Marker vaccines and the impact of their use on diagnosis and prophylactic measures


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Summary
Molecular biology and technical advances in DNA recombination have ushered in a new era in vaccinology. This article examines the recent development of specific marker vaccines and examines the impact of their use on the diagnosis and prevention of major infectious diseases. Gene-deleted vaccines, DIVA strategies (differentiating infected from vaccinated animals) and similar methods have been successfully applied in the control and eradication of Aujeszky’s disease, infectious bovine rhinotracheitis, classical swine fever, foot and mouth disease and, recently, avian influenza. The efficacy and performance of existing marker vaccines and their companion diagnostic tools (which should be assessed by an independent body) are discussed, as are the ways in which these tools are deployed by competent authorities. The limits and the advantages of the use of marker vaccines are carefully analysed in the light of practical experiences. Although these vaccines can limit the speed and the extent of virus dissemination and thus reduce the number of animals slaughtered, marker vaccines are no substitute for sanitary measures. Early detection and warning systems and the quick implementation of sanitary measures, including stamping out, remain key issues in the control of highly contagious diseases.

Keywords
Introduction

Molecular biology and technological advances in deoxyribonucleic acid (DNA) recombination have ushered in a new era in vaccinology. In particular, ‘deleted’ vaccines, used in conjunction with an appropriate diagnostic kit, have emerged over the past ten years, enabling infection-specific antibodies to be recognised regardless of an animal’s vaccination status. The first such vaccines were used to protect pigs against Aujeszky’s disease (AD). The same principles were subsequently applied to the development of vaccines against infectious bovine rhinotracheitis (IBR). For classical swine fever (CSF), subunit proteins were obtained from baculovirus recombinant and the resulting vaccine obtained a European marketing authorisation, re-launching the debate on whether or not to use sanitary measures or medical prophylactic treatments. The same principle is also at work when the detection of antibodies to non-structural proteins (NSP) is used to identify animals infected with foot and mouth disease virus (FMDV), whether or not they have also been vaccinated. Furthermore, more recently, recombinant vaccines have been used to protect birds against avian influenza (AI).

An historic example: marker vaccines used against Aujeszky’s disease

Aujeszky’s disease virus (ADV) belongs to the subfamily *Alphaherpesviridae* of the family *Herpesviridae*, which infect the central nervous system and other organs (such as the respiratory tract) in virtually all mammals, except humans and the tailless apes. It is associated primarily with pigs, the natural host, which remain latently infected following clinical recovery. After primary infection, most pigs develop clinical signs, depending on their age. In naive piglets, nervous signs are observed and the mortality can be very high. In sows, reproductive disorders are induced after infection. In fattening pigs, general clinical signs, such as fever and loss of appetite associated with respiratory disorders of varying severity, are observed. Silent infection can also occur.

Currently available Aujeszky’s disease marker vaccines

Advances in molecular biology have contributed to better knowledge of the genome of existing vaccine strains. By studying conventional vaccine strains, it was found that certain coding sequences of the single-sequence short section of the Bartha strain of the ADV had been deleted. These sequences, situated in enzymatic restriction fragment *BamHI* no. 7, code for two structural glycoproteins: gE and gL. Accordingly, the Bartha strain, when isolated under natural conditions, does not express gE, which makes it possible to distinguish vaccinated pigs from infected pigs, provided, of course, that the corresponding enzyme-linked immunosorbent assay (ELISA) kits are used. ELISA kits make it possible to detect anti-gE antibodies in the serum of pigs, by using monoclonal antibodies that are very specific to certain antigenic determinants of gE, as described by van Oirschot et al. (129, 131).

Subsequently, knowledge about the molecular biology of mutants of the ADV led to a better understanding of the functions of the viral glycoproteins. The first factor of virulence that was identified in the herpes virus was the thymidine-kinase enzyme, which allows the virus to replicate itself in the central nervous system. Later, the virulence of strains of the ADV not expressing the glycoprotein membrane gE was seen to have diminished considerably compared with that of field viruses (9). This gE would therefore appear to play a major role in the spread of the virus within the nervous system, with the infection spreading both through the olfactory tract and trigeminal cavity (82). This knowledge has made it possible to develop new vaccines by means of genetic recombination, which modifies the genome of the vaccine strains in order to excise, remove or delete certain sequences that code for glycoproteins and prevents their expression. These proteins do not induce antibodies in vaccinated animals and so are used as serological markers for infection by wild-type viruses. The functions of these same proteins are often partially responsible for the virulence of field strains (such as gE); their non-expression helps to reduce or eliminate the pathogenicity of these vaccine strains, which always express the major glycoproteins (gB, gC, gD), thus inducing protective immune responses in vaccinated or infected pigs.

Another generation of vaccines, not yet on the market, has appeared, which uses live vaccine strains of the genetically modified ADV as an expression vector of the gene coding for the immunogenic proteins of other viruses, such as CSF (135). These ‘hybrid’ viruses protect the vaccinated animal against both AD and CSF. Moreover, in-depth knowledge of the molecular biology of the ADV has led to the creation of recombinants that cannot be shed by the vaccinated animal in an infectious form. Such recombinants can, however, spread from one cell to another in the inoculated organism, as do conventional live vaccine strains, but in rather limited sites (53).

Finally, one should not overlook the considerable progress that has been made with immunological adjuvant technology, even though this is not directly linked with molecular biology. We have seen the emergence of vaccines...
against AD that are produced by tank-mixing a live attenuated strain with an adjuvant comprised of mineral oils. At the same time, the nature of the oils used in adjuvant composition has evolved, as has emulsion technology, making the vaccines increasingly immunogenic and considerably reducing local reactions at the site of injection.

ELISA kits which are available commercially use indirect or competitive techniques for measuring antibody levels. When marker deleted vaccines are used, several types of kits can be employed to detect specifically the infected vaccinated animals; the ELISA tests that detect gE antibodies are the most commonly used in the field in Europe, whereas tests that detect gG (NSP) or gC antibodies are used more frequently in the United States of America (USA) (89, 144). A combination of several kits is often used in the framework of control programmes against AD, such as gE kits associated with kits that detect gB antibodies or antibodies against the whole viral proteins. The latter two kits are used mainly in unvaccinated herds or regions where vaccination is not carried out, but they can also be used to interpret the herd status in regard to infection (gE+; gB+) or vaccination (gB+; gE–). As well as testing sera, the ELISA can be adapted to test filter paper disks that have been moistened with a small quantity of blood obtained by puncturing a superficial vein (144). This technique is convenient for testing large numbers of pigs. The disks are air-dried before shipment to the laboratory. Moreover, muscular exudates can also be used as alternative biological samples as these kits have been evaluated taking serum samples as the reference; the individual sensitivity of the test was 93.2% and the individual specificity was 98.3% (64).

Requirements for the detection of gE, gB or global viral antigen have been defined by several competent authorities. When comparing different serological tests that detect AD antibodies, the sensitivity of ELISA tests appears very good and the blocking ones often appear a little more sensitive than indirect ones, allowing earlier detection of more than 95% of infected pigs (88).

Marker vaccines used against bovine herpesvirus-1

Bovine herpesvirus-1 (BoHV-1), classified as an alphaherpesvirus, is a major pathogen of cattle. Primary infection is accompanied by various clinical manifestations such as rhinotracheitis, pustular vulvovaginitis, abortion, and systemic infection in neonates. Following clinical recovery, a life-long latent infection is established in the nervous sensory ganglia of infected animals.

BoHV-1 is a pathogen that is found throughout the world and which displays significant differences in regional incidence and prevalence depending on geographical location and breeding management (1). BoHV-1 is responsible for significant financial losses incurred through disease and trading restrictions within the cattle industry, prompting the development of control programmes in North American herds. Based on serological surveys, several studies have aimed at identifying the risk factors for BoHV-1 seropositivity. Some of them are well characterised, e.g. age, sex (males are more frequently positive than females) and herd size (11, 107). Direct animal contacts, such as purchase of cattle and participation in cattle shows were also found to be important risk factors for the introduction of BoHV-1 (132, 133, 134). Other factors such as farm density or cattle density may increase the risk of BoHV-1 introduction (139). As reported for other diseases caused by herpesviruses in man and animals, the latency-reactivation cycle has a deep epidemiological impact since it is responsible for the maintenance of BoHV-1 in a cattle population. BoHV-1 infection of new generation cattle by latent carriers submitted to reactivation/re-excretion stimulus can occur at several different times, e.g. at birth (118), at mating, during transport (117) or following the introduction of heifers into a group of dairy cows. Therefore, the detection of BoHV-1 latent carriers is the main concern in the setting up of BoHV-1 control programmes. Moreover, sanitary measures must be taken to prevent the introduction of seropositive animals or even animals originating from a seropositive herd in order to improve vaccine programme efficacy.

Depending on the seroprevalence of BoHV-1, eradication programmes are based either on the detection and the culling of seropositive animals, or on the repeated vaccination of infected herds. The use of vaccines to control BoHV-1 infections has evolved over the last few decades: previously they were used simply as an effective means of reducing the clinical impact of the disease, whereas nowadays vaccination programmes are implemented with the additional intent of preventing transmission, although this is not as readily achievable as a reduction in clinical signs. Indeed, BoHV-1 vaccines are not efficacious at preventing BoHV-1 infection and establishment of latency. Moreover, vaccine schemes must be accompanied by strict management measures to prevent the reintroduction of BoHV-1 into the cattle herd. Therefore, BoHV-1 control programmes may take a long time to eliminate this well-adapted virus infection of cattle.

Currently available and future bovine herpesvirus-1 marker vaccines

In Europe, several countries have initiated control programmes aimed at BoHV-1 elimination and some
countries are already BoHV-1-free (Finland, Sweden, Denmark, Switzerland and Austria). In this context, the ability to differentiate infected from vaccinated animals (known as the DIVA strategy) was critical for preventing trading restrictions in Europe. To set up the DIVA strategy, marker vaccines and reliable companion tests were developed.

On the one hand, the marker vaccines must include BoHV-1 antigens able to induce a protective immune response. On the other hand, they consist either of subunit vaccines or of BoHV-1 strains from which a gene encoding a non-essential glycoprotein has been deleted. The deleted glycoprotein must be expressed by all BoHV-1 field strains and induce a detectable immune response. It should also be a virulence factor of BoHV-1 in order to ensure a further attenuation of the marker vaccine strain by the deletion of its gene.

Non-essential BoHV-1 glycoproteins gC, gE, gl, gG and gM may be deleted to construct BoHV-1 marker vaccines. Four candidate deletion mutants (BoHV-1 gC-, gM-, gl- and gG-null mutants) do not correspond to the above-mentioned requirements. BoHV-1 gC-null mutants retain virulence in the natural host (56) and BoHV-1 gM-null mutants have never been tested in vivo (61). BoHV-1 gl-null mutants are not sufficiently immunogenic (56). BoHV-1 gG-null mutants are easily reactivated from latency (56) and gG-specific antibody tests are not available.

Evidence of consistent results led to the selection of the BoHV-1 gE-null mutant as a candidate BoHV-1 deleted mutant for use as a marker vaccine. First, it was shown to be immunogenic (56) and to possess very little residual virulence (127). Moreover, BoHV-1 gE was shown to be expressed in a very large subset of BoHV-1 field strains (101) and a gE-specific antibody test has been developed as a companion test for the differentiation of vaccinated from infected animals (66, 130). The development of a BoHV-1 marker vaccine took advantage of the knowledge gained from ADV control and marker vaccines based on ADV gE-null mutants have successfully been developed for the control of ADV (137).

Several studies have been conducted, and others are still in progress, to produce new generation vaccines against BoHV-1. Ideal marker vaccines should combine high levels of safety and efficacy. Several subunit vaccines have been tested. They consist mainly of glycoproteins B, C or D expressed in different systems such as transfected cell cultures (126), recombinant baculoviruses (125), recombinant adenoviruses (42, 43, 100, 148), or recombinant tobacco mosaic viruses (96). The gD-based subunit vaccines are the most efficacious at reducing clinical disease and virus excretion when they are formulated with effective adjuvants. For example, chitosans (42) and CpG oligodeoxynucleotides (54, 86) are new adjuvants that significantly enhance the protective immune response, as evidenced by increased neutralising antibody titres and reduced clinical disease and viral shedding following challenge. The latest vaccine approaches consist of plasmid DNA vaccines containing sequences encoding for the three major immunodominant BoHV-1 glycoproteins gC (50), gB (71) or gD (87, 120, 121). These constructs could potentially be administered by needle-free delivery methods which would help to prevent losses due to the tissue damage that classical vaccine delivery methods can cause (54, 124).

The biological properties of glycoprotein E (gE)-deleted bovine herpesvirus-1 marker vaccines

As for the majority of the BoHV-1 vaccines, marker vaccines are very effective at preventing clinical signs after challenge with highly virulent strains (55). However, none are able to fully prevent infection by the challenge strain, which establishes a latent infection, and might be re-excreted following a reactivation stimulus. New vaccine formulations and protocols have therefore been developed in order to improve the viral protection. Equivocal results were obtained when the two forms (inactivated and live attenuated) of the same marker vaccine were tested. When it was administered twice to seronegative cattle, the attenuated marker vaccine induced a better viral protection than the inactivated marker vaccine after challenge (12). However, the inactivated vaccine was more efficacious at reducing virus excretion after reactivation of latently infected calves than the live attenuated vaccine (13). An interesting approach was to combine the use of the attenuated vaccine as the priming dose and the inactivated vaccine as a booster injection to complete the primary course of vaccination. This kind of protocol was shown to be the most efficacious at reducing virus excretion following challenge (44, 58). The immune status of a BoHV-1 latent carrier is the key factor controlling viral re-excretion following a reactivation stimulus. Therefore, latent carriers must be repeatedly vaccinated at regular 6-month intervals in order to maximally decrease the risk of re-excretion (34).

The efficacy of the DIVA vaccines was demonstrated in two field trials. In the first trial, a significant decrease in the number of gE seroconversions was observed in herds where the gE-deleted vaccines were used (77). The second study demonstrated that repeated vaccination using either inactivated or live attenuated gE-deleted BoHV-1 vaccines is efficacious at reducing the incidence of gE seroconversion in dairy cattle and consequently the herd prevalence of gE-positive animals (34). This study showed the superior efficacy of a protocol whereby all of the herd is vaccinated together at regular 6-month intervals
compared to protocols where all of the herd is vaccinated, but not all at the same time (34).

Three safety concerns about the live attenuated gE-deleted vaccine have to be addressed. First, gE-null BoHV-1 was demonstrated to establish a latent infection (67, 128) and to be re-excreted following experimental stimuli (67, 105) and in field conditions (35). However, there is so far no evidence that this deletion mutant can persist in the cattle population (78).

A second concern rose from the production of seronegative BoHV-1 latent carriers following the use of BoHV-1 marker vaccines in passively immunised calves. Indeed, passively acquired colostral immunity interferes with an active antibody response following infection. It has been experimentally demonstrated that gE-negative BoHV-1 vaccine, when used in passively immunised calves, gives rise to seronegative vaccine virus carriers (67).

The third concern is the potential for BoHV-1 recombinants to arise after co-infection of animals with a replicative gE-deleted BoHV-1 strain and a virulent BoHV-1 field strain (115, 116). One field observation and two experimental findings underlie this concern:

a) the isolation of a gE-deleted BoHV-1 vaccine strain in cows vaccinated eight months before (35)

b) the frequent appearance of BoHV-1 recombinants in co-infected calves (105)

c) the experimental isolation of a virulent gE-deleted BoHV-1 recombinant (84, 85).

**Properties of companion diagnostic tools**

Even if the DIVA strategy has been demonstrated to be efficacious, it presents some weaknesses (7). Indeed, the strength of the tool is fully dependent on the capacity of the diagnostic test to detect BoHV-1 gE-specific antibody. But the sensitivity of the only available gE-specific ELISA is around 70% (62, 97). This rather low level of sensitivity has a 30% false-negative rate in individual tests but it remains sufficiently high for use at infected-herd level. The problem of the low sensitivity of this test is compounded by the weak level of the delayed immune response raised against BoHV-1 gE, which means that it can be as many as 42 days after infection before gE antibodies can be detected (7). The specificity of the gE-specific ELISA test is 92% (62). Although it is an acceptable level in the first steps of a control programme, this lack of specificity will be responsible for several misleading false positive results in herds where BoHV-1 has been eradicated. In these herds, a serial combination of serological tests should be performed. The Danish test system (consisting of a blocking and an indirect ELISA), which was used in the BoHV-1 eradication programme in the Netherlands, has a very high sensitivity (> 99.0%) and a very high specificity (> 99.9%) (29). Finally, a useful approach for dairy cattle herd monitoring is the regular serological testing of milk tank samples (140). A positive result is obtained when 15% of the dairy cows are seropositive towards BoHV-1. This level of seropositivity is rapidly obtained in cases of BoHV-1 introduction into a previously negative herd.

**Marker vaccines used against classical swine fever**

Classical swine fever, previously known as hog cholera, is still a serious threat for the domestic pig population as it is a highly contagious viral disease of worldwide importance. Pigs and wild boars are the only natural reservoir of CSF virus (CSFV). CSFV, bovine viral diarrhoea virus (BVDV), and border disease belong to the genus *Pestivirus* of the *Flaviviridae* family. These are small, enveloped, positive single-stranded ribonucleic acid (RNA) viruses. The piglets develop more evident clinical signs than the adults. The usual clinical sign is hyperthermia, which is usually higher than 40°C in piglets (which pile together in the corner) but in adults it can be lower (39.5°C). The first usual signs of the acute form are anorexia, lethargy, conjunctivitis, respiratory signs and constipation followed by diarrhoea. The chronic form of the disease is generally fatal. Often the infected pigs present jaundice and cyanosis before death (65).

**Currently available classical swine fever vaccines**

Two live attenuated vaccines have been used successfully for many years. The live vaccines include the Chinese strain also known as C strain vaccine, which was attenuated by serial passages in rabbits and later adapted to cell cultures (136), and the Thiverval strain derived from the virulent Alfort strain through more than 170 serial passages at 29°C to 30°C (4, 63). These traditional live vaccines induce a high level of protection against the development of clinical signs and neutralising antibodies at two weeks post challenge. Dewulf et al. (32) demonstrated that no virus transmission was detectable even when the pigs were challenged on the same day as the vaccination. The vaccinal protection lasts at least six to ten months whatever the immunisation route used: intramuscular or oronasal (57, 114). The main problem of using these live vaccines is that it is impossible to distinguish antibodies that are the result of vaccination from those that are due to natural infection.

To clear up this difficulty, different teams have worked on the development of marker vaccines. CSFV envelope
glycoprotein E2 is the major protective immunogenic protein responsible for eliciting neutralising antibodies and conferring protective immunity against the virus and it has been demonstrated to be a highly conformational dependent immunogenic protein (70, 149). Different types of subunit or marker vaccines have been developed as non-replicative ADV expressing the E2 of CSFV (94), or live vectors such as porcine adenovirus. DNA vaccines have also been developed (3) but as yet these do not induce any real protection in pigs. Different vaccine schemes or combinations have been used such as: a prime-boost strategy (DNA-adenovirus) (52) or a co-administration with some interleukin (IL) recombinant protein such as IL-3, 12 or 18 or via a DNA vector (2, 141).

At the present time, only two E2 recombinant protein subunit vaccines produced in the baculovirus expression system have been licensed for market use. The efficacy of these two commercially available E2 marker vaccines has been extensively assessed in different vaccination/challenge and transmission trials. The results of these experiments were rather variable. A single vaccination with a vaccine dose of 32 µg of E2 in a water-oil-water adjuvant prevented clinical signs and mortality following a CSFV challenge three weeks after vaccination (15). At least 14 days were needed to obtain clinical protection in growing pigs vaccinated with a single dose (14, 123), but in the case of earlier challenge, no protection against the disease and no reduction of virus shedding has been demonstrated (123). The ability of the two marker vaccines to prevent transplacental transmission of CSFV has also been evaluated. The results showed that with a double vaccination, virus spreading by transplacental infection under the conditions of emergency vaccination could not be prevented in most of the vaccinated animals and could lead to the carrier sow syndrome and, consequently, to the late onset form of CSFV (30). Based on the results of using the double vaccination protocol on pregnant gilts challenged 46 days after the second immunisation, it was concluded that double vaccination with an E2 subunit marker vaccine protects pregnant gilts from the clinical course of the disease but does not prevent horizontal or vertical spread of the CSFV (31). Despite the fact that these results indicated that the efficacy of these vaccines was not ideal, their use in an emergency vaccination protocol has not been banned by the European Commission (EC).

Properties of companion diagnostic tools and their sensitivity and specificity limits

Discriminatory companion ELISA tests are based on the detection of antibodies to the E\textsuperscript{m} protein. In 1999, sixteen national swine fever laboratories participated in testing the discriminatory ELISAs. The two available kits were tested for sensitivity, specificity, reproducibility and practicability. Reference sera (CSFV and BVDV antibody positive) and field sera were used as well as sera from the weaner and sow experiments carried out during the marker vaccine trial. Both discriminatory ELISAs were less sensitive than conventional CSF antibody ELISAs, although there was considerable variation between them. Neither discriminatory ELISA consistently detected the marker-vaccinated, CSF-challenged weaner pigs correctly as ‘CSF positive’, although CSF-challenged pregnant sows were identified correctly. The limitations of these discriminatory ELISAs would prevent the use of the two licensed marker vaccines under emergency field conditions (40).

In 2003, the EC supported another large-scale inter-laboratory trial to assess the performance of a new version of a companion E\textsuperscript{m} ELISA test. It was concluded that even if the specificity and the sensitivity of the test was better than the previous kits tested in 1999 (40), there was still a need for more reliable tests to be sure that a vaccinated pig has not been infected and is not a virus carrier.

Marker vaccines used against foot and mouth disease

Foot and mouth disease is a highly contagious disease of domestic and wild cloven-hoofed animals including cattle, sheep, goats and pigs. It is caused by a virus (FMDV) of the genus Aphthovirus, family Picornaviridae and exists as multiple serotypes and subtypes; it causes severe economic losses through decreased livestock productivity and trade restrictions. The virus is widely distributed and the disease is completely absent only in the European Union (EU) and in the Australasian and North American continents.

Current vaccines and their biological properties in the framework of eradication and control

In areas with endemic FMD, vaccination is commonly used in conjunction with zoosanitary measures to minimise losses and reduce virus circulation. In FMD-free countries, such as those of the EU and North America, the policy for controlling outbreaks has been primarily based upon ‘stamping out’, i.e. slaughtering of infected and contact animals together with restrictions on the movement of animals and animal products. Nevertheless, provision is retained to vaccinate under emergency circumstances where an outbreak is or threatens to become extensive. During and after the 2001 FMD outbreaks in Europe there was a growing desire to place more emphasis on a ‘vaccinate-to-live’ policy to reduce reliance upon large-scale slaughter of herds at risk of becoming infected. According to such a policy, the stamping out of infected premises and the imposition of movement restrictions would be accompanied by a limited period of emergency
vaccination of surrounding herds, followed by serosurveillance to detect and eliminate infected animals not identified on the basis of clinical disease or epidemiological tracing. In support of this policy, new regulations have been approved by the World Organisation for Animal Health (OIE) and the EU so that countries using this approach can now regain their FMD-free status six months after the last infection has been reported rather than one year later as was previously the case (39, 145).

Current FMD vaccines are produced by infecting susceptible cell cultures (most frequently baby-hamster kidney cell lines) with virulent FMDV, followed by chemical inactivation with binary ethylenimine and purification by ultra filtration. At formulation, antigen is mixed with either an aluminium hydroxide and saponin adjuvant to make an aqueous vaccine for use in ruminants or is emulsified in oil to make a vaccine for the immunisation of pigs and ruminants. These vaccines have been used successfully for decades to control FMD and regular mass vaccination, mainly of cattle, has helped in the eradication of the disease in some regions such as Europe and South America. Emergency vaccine banks have been established by national and international agencies, holding reserves of concentrated unformulated antigen frozen over liquid nitrogen (36). These may be formulated at higher doses than would be used for prophylactic vaccination so as to induce a rapid onset of immunity after administration as a single dose.

Nevertheless, there are a number of concerns and limitations with the use of conventional vaccines in emergency control programmes. Their production requires the growth to high titre and subsequent complete inactivation of virulent strains of FMDV. Although this is conducted within high containment facilities there is still the potential for escape of live virus from these facilities or for inadequate inactivation of virus and these concerns have led some FMD-free countries to prohibit vaccine manufacture on their territory. Conventional FMD vaccines are more difficult to standardise than vaccines produced by synthetic or recombinant techniques and final product testing for safety and efficacy still requires in vivo testing in animals. As mentioned earlier, most vaccines are prepared from concentrated cell culture supernatants from FMDV infected cells and therefore contain variable amounts of viral NSP. Induction of antibody to NSP by vaccines ‘contaminated’ with residual amounts of these proteins makes it difficult to identify infection in vaccinated populations by the use of NSP antibody tests (see ahead). In addition, a consistent cold chain is required in the field for the vaccine to remain efficacious. Although conventional vaccines can prevent clinical signs and spread of the disease in vaccinated animals, they do not induce a sterile immunity and therefore may not prevent virus-exposed animals from becoming acutely infected, and a proportion of such animals will become persistently infected virus carriers (25, 90, 91) whose presence jeopardises recovery of FMD-free status. Moreover, full protection takes time to develop and is short-lived without repeated booster doses (27, 37). Even when emergency vaccine was administered with a ten times greater antigen payload than the normal dose it could not fully protect vaccinated cattle from a severe challenge at ten days post vaccination (Cox and Barnett, unpublished results).

**Possible future vaccines**

Much work has been done on the development of alternative vaccines, including subunit vaccines based on highly immunogenic FMDV proteins or peptides and DNA vaccines (10, 26, 33, 41, 59, 60, 69, 99, 109), but their immunogenicity has been found to be much lower than that of conventional FMD vaccines.

Efforts to produce attenuated FMD vaccines by the adaptation and further passage of FMDV in non-susceptible hosts have been unsuccessful due to the reversion of the attenuated viruses to virulent forms (76). Targeted deletion of the L<sup>meu</sup> gene, which is not essential to virus replication, produced a vaccine that induced a good FMD-specific neutralising antibody response, but could not protect fully (23, 79). Although a recombinant virus in which the RGD receptor binding site was deleted induced protection in natural hosts (74), with such a virus there is potential for selection of virus variants that could enter cells by utilising other receptors (5, 6).

Another approach has been to produce a vaccine which expresses the entire virus capsid, and therefore all of the immunogenic sites present on intact virus, but lacks the infectious nucleic acids (8, 48, 49, 68, 102). Using this strategy, the Plum Island Animal Disease Center in the USA (47, 80, 83, 147) inserted the complete capsid coding sequences of FMDV into a live replication-defective human adenovirus vector, along with the FMDV 3C protease needed for capsid assembly. One parenteral inoculation with this vaccine induced antibody and clinical protection within seven days. The same adenovirus vector expressing the cytokine porcine interferon-alpha could protect animals from FMD for three to five days within one day of vaccination (22, 47).

**Properties of companion diagnostic tools and their sensitivity and specificity limits**

Conventional FMD vaccines are mainly comprised of viral structural proteins (SP) plus RNA and contain only trace amounts of viral NSP synthesised during viral replication. Furthermore, the amount of residual NSP present in vaccines can be reduced by additional purification steps during antigen preparation. Therefore, conventional vaccines elicit a mainly anti-SP antibody response, whereas
infection with replicating virus elicits antibodies to both SP and NSP. Consequently, serological methods which detect antibodies to FMDV SP such as the liquid phase blocking ELISA (51), the solid phase competition ELISA (73) or the virus neutralisation test (45) cannot distinguish between infection and vaccination with conventional vaccines. However, ELISAs that measure antibodies to different NSP (3ABC, 3AB, 3A, 3B, 2A, 2B and 2C) can be used as marker tests to detect infection in conventionally vaccinated animals (17, 26, 28, 75, 98, 103, 106, 108). Incomplete purification of vaccine antigen as well as multiple vaccination increases the likelihood of inducing NSP antibodies, but the latter will not arise after emergency vaccination of previously naïve animals.

To date, the most promising NSP tests have been those that detect antibody to NSP 3ABC, and the OIE index method is an indirect 3ABC screening ELISA (first developed in South America [NCPanafotisal]) supported by a confirmatory immunoblotting test against NSP 3A, 3B, 2C, 3D and 3ABC (143). A number of commercial 3ABC ELISA test kits have recently become available and their sensitivity and specificity were compared to one another and to the index screening method at a workshop in Brescia (Italy) in 2004 (16). The specificity of the tests ranged between 97% and 98%, including when used to test cattle that had been given a single dose of European vaccine. All of the tests were highly sensitive for detection of infection in unvaccinated cattle, whereas the sensitivity of the tests to detect viral carriers in vaccinated and subsequently infected cattle ranged from 68% to 94% depending on the test used. The workshop concluded that two tests performed comparably to the OIE index method (Ceditest® FMDV-NS, Cedi Diagnostics B.V. and 3ABC trapping-ELISA, IZS-Brescia) of which the Ceditest® is the only one available as a commercial kit.

Guidance on how to carry out post-outbreak serosurveillance for FMD in vaccinated populations is provided by the OIE Terrestrial Animal Health Code (Terrestrial Code) (145) and by the European Directive on FMD control (39). The Terrestrial Code requires that herds containing seroreactors must be followed up to determine whether these contain infected animals or not, finding evidence of infection at any stage automatically invalidates freedom from infection status. The European Directive on FMD control specifies that serosurveillance should be carried out at least one month after an outbreak has finished or one month after the last use of vaccine, whichever is the later. Further, it states that the entire vaccinated population should be sampled and tested or enough should be sampled and tested to give 95% confidence to detect a within-herd prevalence of infection of 5%.

The problem of some vaccinated animals becoming carriers without seroconverting to NSP can be overcome by interpreting NSP test results on a herd basis (104), although this still leaves a lack of certainty over freedom from infection in small herds (46, 92), since a test with 80% sensitivity at the individual level requires at least two infected animals in the herd to be sampled to have 95% confidence of detecting at least one of them (93). Imperfect test specificity can be partly overcome by retesting positive samples. For example, with the Ceditest® at the Brescia workshop, discounting the positive results that were not confirmed on Cedi retest increased the specificity to 99.2% and decreased the sensitivity from 86.4% to 85.1% (93). A further increase in test specificity could be achieved by a second retesting of Ceditest-confirmed positive samples using another non-covariant, commercially available NSP assay (SVANOVIR™ FMDV 3ABC-Ab ELISA, Svanova, Upsala, Sweden), resulting in an overall specificity and sensitivity of 99.98% and 71.2% respectively (93).

Foot and mouth disease is therefore a good example of where advances in vaccine production technology to reduce contamination of vaccines with NSP antigens, together with advances in diagnostic techniques to detect antibody to these antigens, have resulted in the development of marker vaccines and companion diagnostic tests that are sufficiently robust that they have resulted in amendments to FMD control policy such that a policy of ‘vaccinate to live’ is now supported in appropriate circumstances. Improved FMD marker vaccines and tests may be available in the future. For example, the experimental adenovirus vectored vaccines described above express recombinant viral capsids that are devoid of NSP 3D, which is a protein that elicits a strong antibody response but cannot be eliminated from conventional vaccines by purification (81). This would enable use of a companion marker test for infection based on detection of anti-3D antibody (47). Testing of saliva for FMDV-specific IgA is also a promising tool for detection of infection in animals given conventional vaccine by the parenteral route (90).

**Marker vaccines used against avian influenza**

Avian influenza viruses all belong to the **Influenzavirus** A genus of the Orthomyxoviridae family. They are enveloped, negative-stranded RNA viruses with a segmented genome, consisting of 8 genes (PB1, PB2, PA, HA, NP, NA, MA and NS). AI viruses may be classified on the basis of the severity of the clinical signs they cause in susceptible birds. Low pathogenicity AI (LPAI), may be caused by viruses belonging to all 15 haemagglutinin types (H1-H15) and produce a mild disease in susceptible poultry characterised by respiratory and enteric signs that are often associated in breeders and laying hens with reproductive disorders. Some LPAI viruses are termed
mildly pathogenic AI viruses. Highly pathogenic AI (HPAI) is, in contrast, a systemic viral disease of poultry with mortality that approaches 100% in many gallinaceous birds. The clinical disease HPAI is caused only by viruses of the H5 and H7 subtypes that contain multiple basic amino acids at the deduced sequence of the cleavage site of the precursor of the haemagglutinin molecule. The main clinical signs presented by HPAI-infected poultry are anorexia, depression, cessation of egg-laying, followed by complete reluctance to move and tremors of the head, paralysis of the wings and incoordination of the leg movements (19).

**Marker vaccines and companion diagnostic tests for notifiable avian influenza**

Increased knowledge of AI occurrence and epidemiology has driven a revision process of the definition of AI for international trade regulations laid down by the OIE. The revised Chapter on AI now reads ‘For the purposes of this Territorial Code, avian influenza in its notifiable form (NAI) is defined as an infection of poultry caused by any influenza A virus of the H5 or H7 subtypes or by any AI virus with an intravenous pathogenicity index (IVPI) greater than 1.2 (or as an alternative at least 75% mortality) as described below. NAI viruses can be divided into highly pathogenic notifiable AI (HPNAI) and low pathogenicity notifiable AI (LPNAI)’ (146). The aim of this section is to review currently available marker vaccines and companion diagnostic tests for NAI viruses of the H5 and H7 subtypes.

Research in AI vaccinology has only recently become a field of interest for pharmaceutical companies and for research institutions, and for this reason the selection of products and performance of companion diagnostic tests are not adequate to cover fully all the complex field situations this infection may cause.

Antigenic cross-reactivity between strains of the same H subtype is believed to occur even between strains belonging to different lineages. However, how cross-protection will be influenced by immunological pressure generated by the variety of seed strains is currently unknown (112). Similarly, the occurrence and extent of antigenic drift in this situation is impossible to predict, but could become a significant issue in the future.

**Inactivated conventional vaccines and companion diagnostic tests or systems to reveal field exposure**

These vaccines are based on a preparation containing inactivated virus grown in embryonated eggs. The seed strains that are currently being used are field isolates collected from natural outbreaks, selected without defined criteria. For this reason they may contain seed viruses of either high or low pathogenicity, although the OIE guidelines indicate that LPNAI strains should be used (146).

In order to be efficacious, inactivated conventional vaccines must contain a seed virus of the same H subtype as the field strain against which vaccination is directed, while the subtype of the other surface antigen, the neuraminidase protein (N), is virtually irrelevant with regard to protection (111). Thus, vaccines may contain a seed virus of the same subtype as the field strain (H5N1 vaccine to combat an H5N1 field challenge) or may be of the same H subtype but of a different N (H5N9 vaccine to combat an H5N1 field challenge). The latter is known as heterologous vaccination.

Currently there are two methods of detecting field exposure with this type of vaccine that have been evaluated in the field with satisfactory results.

The introduction of unvaccinated sentinels into the shed has been used as a method of identifying field exposure within a vaccinated population, regardless of the strain used in the vaccine. This system requires the identification of unvaccinated birds and regular clinical inspections in conjunction with serological testing to detect LPNAI, HPNAI being clearly visible as clinical pathology in the sentinels. The system is deemed to be valid, but requires preparatory work and is more time consuming, especially when the number of herds to be vaccinated is very high, and particularly when birds that are not confined to cages need to be identified within the flock. Furthermore, the risk that sentinel birds could be substituted with naive birds in order to escape restriction policies exists.

In the case of heterologous vaccination, it is possible to use the diversity between the N antigen contained in the vaccine and the one in the field as a natural marker system. This DIVA system was developed in Italy in 2001, and has been successfully used to combat multiple introductions of NAI (18). The system is based on the detection of antibodies to the N protein of the field virus, which represent evidence of field infection. Currently, only an indirect immunofluorescence antibody test has been fully validated (20, 21).

An encouraging system that can be used as a companion test to vaccines containing homologous or heterologous seed strains, is based on the detection of anti-NS1 antibodies (122). This system is based on the fact that the NS1 protein is synthesised only during active viral replication and is therefore not present in significant amounts when inactivated vaccines, that do not replicate in the bird, are used. Birds that are vaccinated with such
vaccines will develop antibodies to the NS1 only following field exposure (38). Full and field validation of this system under different circumstances is still in progress (H. Chen, personal communication) and should be made available before this system is recommended. No diagnostic kit has been validated to date.

**Engineered vaccines and companion tests**

Engineered vaccines include all vaccines that are not natural isolates or natural reassortants. These include recombinant live vectored vaccines and vaccines based on reverse genetics and recombinant proteins.

Recombinant live vectored vaccines are based on inserting an H5 or H7 haemagglutinin gene in a suitable, replicating vector which will, during its replication, induce the production of antibodies to the haemagglutinin of the influenza gene. Fowlpox-based recombinant viruses have obtained marketing authorisations and are being used currently in several countries. Newcastle disease virus (NDV)-based recombinants have been developed by several groups (H. Chen, personal communication; 113, 138), with a product developed by Chinese researchers being used currently in the field in the People's Republic of China. An infectious laryngotracheitis-based product has also been described (72).

All these preparations, with the exception of the one developed by Swayne (113), offer protection from clinical signs and reduce shedding levels. However, for most of these products, their efficacy in the presence of pre-existing antibodies due to natural infection with a field strain of the vector virus, e.g. NDV or fowlpox virus remains to be established (111, 113).

The greatest advantage to the use of these products is that companion diagnostic tests directed to detect antibodies to any viral proteins other than the haemagglutinin may be used to identify field-exposed flocks. Thus, the agar gel immunodiffusion or ELISA tests directed to the detection of antibodies to the NP or M proteins, can be successfully used, and enable the detection of field challenge caused by any influenza A virus. Tests directed to the detection of antibodies to the N protein, identify field exposure only to viruses of known N subtype.

Inactivated vaccines generated by reverse genetics have recently been developed in the USA and in the People’s Republic of China (119). The seed strain contained in the vaccine is basically a synthetic virus, completely engineered in the laboratory. These viruses contain a backbone derived from a virus that has high replication capacities (A/Puerto Rico/8/XX) with the two genes encoding for surface antigens (H and N) derived from contemporary viruses. This combination of genes allows on the one hand an excellent replicative efficiency – which ensures high titred, consistent virus yields during production – and on the other, suitable surface antigens. Since these vaccines have the same properties as conventional inactivated vaccines, the same companion tests apply.

Recombinant protein-based vaccines are synthesised in the laboratory by expressing the haemagglutinin in a suitable system, for example baculoviruses, plants and yeasts (110). Several prototypes have been generated and have been tested in the laboratory with encouraging results, however, probably due to the cost of production, they have not attracted any commercial interest to date. The use of any of these ‘engineered’ vaccines in the field would have several advantages, including the possibility of using a variety of companion diagnostic tests, as is the case with recombinant vectored vaccines – and of course, of updating the haemagglutinin component should this be required.

In summary, to date, only conventional inactivated (containing natural or synthetic strains) and recombinant live vectored vaccines are available for use and can be coupled with a suitable companion diagnostic test. Both categories have some advantages and limitations in their application in the field, and certainly, considering the complexities of these vaccines and the need to extend their use, more research is needed to optimise products and companion tests in order to tackle the current limitations to their use in the field.

**Limitations and advantages of the use of marker vaccines**

In spite of the major progress that has been made as a result of the development of marker vaccines, it would be a mistake to consider that their use could simply replace sanitary prophylactic measures. Indeed, past experience is very useful for assessing the limits and the advantages of the use of these marker vaccines, which could be a powerful tool in a set of measures to control and eradicate a contagious disease. However, the use of such vaccines has to be adapted to the epidemiological situation, the contagiousness of the disease concerned and to the presence or absence of conditions with the capacity to influence the spread of infection. To control a disease, the key point is to detect clinically inapparent infected animals (healthy carriers) which can infect in-contact susceptible animals. When vaccination is used, the critical stage of alert induced by the appearance of clinical signs is removed or suppressed. For this reason, such vaccines have to be as efficient as possible, not only to protect vaccinated animals against clinical signs, but also to
prevent, as far as possible, the excretion of the virus by vaccinated and subsequently infected animals. Moreover, the sensitivity of the diagnostic kits should be as high as possible to reduce to the greatest possible extent, the probability of false negative results; indeed, in such a strategy, the epidemiological consequences of false positive results are less significant than false negative results, as positive results are generally confirmed (in a second, complimentary phase) by a reference laboratory using another diagnosis tool.

The longest experience of using marker vaccines has been accumulated in relation to the control and eradication of AD. In this case, the use of deleted marker vaccines has represented a considerable advance in programmes to control the disease in several countries.

First, these vaccines have made mass vaccination possible, whilst retaining the means for serological detection of infection. This has enabled vaccinated herds which subsequently become infected to be pinpointed so that the necessary measures can be applied to prevent the field virus from spreading further.

Second, it has become possible to implement sanitary measures in a gradual manner in vaccinated, infected herds, by culling the infected sows at varying speeds, as required. These infected sows are detected through serological screening using the ELISA technique, which enables vaccinated pigs to be distinguished from those that have been vaccinated and then subsequently infected.

This means that vaccination has a combined effect which allows a programme of prophylactic treatment to be carried out in total safety. Mass vaccination, conducted several years in succession, limits the quantity of virus shed into the air by the infected pigs, thereby considerably reducing the probability and scale of the air-borne spread of contagion between herds (95, 137). Furthermore, systematic vaccination avoids economic losses due to a poorly controlled infection. Consequently, after several years of vaccination in a country or region, and the introduction of sanitation measures into the infected herds and the continual culling of the oldest infected sows, the prevalence of infection gradually diminishes; in addition, the incidence of infection remains very low and is kept under control. However, the cost of vaccination must be taken into account when calculating the total cost of a prophylactic treatment.

Authors compared the cumulative costs, over ten years, of various measures for controlling AD in northern Germany, following the introduction of prophylactic treatment (Table 1). Of the five possible strategies, the most economical is based on systematic vaccination, followed by screening of infected herds and the slaughter of sows presenting infection-specific antibodies.

Of course, this is a cumulative cost which takes all costs into account: those of the State, those of trade organisations and those of breeders. The authors note that the prevalence of infection diminishes during the first two years, but that vaccination alone is not enough to eliminate infection; during the final years of the programme the ADV persisted in a small number of herds. After 42 months of vaccination, few herds still harboured infected breeding animals. The detection and elimination of these breeding animals lead to a sharp drop in the prevalence of infection in breeding herds, whilst the risk of infection in fattening farms (or of fattened animals in other farms) becomes zero (142).

As a result of past and present experience, it has become possible to develop a strategy for using vaccines to control AD. In countries that have sufficient economic resources to envisage eradication of the infection, there are two possible options:
- where the prevalence of infection in a given territory is high, or there is a high density of pig herds, mass vaccination with effective deleted vaccines is the only means of reducing prevalence; however, although these measures are necessary, they are not in themselves sufficient to eradicate the infection. Identification, screening and culling of the infected breeding animals appear to be essential to successful eradication whilst

<table>
<thead>
<tr>
<th>Strategy</th>
<th>Cumulated cost over ten years (in thousands of euros)</th>
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<tbody>
<tr>
<td>Vaccination of sows twice per year</td>
<td>18,085</td>
</tr>
<tr>
<td>Vaccination of sows three times per year</td>
<td>18,143</td>
</tr>
<tr>
<td>Vaccination of sows three times per year, and of pigs for consumption once per year</td>
<td>13,534</td>
</tr>
<tr>
<td>Vaccination of sows three times per year, and of pigs for consumption once per year. Serological controls and slaughter of pigs presenting infectious antibodies (where prevalence is &lt; 10%)</td>
<td>9,907</td>
</tr>
<tr>
<td>Control and slaughter of pigs possessing infectious antibodies</td>
<td>19,342</td>
</tr>
</tbody>
</table>
continuing to systematically vaccinate the animals at least two years after elimination of the last infected pig. In the latter case, it is advisable to control the movements of piglets, pigs for consumption and breeding animals as much as possible;

– by contrast, in regions with a low herd density and low prevalence of AD, serological screening and the culling of infected breeders or total slaughter of certain herds, appear to be the most effective, and in some cases the most economical, measures for achieving eradication. Such measures have been successfully introduced, for example, in Denmark, the United Kingdom, Sweden and several regions of France.

However, independently of the performances of the vaccines and the companion kits (which are key issues in determining the use of such tools), other examples show that the use of marker vaccines would not have had a significant impact on the control of the situation. When a serious CSF epizootic hit several European countries in 1997, many people believed that the use of these new generation serological marker vaccines could prevent a further animal health catastrophe. However, an analysis of the situation that existed when the first CSF outbreaks appeared in the Netherlands revealed that more than 22 herds were already infected when the primary outbreak was identified in the region of Venhorst on 4 February 1997. The situation rapidly became dramatic for the region because farmers had already sold piglets before the veterinary administration could isolate the infected zone. This led to a rapid spread of the infection in the south of the country.

Under such circumstances, the use of a serological marker vaccine would not radically alter the basic nature of the problem, as it does not obviate the need to identify potentially infected animals and to take a sample of serum before any animals are transported, in other words, to strictly control the movement of pigs. Indeed, at the start of an epizootic, the success of control measures depends on their being rapidly implemented after the appearance of the first outbreak and before extensive, undetected spread has occurred. Vaccination is no substitute for basic measures to control contagious diseases.

So, as a general rule, as long as CSF has not been eradicated in the world, there is still a great risk of reintroduction of the virus in free areas. Farmers and veterinarians are in the best position to detect a new introduction of CSFV, so they should receive training in the detection of clinical signs and remain extremely vigilant. A non-vaccination policy is logical in disease-free states but emergency vaccination may be considered in contingency plans to avoid destroying millions of pigs. When outbreaks occur, the use of the traditional live C strain vaccine is as effective at preventing the spread of virus as culling the neighbouring herds. This strategy can be used after the start of an epizootic when there are too many outbreaks occurring at the same time, but this means that the pigs are seropositive and leads to their destruction. For this reason, the development of efficacious marker vaccines and reliable discriminatory tests should be encouraged. As it has been demonstrated that 14 days are necessary to induce good protection with the available subunit E2 vaccines, their use could be envisaged when several outbreaks occur at the same time and the use of strict sanitary prophylactic measures alone may not be enough to control the disease: vaccinating pigs in the zone around the outbreaks would allow the movement of pigs and prevent mass culling. As these E2 subunit vaccines do not prevent vertical transmission, their use must be limited to growing pigs. Simulation models will also be useful tools for choosing the best control measures to apply, depending on the epidemiological situation.

At the start of an epizootic, in regions with a high density of pig herds, ring or zonal vaccination can also be envisaged in order to prevent the virus from replicating too rapidly and to limit the cost of preventive slaughter. However, in this case, transmission of the virus must be limited and control measures must be properly applied and effective. Such an approach is particularly pertinent for highly contagious diseases such as FMD in those circumstances under which the air-borne transmission is one of the main epidemiological factors in the spread of the virus. So, if the first outbreaks appear in an area with a high density of susceptible herds and under epidemiological conditions that favour air-borne spread, ring vaccination, implemented on the basis of the results of models and assessment to determine the risks and directions of spreading, could be useful in limiting the speed and the extent of the virus dissemination (24). However, due to the ability of vaccination to mask the appearance of clinical signs without preventing infection, vaccinated herds, even with a serological monitoring programme, represent a greater risk for undetected spread than unvaccinated herds, where monitoring can be based on clinical inspection alone.

In the case of AI, the use of marker vaccines has proved very effective in controlling LPN/AI infection in turkeys and poultry (18). Nevertheless, the difficulty of implementing such a strategy will depend on the species in which the vaccines are intended to be used and, consequently, on the efficiency of one type of vaccine in regard to one particular species. The efficiency of a vaccine and the performance of a companion diagnostic kit cannot be extrapolated from one avian species to another, so the use of marker vaccines will depend on the availability of validated data on the performance of the vaccine and companion diagnostic kit in the avian species that is to be vaccinated.
In regard to the HPNAI strains, some Asian countries have implemented a massive vaccination campaign due to the high prevalence of infection in the domestic flocks. In some countries, such as Vietnam, this strategy seems to have given good results, but the lack of validated data prevents drawing definitive conclusions on the best strategies to use in the face of such complex and difficult animal health crises.

Conclusion

The power of the combination of a marker vaccine and a companion diagnostic kit for the control of an infectious disease depends not only on the performances of the vaccine and the kit themselves but also on the way in which these tools are deployed by the competent authority for use in the field. Ideally, both the vaccine and the diagnostic kit should be scientifically assessed by an independent body following submission of a dossier of data in support of an official application for a marketing authorisation. With respect to tests, at least for those used in the context of official control and eradication campaigns, national or international Reference Laboratories should control the quality of each batch released onto the market. If several diagnosis laboratories are involved in the diagnosis and the surveillance of the infection and the disease, a Reference Laboratory should organise proficiency tests, the results of which should be used to deliver an agreement to the diagnosis laboratories to allow them to carry out the diagnostic tests. Regulatory systems already exist in most regions of the world (e.g. EU, USA, Japan) to control the quality of vaccines released onto the market. The situation is more varied with respect to companion diagnostic tests as not all regions or countries have legislation governing their marketing. Adequate validation of tests as ‘fit for purpose’ and appropriate quality control, either by the manufacturer themselves or by official Reference Laboratories, are necessary to ensure that the test performs as claimed and that all batches reaching the market are of consistent, high quality. In addition, where testing is carried out by multiple laboratories, experience has shown a clear need for quality assurance of the testing performed in all participating laboratories. This can be achieved by a combination of laboratories working to recognised quality standards, backed up by external accreditation, and participation in regular proficiency tests conducted by an appropriate Reference Laboratory. Only those laboratories reaching the required standards should be certified as competent to be involved in national disease eradication campaigns.

A successful programme can be based on vaccination, but should also include sanitary measures. Furthermore, when vaccination is part of a control programme, it should be implemented only for a certain period of time. Most of the time, when the prevalence of the infection decreases significantly and when the epidemiological unit is correctly protected from outside introduction of the agent, vaccination should be replaced by sanitary measures.

Les vaccins à marqueurs et les conséquences de leur utilisation sur le diagnostic et la prophylaxie

P. Vannier, I. Capua, M.F. Le Potier, D.K.J. Mackay, B. Muylkens, S. Parida, D.J. Paton & E. Thiry

Résumé

La biologie moléculaire et les avancées techniques liées à la recombinaison de l’ADN marquent le début d’une nouvelle ère en vaccinologie. Les auteurs examinent le développement récent de vaccins à marqueurs spécifiques ainsi que les conséquences de leur utilisation sur le diagnostic et la prévention des principales maladies infectieuses. Les vaccins obtenus par délétion de gène, les stratégies DIVA (visant à différencier les animaux infectés des animaux vaccinés) et d’autres méthodes similaires ont été appliqués avec succès pour
Resumen
La biología molecular y, en particular, los adelantos registrados en la técnica de recombinación de ADN, han marcado el comienzo de una nueva era para la vacunación. En este artículo se examinan las vacunas marcadoras específicas, de reciente desarrollo, y las repercusiones de su administración en el diagnóstico y la prevención de importantes enfermedades infecciosas. Se han obtenido resultados satisfactorios en materia de control y erradicación de la enfermedad de Aujeszky, la rinotraqueítis infecciosa bovina, la peste porcina clásica, la fiebre aftosa y, más recientemente, la influenza aviar con vacunas producidas mediante delección de genes, estrategias para diferenciar entre animales vacunados e infectados (estrategias DIVA) y otros métodos similares. Los autores también analizan la eficacia y los resultados obtenidos con las vacunas marcadoras existentes y los equipos de diagnóstico asociados (que deberán ser evaluados por un organismo independiente), así como la forma en que las autoridades competentes las utilizarán. Asimismo, examinan detalladamente las limitaciones y las ventajas de la administración de vacunas marcadoras a la luz de la experiencia adquirida en la práctica. Pese a que pueden limitar la velocidad y la importancia de la diseminación del virus y, por consiguiente, reducir el número de animales sacrificados, las vacunas

Mots-clés

Vacunas marcadoras y sus consecuencias sobre el diagnóstico y medidas de profilaxis

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marcadores no pueden sustituir a las medidas sanitarias. Los sistemas de detección y alerta rápidas y la aplicación inmediata de medidas de profilaxis, incluido el sacrificio sanitario, siguen siendo decisivos para controlar las enfermedades altamente contagiosas.

**Palabras clave**

## References


