced in inbred pigs of the dd haplotype. The reason(s) for this discrepancy is not clear, but these findings suggest that a live-attenuated ASFV vaccine may not protect 100 per cent of animals in the field. Another concern about ASF vaccines is related to the genetic diversity of strains circulating in some countries and the ability of one attenuated strain of ASFV to protect against heterologous strains. Recent studies have demonstrated cross-protection against heterologous ASFV strains in pigs immunized with low-virulence ASFV OURT88/3 [44].

Protection induced by low-virulence isolates of ASFV does not appear to be mediated by antibody but is dependent upon CD8+ T cells [43]. Therefore, knowledge of the ASFV proteins recognized by protective CD8+ T cells in pigs expressing different MHC class I genes, and the development of an antigen-delivery system that will prime a protective CD8+ T-cell response provides the opportunity to develop a safe and effective recombinant virus vectored vaccine. The prospects for vaccine development have been reviewed further by Costard et al. [44].

8. BLUETONGUE VACCINES

BT is an acute haemorrhagic fever of domesticated and wild ruminants virus caused by BT virus (BTV). The virus is transmitted between ruminant hosts by the bites of certain species of Culicoides, which become infected after feeding on an infected host. BTV can also be transmitted via seminal fluid and across the placenta, resulting in foetal abnormalities. BTV infection of ruminants occurs throughout much of the temperate and tropical regions of the world, coincident with the distribution of Culicoides biting midges, and in recent years the virus has spread beyond its prior upper northern limit and caused devastating disease in naive sheep and cattle in southern, central and northern Europe. Both live-attenuated and inactivated BTV vaccines are available [45], and although they have a number of limitations, the successful control of the BTV-8 outbreak in the UK was attributed to the high level of coverage achieved with an inactivated vaccine in previously affected areas, together with cooler temperatures in the UK in 2008 [12].

Twenty-six distinct serotypes have been identified so far, which do not induce cross-protective immunity, with the possible exception of animals sequentially infected with multiple serotypes [46]. The threat posed by the range of different BTV serotypes has highlighted the need for more widely cross-protective and DIVA BTV vaccines. Although inactivated vaccines are safe if properly produced, DIVA strategies are not currently available. However, ELISAs for the detection of antibodies to non-structural proteins (NS1 or NS3) show promise for the development of a DIVA strategy [47,48]. As vaccination requires large amounts of inactivated antigen and the transient duration of immunity induced by inactivated vaccines means that booster vaccinations are required, production costs are high. Modified live virus vaccines, which are cheap to produce and generate protective immunity after a single dose, are unsuitable at present for the development of a DIVA strategy. Furthermore, they can induce viraemia and severe clinical disease in certain susceptible breeds of sheep [49], with the potential to be spread by vectors causing disease outbreaks in their own right, and to produce novel virus strains as a result of gene reassortment with wild-type viruses.

Alternative vaccines with DIVA potential have been developed using poxvirus vaccine vectors expressing BTV major structural proteins VP2 and VP5. Vaccination with recombinant poxviruses expressing VP2 and VP5 together induced greater protection in sheep than vaccination with poxviruses expressing either protein alone [50,51]. Although vaccination with recombinant capripox expressing the BTV core protein VP7 induced only partial protection in sheep against a heterotypic BTV strain, this finding highlights the potential of VP7 for the development of more broadly protective vaccines [52]. VLPs, produced by the expression of all four major structural proteins of BTV (VP2, VP5, VP7 and VP3) in insect cells, using a recombinant baculovirus vector, are highly immunogenic and confer protective immunity in sheep [53]. These VLPs mimic the whole virus structure but do not contain any BTV nucleic acid. However, as a number of different BTV serotypes are in circulation, a drawback to the production of VLPs is that they require the expression of these four proteins from each serotype. This problem has been partially overcome by the development of a new baculovirus expression strategy which allows pre-integration of the genes encoding the relatively conserved BTV inner capsid proteins (VP7 and VP3) from one serotype at one baculovirus locus and those encoding the outer capsid proteins (VP2 and VP5) from other BTV serotypes at a different locus [54].

The recent development of a reverse genetics system for BTV [55] has provided the opportunity for a third approach to novel BTV vaccines compatible with DIVA tests, the construction of a disabled infectious single cycle vaccine. Such a vaccine would consist of genetically engineered BTV containing a deletion in a gene essential for virus replication and which can propagate in cells, in vitro, that constitutively express the deleted gene, but which will not be able to propagate in animals.

9. RIFT VALLEY FEVER VACCINES

Rift Valley fever virus (RVFV) is a negative-sense, single-stranded RNA virus, with a tripartite genome. RVFV is transmitted by mosquitoes and causes large outbreaks of disease in ruminants and humans in Africa and the Arabian Peninsula. Humans develop
<table>
<thead>
<tr>
<th>Virus</th>
<th>Viral determinants that induce protection</th>
<th>Host responses that mediate protection</th>
<th>Existing licensed products</th>
<th>DIVA availability</th>
<th>Promising prototypes reported in refereed publications</th>
<th>Comments</th>
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<tr>
<td>FMD</td>
<td>capsid</td>
<td>antibody provides serotype-specific protection; role of T cells less clear</td>
<td>killed cell culture-grown virus</td>
<td>by detection of antibodies to viral non-structural proteins; used extensively in South America</td>
<td>recombinant adenovirus-vectored; DNA; VLPs</td>
<td>baculovirus-expressed VLP for serotype Asia 1 and synthetic peptide for serotype O (pigs only) licensed in China [61]; human adenovirus-vectored vaccines in clinical trials in USA</td>
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<td>PPR</td>
<td>H or F surface glycoproteins (RPV N confers partial protection against RPV [62])</td>
<td>antibody provides protection; role of T cells less clear [62]</td>
<td>live attenuated</td>
<td>not available with licensed products</td>
<td>recombinant sheep and goat pox vectored</td>
<td>DIVA by detection of antibodies to viral N protein possible with new vaccine candidates</td>
</tr>
<tr>
<td>ASF</td>
<td>unknown</td>
<td>CD8$^+$ T cells important serotype-specific protection by antibody; T cells may also confer protection [63,64]</td>
<td>none</td>
<td>not investigated</td>
<td>live attenuated</td>
<td>limited cross-protection between heterologous strains</td>
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<tr>
<td>BT</td>
<td>external VP2 and VP5 proteins; VP7 core protein</td>
<td>antibody is protective [65]; role of T cells less clear</td>
<td>killed cell culture-grown and live attenuated virus (for animals; no licensed human vaccine)</td>
<td>not with licensed products</td>
<td>rationally attenuated; recombinant sheep pox, adenovirus, Newcastle disease virus and Sindbis vectored; DNA; VLP [66]</td>
<td>DIVA by detection of antibodies to viral NSs protein possible with new vaccine candidates</td>
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<tr>
<td>RVF</td>
<td>structural glycoproteins Gn and Gc; partial protection by Nc in mice [58]</td>
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Table 1. Progress in vaccine development.
an acute febrile illness, followed by a fatal haemorrhagic fever, encephalitis or ocular disease. Ruminants experience abortions during outbreaks and there is a high mortality in young animals. Both inactivated and live, attenuated RVFV vaccines have been used to control RVF in ruminants. However, sustained vaccination with inactivated vaccines is required to protect against abortion, and production of the vaccine is expensive and hazardous to humans. Live, attenuated RVFV vaccines confer good protection three to four weeks after a single inoculation, and a single administration is said to confer lifelong protection. However, protective efficacy in young animals is compromised by the presence of maternal antibodies. Furthermore, live-attenuated vaccines can induce a low percentage of abortion and teratogeny in sheep, and some vaccine strains may revert to virulence.

The development of a reverse genetics system for RVFV has provided the opportunity to develop a rationally attenuated RVF vaccine compatible with a DIVA strategy. RVFV lacking the non-structural genes NSs and/or NSm are highly attenuated in rats and confer protective immunity against lethal RVFV challenge [56]. However, the efficacy of these mutant viruses in ruminants has not yet been evaluated. Recombinant virus vectors, such as a Sindbis virus replicon or the sheep pox virus responsible for lumpy skin disease, engineered to express the surface glycoproteins of RVFV, induced neutralizing antibodies in sheep, and the latter recombinant vaccine protected sheep against RVFV challenge [57,58]. Non-infectious RVFV VLPs have also been produced in insect and mammalian cells [59,60], and immunization with VLPs conferred protection against lethal RVFV challenge in mice [59].

10. CONCLUSIONS
Threats from emerging and re-emerging viral diseases of animals are set to continue and vaccines will remain the foremost tool for their control in the foreseeable future. Although traditional methods for vaccine development involving viral attenuation or inactivation have resulted in products that have been enormously effective, there remain important diseases for which vaccines developed in this way are either ineffective or suboptimal. There are also differences in requirements for vaccines during emergency and prophylactic use. Progress in vaccine development for some important veterinary livestock diseases is summarized in table 1. An explosion of possibilities has arisen for vaccine development. However, the limited facilities to test animals and ethical concerns over this type of activity underline the high priority for work to develop better tools to investigate and understand the immune systems of livestock species in order to arrive at better correlates of protection.

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