

Veterinary vaccines and their use in developing countries

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Summary

The burden of infectious diseases in livestock and other animals continues to be a major constraint to sustained agricultural development, food security, and participation of developing and in-transition countries in the economic benefits of international trade in livestock commodities. Targeted measures must be instituted in those countries to reduce the occurrence of infectious diseases. Quality veterinary vaccines used strategically can and should be part of government sanctioned-programmes. Vaccination campaigns must be part of comprehensive disease control programmes, which, in the case of transboundary animal diseases, require a regional approach if they are to be successful. This paper focuses on the salient transboundary animal diseases and examines current vaccine use, promising vaccine research, innovative technologies that can be applied in countries in some important developing regions of the world, and the role of public/private partnerships.

Keywords

Anthrax – Biotechnology – Bluetongue – Brucellosis – Contagious bovine pleuropneumonia – Foot and mouth disease – Mycoplasma – Rift Valley fever – Vaccination – Vaccine.

Introduction

The growing demand for livestock products (fuelled by population growth, increased urbanisation and greater purchasing power of individuals in developing or middle-income countries) coupled with the necessity of complying with the standards of trade agreements, mean that governments must improve animal health in their countries, particularly as it relates to infectious disease control (27, 28, 40), limits on residues in commodities, and animal welfare (13). Recent assessments show that infectious diseases will continue to be a major constraint to sustained international exports in livestock commodities from developing countries unless targeted sanitary measures are instituted in those countries to reduce the burden of these diseases (68). This paper addresses vaccines for selected epidemic diseases of livestock, examines historical and current trends for prophylaxis to

improve animal production in the high-risk and endemic areas, and highlights some opportunities and recent advances in vaccine research. Excellent vaccines used in a less than optimal vaccination strategy will fail to truly curb the incidence of disease. Furthermore, transboundary animal disease containment and control (for eventual eradication) require regional approaches and 'buy-in' from the public and private sector (including smallholders that raise animals to meet their own needs), but developing such regional vaccination strategies – based on quality, effective vaccines – requires well equipped and proficient diagnostic laboratories linked to reliable veterinary epidemiological units. Vaccines and vaccination must complement other aspects of disease prevention and control, namely, enabling legislation, open and risk-based surveillance, diagnostic proficiency, early response, transport and market regulations, compliance, and communication.

Veterinary vaccines for selected transboundary animal diseases

All transboundary animal diseases, including those selected for discussion here, have the following defining characteristics:

- they are of significant economic, trade and/or food security importance for a considerable number of countries
- they can easily spread to other countries and reach epizootic proportions
- their control and management, including exclusion, requires cooperation among neighbours, whether these be local, provincial, national, or regional (44).

Vaccines for livestock offer an important and, at times, an essential tool for progressive control of a given transboundary animal disease, but they require complementary actions:

- enabling legislation
- surveillance
- investment for diagnostic proficiency and capability
- early response
- coordination among several agencies
- management of livestock transport mechanisms
- market inspection and hygiene compliance
- public communication.

Foot and mouth disease

Foot and mouth disease (FMD), a highly contagious viral disease of mammals of the order Artiodactyla, is still considered globally as one of the most economically important diseases and is a threat to livestock production and agricultural development. Despite the fact that there are numerous viruses (serotypes) that cause clinical disease characterised by a variety of lesions and a drop in productivity, the most important aspect of FMD is its impact on trade in animals and animal products (8, 61, 93).

Since the beginning of the 21st Century, FMD has occurred in almost two thirds of the Member Countries of the World Organisation for Animal Health (OIE), either in an epizootic or enzootic form, causing varying degrees of economic losses. However, some of the major livestock-producing regions of the world, including North America, Western Europe, Oceania and some parts of South America

and Asia, are recognised as free of the disease at present. Due to increased global trade and movement, FMD has shown great potential in recent years for sudden and unanticipated international spread. The evolution of the pandemic Pan-Asia strain of type O FMD virus in recent years and the introduction of SAT types to the Arabian Peninsula are good illustrations (61, 93).

The epidemiology of FMD is characterised by the relative stability of the virus, its ability to survive outside living animals, the rapid growth of the virus, the small quantities of virus required to initiate the infection, the existence of asymptomatic carriers and, in sub-Saharan Africa, the persistence of the infection in wildlife (61, 93).

The first FMD vaccine, developed in 1938 by Waldmann and Köbe, was based on formaldehyde inactivated virus harvested from tongues of artificially infected cattle, collected at the height of the clinical disease and adsorbed on aluminum hydroxide. The large-scale production of the FMD vaccine started with the Frenkel vaccine in the late 1940s, using bovine tongue epithelium collected from abattoirs as *in vitro* culture system. Although this approach lasted through the early 1990s, one of its major disadvantages was the inability to guarantee freedom from bacteria or yeast or any form of contamination (8), nor guarantee the standardisation of the primary amplification mechanism (i.e. primary culture versus cell culture).

The finding, by Mowat and Chapman in 1962, that FMD virus could multiply efficiently in a baby hamster kidney (BHK) cell line opened the door to the cell-based production of FMD vaccine in suspension and monolayer cultures (8). Vaccines currently used against FMD throughout the world, including Africa and South America, often contain one or more serotypes that have been grown in large volume in BHK cell culture and then inactivated using aziridine compounds (usually binary ethyleneimine) (2, 31, 86). The virus harvest is then concentrated and formulated with an adjuvant (either saponin/aluminum hydroxide gel or various oil emulsions) to potentiate the immune response of the host. Such vaccines have been used successfully for decades to eradicate FMD in different parts of the world.

Vaccination has proven to be a very effective way of controlling and eliminating FMD from certain regions of the world, such as Western Europe and parts of South America (58, 86). Different forms of vaccination programmes are implemented in different regions of the developed world, with varying challenges to their success. One key challenge is the availability and high cost of the vaccine. Because the vaccine needs to contain a large quantity of specific antigen (1 µg per dose or perhaps closer to 5 µg per dose) and the production of large volumes of FMD virus needs to be conducted in a biosecure facility that will prevent virus escape into the

environment, they are expensive to produce. Most production plants are owned by multinational biopharmaceutical companies, usually driven by profit rather than disease control or eradication imperatives. Furthermore, the duration of immunity induced is short and booster inoculations need to be administered at 4 to 6 monthly intervals in most animals, including young cattle. In swine, aqueous-based vaccines are ineffective, and the application of oil-based technology to protect swine and prolong immunity to bovids (i.e. boosters every 6 to 12 months) offered great advantages in the 1980s when first applied widely in South America. Oil-based FMD vaccines have been shown to be very effective as emergency vaccines for pigs (34, 85).

Another challenge in the control of FMD is the debatable situation of 'asymptomatic carriers': ruminants vaccinated against FMD may be protected from developing clinical disease but are not necessarily protected from infection and some vaccinated animals may become persistently infected following challenge (1). However, the precise epidemiological role of the persistently infected animal in the maintenance of the disease and their responsibility for disease outbreaks in susceptible species has been an issue of much debate over the past 80 years. The epidemiology of FMD in endemic regions of sub-Saharan Africa has unique features that render the control of the disease extremely complex: firstly, the prevalence of six of the seven FMD serotypes and secondly, the reservoir role played by wildlife, mainly free-living African buffalo (*Syncerus caffer*) populations infected with the three SAT-types of FMD virus, i.e. SAT1, SAT2 & SAT3 (99). Little is known about the sylvatic maintenance of the virus in Asia and South America.

The significance of viral diversity (and thus antigenic diversity) as a complicating factor in effective vaccination against FMD in Africa is frequently ignored. Immunity is induced only to virus serotypes and subtypes included in the vaccine. In addition to the large number of serotypes prevalent on the African continent, sub-Saharan Africa is the only region of the world where the SAT serotypes of FMD virus are endemic, with widely distributed serotypes O and A, and serotype C being detected in Kenya. SAT2 (102), SAT1 (10) and serotype A (57) have been shown to harbour considerable nucleotide sequence diversity, giving rise to lineages with >20% sequence divergence. These divergences have been shown to be associated with considerable geographically based antigenic variation (98), corresponding to different topotypes within the occurring serotypes. An effective and systematic progressive FMD control programme using vaccination, in Africa or other endemically affected regions, should therefore include vaccine strains that are likely to protect against challenges by field viruses occurring in specific localities. The development of such control programmes is hindered by the fact that most outbreaks of FMD in Africa and Asia are

not investigated thoroughly enough with respect to the occurrence of intratypic variants.

The role of the African buffalo and other wildlife species in the persistence of FMD in sub-Saharan Africa has not been well studied beyond southern Africa, despite the fact that in some other regions there are large numbers of wildlife (99). However, for logistical reasons it is still difficult to envisage in the foreseeable future the scenario in which vaccines would be used against FMD in wildlife, except perhaps under special circumstances, e.g. in zoos or game ranches.

International regional collaboration has proven successful in the progressive control of FMD, e.g. the work of the Pan American Foot-and-Mouth Disease Centre in South America and the European Union FMD Commission (47, 86). Sadly, no similar organisations have been operational on the African continent. A number of southern African countries have, however, been successful in instituting control mechanisms that have proven successful in controlling FMD, these include Botswana, Lesotho, Namibia, Swaziland and South Africa. In 2003, a meeting of the National Chief Veterinary Officers of the Southern African Development Community (SADC) agreed on a 20-year framework for the progressive control of FMD in the SADC region and early steps are being taken towards this objective, under the aegis of the SADC Livestock Technical Committee (71). Similarly, there are some encouraging early steps being taken in Asia towards regional cooperation in FMD control, such as the Southeast Asia FMD Campaign, the Indian FMD control project and some projects in the Greater Mekong Delta. So far, however, there is no international mechanism for galvanising national and regional efforts towards coordinated progressive control of FMD in a manner akin to the concerted effort for global rinderpest eradication begun in the late 1980s.

Given the complexity of FMD epidemiology in Africa, broader control approaches will have to be designed, taking into account aspects of movement control, diagnostics, training and effective vaccines and vaccination programmes. Effective vaccine and vaccination strategies of the future should address the following needs:

- a) broad spectrum coverage, even within a serotype (such vaccines should protect against all topotypes within a serotype, especially those of the SAT FMD viruses)
- b) differentiation between vaccinated and infected animals
- c) vaccines that can provide durable protective immunity (beyond 12 months in a developing country setting)
- d) vaccines and a vaccination strategy for wildlife, if feasible (i.e. oral vaccination with proven efficacy in a controlled challenge setting)

e) genetic typing and geographical overlay maps for all possible serotype and topotype variants to better design appropriate vaccines, while developing effective and appropriately financed vaccination programmes.

Lubroth and Brown concluded that differentiation between vaccinated and infected animals, through post-vaccination serological monitoring and analysis of virus circulation, would depend upon improved quality control of FMD vaccines to ensure the elimination of non-structural proteins (NSP) during vaccine production and formulation (62), recently established as a standard by the OIE. Subunit FMD vaccines that lack any of the FMD NSP, including the highly immunogenic 3D protein, could be produced as a spin-off from conventional production. Such vaccines would only contain antigenic portions of the viral genome required for virus neutralisation and elimination, so clear distinctions could be made between vaccinated and infected animals using complimentary NSP-based assays (7, 67). Given the considerable impact of FMD on trade in animals and animal products (and sometimes also on trade in other products such as straw or alfalfa), the speedy recovery of disease-free status in many developing countries becomes an imperative. It is critical to differentiate vaccinated from infected animals. The exclusion of NSP from FMD vaccines means that vaccination, in combination with post-vaccination serological surveys and appropriate measures such as improved animal management, has become a feasible way of combating the disease in developing countries. Such vaccines could be obtained either through improved antigen purification during the production of inactivated FMD vaccines (7) or through the use of live vectored vaccines expressing only the empty FMDV capsid (67). The same research groups that are working on developing such vaccines, in an attempt to generate an early protection or prophylactic antiviral treatment, have successfully used expressed porcine type 1 interferon (IFN α /b) in swine to stimulate early protection prior to the vaccine-induced adaptive immune response (50, 66). With the advent of new technologies in vaccine development, it is expected that some of the genetic diversity and virus persistence problems associated with FMD might be addressed. Developments in adjuvant technology have already resulted in more effective vaccines against FMD. Recombinant deoxyribonucleic acid (DNA) technology, recombinant protein and/or DNA-based vaccines are being used in various heterologous systems to test different new generation vaccines (2, 3, 30).

Rift Valley fever

Rift Valley fever (RVF) is an insect-borne, multi-species zoonotic viral disease of livestock whose causative agent was first isolated in the 1930s. It had been exclusively confined to the African continent, but RVF spread to the

Middle East in 2000. It is considered a threat to other countries in the region such as Iran and Iraq, and possibly Pakistan and India (25). The disease also features on most lists of potential biological warfare agents due to its severe zoonotic nature.

The occurrence of the disease is usually reliant on the presence of susceptible animals, a build-up of the mosquito vector population (usually associated with heavy rains) and the presence of the virus. Since the development of the live attenuated Smithburn vaccine, vaccination has been used for the control of RVF in southern and East Africa.

There are currently two types of vaccines used for the control of RVF in domestic animals: a live attenuated vaccine and an inactivated vaccine. All currently used live attenuated vaccines are based on the Smithburn isolate, which was derived from mosquitoes in Western Uganda in 1944 and passaged 79-85 times by intracerebral inoculation of mice (this resulted in loss of hepatotropism, acquisition of neurotropism and the capacity to immunise sheep safely when administered parenterally) (90). The 103 and 106 mouse brain passage levels of the virus are used to produce the vaccine in cell culture in South Africa and Kenya respectively, using BHK cells. Millions of doses of this vaccine have been produced by Onderstepoort Biological Products (OBP) in South Africa since 1952 and by the Kenya Veterinary Vaccines Production Institute since 1960 and have been widely used in Africa (54, 94).

Rift Valley fever vaccines based on the Smithburn virus have several disadvantages: they may induce abortions, teratology in the foetuses of vaccinated animals, hydrops amnii, and prolonged gestation in a proportion of vaccinated dams. Being a live vaccine, the vaccine cannot be used during an outbreak. Even in endemic areas vaccination is often not sustained during years in which there have been no outbreaks. To address these problems, and also the poor antibody response to the Smithburn vaccine in cattle (5), an inactivated vaccine was developed and has been used for years in South Africa; it is suitable for use in all livestock species (including pregnant animals) and can be used during outbreaks. The inactivated RVF vaccine makes it possible to vaccinate cows that can then confer colostral immunity to their offspring. Given the poor immunogenicity of this vaccine in cattle, it requires a booster three to six months after initial vaccination, followed by annual inoculations (5).

Table 1 summarises the advantages and disadvantages of the different types of RVF vaccines.

The shortcomings of these inactivated and live attenuated vaccines have led to research into alternative new generation vaccines. A lumpy skin virus expressing the two immunogenic glycoproteins of RVF virus has been tested

Table 1
Comparative evaluation of different Rift Valley fever vaccines

Vaccine	Strain	Advantages	Disadvantages
Inactivated	Pathogenic field strain	Safe in pregnant animals Can be used in outbreaks	Short-term immunity Multiple vaccinations required Risk of handling virulent strain during production Colostrum immunity is poor Sheep better protected than cattle 100 × more antigen required than for live attenuated Long lead time for production and limited shelf life
Live attenuated	Smithburn	Highly immunogenic Single dose Good immunity (within 21 days) Effective and easy to produce Safer production	Only partially attenuated Teratogenic for foetus Potential risk of reversion to virulence Not advisable for use in outbreaks Theoretical possibility of transmission by mosquitoes?
Live attenuated	MP12	Effective and easy to produce Safe production	Teratogenic for foetus Abortion in early pregnancy Not available commercially
Avirulent natural mutant	Clone 13	Safe in pregnant animals Safe in outbreaks Produced as standard freeze-dried live vaccine Safe, effective and easy to produce	No registered vaccine yet available No large-scale field data yet available

in the laboratory and, to a limited extent, in target animals (103).

A live attenuated candidate vaccine strain, the MP12 (developed by mutations of a human isolate in the presence of the mutagen 5-fluorouracil) has been tested extensively and shown to be safer than the Smithburn vaccine (69). However, despite showing good immunogenicity in late pregnant ewes and young lambs, when tested in a more extensive vaccination trial the MP12-based vaccine resulted in abortions and/or severe teratogenicity when administered between day 35 and 50 in gestating ewes (54, 55). Earlier, an avirulent RVF virus isolated from a non-fatal case of RVF in the Central African Republic had been passaged in mice and Vero cells, and then plaque purified in order to study the homogeneity of virus subpopulations. A clone designated 13 did not react with specific monoclonal antibodies and when further investigated was found to be avirulent in mice yet immunogenic. The attenuation appeared to be the result of a large internal deletion in the NSs gene (70).

Clone 13 has been used to produce a vaccine that has been extensively tested in South Africa, with very good safety and efficacy results in cattle and sheep. The safety was shown in trials conducted in sheep synchronised for oestrus and artificially inseminated. After confirming pregnancy on day 30, all the ewes were vaccinated with a high dose of the Clone 13 vaccine (10^6 MICLD₅₀ [mouse

intracerebral lethal dose]): 7 on day 50 and 4 on day 100 post vaccination. Four pregnant cows were also vaccinated with the same dose of vaccine. None of the ewes or cows showed clinical signs of disease and no abortion occurred, with all dams giving birth to healthy offspring. While all unvaccinated control ewes aborted after virulent challenge, all ewes vaccinated with Clone 13 vaccine containing at least 10^4 MICLD₅₀ virus antigen gave birth to healthy lambs and did not show any clinical signs that could be associated with RVF (54).

Ongoing trials are being conducted in Africa with Clone 13-based RVF vaccine. Though still preliminary, the novel master seed for vaccine production appears to be a better alternative to the Smithburn-based vaccines, since a non-teratogenic vaccine will make it possible to envisage vaccination programmes in endemic regions of RVF where the unknown pregnancy status of animals will not be a constraint.

Bluetongue

Bluetongue is an arthropod-borne viral disease of sheep and cattle, caused by one or many of the 24 known serotypes of the bluetongue virus (BTV). The virus has been recognised as an important aetiological agent of disease in sheep in South Africa, and until 1943 was

believed to be restricted to Africa, south of the Sahara. The disease has since been identified in several countries outside Africa, such as Cyprus, Israel, the United States of America (USA), Portugal, Pakistan, India, Italy, France, Spain, the People's Republic of China, Malaysia, Bulgaria, Australia, Argentina, and, most recently Kazakhstan, as well as North African countries including Morocco and Tunisia. In 2006, the disease was reported for the first time in some northern European countries (Germany, Belgium, and the Netherlands). Bluetongue virus commonly occurs between latitudes 35° S and 40° N, but the virus has also been detected further north at beyond 48° N in Xinjiang, China, western North America and in Kazakhstan (35).

The factors contributing to the spread of BTV include animal migration and importation, extension in the distribution of its major vector, *Culicoides imicola*, involvement of novel *Culicoides* spp. vector(s), the ability of the virus to overwinter in the absence of adult vectors, and its persistence in healthy reservoir hosts such as cattle and some wild ruminants. The eradication of BTV from endemic regions of Africa is virtually impossible due to the role played by the widely distributed *Culicoides* spp. midge vectors, the multiplicity of serotypes that may circulate at any point in time, and the presence and ubiquitous distribution of reservoir species, both known and unknown. However, most indigenous breeds of sheep in sub-Saharan Africa are resistant to the disease.

Strategies for the control of BT depend on whether they are aimed at outbreaks of the disease in endemic areas or in areas where the disease is not usually present. In the latter case, the aim is usually eradication, whereas in endemic areas attempts can only be made to limit the occurrence of the disease and its economic impact, through vaccination. The initial BT vaccine was developed more than 50 years ago in South Africa and has been improved over that time to currently include 15 of the 24 serotypes known to occur in southern Africa (101). The current vaccine consists of live attenuated, cell-adapted, plaque-purified viruses in three pentavalent vaccines, which are administered separately at 3-week intervals. After two to three annual immunisations, most sheep are immune to all serotypes in the vaccine. An average of 8 million doses is used annually in South Africa, while a limited number of sheep are vaccinated in other southern African countries. Some concerns about the current vaccine have been raised in recent years. These include:

- the teratogenicity of attenuated BT vaccine strains resulting in brain defects in the foetus when administered during the first half of gestation (hence the recommendation not to administer the vaccine during the first half of pregnancy in ewes)
- the risk of reassortment and recombination between attenuated and virulent strains in the field

- the risk of transmission of attenuated viruses by vector midges or their release in the environment.

The risk of reassortment in the field is minimised by the long interval between the recommended vaccination period (i.e. late winter, early spring) and the BT season (summer), which would make the co-circulation of vaccine and virulent wild-type viruses unlikely.

Since the isolation of VP2 protein and the demonstration of its ability to induce protective immunity (53), and the subsequent discovery that other BT-viral proteins could also elicit protective immunity, different expression systems and combinations have been explored and tested in sheep with varying results. These have included baculovirus expression systems which use insect cell cultures and produce BTV-like particles (VLPs [virus-like particles] and CLPs [core-like particles]) (80). While eliciting protection against virulent challenges and having the advantage of easy discrimination between infected and vaccinated animals, the big challenge to date of new generation BT vaccine candidates is the difficulty of scaling up the production of these different vaccine antigens at affordable costs. The difficulty in achieving simultaneously protection against multiple serotypes, as is the case of BT in southern Africa, is another challenge to overcome.

During recent outbreaks of BT in the Mediterranean region, monovalent and various multivalent BT vaccines were custom-made and used as emergency vaccines by some affected countries (29). Concern about the importation of exotic virus vaccines – though attenuated – for use in a novel environment with unknown residual virulence, and differences in the genetics of sheep and their susceptibility to BTV have lead countries to opt for the inactivated or other forms of non-replicating vaccines. While inactivated BT serotype 2 and 4 vaccines have been developed (12, 29), produced and used, no recombinant-derived vaccine has yet been produced commercially.

African horse sickness

African horse sickness (AHS) is a vector-borne viral disease affecting all equidae, and resulting in high mortality in susceptible horses. Several of the nine known serotypes of the virus can occur during the outbreak season in southern Africa, the only region of the world where all the serotypes have been isolated. While AHS causes severe clinical disease in horses, with high morbidity and mortality, donkeys, zebras and mules usually suffer milder forms of the disease but may act as amplifiers of infection as they serve as sources of blood meals for haematophagous vectors. Probably on account of its larger number of horses and the rather temperate climate as compared to other countries in the region, South Africa has definite seasonal occurrence of AHS, with the first cases usually noticed

towards midsummer, and the disease disappearing abruptly after the onset of cold weather in autumn (23).

African horse sickness appears to be endemic in the tropical regions of Africa. The Sahara Desert forms a formidable barrier against northward spread, but on occasion, AHS outbreaks in northern African countries have occurred in regions with high concentrations of horses such as Morocco, the Horn of Africa (particularly Ethiopia and Eritrea) and across the Sahel of West Africa (in countries such as Senegal). The disease has also occurred outside Africa on a few occasions, the most notable being the major outbreak in the Near and Middle East from 1959 to 1963 and in Spain (1966 and 1987 to 1990) with subsequent extension into Portugal and Morocco.

After its first reported occurrence in South Africa in 1719, and the subsequent repeated outbreaks, including the most serious 1854 to 1855 outbreak in the Cape Colony that resulted in the death of 40% of the horse population, it became clear that vaccination would be the only way to protect horses in South Africa. The quest to study the causative agent of AHS and develop a vaccine was one of the reasons behind the establishment of the Veterinary Research Institute at Onderstepoort (north of Pretoria, South Africa) in 1908. The earliest attenuated vaccine was derived from subpassaging the virus some 100 times in embryonated eggs. Subsequently, additional serotypes of AHS virus have been identified and similarly included in the vaccine. Further changes were made to the attenuation process, by reducing the number of egg passages with subpassaging using suckling mice brains or through a cell line.

However, in the early 1980s problems were encountered, with some of the mice brain attenuated strains causing neurological problems in vaccinated horses. This led to the withdrawal of serotype 4 from the vaccine for a period, and a decision that all strains should be cell culture attenuated. Serotype 4 was re-introduced into the multivalent two-dose vaccine, but serotype 5 was withdrawn, and to date has remained absent from the current live attenuated vaccine. Several attempts were made to further attenuate serotype 5 by adaptation to a cell line, but the strain remains neurotropic when administered in combination with the other vaccine strains to horses.

In South Africa, around 150,000 doses of AHS live attenuated vaccine are used annually for a horse population of 350,000. Most horses and other equidae in rural disadvantaged communities do not receive vaccines. An increase in the occurrence of outbreaks in recent years, which have involved horses in these communities has called for the need to expand vaccination into such communities in order to break the cycle of spread of the infection. The recent outbreaks of AHS in South Africa

have also been characterised by either the re-emergence of serotypes that had been dormant for a few years, such as AHS serotype 5, or the appearance of virulent strains of serotypes that had previously been considered to be mild, such as AHS serotype 9. These events highlight the need for alternative and more comprehensive vaccines. Non-replicating, inactivated or recombinant polyvalent AHS vaccines with proven safety and efficacy are therefore required.

Inactivated AHS 4 vaccines have been extensively studied since the first commercial vaccine was produced in the early 1990s (52). Besides limited use in Spain, no large-scale use has since taken place. No work has been conducted in a multisero-type environment to determine the level of protection that could be achieved against all serotypes or different strains.

Through DNA recombinant technology, different subunit and CLPs have been tested as candidate vaccines. Indications from different studies are that such vaccines might require the incorporation of multiple viral proteins in order to stimulate protective immunity (37, 81). One such study was conducted by Martinez-Torreceda *et al.*; results indicated that VP7 was required for the baculovirus-expressed VP2, VP5 and VP7 combination to induce neutralising antibodies and protection (64).

Contagious bovine pleuropneumonia

Contagious bovine pleuropneumonia (CBPP) is an insidious pneumonic disease of cattle caused by *Mycoplasma mycoides* subspecies *mycoides* small colony variant (MmmSC). Phylogenetically, the organism is a member of the *Mycoplasma mycoides* cluster, which are pathogens of ruminants. Contagious bovine pleuropneumonia is primarily a disease of cattle with *Bos taurus* and *B. indicus* breeds being fully susceptible. The water buffalo (*Bubalus bubalis*) has a lower level of susceptibility and the African buffalo (*Syncerus caffer*) is not affected by the disease (97). The disease is characterised by the presence of sero-fibrinous interlobular oedema and hepatisation giving a marbled appearance to the lung in acute to subacute cases, and capsulated lesions (sequestra) in the lungs of some chronically infected cattle. Joint infections are common in calves. The occurrence of sub-acute/sub-clinical infections and chronic carriers after the clinical phase of the disease, creates major problems in the control of CBPP. With the exception of South America and Madagascar, the disease has occurred in most parts of the world at some point in time (97).

Contagious bovine pleuropneumonia is endemic in numerous areas of Africa, and is suspected to occur occasionally in the Middle East and possibly in some parts of Asia. North America, Europe, Australia and most parts

of Asia have eradicated the disease through slaughterhouse inspection and traceback methods, test and slaughter, and animal movement control. In the case of Australia, the adjunct, judicious use of vaccines was employed. In Africa, CBPP is found in an area south of the Sahara desert, from the Tropic of Cancer to the Tropic of Capricorn and from the Atlantic to the Indian Ocean. In the early seventies, the CBPP disease situation appeared to be under control. However, after almost 20 years of respite, CBPP made a spectacular comeback on two major fronts – one in the east of the African continent and the other in the south. Almost at the same time, there was a resurgence of the disease in previously known endemic areas of West Africa. It is endemic in the pastoral herds of much of western, central and eastern Africa, Angola, northern Namibia and Zambia. Botswana, Lesotho, Madagascar, Malawi, Mauritius, Mozambique, southern Namibia, South Africa, Swaziland and Zimbabwe are currently (2006) free from the disease (42, 96). Contagious bovine pleuropneumonia represents a major constraint to cattle production in Africa and is regarded as the most serious infectious animal disease affecting cattle, now that rinderpest is almost eradicated (with the possible exception of the Somali ecosystem) from the continent.

In the 1960s and 1970s, sustained research on CBPP vaccines in Kenya (Muguga), Chad (Farcha), Nigeria (Vom) and other African countries, coupled with a large multi-donor funded international campaign – known as Joint Project 16 – resulted in the disappearance of clinical disease from most parts of Africa. Various strategies have been used in the continent to control CBPP and these include the stamping-out of infected herds and targeted/mass vaccinations coupled with the use of antibiotics (although official policy on using antibiotics to control CBPP suggests the contrary). In view of the epidemiological situation of the disease, most CBPP endemic countries control the disease by vaccination, which is usually carried out by the official Veterinary Services. In the past, vaccination was practised in some parts of Africa by using traditional methods such as placing a piece of diseased lung tissue under the skin on the bridge of the nose of susceptible cattle (96). In modern times, immunisation has been refined by attenuation of the immunising agent. Attenuated vaccines against CBPP have been developed by multiplying the CBPP agent in heterologous host such as embryonated chicken eggs and later by serial subculture on artificial *Mycoplasma* growth media (51, 88). The efficacy of live vaccines is directly related to the virulence of the original strain of MmmSC used for their production. Attenuated virulent strains of MmmSC stimulate the best immunity (65) but they also induce the most severe and undesirable local and systemic reactions, which may even result in the death of the animal. The live attenuated vaccines currently in use are a compromise between virulence, immunogenicity and safety. In the past, it was thought that if vaccination were

to be effective, a local lesion had to be produced at the site of inoculation. This led to strong resistance from farmers, who feared their cattle would die as a result of post-vaccinal reactions. Among the many vaccinal strains developed during the CBPP outbreaks in Australia and Africa almost 40 years ago, those currently in use in Africa are the T1 vaccine strain, isolated in Tanzania and passaged 10 times in embryonated eggs and then 44 times in broth cultures (T1/44), and its streptomycin-resistant derivative, T1/SR (15, 76). Broth culture vaccines have been replaced by lyophilised vaccines. The combined rinderpest/CBPP vaccine (Bisec) was actively used in many parts of West Africa to control the two diseases. The discontinuation of rinderpest vaccination and the disuse of Bisec have contributed in large part to the resurgence of CBPP in parts of West Africa, because many countries were no longer able to pay the recurrent costs involved in getting vaccine teams into the field to continue vaccination against CBPP. Vaccination failures with T1/SR vaccine in Botswana and parts of East Africa prompted concerns about the efficacy and/or identity of the strain being used for vaccine production. The question of identity was resolved by the use of molecular epidemiological tools which allowed its characterisation and distinction from the KH3J vaccine strain (60). The two predominant vaccine strains of T1/44 and T1/SR were re-evaluated for efficacy and immunogenicity (95). In these studies naïve cattle were vaccinated with vaccine containing the minimum dose of 10^7 viable mycoplasma organisms per dose, as recommended by the OIE (104), in three locations in Africa (Cameroon, Kenya, Namibia) and challenge studies were carried out. Primary vaccination induced a protection rate of 40% to 60% wherever it was used. The duration of immunity was found to be longer with T1/44 than with T1/SR and revaccination after one year enhanced the level of protection (80% to 90%).

Mycoplasmas are prone to frequent genetic variations, so to prevent varying levels of immunogenicity in the final product great care should be exercised in CBPP vaccine production so that the cloning procedures do not cause antigenic drifts (22, 63). Simple modifications to current accepted protocols include:

- a) buffering the growth medium so that neutral pH is maintained thereby attaining the minimum of 10^7 viable mycoplasma organisms per dose;
- b) including a pH buffer in the vaccine so that deleterious acidification would be visibly recognised and ineffective or damaged vaccine discarded;
- c) changing the reconstitution method for freeze-dried vaccine to exclude 1 M $MgSO_4$ which could be substituted with phosphate buffered saline. It has also been argued that improving the quality of the existing CBPP vaccines is more likely to deliver significant beneficial effects than

developing a new generation of vaccines, which could be an expensive and time-consuming process.

The thermostability of CBPP bacterins has been an area of great concern considering that the vaccines have to be transported over long distances to remote areas where cold chain maintenance is difficult. A new live vaccine dehydration and preservation technology, called *xerovac*, was developed at the Pan African Vaccine Centre (PANVAC) in Ethiopia (107). This method, which mimics the survival strategies of cryptobiotic organisms, could be applied to vaccine production to yield thermotolerant vaccines. Litamoi *et al.* demonstrated that rapid dehydration of CBPP vaccine in an excipient composed of a high concentration of trehalose (25%) following the *xerovac* method rendered the CBPP vaccine product more heat tolerant than similar vaccines prepared by conventional methods (59). Trehalose is one of the most chemically unreactive and stable sugars and is very stable to hydrolysis. They also demonstrated that the addition of chitosan as a mycoplasma precipitating agent, conferred additional heat resistance to the vaccine, although the titre of mycoplasma dropped (due perhaps to the greater need to homogenise chitosan and mycoplasma cultures) and there was a consequent titre loss due to the fragility of mycoplasmas. These findings require further studies to establish a more suitable MmmSC culture medium with low solute concentration, and substitution of glucose with trehalose in the culture medium for CBPP vaccine production. The establishment of an independent laboratory, such as PANVAC under the auspices of the African Union, would be in a position to certify CBPP and other animal disease control vaccines and contribute to quality control and a better CBPP vaccine product (59, 107).

Haemorrhagic septicaemia

Haemorrhagic septicaemia (HS) is a peracute to acute highly fatal bacterial disease, principally of cattle and water buffalo (*B. bubalis*), caused by specific types of *Pasteurella multocida*. The disease is endemic throughout southern and south-eastern areas of Asia and many regions of Africa. Haemorrhagic septicaemia was traditionally regarded as being caused only by serotype B or E, but other serotypes are recognised as causing the disease, particularly serotypes B:1 and B:3,4 (9). The two common serotypes of *P. multocida* associated with the disease are types B:2 (in Asia) and E:2 (in Africa). The Asian B:2 serotype has also been associated with sporadic septicaemic disease in pigs. In Egypt and Sudan, the presence of both B and E serotypes has been reported (89). Outbreaks of HS are usually limited in extent and tend to be associated with conditions of stress. The Asian form of HS occurs in countries with high seasonal rainfall and is usually endemic in marshy zones or along river deltas. The diagnosis of HS depends on the isolation of the causative

organism, *P. multocida*, from the blood or bone marrow of a dead animal by culture and biological methods, and the identification of the organism by biochemical, serological and molecular techniques. Since HS is primarily a bacterial disease, it should theoretically lend itself to effective antibiotic therapy. However, treatment is constrained by a number of factors. The acute nature of most cases of the disease limits the efficacy of antimicrobial therapy of sick animals, but it can be effective if they are detected and treated in the early stages of the disease. As the disease occurs in places with substandard husbandry practices, most cases escape early detection. Vaccination, therefore, appears to be an alternative effective control option. A solid, long-lasting immunity is conferred on animals that recover from the natural disease, which persists longer than that induced by vaccination (26).

Haemorrhagic septicaemia is preventable using vaccines containing the causative bacterial agent. However, since *Pasteurella* is a poor immunogen, a large amount of antigen (whole bacterial cell) has to be administered. This procedure occasionally leads to endotoxic shock (26). There are three types of bacterins used against HS: alum-precipitated vaccine, oil-adjuvanted vaccines and formalinised bacterins. Some significant success in the control of HS has been achieved in Asian countries by the immunisation of buffaloes and cattle with alum-precipitated or oil-adjuvant bacterins (19). Immunity is, however, of short duration, lasting from six to nine months on primary vaccination and 12 months after secondary vaccination. Large-scale vaccination of cattle against HS is not practised in many countries of Africa. An outbreak of HS in Zambia in 1979 was largely controlled by using formalinised bacterin obtained from Sudan (45). It is advisable to use bivalent vaccines (B and E) in Eastern and Central Africa because of the presence of both B and E serotypes. Despite the fact that the alum-precipitated vaccine is known to provide immunity of short duration, it is still the most common vaccine in use, since it is the easiest vaccine to inject. The oil-adjuvant vaccines, though known to be more potent, are difficult to inject on account of their high viscosity. During the past decade, a considerable amount of research has been done in South Asia aimed at producing oil-adjuvant vaccines of low viscosity. It is known by one of the authors (W. Amanfu) that Sri Lanka and Indonesia have successfully used lower levels of lanoline as the emulsifying agent in an effort to reduce viscosity. Malaysia has modified the use of the alum-precipitation technique to concentrate broth cultures in order to reduce the dose volume of the oil adjuvant in the vaccine formulation, which they believe will facilitate injection (unpublished data). In India, at least one vaccine producer is marketing a combined FMD-HS-Blackquarter oil-adjuvant vaccine.

A live heterotypic vaccine made with *P. multocida* serotype B:3,4 isolated from a fallow deer (*Dama dama*) in the

UK (56) protected cattle against a serotype B:2 challenge and conferred immunity against HS for one year in cattle vaccinated subcutaneously (72). The intranasal vaccine has been recommended by the Food and Agriculture Organization of the United Nations (FAO) as a safe and potent vaccine for use in Asian countries based on trials in Myanmar. However, there is no report of its wide-scale use in other countries and inactivated bacterin preparations are preferred. The safety, efficacy and cross-protection of a live intranasal HS bacterin containing *P. multocida* serotype B:3,4 were further tested in young cattle and buffaloes in Myanmar (73). In this study, the administration of 100 times the recommended dose to 50 cattle and 39 buffalo calves was innocuous. Seven months after vaccination, all 39 buffaloes were protected and 12 months after vaccination, three out of four buffaloes were protected against a subcutaneous challenge with serotype B:2, with vaccinated cattle developing serum antibodies detectable by the passive mouse protection test. The serum of vaccinated cattle cross-protected passively immunised mice against infection with *P. multocida* serotypes E:2, F:3,4 and A:3,4. This finding could be studied further in parts of Africa where both serotypes B:2 and E:2 occur. The recent adoption (2005) of standards by the OIE on the requirements for vaccines and diagnostic biologicals for HS should provide the necessary technical platform for the production and quality assurance of HS vaccines (105).

Brucellosis

Brucellosis is caused by bacteria of the genus *Brucella* which are Gram-negative non-spore-forming non-encapsulated coccobacilli or short rods with rounded ends. The disease is characterised by abortion, retained placenta, orchitis and infection of accessory sex organs in males. Arthritis and hygromas may be seen, especially in cattle. The disease is prevalent in most countries of the world, and primarily affects cattle, buffalo, pigs, sheep, goats, dogs and some wild terrestrial and marine mammals. As a zoonotic disease, brucellosis is of serious public health significance, particularly those infections caused by *Brucella melitensis*, which in man has the most virulent characteristics of all the brucellae. In cattle, brucellosis is primarily caused by *B. abortus*, but in some countries, particularly in southern Europe and in the Middle East, *B. melitensis* has been implicated as a cause of abortions especially when cattle are kept in close contact with infected sheep or goats. Occasionally, *B. suis* may infect the mammary gland of cattle, but this has not been associated with abortions in cattle. *Brucella melitensis* commonly causes caprine and ovine brucellosis, whilst *B. ovis* causes ram epididymitis. *Brucella suis*, which causes porcine brucellosis, consists of five biovars. Brucellosis in pigs is characterised by an initial bacteraemia followed by the development of chronic lesions in the bones and reproductive organs. Other brucellae organisms of

significance are *B. canis*, which causes epididymitis and orchitis in male dogs and metritis in bitches, and *B. neotome*, which was isolated in rodent species in the USA. Most of the serological tests for isolation of smooth *Brucella* spp. infections (*B. abortus*, *B. melitensis*, and *B. suis*) have been developed to detect antibodies directed against antigens associated with the smooth lipopolysaccharide (S-LPS) and are shared by all the naturally occurring biovars of *B. abortus*, *B. melitensis*, and *B. suis* (24). Since *B. abortus* antibodies may cross-react with those against *Escherichia coli* O157 and *Yersinia enterocolitica* serovar 0:9, false positive reactions may be important during the final stages of an eradication programme. In rough brucellae such as *B. canis* and *B. ovis*, specific antigens associated with the rough lipopolysaccharide (R-LPS) have to be used for the diagnosis of infections caused by these organisms.

Cattle vaccines

The most widely used vaccine for the prevention of brucellosis in cattle is the *B. abortus* Strain 19 (S19), which remains the reference vaccine to which other vaccines are compared. The standards and administration of S19 vaccine in cattle, have been well described (106). Briefly, the S19 vaccine is used as a live bacterin and is normally given to female calves between 3 and 6 months of age as a single subcutaneous dose of $5-8 \times 10^{10}$ viable organisms. A reduced dose of from 3×10^8 to 3×10^9 organisms can be administered subcutaneously to adult cattle. Some animals may develop persistent antibody titres, may abort and excrete the vaccine strain in the milk. Alternatively, the vaccine may be given at any age as two doses of $5-10 \times 10^9$ viable organisms, given by the conjunctival route. This produces protection without a persistent antibody response and reduces the risks of abortion and excretion of live vaccine strain *B. abortus* in the milk. Vaccination with S19 bacterin increases resistance to *B. abortus* but does not induce sterilising immunity. If an animal is infected, vaccination will not cure the infection. The increase in resistance following vaccination has been termed *relative immunity* since it is estimated to be only about 70% effective against field challenge by preventing unrestricted multiplication of *B. abortus* in the uterus and mammary gland (74). The main disadvantage of S19 vaccination is the induction of post-vaccinal antibodies that are detected in serological tests. Currently, there is no single fully validated test that can be used to distinguish between antibodies due to infection and those due to vaccination, although newer tests and combinations of tests have been developed to attempt to overcome this problem (49).

Brucella abortus rough strain vaccine RB51 has been the official vaccine used in the USA since 1996 for the prevention of brucellosis in cattle (87). Protocols for use of this bacterin in the USA have been reviewed (49). However, some countries in Latin America have officially

adopted the use of RB51, but use different regimens. The vaccine strain is a rough rifampicin-resistant mutant of *B. abortus* strain 2308, of the virulent *B. abortus* biovar 1 strain. This mutation has been described as very stable, with no reversion to smoothness *in vitro* or *in vivo*. Some studies have been conducted in cattle to compare the vaccine potency of RB51 with that of S19, with a general conclusion that RB51 does not significantly induce a higher degree of protection than S19 and its superiority to S19 still remains controversial (21). An advantage of using RB51 vaccine, however, is that antibodies induced by its administration are not detected by the currently prescribed serological methods. *Brucella abortus* S19 and RB51, although attenuated strains, are still capable of causing disease in humans, and in the case of RB51, the organism is resistant to rifampicin, one of the drugs of choice for treating human brucellosis.

Strain 45/0 of *B. abortus* was isolated in 1922 and after 20 passages in guinea pigs a rough derivative isolate named 45/20 was obtained. Strain 45/20 has been used as an inactivated vaccine incorporating an oil adjuvant, but this is not as protective as S19. In addition, large unsightly granulomas developed after use in some instances and its use has been discontinued (49).

Small ruminant vaccines

In small ruminants, the *B. melitensis* Rev. 1 Elberg strain has been recognised as a superior bacterin, compared to *B. abortus* S19 and *B. suis* S2 (11). The Rev. 1 bacterin not only protects against *B. melitensis* but it also protects other animal species against *B. abortus* or *B. suis*. In spite of this, the traditional approach is still dominant: homologous vaccines, e.g. *B. abortus*, *B. melitensis*, and *B. suis*, are administered to each of their principal hosts, respectively. Due to the ability of Rev. 1 to induce abortion and the fact that the organism can be excreted in the milk, it is suggested that the vaccine be administered subcutaneously prior to the first gestation at 3 to 7 months of age. This regimen could lead to long-lasting persistence of specific antibodies, which could create serious problems in the serological diagnosis of the disease (43). When used as a flock vaccination programme, inoculation of *B. melitensis* Rev. 1 greatly decreases the prevalence of brucellosis in goats and sheep, and hence reduces brucellosis in human populations. When Rev. 1 is administered by the conjunctival route, the immunity conferred is similar to that induced by the standard method, but the serological response evoked is significantly reduced (4). Production of *B. melitensis* Rev. 1 vaccine is based on a seed lot system where master seed cultures must be obtained from OIE/FAO Reference Laboratories, and must conform to minimal standards for viability, smoothness, residual infectivity and immunogenicity.

Future outlook: novel biotechnological vaccines

Vaccination is one of the most important and cost-effective methods of preventing infectious diseases in animals (reviewed in 79). Currently, the majority of licensed bacterial and viral vaccines are either live attenuated or inactivated. Live attenuated vaccines are very efficient in inducing long-lasting immunity via cell-mediated and humoral immune responses. These vaccines, however, do have disadvantages when given to pregnant and immunocompromised animals, and some have the potential to revert back to virulence (i.e. RVF). Inactivated vaccines cannot replicate and are thus non-infectious but also lack the ability to induce a long-lasting and comprehensive, especially cell-mediated, immune response. They are thus often regarded as inferior in stimulating immunity in comparison with live attenuated vaccines, although their negative effects are less severe.

Because of the globally increasing qualitative and quantitative demands for livestock and their products, vaccine producers are increasingly being required to fulfil a set of prescribed specifications. These include ensuring that the protective antigens used during the production of validated attenuated vaccines are free from pathogen-associated toxins and immunosuppressive components and are capable of eliciting long-lasting immunity. Recombinant subunit, DNA and non-pathogenic virus-vectored vaccines are currently the most cost-effective methods of producing antigens that are free from the exogenous materials that are associated with conventional vaccines.

Live vaccines

Live vaccines can be regarded as the most successful category of vaccines available and include not only conventional attenuated vaccines, but also gene-deletion attenuated and recombinant virus-vectored vaccines. The advantages of attenuated vaccines include their low reactivity and their ability to induce protective systemic, as well as mucosal, immune responses. In addition, there are low manufacturing costs due to minimal downstream processing and the fact that adjuvants are not required for their formulation. The overall safety profiles of conventional attenuated live vaccines fall short of what is desired from an ideal vaccine. Conventional live bacterial and viral vaccines are produced by selecting attenuated mutants which have the capacity to induce infection but have a reduced or non-existent ability to induce disease. In most attenuated vaccines, attenuation is achieved by blind serial passage in heterologous tissues (cell cultures, eggs, laboratory animals or broth cultures). But also mutation is induced spontaneously either by chemical treatment,

heating or spontaneous mutagenesis (and clonal selection). It is important to realise that mutational attenuation is an uncontrollable process and the induced mutations are rarely characterised at the genomic level. Therefore, it is very difficult to control the degree of attenuation. Reversion to virulence is the greatest potential risk of attenuated live vaccines, causing not only potential disease, but also possible shedding of organisms into the environment. Despite these risks, there are many examples of safe and effective attenuated veterinary vaccines in current use. The global eradication of rinderpest is about to be achieved through the wide use of such live attenuated vaccines of undetermined genetic definition. Recent advances in genetic engineering have not only enabled the identification of genes associated with pathogenicity or virulence in infectious organisms, but have also allowed for the deletion or inactivation of selected target genes, thereby increasing the safety profile of candidate vectored vaccines. The first commercial live gene-deletion attenuated vaccine was a glycoprotein E deleted (gE-) pseudorabies (Aujeszky's disease) vaccine that is currently used for eradication programmes in Europe and the USA. More recently, a gE-gG-TK- attenuated pseudorabies vaccine has been developed. It seems highly likely that these vaccines will become more prevalent in the future once they are perceived as being safer than conventional attenuated vaccines, as their degree of attenuation can be more effectively managed. The delivery of heterologous antigens via a recombinant live vector offers significant advantages as part of a comprehensive vaccination strategy, in that the recombinant organism would only induce a mild infection and subsequently induce immune responses to the gene derived from the pathogenic organism (91). Regardless of the vector organism used, expression of the heterologous (protective) antigen by the recombinant vector is the key to effective antigen presentation and induction of an adequate immune response. Currently, it is possible to use viruses, bacteria and even parasites as live recombinant vectors. The first recombinant live viral vector evaluated was vaccinia virus, a poxvirus that has since been used to express viral, bacterial and parasitic antigens that have been reported to elicit protective immunity in several animal disease models. Vaccinia virus demonstrated efficacy as a recombinant live viral vector in experimental trials when virus expressing rabies gG as a surface antigen was orally delivered to foxes and other wild animals. Vaccinia virus expressing pseudorabies gD as a surface antigen delivered intramuscularly induced an effective immunological response very similar to a conventional inactivated vaccine. Currently, a number of pox-based vectored vaccines are being marketed. Viruses with large genomes, such as vaccinia and adenovirus, are better candidates for recombinant viral vectors than smaller genome viruses, due to the fact that they can accommodate substantially larger inserts of foreign DNA while retaining their infectivity. These viruses present a cost-effective option for vaccination strategies since their

genomes have been sequenced and because commercial expression vectors are available and can be cultured to very high titres resulting in reduced production costs. An additional advantage of using recombinant live vaccines is the possibility of producing multivalent vaccine, which means that more than one disease can be addressed at the same time and the cost of vaccination campaigns can be reduced. This is important for developing countries where infrastructures are poor.

Despite very promising results with live vectored vaccines there are also some concerns. The major concern is that live vector vaccines will not induce adequate immunological responses in animals that have pre-existing antibodies against the vector, with some exceptions. Genes of interest or their fragments, can not only be added but also deleted from a genome. The first gene-deleted live attenuated pseudorabies vaccine was a naturally occurring mutant, lacking glycoprotein E (gE-), known as the Bartha vaccine, which has now been used for decades in controlling pseudorabies virus in pigs. The Bartha vaccine was subsequently recognised for its ability to differentiate vaccinated animals from naturally infected animals, effectively making it the world's first marker vaccine. Indeed, in order to evaluate the effectiveness of a disease eradication programme, an insertion, mutation, or detection method is required in order to differentiate vaccinated (immune) animals from naturally infected animals (DIVA). This is achieved by developing vaccines that lack one or more antigens, or that have one or more extra antigens, or that have one or more detectable changed antigens, thereby inducing an antibody response (or lack thereof) in vaccinated animals that could subsequently be used to serologically differentiate between infection that was induced by a wild-type infection and infection induced by vaccination. These DIVA vaccines are important tools in assessing the effectiveness of vaccination programmes, without the time-consuming and near impossible task of individually evaluating infectivity, transmissibility and susceptibility within a vaccination programme. With the availability of recombinant technologies and sequenced pathogenic genomes the identification of potential markers is more feasible than ever before. It seems logical that vaccines developed in the future will increasingly embrace DIVA principles in order to provide a much needed tool for livestock disease prevention or eradication programmes. Currently, numerous national animal disease eradication programmes based on the serological confirmation of infection alone require the destruction of herds to limit the spread of disease. Due to a reliance on serological confirmation for absence of disease or infection, a vaccination programme is actually incompatible with surveillance. Thus, vaccination programmes are often banned, along with animals originating from countries vaccinating for certain diseases. However, the application of the DIVA marker technology creates a compatibility between surveillance and

vaccination programmes, allowing vaccination to play a large and extremely significant role in the eradication of these diseases. Several marker vaccines are already commercially available, and their role and contribution in disease eradication appears promising. For them to be embraced by the industry, however, requires support from governments and the livestock industry to ensure that the biopharmaceutical industry develops these vaccines. The move to eradicate diseases is not only driven by the financial considerations of the producer, but is often politically motivated: once a country is declared disease-free, the country can use this disease-free status as an effective trade barrier, making such vaccines even more attractive. The upcoming technology of reverse genetics, which relies on full-length genetic sequences created *de novo*, offers great promise in vaccinology, as precise and stable manipulations can be made, with control of the transcription and translation processes.

Inactivated vaccines

Molecular biology and genetic engineering have had an enormous impact on vaccine development by providing the tools and techniques to produce a single protein in a prokaryotic or eukaryotic system. Furthermore, if the protein is produced in prokaryotic systems, it can be tailored in such a way that the protein of interest is either expressed on the surface of the bacteria, in the periplasm, as insoluble inclusion bodies or secreted into the media. The recombinant approach to subunit vaccines is to clone the gene that encodes the protective antigen into a secondary, preferably non-pathogenic, organism that is capable of expressing the immunogen in its native form or with minimal alterations. This protein can then be expressed and harvested using traditional bacterial antigen production methods, or delivered by a live non-pathogenic vector. Recombinant subunit vaccines eliminate the risks associated with handling pathogenic organisms as well as the risks associated with live or inactivated products either reverting or still possessing a pathogenic or virulent state due to incomplete inactivation or attenuation. In all subunit vaccine approaches, the identification of proteins or epitopes involved in eliciting a protective immune response is crucial. Enormous advances in computer modelling and bioinformatics have made the rapid identification of protective or critical epitopes possible, including cross-species identification of functionally similar proteins. The power of recombinant technology lies not only in single protein or epitope subunit vaccines, but also in generating fusion epitopes. The possibility even exists that multiple protective epitopes could be cloned from a variety of pathogens to create a single protein. This 'string of beads' vaccine should be capable of inducing protective immunity to a wide range of viruses in a single subunit. The combination of genomics, bioinformatics and recombinant technology has even allowed for the development of vaccine candidates before the pathogen

could even be cultured (78). As an example, it is still not possible to culture (human) hepatitis B virus, yet a human vaccine has been available for over a decade.

Commercial production of a recombinant subunit vaccine requires the selection of an appropriate expression system based on the nature of the protein being expressed. Critical factors in the selection of a biopharmaceutical expression system include the production of an immunologically protective epitope, affordable protein production, affordable extraction and cleanup, minimal immunological interference from host proteins and minimal pyrogen production. For the production of non-glycosylated proteins, bacterial expression systems are excellent candidates. Organisms such as *E. coli* and *Salmonella typhimurium* have been used extensively for the expression of a wide variety of foreign genes and as a result many production, stabilisation and optimisation strategies have been described. While prokaryotic expression is efficient and affordable for the production of a broad range of immunogens, including a few natively glycosylated proteins, production of many viral glycoproteins in prokaryotic systems does not result in protective immunity due to the lack of glycosylation, despite producing significant immune responses. Additionally, the presence of lipopolysaccharides and other pyrogens leads to various complications, including interference and possible injection-site reactions. Thus, for the expression of glycoproteins and other modified proteins, eukaryotic expression systems are far more suitable. Examples of such systems include those that use yeast, insect cells, plants and mammalian cells, which have been systems of choice for producing many immunogens. Although expression of proteins in mammalian cells is generally expensive, for some viral glycoproteins availability of such expression systems is critical. This is especially true where post-translational modification such as glycosylation of nascent proteins is important for proper folding and generation of specific epitopes. Viral expression systems remain the preferred method of commercial production of native glycoproteins. This technology was originally demonstrated with vaccinia virus as the vector, but almost any virus can be used as an expression system for producing either whole proteins or epitopes.

The molecular breakthroughs in cell transformation and gene therapy have contributed to the development of the new field of DNA vaccinology, with its enormous potential for providing safe, inexpensive and effective DNA-based vaccines. The basic concept of DNA vaccine use is the delivery of plasmid DNA, which encodes for immune stimulating and protective proteins, into the cells of the host animal, where direct transcription and translation occurs – effectively transforming the vaccinated animal into a bioreactor for the production of its own vaccine (36). The fact that the protein is produced within the host means the vaccine should be correctly modified post-

translationally and as such should possess the authentic conformational and chemical structure (i.e. glycosylation) and, thereby, functional and immunostimulatory epitopes. Despite the fact that the antigen is produced by the host's cellular machinery, the immune system recognises the protein as being foreign and mounts both a cellular and humoral immune response very similar to one induced by live vaccines or in animals that have recovered from natural infection. Although CpG motifs, which are commonly present in DNA vaccines, induce a wide range of cytokines, it seems that Th1-like or Th2-like responses are not equally proportional, with Th1 induction exceeding Th2-like responses.

The type of immunological response that follows vaccination depends not only on the gene being introduced but also on the site of administration and the method of delivery. One of the major problems of current vaccines is their inability to elicit active immunity in neonates possessing passively acquired maternal antibodies. DNA vaccines have been shown to effectively circumvent maternal antibody interference. Furthermore, since the mucosal immunological system functions as an inter-related system, the delivery of a DNA vaccine at one mucosal site should induce at least partial immunity at other mucosal sites and may in fact serve to prime the immune system prior to vaccination with a traditionally delivered, inactivated or subunit vaccine. A large number of experimental DNA-based veterinary vaccines have been evaluated in a number of species with varying degrees of immunological response and efficacy against challenge. Despite promising results, some questions have been raised regarding the safety of DNA vaccination. Possible integration of plasmid DNA into the host genome and potential risk of malignancy and integration of foreign genes into germ cells could potentially lead to vertical gene transmission and expression. Additionally, the potential exists for the development of antibodies against the DNA vaccine itself and thus for the induction of autoimmunity as a consequence, although no evidence exists for such an event occurring, even when milligram quantities are successively injected. Despite the lack of scientific evidence to substantiate these plausible risks from DNA vaccination, it would be unwise to rule them out entirely and it is reasonable to expect regulatory authorities to impose cautionary measures in order to avoid these situations.

Vaccine formulation and delivery and stimulation of mucosal immunity

Subunit or inactivated vaccines require specific adjuvants in order to elicit an immunological response tailored to mimic responses induced by natural infection. It has been generally accepted that the optimal protective response is achieved when the vaccine is administered via the same route by which the infection enters the body (32, 33). The

correct formulation is therefore essential in the development of an effective vaccine, as the adjuvant must be compatible with the route of administration and complementary to the antigen. Today's highly efficacious and safe vaccines, be they recombinant or conventional inactivated vaccines, would not be available if it were not for the adjuvants and delivery systems developed over the past three decades. A variety of chemical and biologically derived compounds have been added to vaccines in order to increase the elicited immunological response, including aluminium salts, mineral oil, cholera toxin and *E. coli* labile toxin. More recently, several classes of compounds, including immunostimulatory complexes (ISCOMs), liposomes, virosomes and microparticles have been employed to act as antigen delivery vehicles and they are proving to be potent adjuvants, greatly enhancing the magnitude and the duration of the immunological response to the formulation. An efficacious response to non-replicating vaccines (subunit and inactivated) is entirely dependent on the adjuvant. Vaccines have traditionally been administered via intramuscular or subcutaneous injection. However, both intramuscular and subcutaneous injection routes share a disadvantage in that although they can induce comprehensive systemic responses, only poor mucosal immunity is elicited. Mucosal immunity and the production of local IgA antibodies are central to prevention of pathogen penetration of mucosal surfaces, the major route of infection for numerous diseases (e.g. FMD, classical swine fever, brucellae, influenzas, etc.). Administration of vaccine onto mucosal surfaces such as those in nasal passages, eyes, lungs and the gastrointestinal tract is an effective way of inducing mucosal immunity. It is important to emphasise that all mucosal sites are interconnected by a common mucosal immune system and that the administration of protective antigens at one primary site will stimulate antigen-specific lymphocytes which migrate and provide immunity at other mucosal sites, regardless of the site of induction. In veterinary vaccinology, the delivery of live attenuated organisms to mucosal surfaces has proven to be very effective.

Public/private partnerships

Private industry/Government partnerships

Since 1999 several studies have predicted a growing demand for livestock products due to population growth, increased urbanisation and economic growth, primarily in what are now developing or middle income countries (27, 28, 40). A combination of growth in animal densities, intensification of production methods, the globalisation of trade, climate change and the movement of people and animals is increasing the risk of international spread of infectious animal diseases. Recent assessments point to the

burden of infectious diseases continuing to be a major constraint to sustained international trade in livestock commodities from developing countries unless targeted sanitary measures are instituted in those countries to reduce the burden of infectious diseases (16, 38, 39, 75, 82, 84).

Varying levels of disease risk in different countries mean that there are likely to be differences between the specifications and requirements for vaccine use in the Organisation for Economic Co-operation and Development (OECD) countries – where major infectious diseases (e.g. FMD) are generally absent – and those required by developing countries for progressive disease control from an endemic status. For example, in the case of FMD vaccines, the requirement for OECD countries is likely to be for vaccines prepared from highly purified and concentrated antigens with highly purified or synthetic adjuvants or immunomodulators. Such vaccines would be required to be powerful enough to block infection and induce a rapid onset of immunity or immediate (but not necessarily long-lasting) protection (50, 61, 62, 67, 93). By contrast, the vaccines needed for controlling endemic FMD in developing countries are similar to the type of moderate potency vaccines that were successfully used in Europe and South America some 20 years ago to control FMD and which induce long-lasting immunity, i.e. 12 months or longer (6, 7, 30, 48). A second example is the growing tendency for OECD countries to move away from the use of live attenuated vaccines, especially those derived from RNA viruses, for fear of mutation/recombination and reversion to virulence, whereas these have been the bedrock for disease control programmes and are likely to continue to be the most cost-effective tools for disease control in developing countries in which the disease has endemic status. Such differences in intrinsic specifications are likely to weigh heavily in considerations for technology transfer from OECD countries, or from Middle Income Countries such as those in South America, to vaccine manufacturing units in the Low-Income-Food-Deficit-Countries (LIFDCs). The technology transfers are likely to be facilitated through judicious public/private sector partnerships in which individual livestock owners may have to pay for the vaccine, but also have access to a better future through the establishment of associations or cooperatives, methods to increase commodity manufacture, and improved production and animal hygiene methods. This means that livestock owners can therefore afford more from the service industries (i.e. veterinary care, purchase of preventive medicine) and will experience a general improvement in their livelihoods. Technology offers opportunities for the commercial sector in the LIFDCs as long as stakeholders or shareholders (investment) are patient and hold a medium to long-term vision. Should commercial vaccine selling interests be based on immediate purchases – profits are likely to be short-lived.

An additional factor in vaccine demand, and therefore public/private partnership opportunity, is likely to be in the range of vaccine requirements. For example, the requirement for vaccines in OECD countries is likely to be for a limited range of vaccine types, targeting only those highly contagious diseases, such as FMD, that pose a high risk of spread either through globalised trade or factors associated with climate change (e.g. RVF, bluetongue, AHS). Countries of sub-Saharan Africa are likely to have a requirement for a much wider range of vaccines than even in other developing regions of the world for the simple reason that Africa has the heaviest burden of infectious and protozoal diseases in the world (84). For this reason, the future demand for vaccines in developing countries is not likely to be one that can be addressed simply by importing vaccines produced in, and for the needs of, OECD countries or by relocating vaccine production plants from OECD to developing countries. Future vaccine requirements in developing countries are likely to be met in a variety of ways, including technology transfer, development of vaccines that are specifically tailored for the disease epidemiology in developing countries, and investment at the local level, as has been the case in Latin America – though at times this investment has focused on making profits in the short-term rather than on building longer-term relationships between farmers and the vaccine-production industry. What would be beneficial are public/private partnerships which take into account the socio-economic dynamics and recognise the requirement for public service commitment in a commercially profitable environment.

Public/private partnerships for vaccine manufacture

The premise is that vaccine production and marketing are most sustainable when undertaken in some form of commercial environment. In developing countries, experience has shown that neither relying on purely market forces nor on governmental production and free distribution of vaccines in total disregard of commercial practices is sustainable. Vaccine production and distribution in a conventional governmental department setting tends to focus on the number of doses; it often does not adhere closely to good manufacturing processes or quality assurance programmes and there is little focus on return on investment and/or technology renewal. Consequently, there is a tendency to persist with out-dated technology, and little effort is made to ensure purity, efficacy, or potency of the product. Almost invariably, government-led production does not target the export market because of the need to meet demanding quality standards, so all that remains is the non-lucrative domestic market. By contrast, the private sector will only concentrate on those vaccines that guarantee high profitability. So there is an increasing need in developing

countries for public/private partnerships for vaccines. Similarly, in the OECD countries public/private partnerships would be valuable in aspects of research and development, though in recent history production, marketing and delivery have been relegated fully to the private sector, with some exceptions such as exotic or foreign transboundary animal diseases.

For any partnership to flourish there needs to be either a convergence or complementarity of interests and objectives. Government objectives for vaccines revolve around national animal health programmes and the need for quality assured vaccines that are affordable, efficacious and safe. The objective for vaccine production companies is to generate profit from the sale of licensed vaccine products. Where there is an assured high volume demand for particular vaccines (e.g. FMD vaccines in South America) there is good incentive for the private sector to invest in vaccine manufacturing plants, since it is reasonably certain that they will recoup their investment. It would then be the government's role to regulate and ensure the quality control of the end product and monitor the efficiency and efficacy of vaccine use, rather than invest in production themselves. In this case, the government/private sector partnership is complementary, as the government requires a sustained supply of efficacious and affordable vaccines from the private sector, while the private sector is satisfied to have a government-guaranteed demand within the competitive environment of different vaccine suppliers.

However, increasingly, the private sector does not have sufficient confidence of a guaranteed high volume market, either because of market uncertainty or because the market is simply too narrow, and although it may be of strategic importance to the country it is unlikely to be very profitable. Therefore, the increasing tendency in developing countries has been for governments or other sections of the quasi-public sector (e.g. livestock farmers' cooperatives or associations) to either set up parastatal companies with commercial autonomy or to seek some form of partnership with commercial enterprises for technology transfer and/or for the management of vaccine production. Haigh has described the different forms of partnerships (51), they include the following:

a) A self-contained parastatal local company that is empowered to seek targeted alliances with private enterprise for the purpose of acquiring some specific technology and, in addition, to either manufacture under licence or to act as a distribution agent for a foreign company. An example of this format is Onderstepoort Biological Products in South Africa.

b) A second category of partnership is one in which either a government department or a farmers' cooperative or association sets out to produce quality vaccines for its

members and seeks an agreement with a foreign established vaccine manufacturing firm to provide a comprehensive technology transfer service that would include design, construction and procurement of the necessary equipment and engineering services, training of staff, commissioning of the new vaccine plant and a post-launch consultancy service for a limited period. Examples of this category include the Indian Dairy Development Corporation and VECOL in Colombia, both of which manufacture FMD vaccines.

c) A third category is one by which the recipient government sets up a vaccine trading company and then seeks a technology transfer arrangement with a reputable vaccine manufacturing firm. The arrangement will include an agreement for long-term technical management support, with options for periodic up-dating of the technology by the contracted firm. The Botswana Vaccine Institute is such an example.

Whatever format is selected, there must be guidelines which the technology purchasing government and the technology vendor need to take into account in order to ensure a successful and financially sustainable partnership (51).

It must also be stressed that the partnership may be involved in the manufacture of non-profitable vaccines. It is important that the government partner does not force the commercial partner to produce vaccine at a loss. The most appropriate point for the government to intervene is at the point of sale, i.e. to provide subsidies to the vaccine users (the livestock farming community) so that they can afford vaccines whose prices reflect the true cost of production.

Public/private partnerships for vaccine technology development

Traditionally, the discovery work for vaccines was often undertaken in governmental research establishments. Private industry picked up promising lines of research to develop them into a vaccine product. Foot and mouth disease vaccines provide a prime example of government/private industry development of vaccine technologies. The culture of bovine tongue tissues, the culture of FMD virus in suspended bovine tongue tissues, the inactivation of the virus with formalin and subsequent formulation into an immunogenic vaccine were all done in a government research establishment, in Amsterdam in the Netherlands (46). However, the scaling up of this technology to industrial proportions for the production of millions of doses of vaccines became possible when the technology was taken up by commercial enterprises in Europe and Argentina (7). Furthermore, modern FMD vaccines arise from the culture of the virus in BHK cells.

The technology evolution for this type of vaccine also followed the same pathway, i.e. discovery research being done in government establishments and process development in industry. Thus, the establishment of BHK cells as a continuous culture, the demonstration of their susceptibility to FMD virus, the adaptation of BHK cells to continuous culture in suspension, plus a reconfirmation of their susceptibility to FMD virus and the demonstration of first order kinetics inactivation of FMDV by aziridine compounds were all first discovered in governmental or inter-governmental research establishments (2, 14, 17, 18, 20, 92). The volume of FMD vaccine production is now larger than that of any other vaccine (human or veterinary), and this was achieved because government discoveries were developed and industrialised by private companies (initially Wellcome and later other commercial enterprises in Europe and South America [77]).

As already shown, the demand for conventional vaccines is likely to be predominantly in developing countries, especially for those diseases that are transboundary in nature and considered 'exotic' by most OECD countries. Controlling these epidemic diseases in their endemic area would decrease the risk of their extension. Therefore, there is likely to be a need for governments in developing or middle income countries to invest in and import vaccine technology development with a view to licensing such technologies to the private sector of public/private partnerships. Other tendencies are likely to be the so-called 'south-to-south' technology transfer (cooperation between developing countries with similar levels of development) and partnerships in vaccine production and licensing.

Recently, there has been a sharp drop in the amount of research being carried out on vaccines for tropical animal diseases, whether based on conventional or recombinant DNA technology. The net result is that there are no vaccines against such critical diseases to human and animal welfare as African swine fever, malignant catarrhal fever, trypanosomosis and other blood parasite diseases. Moreover, some of the available vaccines, such as those for CBPP, are far from optimal for the purpose. At the same time, there is only very limited international motivation to create the animal disease equivalent of the Global Research Forum for Vaccines. This alliance of governments, the private sector and international development agencies, which has been spearheaded by the World Health Organisation (WHO) since 1996, was established to coordinate research on otherwise non-commercially attractive vaccines for human infectious tropical diseases, including some zoonoses. It should be noted, nonetheless, that recent initiatives such as GALVmed (Global Alliance for Livestock Veterinary Medicines) or the European Technology Platform for Global Animal Health (ETPGAH) have been set up as global not-for-profit pharmaceutical outreach programmes funded through private/public

partnership principles. The objectives of these initiatives are to improve access to pharmaceuticals, vaccines and diagnostic products and applied research.

Public/private partnerships for vaccine distribution and accessibility

Access to vaccines and other veterinary products for local communities of the developing world, initially under government control, has almost disappeared in most countries, often on account of dwindling resources, general deterioration of infrastructure and reforms such as the decentralisation of Veterinary Services and the cost-recovery approach. This problem is compounded by the cold chain requirement for most vaccines and the limited extension activities in sub-Saharan Africa and South Asia. Public/private partnerships could facilitate access to vaccines for rural livestock owners, either through the support of big corporations, or through funding that encourages the establishment of local distributors. The most notable example is the work of the National Dairy Development Board of India, which not only produces vaccines through its subsidiary, Indian Immunologicals, but also ensures wide distribution of vaccines, including FMD vaccine, to all members of the national dairy cooperative throughout India.

In sub-Saharan Africa some promising initiatives are beginning to emerge. For example, the initiative of the International Rural Poultry Centre, an Australian non-governmental organisation (NGO), working together with a mining company in Chibuto, Mozambique, is an example where the private interest, the mining company, through facilitation by an NGO, is assisting the government and the communities to control poultry diseases and distribute Newcastle Disease vaccine produced by the National Veterinary laboratory, thus creating a market for the latter. The establishment of local distribution networks is one of the approaches that is being developed by GALVmed to improve access to veterinary pharmaceuticals, including vaccines, in developing countries.

Conclusions

Several groups and authorities have proposed that the global management of high impact animal diseases is best tackled through programmes which focus on controlling diseases at source, i.e. in developing countries (39, 44, 83). The approach to disease control/management in developing countries is one that is likely to be driven by risk-based surveillance and risk management principles, whether this is for the progressive control of diseases identified at the national or regional level as of strategic

importance or whether it is for tactical intervention to halt or prevent the spread of infectious diseases beyond pre-determined acceptable levels of risk. Whether the objective is to solely to protect animal health or to protect human health via the control of zoonoses and food-borne infections, the dominant motivating factor for disease control is likely to be market access. Several approaches have been advocated to promote animal health management that enhance access to livestock commodities for developing countries, without undue risk of disease transmission. These have included bench-marking progress in animal health so that developing countries can

progressively access market opportunities (local, regional to international) as their animal health status improves and in-country processing of animal products to reduce the risk of distant transmission of serious infections, particularly where the slaughter animals are sourced from low-disease-risk zones within exporting countries (41, 84, 100). The net effect is that there is likely to be an increasing demand for vaccines of assured efficacy and safety.



Les vaccins vétérinaires et leur utilisation dans les pays en développement

J. Lubroth, M.M. Rweyemamu, G. Viljoen, A. Diallo, B. Dungu & W. Amanfu

Résumé

Les maladies animales infectieuses, notamment celles qui affectent les animaux d'élevage constituent, encore aujourd'hui, une entrave au développement durable de l'agriculture, à la sécurité sanitaire des aliments et à l'accès des pays en développement et en transition aux bénéfices économiques générés par le commerce international des produits de l'élevage. Ces pays doivent donc prendre les mesures ciblées appropriées pour limiter les risques d'apparition de maladies infectieuses sur leur territoire. L'utilisation stratégique de vaccins vétérinaires de qualité peut et doit faire partie des programmes approuvés par les gouvernements. Les campagnes de vaccination doivent s'inscrire dans le cadre de programmes planifiés de lutte contre les maladies ; s'agissant de maladies animales transfrontalières, ces programmes ne réussiront que s'ils ont une envergure régionale. En centrant leur propos sur les principales maladies animales transfrontalières, les auteurs décrivent l'utilisation actuelle des vaccins, les travaux de recherche prometteurs et les technologies innovantes qui pourront être appliquées dans les pays appartenant aux grandes régions en développement de la planète. Ils étudient également le rôle des partenariats public/privé dans ce domaine.

Mots-clés

Biotechnologie – Brucellose – Charbon bactérien – Fièvre aphteuse – Fièvre catarrhale du mouton – Fièvre de la Vallée du Rift – Mycoplasme – Pleuropneumonie contagieuse bovine – Vaccin – Vaccination.



Las vacunas veterinarias y su utilización en los países en desarrollo

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Resumen

Las enfermedades infecciosas del ganado y otros animales siguen constituyendo un pesado lastre para lograr la seguridad alimentaria y un desarrollo agrícola duradero, y para que los países en desarrollo o en transición participen de los beneficios económicos que reporta el comercio internacional de productos ganaderos. En dichos países deben aplicarse medidas destinadas específicamente a reducir la aparición de enfermedades infecciosas. Empleadas de modo estratégico, las vacunas veterinarias de calidad pueden y deben formar parte de programas avalados por el gobierno de cada país. Las campañas de vacunación deben estar inscritas en programas generales de control zosanitario, cuyo éxito, en el caso de enfermedades animales transfronterizas, exige trabajar a escala regional. Los autores, centrándose en determinadas enfermedades animales transfronterizas de especial relevancia, pasan revista al uso actual de las vacunas, así como a las investigaciones prometedoras en la materia, a tecnologías novedosas que pueden aplicarse en importantes regiones en desarrollo del mundo y a la función de las alianzas publico-privadas.

Palabras clave

Biotecnología – Brucelosis – Carbunco bacteridiano – Fiebre aftosa – Fiebre del Valle del Rift – Lengua azul – Micoplasma – Perineumonía contagiosa bovina – Vacunación – Vacuna.



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