Development and performance of inactivated vaccines against foot and mouth disease

S.J. Barteling

Consultant, Veterinary Vaccines; formerly Head of the Department of FMD Vaccine Development and Production, ID-Lelystad, The Netherlands and Head of the Community Co-ordinating Institute for Foot and Mouth Disease (European Union)

Present address: Nieuwe Keizersgracht 438, 1018 VG Amsterdam, The Netherlands

Summary

The historical background of foot and mouth disease (FMD) vaccine production is briefly described. Improvements achieved through the use of monolayer and suspension cultures are outlined. Elements that are crucial in the production of modern vaccines are discussed, such as inactivation of viral antigen, successive concentration and purification of the antigen and the final formulation of the vaccine. Storage of concentrated antigen at ultra-low temperatures creates greater flexibility for the producer and has also enabled national and international organisations to establish vaccine banks.

The purification of FMD viral antigens, including the removal of non-structural proteins (NSPs), enables the immune responses of vaccinated animals to be distinguished from the responses of animals infected with live FMD virus. Consequently, the combined use of purified vaccine and tests for the detection of antibodies against NSPs essentially provides a marker system to distinguish between vaccinated animals that subsequently become infected and those that have not.

Bearing in mind the good record of modern vaccines in the control of outbreaks and the possibility of screening vaccinated herds for carriers, the author proposes that the OIE reconsider the differences between the requirements for regaining export status following the use of stamping-out as opposed to vaccination in outbreak situations.

Keywords


Introduction

Foot and mouth disease (FMD) occurs in many countries of Asia, Africa, the Middle East and South America (30, 68, 79). In the past, the United Kingdom (UK), Ireland, countries of Scandinavia, Japan, Canada and the United States of America were able to control the disease by ‘stamping-out’. The UK applied this policy by law as early as 1892 (42). In most other countries of Europe, control only became possible after the Second World War, when sufficient vaccine became available, firstly through the introduction of the Frenkel culture technique and later through virus production in baby hamster kidney (BHK) line cells. The quality of vaccines incorporating alhydrogel and saponin as the adjuvant gradually increased up to 1991, when vaccination was discontinued in all countries of the European Union. In South America, the compulsory vaccination of cattle served for many years at best to maintain a status quo of limited outbreaks (30). This was due to the poor quality of the vaccines used and the consequent negative attitude of farmers. However, from the 1980s, all vaccine batches were controlled by independent, government-controlled laboratories, and oil-based vaccines that offer a longer lasting immunity, were gradually introduced. Farming communities regained their trust in vaccination and cooperated. As a consequence, FMD outbreaks became a sporadic event in extensive areas of South America. In the 1990s, many countries achieved freedom from FMD and obtained the status...
of FMD free without vaccination’ from the Office International des Epizooties (OIE: World organisation for animal health) (30, 79).

The history of the disease in South America demonstrates that for the control of widespread disease, the quality of the vaccines employed is crucial for the success of an eradication programme.

The critical elements for the production of quality vaccines are discussed below.

Historical background of FMD vaccine production

After Sabin developed an attenuated poliomyelitis vaccine, much research was carried out along the same lines for FMD in the 1950s and 1960s. The basic approach was to passage FMD virus in vitro culture systems to select a virus population that induced an immune response to the virus without causing disease. Many such vaccines have been developed in the medical and veterinary fields. Production of attenuated (also referred to as ‘modified-live’) virus vaccines in cell cultures is relatively simple and cheap because only a small quantity of infectivity per dose of vaccine is required. However, difficulties were encountered in effecting viral attenuation for all animal types (species, breeds, sexes and ages) in which the vaccines were to be used. The problem of reversion to virulence was a further complicating issue (30, 68). Thus it was difficult to ensure safe attenuation of FMD virus for all varieties of animals that needed to be vaccinated in all circumstances. Outside the risks of back-mutation to virulence, there is the risk of contamination with other viruses, e.g. introduced by contaminated calf serum used in the culture medium. These difficulties resulted in the use of attenuated vaccines being abandoned in the late 1960s and early 1970s in most parts of the world.

The alternative was to use viruses that had been chemically inactivated (i.e. ‘killed’) as the basic immunogen in FMD vaccine. Upon injection into animals, antibodies against the surface proteins of the virus are induced that protect against future infection.

There are three crucial requirements for production of inactivated virus vaccines. Firstly, the viral antigen must be produced in large quantities because each dose of vaccine needs to contain high levels of inactivated virus to be effective. Secondly, the virus preparation must be inactivated in such a way that no residual infectivity remains but at the same time the immunogenicity of the surface proteins of the virus needs to be preserved. Thirdly, an adjuvant must be added to the vaccine to potentiate the antibody response to viral proteins.

The first inactivated vaccines against FMD employed the Waldmann formulation developed in Germany. This essentially involved artificially infecting cattle and then killing the animals at the height of clinical disease and harvesting the tongue epithelium to provide infective virus. Al(OH)3-gel was then added to the virus preparation which was then inactivated with formaldehyde, the virus preparation clarified by chloroform-treatment and filtered through porcelain candle filters (14, 68, 87).

In the late 1940s, Frenkel developed a large-scale in vitro culture system using freshly cut slices of normal bovine tongue epithelium obtained from an abattoir (44). The harvested epithelium was subsequently infected with FMD virus to produce large quantities of newly replicated virus harvest which was mixed with chloroform, clarified by centrifugation and filtered through Seitz filters. Thereafter, the method was similar to that developed by Waldmann (44, for a review, see 14 also). The Frenkel-type vaccine worked very well (19). This method was adopted by a number of laboratories outside the Netherlands (42, 43, 68).

The main disadvantage of the Frenkel system is that during culture some contamination with bacteria and yeast cannot be avoided. In addition, because of the uncertainty of the source of the substrate (e.g. risks of bovine spongiform encephalopathy [BSE]), this method cannot fulfill the requirements for good manufacturing practice (GMP). Nevertheless, the Frenkel method continued to be used in several European laboratories until the discontinuation of vaccination in 1991.

In 1962 Mowat and Chapman (58) reported that FMD virus multiplied efficiently in a BHK cell line (77). These cells were subsequently adapted to growth in suspension culture (27, 28, 31, 82) which enabled relatively easy ‘scaling-up’ to an industrial level (66, 81). This production system provides the basis for almost all FMD vaccine manufactured in the world at present.

Some FMD outbreaks in Europe have been clearly linked to vaccination and/or vaccine production plants, either because the virus contained in the vaccine was not completely inactivated (12, 88), or, it escaped from production or test laboratories by other means (18, 48).

Formaldehyde was used to inactivate FMD virus vaccine for many years but suffers from the disadvantage that the kinetics of the inactivation process is not first-order. For this reason, formaldehyde was replaced by aziridin compounds, in particular binary ethylene-imine (BEI) (3, 4, 25, 26).

Difficulties in Germany and the Netherlands were encountered in the 1960s with controlling FMD outbreaks in pigs using vaccine that was available at that time. Eventually, the use of an eight-fold concentrated Frenkel-type vaccine enabled control of the outbreaks (20). However, it was clear that aqueous
aluminium hydroxide vaccines were not ideal for the protection of this species. Therefore, other adjuvant formulations were tried. It was discovered that antigen emulsified in mineral oil (45) protected pigs well (11, 53, 56). Others found that di-ethyl amino-ethyl dextran was effective in pigs even if added to vaccines containing aluminium hydroxide (49, 89). However, double-oil emulsion vaccines are currently preferred for the vaccination of pigs and in outbreak situations, because they can be used for the protection of all species.

Key elements in foot and mouth disease vaccine production

The production environment

The ease with which FMD virus can spread requires that all work with live virus must be carried out in a containment facility and under a regime that prevents the escape of the virus into the environment.

For GMP requirements, but also to ensure virus containment, all steps of the production process should be conducted in a closed system consisting of stainless steel vessels interconnected by piping. As even safety systems can fail, a strict regime for all staff and maintenance of ‘awareness’ of the risks are essential.

Inside the ‘high-containment’ areas of the production facility only work on live virus should be conducted. All other work, e.g. cultivation of the cells and vaccine formulation should be performed in areas of the facility where exclusion of live virus can be guaranteed. Inactivated antigen, the safety (i.e. freedom from FMD virus) of which has not yet been verified, must be processed in a separate part of the building, also under containment. Movement of people between areas of the facility where live virus may be present and ‘clean’ areas needs to be strictly controlled. Following the principle ‘keep the devil inside the bottle as much as possible’, and, also, ‘where there is no virus it cannot escape’, it is essential that ‘open’ work with live virus in the production facility is conducted in biological safety cabinets only.

In principle, the status of a country or zone ‘free of FMD without vaccination’ should not be compatible with the presence of FMD production facilities unless they are guaranteed secure. Therefore, it may be advisable that in future, an international organisation such as the OIE not only sets detailed requirements for the handling of live FMD viruses, but also carries out inspection on compliance.

Good manufacturing practice

In modern veterinary medicine, products must be produced in compliance with GMP. All substrates that are used in the production process (e.g. components of culture media, cells and vaccine seed viruses) must fulfil quality criteria and each step of the production process must be validated. In addition, buildings and equipment must fulfil minimum standards for finish and performance. Such a system requires licensing and inspection by a relevant authority (62).

Once procedures are validated and accepted they must be described in standard operation procedures (SOPs). Thus, for every production step a SOP must be available and followed exactly and may only be changed or replaced after the new procedure has been validated. All this must be guided and certified by independent quality assurance (QA) and quality control (QC) officers, while QC tests must be carried out independently from production.

Cell cultures

Primary cell cultures are no longer acceptable for use in vaccine production. Besides the possibility that ‘hidden’ viruses may contaminate the vaccine, there is the fear of the presence of prions that cause spongiform encephalopathies. The latter proteins will not be eliminated by the inactivation procedure. Therefore, cells of a well characterised cell line should be used and serum and/or other biological products used for the culture medium should originate from countries free of BSE.

As outlined above, most vaccine producers now use suspension cultures of the BHK cell line (66, 81). Before the cells are used for virus production, the cell growth medium containing calf serum is generally replaced by serum-free medium. Alternatively, poly-ethylene glycol (PEG)-treated serum, from which gamma globulins are removed, may be used in the cell culture growth medium in which case it may not be necessary to use serum-free medium during the virus replication phase. If, after inactivation of the virus, the antigen is concentrated by PEG precipitation, there will be little or no co-precipitation of serum proteins and a purified or partially purified product thus obtained (8, 14).

Seed virus

Selection of vaccine strains

There is a widely held idea that because vaccine strains should be antigenically as close as possible to the strains of virus circulating in the field, local field strains should be used in the manufacture of effective vaccine. This is a misconception. Firstly, many field strains are difficult to adapt to grow in BHK suspension cultures (70). They may not be stable or, after adaptation, their antigenicity may be changed (23, 55). Secondly, it may take a considerable period of time to adapt a new strain for commercial production and complete all the test and validation procedures necessary. Furthermore, some standard vaccine strains perform better and elicit protection against a wider range of intratypic variants than others (22, 70). Thus the desire to incorporate new field strains immediately into commercial vaccines needs to be tempered by the realities. For certain countries or regions in South America, only selected
vaccine strains are permitted by the laboratory of the Pan-American Center for Foot-and-Mouth Disease (PANAFTOSA: Centro Panamericano de Fiebre Aftosa) in Rio de Janeiro in close cooperation with national laboratories and national Veterinary Services. Some strains, e.g. the O₁ BFS-related O₁ Campos, have been used successfully for decades.

Preparation of seed virus

Once the necessary vaccine strains have been identified, they need to be adapted for growth in BHK-suspension cultures. However, most FMD virus strains multiply more readily in BHK cells grown in monolayer than in suspension. The fundamental background to that phenomenon is unknown. The usual approach is to first passage the virus in BHK-monolayer cells. When a rapid (e.g. overnight) cytopathogenic effect occurs, the virus can be further adapted to suspension cultures, until viral replication is optimal. Successively, a ‘mother’ seed-stock and a ‘master’ seed-stock should be prepared. However, passages should be kept to a minimum to minimise the possibility that mutations occurring during passage will result in a vaccine strain that induces an immune response that differs from that of the parent strain. After these stocks have been tested for extraneous agents (e.g. other viruses and mycoplasmas) the master stock can be used to produce a working stock that is stored in aliquots at −70°C. When they have been titrated they can be used at a fixed multiplicity of infection (moi), e.g. 1,000 infectious units per cell. Better standardisation is obtained if a large working stock is prepared, concentrated by ultra-filtration or by precipitation with polyethylene glycol and stored at −70°C. Aliquots can serve for an entire series of production batches.

Quantification of antigen

An ‘in process control’ of each step of vaccine production is required by GMP. The crude virus harvest (including between filtration steps), following inactivation and additional antigen processing steps must all be checked for yields of live virus or inactivated antigen as well as sterility.

Biological tests

Prior to the early 1950s the only methods available for quantification of live FMD virus were titration in cattle tongue (46) and in suckling mice (75). In the 1960s, several in vitro methods became available. Primary foetal calf thyroid cells are the most sensitive cells for detection of FMD virus (65, 76). However, these cells are not always available. A good alternative is IB-RS-2 cells (35) or BHK-monolayer cells, either in tubes or, more conveniently, in micro-titre plates (86). Plaque titration is performed in BHK-monolayer cells with agar overlay (2), or in an agar cell suspension plaque assay in which cells are derived from a large cell stock, stored over liquid nitrogen (6). The method is sensitive and reproducible. Although these biological tests do not indicate much about the antigenic mass required for vaccine formulation, they are required for titration of seed virus and to enable standardisation of the moi. Titration of infectivity (e.g. by plaque assay) is also required for verifying that the kinetics of virus inactivation are within acceptable limits.

Quantitative sucrose density gradient analysis

For years the only means for estimating the antigenic mass of virus harvests was by complement fixation assay (24). A more accurate measure is now enabled by the fact that intact FMD virions have a sedimentation coefficient (S) of 146. So the 146S content of viral harvests is currently estimated (in micrograms per ml) by quantitative sucrose density gradient analysis and continuous flow UV spectrophotometry (10, 37, 41).

Inactivation and ‘in process’ safety testing

Virus inactivation and follow-up safety tests are the most critical steps in the preparation of inactivated FMD vaccines. As outlined above, some FMD outbreaks in Europe have been linked to vaccination, indicating that the virus contained in the vaccine was not always properly inactivated (18, 48). Inactivation of FMD virus by formaldehyde does not follow first order kinetics and may result in ‘tailing-off’ of the inactivation process (12, 25). Vaccine producers also did not realise initially that components in the medium may interfere with the inactivation process (12). For example, the presence of protein digests such as lactalbumin hydrolysate and Tris-buffer inhibit the activity of formaldehyde. Consequently, in the 1980s most vaccine producers changed to inactivation using aziridins, BEI in particular, to provide a safer product (3, 4). Furthermore, because some virus particles may escape inactivation if they do not come into contact with the inactivant, e.g. at ‘dead’ spots inside tubes or valves, it is essential that the virus-inactivant mixture be pumped into a second vessel where the inactivation process is completed (14, 32).

Safety tests can fail to detect low levels of residual infectivity following viral inactivation. The cattle test (injection of small quantities of the inactivated antigen or vaccine into the tongue epithelium of several animals) as prescribed by the European Pharmacopoeia (EP) (32) has often been considered as final proof of safety. However, in the three cattle prescribed by the EP, only 6 ml of vaccine can be tested. The statistical validity of testing a 6 ml sample of a large volume of inactivated virus (e.g. >1,000 l) can be shown to be inadequate. The safety of an antigen/vaccine batch can be controlled more effectively by verifying the inactivation kinetics. Logarithmic plots of residual infectivity against time of inactivation reaction should be linear. The straight line so derived enables extrapolation to the end-point of the inactivation period. Therefore, inactivation kinetics of every vaccine batch need to be checked. Test samples can be stored frozen for back-up to enable repeat testing in cases where the result is not clear-cut. In addition to the use of inactivation plots, a large sample (representing at least 200 doses of antigen) should be tested in vitro (2).
Under certain conditions the inactivation plots obtained during inactivation with aziridines can also show ‘tailing off’. For instance, inactivation by acetyl-ethylene-imine of FMD virus preparations concentrated by PEG demonstrated this phenomenon (7). This example clearly shows that the estimation of inactivation kinetics, as part of ‘in process control’, is absolutely necessary.

As aziridines are highly toxic, many manufacturers were initially reluctant to introduce this agent. Fortunately, bromo-ethylamine hydro-bromide (BEA), which is relatively harmless, can be used instead. The BEA is transformed into the active substance, ethylene-imine, at pH values above 8 (3, 4). This can be done shortly before use in a closed container connected to the vessel that contains the clarified virus harvest.

Vaccine preparations inactivated by aziridines have been found to be less stable than those inactivated by formaldehyde (60, 69). Batches of Frenkel-type vaccine inactivated with formaldehyde often remained fully protective for 10 years or longer, while the shelf-life of the BHK-derived vaccine inactivated with BEI usually does not last longer than two years. Furthermore, vaccines prepared from concentrated antigens inactivated with aziridine are found to be unstable at the International Vaccine Bank (UK) (38, 39).

The stability of vaccines inactivated with formaldehyde may be due to the cross-linking activity of formaldehyde (13, 59, 69). For the labile SAT 2 vaccine strains, it has been shown that stability increased significantly when, after inactivation with aziridine, the antigen was treated with formaldehyde (59, 69). Recently, a synergistic effect has been reported when formaldehyde and BEI were used simultaneously, resulting in a more than 100-fold increased inactivation rate and, therefore, increased safety (15). Sufficient inactivation can be reached within 8 hours (as opposed to over 40 hours) rendering production schedules more flexible.

Purification, concentration and storage of vaccine antigen

Purification of vaccine antigens serves two purposes. Proteins that may induce allergic reactions are either removed or their quantities significantly reduced (17, 47, 54). Secondly, non-structural proteins (NSPs) of FMD virus are similarly removed or their concentration reduced (U. Bruderer and coll., personal communication). The latter proteins are involved in the replication of FMD virus. Thus vaccines prepared from purified antigen will not induce antibodies against NSPs, while infected animals do (21, 52; U. Bruderer and coll., personal communication). Therefore, in combination with tests able to detect antibodies to the NSPs of FMD virus, differentiation between vaccinated and infected animals can be achieved. For that reason, antigens used in modern vaccines, particularly those stored in vaccine banks and intended for the rapid preparation of vaccines for emergency situations, should be shown not to induce the production of antibodies to NSPs.

In general, purification and concentration of inactivated FMD virus is carried out before the results of bacteriological and viral safety tests are available. Therefore, the vaccine bulk at that stage must be considered to contain live virus and these processes must therefore be carried out in a high-containment environment as indicated above. However, recontamination with live virus must also be precluded. Therefore, the ‘quarantine’ area where viral inactivation takes place must be separated from the rest of the production facility. After concentration and purification in the ‘quarantine’ area, the antigen can be stored at ultra-low temperatures (e.g. at −70°C) until its safety is verified. Thereafter, concentrated antigen can then be stored frozen outside the high-containment part of the facility where it is readily available for vaccine preparation.

As FMD vaccines have a shelf-life limited generally to about two years, vaccine manufacturers prefer storage of highly concentrated, inactivated antigen at ultra-low temperatures (8, 38, 51). The shelf-life of manufactured antigens can thus be extended by many years.

Concentration of FMD virus antigens destined for incorporation into vaccine enables the requisite quantity of antigen required to stimulate an effective immune response to be incorporated into a small dose (<3 ml). Even multivalent vaccines (i.e. those containing antigens of two or more serotypes) can be incorporated into volumes <5 ml.

Two alternative methods are applied for the concentration of the inactivated antigen, namely: ultra-filtration and precipitation by polyethylene glycol (PEG).

Ultra-filtration systems for concentrating FMD virus preparations were pioneered by Strohmaier (78). Later, hollow fibre systems were developed as a refinement (57). However, filter materials with cut-off values of 100,000 D and lower, result in harvests that are insufficiently purified. Conversely, cut-offs of 200,000 D and higher, result in considerable loss of 146S material (unpublished findings).

A single precipitation step with PEG is sufficient to remove allergenic components from BHK vaccines (47). In general, PEG with a molecular weight of 6,000 (PEG 6M) is used. The precipitated antigen is harvested by low speed centrifugation (85). Filtration of the precipitated antigen together with a filter-aid, such as calcified diatomaceous earth, was more convenient than centrifugation (8, 51). Antigen collected in the filter-aid in a horizontal disk-type filter can be eluted in a small volume of buffer that is recycled through the filter. Highly purified products can be obtained after a second precipitation-elution cycle. No anti-NSP activity was detected in the sera of cattle that had been vaccinated repeatedly with vaccines prepared from such antigens (U. Bruderer, personal communication).

Inactivated antigen can also be efficiently precipitated with polyethylene oxide but, to the knowledge of the author, this method has not yet been applied on an industrial scale (1).
For producers, concentration of the antigen and storage at ultra-low temperatures offer the great advantage that stocks of antigen can be created (e.g. for vaccine banks). Production schedules become more flexible and the rapid delivery of large quantities of vaccine is possible when needed.

**Vaccine formulation**

To be effective, inactivated vaccines against FMD require that the purified and inactivated antigen harvest be mixed with an adjuvant that potentiates the immune response. Although numerous substances with adjuvant activity are known, only three are used in FMD vaccines, namely: Al(OH)₃-gel (87), saponin (34, 67), and various types of oil emulsion. The first two types of adjuvant are generally used in combination and such vaccines are usually referred to as ‘aqueous’. Vaccines incorporating oil emulsions are based on the antigen being emulsified in a light mineral oil with an emulsifying agent, mannide-mono-oleate, originally developed by Freund (45). Freund’s original ‘complete’ adjuvant also contained bacterial cell wall components. However, this causes severe adverse effects at the injection site and in veterinary practice therefore, only ‘incomplete’ adjuvant, i.e. without bacterial cell wall components, is acceptable. Some producers emulsify the primary emulsion once more in buffered saline containing Tween 80, which provides a product with low viscosity (60).

Good results have been obtained with oil-emulsion vaccines in South America (29, 30, 79). Immunity lasted for more than six months after primary vaccination. Application of these vaccines in cattle enabled a reduction of the frequency of vaccination from three times a year, to six-monthly intervals in the first two years of life and annual vaccination thereafter.

**Approval of vaccines**

Once vaccine formulation, bottling conditions, and labels fulfil the requirements of the EP (32) or other national or international standards, safety and potency tests must be performed under the responsibility of an independent control authority.

In the classical safety test, the vaccine is inoculated intradermalingually into cattle, the part of the animal that is most susceptible to FMD virus (46). As only small volumes (2 ml per animal) can be tested and only three cattle are used, this test does not guarantee freedom of residual live virus as indicated above. The additional intramuscular injection prescribed by the EP does not contribute to better detection because this route is several orders of magnitude less sensitive than the intradermalingual route (46) or susceptible cell cultures (2, 7). The intra-dermalingual test is still used, largely for psychological reasons ‘the product has been tested in the target animal by injection at the most sensitive spot’. It is also still included in the current version of the EP. However, because of the small volume tested, this test will only reveal gross contamination with live FMD virus, which can be readily detected by other tests.

The existing EP monograph on FMD vaccines is not always clear (9). It is understood that for the new version several of the elements mentioned, including in vivo safety tests, will be amended (36).

The EP prescribes that a potency test in cattle must be performed before a vaccine can be released onto the market (32). Three groups of five cattle are vaccinated: one group with undiluted vaccine and the two other groups with 4- and 16-fold dilutions of the test vaccine prepared in buffer. Three weeks after vaccination, the cattle are challenged with the virus strain incorporated in the vaccine. If the feet and other areas (i.e. other than the injection sites on the tongue) remain free of lesions for a period of 8 days, the cattle are considered protected. Unvaccinated control animals must develop lesions on at least three feet. A level of 3 PD₅₀ based on probit analysis is required for a vaccine to pass the test.

The British Pharmacopoeia (BP) prescribes that the dilutions of vaccine be prepared in the adjuvant resulting in roughly 2- to 3-fold higher protection values compared with those obtained with the EP test (5). Thus the high PD₅₀ values reported for the vaccines stored in the International Vaccine Bank in the UK must be valued accordingly. In addition, one must keep in mind that a vaccine that just passes the 3 PD₅₀ by the BP test is likely to fail the EP test. In the view of the author, vaccine potency testing in Europe needs to be standardised.

In South America a different system is used by the national control laboratories (30). The test requires that 16 cattle be vaccinated with a standard dose of vaccine as prescribed by the manufacturer. Three weeks after vaccination, the animals are challenged with approximately 10³ ID₅₀. Twelve cattle (out of 16) must be protected (Argentina requires 13). If the vaccine does not pass the test the first time and if at least 10 (Argentina requires 11) cattle are protected, the manufacturer may request a second test (paid by the manufacturer). If the test fails again, the entire batch under embargo, often 2 million doses or more, is destroyed by the national control authority.

Since there is a good correlation between serum antibody titres and protection of cattle, vaccine potency can also be assessed by serology (63, 64, 84). A study in this connection was conducted under the supervision of PANAFToSA (64). In this study, the sera from about 1,000 protection tests were used to establish an expected level of protection for each antibody titre determined using an indirect enzyme-linked immunosorbent assay (ELISA).

**Vaccine performance**

There is no doubt that modern FMD vaccines, in combination with other zoo-sanitary measures, can be used both to eradicate endemic FMD and to contain and eliminate FMD outbreaks that occur in normally FMD-free countries or zones (16, 50). A number of studies with potent vaccines have shown that
contact transmission can be rapidly reduced by such vaccines (33, 72) and that within 3-5 days of vaccination of pigs, cattle and sheep, the animals concerned develop effective immune responses (40, 71, 72, 74, 80).

However, two basic issues are currently matters of debate and contention, as follows:

a) the biological significance of the fact that vaccinated animals may in some cases be infected in the face of vaccination and, despite protection from clinical disease, become persistently infected, i.e. carriers

b) the safety of exports of live animals and other animal-derived commodities from countries or zones that practice routine vaccination against FMD.

An issue related to these two points is the period of time that should elapse between the cessation of vaccination following an outbreak controlled by vaccination in a normally FMD-free country and resumption of exports of FMD-susceptible animals and animal products to FMD-free countries or zones. The OIE has already proposed a reduction of the time period to six months, as explained in the introduction to this special issue of the OIE Scientific and Technical Review (83).

The availability of tests, including those for antibodies against NSPs, that can be used to differentiate between animals that are serologically positive to structural proteins of FMD virus as a result of infection, as opposed to vaccination, provides a powerful tool to address these issues. More importantly, these tests can in many, but not all, cases be used to differentiate between animals that have merely been vaccinated and those that have been infected subsequent to vaccination (21, 73, U. Bruderer, personal communication). At present it is accepted that the use of NSP tests can be used to verify absence of infection in vaccinated cattle on a herd basis. Whether this is possible for individual animals is currently uncertain because the sensitivity and specificity of NSP tests have not yet been satisfactorily established. It is therefore vital that this is done as soon as possible.

It is a contention that routine vaccination should not be an automatic constraint on exports of FMD-susceptible livestock and their products to FMD-free countries as implied in the current chapter on FMD in the OIE International Animal Health Code (61). The reason is that such exports have not been shown to have initiated FMD outbreaks in the past. Furthermore, with suitable assurances on the levels of herd immunity prevailing in the exporting country and, subject to risk assessment and mitigation of unacceptable risks factors identified, it is likely that the risk of importation from countries that practise routine vaccination can be reduced to acceptably low levels. This approach is not, however, provided for in the current International Animal Health Code. This is an issue that requires reconsideration by the OIE.

The question of the prevalence and epidemiological significance of carrier cattle in vaccinated populations remains unresolved because there is little data on the issue. It therefore seems obvious that for the time being, individual countries need to decide whether to follow the recommendations laid down in the FMD chapter of the International Animal Health Code or, make their own judgement on the basis of scientific risk analysis.

Field experience has shown that following extended outbreaks controlled by the vaccination of entire livestock populations, the biological significance of vaccinated carrier cattle and the risk they represent is small. What has not been addressed so far is the probability that control by vaccination of limited outbreaks (where only a small proportion of the livestock population involved) (e.g. <10%, as in the Netherlands in 2001) represents an even smaller risk. Assessment of that risk might well demonstrate that it represents an acceptable risk. A reassessment of this issue may lead the OIE and international trade organisations to the conclusion that either stamping-out or vaccination of a limited fraction of the livestock population should have similar consequences for future exports, provided that prior serological screening demonstrates freedom from viral circulation. This would enable responsible veterinary authorities to select the best alternative for eradication of outbreaks without undue concerns about future trade opportunities.

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Mise au point et propriétés des vaccins à virus inactivé de la fièvre aphteuse

S.J. Barteling

Résumé
L’auteur retrace brièvement l’historique de la production des vaccins contre la fièvre aphteuse. Il expose les progrès obtenus grâce aux cultures de lignées cellulaires en monocouche et en suspension. Il passe en revue les aspects essentiels pour la production des vaccins modernes, tels que l’inactivation de l’antigène viral, la concentration et la purification successives de l’antigène, ainsi que la formulation finale du vaccin. Outre la grande souplesse conférée au producteur par le stockage à très basse température de l’antigène concentré, cette technique a également permis aux organisations nationales et internationales de créer des banques de vaccins.

La purification des antigènes du virus de la fièvre aphteuse, y compris l’élimination des protéines non structurales, permet de distinguer les réponses immunes des animaux vaccinés de celle des animaux infectés par le virus de la fièvre aphteuse. L’utilisation conjointe d’un vaccin purifié et d’êpreuves de recherche des anticorps dirigés contre les protéines non structurales fournit donc un moyen pratique de marquage capable de reconnaître les animaux qui ont été infectés à la suite de la vaccination.

Compte tenu des bons résultats obtenus avec les vaccins modernes utilisés en cas d’épizootie et de la possibilité de dépister les animaux porteurs au sein des troupeaux vaccinés, l’auteur suggère que l’OIE reconsidère le bien-fondé de la différence entre les conditions exigées pour la reprise des exportations, d’une part, des pays pratiquant l’abattage sanitaire et, d’autre part, de ceux pratiquant la vaccination pour le contrôle d’une épizootie.

Mots-clés
Banques de vaccins – Bonnes pratiques de fabrication – Fièvre aphteuse – Inactivation virale – Production de virus – Prophylaxie.

Elaboración y propiedades de las vacunamas inactivadas contra la fiebre aftosa

S.J. Barteling

Resumen
El autor repasa los antecedentes históricos de la producción de vacunas contra la fiebre aftosa, exponiendo sucintamente los progresos conseguidos gracias al uso de cultivos de líneas celulares en suspensión o monocapa. Después se centra en los aspectos más importantes de la producción de las vacunas modernas, como la inactivación del antígeno vírico, los procesos sucesivos de concentración y purificación del mismo y la formulación final de la vacuna. El almacenamiento del antígeno concentrado a temperaturas ultra bajas ofrece al
fabricante mayor flexibilidad y a los organismos nacionales e internacionales la posibilidad de crear bancos de vacunas.

La purificación de los antígenos del virus de la fiebre aftosa (proceso que comprende la extracción de las proteínas no estructurales) hace posible distinguir entre la respuesta inmunitaria de ejemplares vacunados y la de animales infectados por el virus vivo. Por ello el uso combinado de vacunas purificadas y pruebas de detección de anticuerpos contra proteínas no estructurales constituye en esencia un sistema de marcaje para identificar los ejemplares vacunados.

Basándose en los buenos resultados obtenidos con las vacunas modernas utilizadas en caso de epizootia, y en la posibilidad de someter los hatos vacunados a pruebas de criba para detectar a los animales portadores, el autor sugiere que la OIE vuelva a examinar la justificación de la diferencia entre las condiciones exigidas para que un país pueda reemprender las exportaciones cuando practica el sacrificio sanitario y cuando practica la vacunación para luchar contra una epizootia.

**Palabras clave**

Bancos de vacunas – Buenas prácticas de fabricación – Control – Fiebre aftosa – Inactivación de virus – Producción de virus.

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**References**


