

Antigen and vaccine banks: technical requirements and the role of the European antigen bank in emergency foot and mouth disease vaccination

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Summary

Antigen and vaccine banks are stocks of immunogenic materials ready to be formulated into vaccines (bulk antigens) or ready to use (vaccines) in case of need by one or more of the parties of the bank. These stocks were primarily developed by foot and mouth disease [FMD] free European countries to control unexpected severe FMD episodes after the cessation of routine vaccination in the 1990s. For various reasons, including the lack of suitable antigens or of discriminatory tests to be used following emergency vaccination, such banks have so far not been developed to control other transboundary diseases, although over the last few years stocks of vaccines have been collected by the European Community to support control measures for bluetongue or classical swine fever.

The FMD virus antigens in the banks are stored at ultra-low temperatures (usually -130°C) to guarantee a shelf life of at least five years compared to a shelf-life of one to two years for vaccines stored at $+4^{\circ}\text{C}$. When concentrated, a 50 l volume of antigens can contain up to 15 million cattle doses as per the standard potency specifications in the OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*. Selecting antigen/vaccine strains for storage in a bank and selecting the appropriate strain(s) to be used in the case of emergency vaccination is the responsibility of FMD disease experts. The paper discusses the role of serological testing for the detection of infected animals in a vaccinated population, which is necessary for the recognition of FMD status. Technical advantages and disadvantages of antigen and vaccine banks in general are also outlined in this article. Finally, the experience of the European Community in organising, renewing, and controlling a sizeable FMD antigen bank since 1993 is discussed, and the use of the European Union (EU) antigen bank for international actions outside the EU is presented.

Keywords

Antigen bank – Control strategy – DIVA method – Emergency vaccination – European Community – Foot and mouth disease – Non-structural protein – Strategic reserve – Vaccine bank – Vaccine strain selection.

Introduction

Nowadays, the terms ‘antigen bank’ and ‘vaccine bank’ are better understood than in previous years by those working in the field of infectious or contagious disease control. The history of the foot and mouth disease (FMD) episodes in 2000 in Japan and South Korea, and the devastating epidemic in 2001 in parts of Western Europe remain in the collective memory of many animal health experts (40, 41). In particular the culling of vast numbers of animals, which was the dominant control strategy in 2001, and the limited use of emergency vaccines available from antigens held in antigen banks have triggered an intensive discussion about the most effective and ethically sustainable disease control strategy.

Known worldwide as vaccine banks, antigen banks or strategic reserves, these collections of immunogenic material ready to be used or ready to be rapidly reconstituted into the final vaccine product have, to date, performed well on several occasions. However, these materials have only been utilised, thus far, for the control of FMD outbreaks in order to protect countries that have been free of the disease without vaccination for a long period of time before the outbreak.

The first mention of strategic reserves was made after the devastating outbreak of FMD in Great Britain in 1967-1968 by a high-level commission established by the British Government and chaired by the Duke of Northumberland to examine the outbreak and make recommendations for the future. One of the Commission’s recommendations was to maintain a stock of FMD vaccine for use if a similar outbreak of FMD occurred again. Following the recommendation of the Commission, subsequently referred to as the Northumberland Commission, the British Government purchased annually several hundred thousand doses of completely formulated FMD vaccine types O, A and C and established the first strategic antigen bank in the world. Because the vaccine was completely formulated, it had to be discarded and replaced at the end of its shelf life. In addition to the establishment of a vaccine bank, the British Government encouraged the private sector to invest in vaccine production through providing financial support to the State Laboratory Animal Virus Research Institute (AVRI, now called the Institute for Animal Health, IAH) in Pirbright in the United Kingdom (UK). Consequently, a centre of excellence for FMD vaccine manufacturing developed within the Institute, and during the following years several scientific and technological breakthroughs by researchers at the Institute contributed to the improvement of FMD vaccines.

During the early 1970s, several European manufacturers developed different technologies to concentrate, purify, and store FMD viruses, which have the valuable

characteristic of being able to resist freezing when mixed with appropriate buffers and preservatives.

In 1974, a French manufacturer published the first patented process for the concentration and purification of the FMD virus prior to inactivation using a chemical named Polyox as the active agent (1).

In 1979, Lei and McKercher (33) published the results of a two-year study in Denmark investigating the production of strategic reserves using a virulent form of the FMD virus precipitated on diatomea filters and ready for the processes of inactivation and formulation. The inactivation of virulent virus concentrates was a lengthy process that was full of difficulties due mainly to the occurrence of virus aggregates. The advantages of establishing strategic reserves using already inactivated bulk antigens, which can more quickly be turned into vaccines than virulent viruses, thus, became rapidly evident.

In early 1979, the United States Department of Agriculture (USDA) decided to establish a large strategic reserve of FMD bulk antigens as an alternate source of protection for the livestock industry. This did not imply a change in the policy recommending stamping out as the primary eradication strategy should FMD ever reach the United States of America (USA). However, the potential for a large-scale outbreak, the impacts of such an outbreak, and the related environmental and animal welfare issues were already identified in the late 1970s and dictated the use of vaccination as part of the eradication procedures. Later, Mexico and Canada joined the Bank, referred to as the North American FMD Vaccine Bank, which is presently located at the Plum Island Animal Disease Center in New York in the USA.

In 1985, another joint FMD antigen bank, designated as the International Vaccine Bank (IVB), was established as a strategic reserve at the AVRI (now the IAH). This reserve was established in response to an agreement signed by the governments of Australia, Finland, Ireland, New Zealand, Norway, Sweden, and the UK. Several years later Malta joined the agreement.

In the early 1990s, as a consequence of the cessation of routine vaccination against FMD in the European Community (followed rapidly by similar bans by other governments in Central and Eastern Europe) there was a high demand for the establishment of strategic antigen banks for use in the event of a reappearance of the dreaded disease. Several governments negotiated contracts with manufacturers to establish their own national reserves. In 1992, the European Union (EU) launched an ambitious programme to store several million doses of important representative strains of the FMD virus (12, 30).

From a regulatory perspective, the establishment of strategic reserves led the European Pharmacopoeia to adapt their procedures regarding the emergency release of vaccines prepared from previously controlled antigens (at that time, standards pertaining to the emergency release of vaccines had not yet been included in the *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals [Terrestrial Manual]* published by the World Organisation for Animal Health [OIE] [45]).

Banks of manufactured bottled vaccines

Keeping stocks of vaccines in bottles ready for use and in appropriate locations is a common preventive measure against health threats which have the potential to become animal health disasters, particularly if sufficient amounts of vaccine would otherwise be unavailable in an emergency situation. There is no need for very specialised premises and all types of vaccines against any disease can be stored if they have been manufactured according to standard marketing authorisation procedures.

The main advantage of bottled vaccines is the availability for immediate use for the full duration of the shelf life of the vaccine. Vaccine banks are normally subjected to regular inspection by or on behalf of the owner and the vaccines can be potency tested at the end of the shelf life, if the owner so wishes, to see if the validity period can be extended. One of the administrative disadvantages of vaccine banks that are comprised of ready-to-use bottled vaccines is the need to renew the stocks at the end of the shelf life of the product (between 12 and 24 months). If renewal orders are received too late by the manufacturer, there is a gap between the expiry date of the current bank and the arrival of new stock. Such interruptions in vaccine validity are potentially problematic in the case of an outbreak because a vaccine with an expired shelf life is not acceptable for use by regulators, veterinarians, or farmers. The products stored within the vaccine bank should be carefully managed by the owner such that fresh vaccine supplies should arrive prior to the expiry of the current vaccine supply in order to prevent gaps in product availability.

Because bottled vaccines are completely formulated, they have to be discarded and destroyed at the end of their shelf life. Environmental concerns make the destruction of large amounts of bottled vaccines difficult and costly. Destruction also requires highly specialised premises. For these reasons, vaccine banks are almost always owned by governments or maintained by international organisations and only occasionally owned by manufacturers, for whom

incorrect sales forecasts could result in the costly destruction of large amounts of expired products. However, rolling stocks of extra quantities of ready-to-use vaccines in countries and regions that carry out routine vaccination is a proven effective tool to respond to outbreaks occurring despite the vaccination programme.

Another disadvantage of manufactured vaccines is their limited use in controlling diseases in which antigenic variation of the pathogens is frequently observed (e.g. FMD, avian influenza), or new combinations of field strains require new combinations of antigens in the composition of the vaccine. The formulation of bottled vaccines is fixed and cannot be adjusted, with the exception of the option to increase the volume of the dose injected if the field strain proves to be different from the vaccine strain; such use could seriously decrease the number of doses available for use as marketed by the commercial supplier.

Banks of inactivated antigens stored in bulk

The technology for storing deep-frozen inactivated bulk antigens over liquid nitrogen has been developed over the past thirty years only for FMD antigens. The reason for this is very likely linked with the necessity for the production of large quantities of FMD vaccines for compulsory vaccination campaigns and for the control of outbreaks in previously free areas. Compulsory FMD vaccination campaigns which are carried out during a fixed and limited period of the year require the delivery of huge amounts of FMD vaccines within a short delay. The control by emergency vaccination of FMD outbreaks in areas where routine vaccination is not carried out, likewise requires the mobilisation of large quantities of vaccines within a short time period that have undergone all required controls prior to use. Freshly manufactured vaccines cannot be produced at a capacity to meet such market demands. Consequently, the solution to this problem was found through the development of a new method for storing stocks of concentrated, inactivated, and often purified antigens that can rapidly be formulated into vaccine for use in vaccination campaigns or in the event of an outbreak. When stored frozen over liquid nitrogen (-130°C), concentrated inactivated FMD antigens have a shelf life of more than five years, which is significantly better than the shelf life of bottled vaccines (Table I).

When required for use, antigens kept frozen above liquid nitrogen are subject to formulation into a registered vaccine and must be manufactured according to the regulatory framework of the final vaccine product (registration dossier, good manufacturing practice [GMP]

Table I
Comparison of the shelf life of foot and mouth disease frozen antigens and of foot and mouth disease vaccines prepared from frozen and fresh antigens

Type of product	Shelf life	Vaccine potency
Frozen antigens in banks	5 years at – 130°C	Equivalent
Vaccine prepared from frozen antigens	12 to 24 months at +4°C *	Equivalent
Vaccine prepared from fresh antigens	12 to 24 months at +4°C *	Equivalent

* Temperatures as indicated in the Marketing Authorisation in force

and requirements for the prevention of the transmission of agents causing spongiform encephalopathy). In the version adopted in May 2006 by the International Committee of the OIE, the FMD Chapter of the *Terrestrial Manual* (available at www.oie.int) describes for the first time the storage and monitoring of antigen concentrates.

The use of vaccine could be the best choice to prevent or control many well-known transboundary diseases, such as highly pathogenic avian influenza, classical swine fever (CSF), African horse sickness (AHS), rinderpest, bluetongue, West Nile fever or Rift Valley fever, etc. Due to a low market demand for such vaccines and, consequently, a low return on investment, vaccine producers have not directed research toward the production of antigens for storage in antigen banks for emergency use. In the early 1990s, in an effort to participate in the control of a severe AHS serotype 4 episode raging in Portugal, Spain and Morocco, a European vaccine manufacturer produced a number of commercial batches of inactivated purified AHS serotype 4 antigen (31, 42) to be stored as frozen antigen in bulk until reformulated into vaccines. Later this manufacturer extended this process, on a small scale, to include several batches of inactivated vaccine against vesicular stomatitis (32). The lack of interest at that time by governments and international organisations to use these vaccines in their disease control policy was responsible for the absence of follow-up studies on the target diseases and for the cancellation of the programme concerning the establishment of vaccine banks for other transboundary diseases.

Technical advantages of antigen banks

As the only operational antigen banks are for FMD antigens, the following sections will deal strictly with FMD antigens; however, all of the technical aspects described can be applied to other frozen antigens, provided they share similar properties.

Compared to the traditional ‘in line’ production scheme for freshly manufactured antigen, modern FMD vaccine manufacturing processes include an inevitable step before the final formulation of the vaccine is completed: freezing of the antigens in a revolving antigen bank (Fig. 1).

The following specified technical advantages of reconstituting vaccines from antigens stored in antigen banks outweigh any of their disadvantages.

The first technical advantage of using antigen banks is the consistency in the manufacturing of the vaccine batches. Several runs of inactivation of several thousand litres of industrial virus harvests can be pooled as raw antigens. Equally, several pools of raw antigens can be processed to obtain highly concentrated and purified batches of bulk antigens, resulting in up to seven million doses at a potency of 6 PD50 (50% protective dose) in a volume as small as 50 l. A concentration factor of approximately 300 is very common; however, this value is not frequently exceeded due to the increased antigen losses that this entails.

Under such manufacturing conditions, production and testing of blends of several batches of consistently

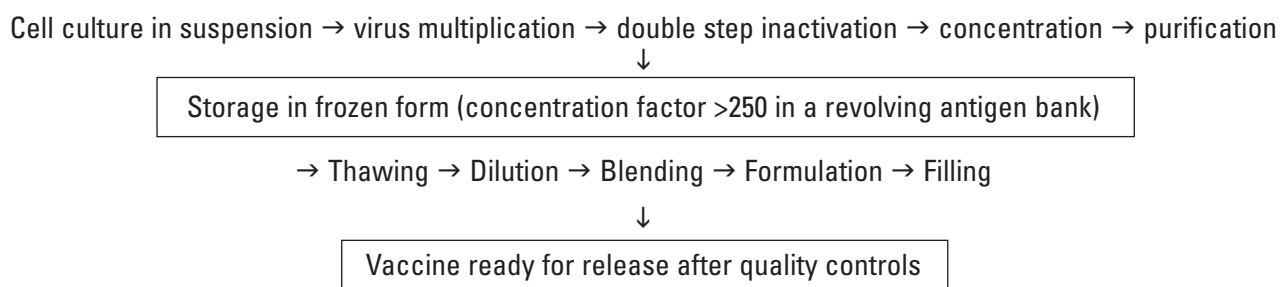


Fig. 1
Modern foot and mouth disease vaccine production scheme, including the storage of frozen antigen (in a revolving antigen bank)

manufactured antigens minimise the number, duration, and cost of quality control tests prescribed in the *Terrestrial Manual* (44) or by the European Pharmacopoeia (28) to assure quality, safety, and efficacy.

The second technical advantage is the possibility to formulate the stored antigens at several different time points, possibly years apart, into the same final vaccine preparation. Additionally, the shelf life of the final product starts from the time the vaccine is formulated without reference to the time that the antigen was produced. Today, between 90% and 95% of FMD vaccines are produced routinely by manufacturers using antigens from antigen stocks, which means that the virus production units and vaccine manufacturing units can operate independently. Thus, at any given time there is a ready-to-use supply of antigens in the antigen bank available to meet the market demand.

The third technical advantage of establishing antigen stocks, applicable to manufacturers of the antigens, is that blends of several batches of monovalent bulk antigens can be formulated into trial vaccines and fully tested before storage. The blends can ensure that any vaccine produced from a given controlled antigen will meet the minimum requirements of the OIE, the European Pharmacopoeia, or other established requirements. During the storage time, periodic tests are conducted to ensure that the antigenic characteristics (antigen content and immunogenicity) of the antigen stocks have not deteriorated (4) (Table II).

The fourth technical advantage is the option to calibrate the final vaccine composition, which is an extension of the third advantage and is commonly used by manufacturers but rarely by bank owners. Starting from the same bulk antigen, several blends made up of different antigen payloads can be tested to adjust the composition of the final vaccine according to the protection level required by the disease situation in the field. Consequently, different compositions of the same bulk antigen can be processed to produce final vaccine preparations with an expected potency ranging from 3 to 10 PD₅₀. This is a true breakthrough for manufacturers who are, therefore, not obliged to wait for the vaccine control results and can adjust the vaccine potency according to the specification required by the contracting party in response to the emergency situation and the immunological relationship of

the vaccine strain to the particular field virus. Consequently, the number of doses available in the antigen bank can vary according to the antigen payload selected to produce the final vaccine preparation, and must therefore always be expressed in relation to the expected potency.

The fifth technical advantage lies in the rapidity with which the antigens can be turned into the final vaccine. Because the antigens have been fully tested before storage it is possible to produce the final vaccine product within a few days of the receipt and registration of an official order. The possibility of the emergency release of vaccines formulated from antigen stocks without waiting for the completion of the quality controls, as permitted by the European Pharmacopoeia providing that the formulation unit complies with the EU GMP requirements, is another major advantage of maintaining antigen banks. Vaccines against FMD are an exception in terms of standard authorisation procedures, which have been outlined in the monograph of the European Pharmacopoeia, but not in the *Terrestrial Manual* at the present time. The European Agency for the Evaluation of Medicinal Products (now known as the European Medicines Agency [EMA]) noted in a Position Paper on Requirements for Vaccines against Foot-and-Mouth Disease, 'The Ph. Eur. monograph "foot-and-mouth disease (ruminants) vaccine (inactivated)" is unique in that it contains a special provision to allow Competent Authorities to release vaccine in the event of urgent need, provided that a trial blend representative of the vaccine to be released has been tested with satisfactory results and provided that the various components of the final blend have passed sterility tests'. Practically, authorisation exception for the early release of emergency vaccine is always used by a client facing an FMD crisis and this explains the very short period of time between the receipt of the order by the manufacturer and the delivery of the vaccine on site (which varies between four and thirteen days according to shipping distance and flight availability).

A sixth technical advantage is associated with the banks that contain highly purified antigen. In-depth purification of bulk antigens has demonstrated the elimination, to a very large extent, of the non-structural proteins (NSP) of the FMD virus (38). Non-structural proteins occur as a result of FMD virus replication and are considered markers

Table II
Quality control scheme currently used for foot and mouth disease antigens in the European antigen bank

Time point	Quality control employed
Prior to storage in bank	Full quality controls according to the marketing authorisation for vaccine release
Each year during storage	Testing of antigen mass (in micrograms) in sample tubes kept with bulk antigens
Mid shelf life and at end of shelf life	Testing of vaccine trial blends from sample vials; vaccine potency is tested on five cattle using a virus neutralisation test

of infection. However, because one copy of the NSP, called 3D or Virus Infection Associated Antigen (VIAA), remains attached to the capsid of a high proportion of virions, complete NSP elimination is not possible. Recently, serological tests have been developed to detect in a vaccinated population those animals that have been infected with replicating FMD virus. These tests rely on the detection of antibodies to the NSP of the FMD virus which are evidence of viral replication in the animal (Table III).

Several authors have published studies on the serology of ruminants after FMD vaccination and infection (5, 35, 36, 37). So far, however, there have only been a few publications on serological investigations following emergency vaccination using vaccines formulated from concentrated inactivated antigens: two of these were presented to the Research Group of the FAO European Commission for the Control of Foot-and-Mouth-Disease (EUFMD) in 1998 (6, 43) and a third to the OIE International Conference on the Control of Infectious Animal Diseases by Vaccination in 2004 (7).

The seventh and last technical advantage of using antigen banks relates to the cooperation between the owners of the banks in assisting each other when outbreaks occur. For example, the EU Antigen Bank (see below) lent several million doses to governments that had made diplomatic requests for vaccine for emergency use in disease outbreaks. The vaccines were used successfully and the vaccine doses were replaced in the EU Antigen Bank a short time later with newly manufactured antigens with a full shelf life.

Such inter-governmental cooperation results in greater efficiency in the global control of FMD using vaccination and allows for greater instant production capacity. Recently, initiatives were launched to create what could be described as a 'global virtual network for antigens from banks' (39) and workshops were organised on the subject by the EU-funded FMD and CSF Coordination Action (a

project that will gather and share information relevant to the control of two of the most important OIE listed diseases, both of which have caused devastating outbreaks of disease in Europe and continue to pose a serious threat; further information is available at www.fmd-and-csf-action.org). However, there are limitations to the sharing and dissemination of information because details on the content of strategic antigen reserves are considered classified information (30).

Technical disadvantages of antigen banks

Difficulties in producing concentrated and purified antigens are not easily overcome since the integrity of the inactivated virus particles (the antigen) has to be maintained during the freezing stage, the storage stage, and the thawing and dilution processes required for vaccine preparation. If the total antigen losses in the final vaccine product are greater than 50% of the initial quantity of virus particles, the process loses much of its advantage and the cost per vaccine dose prepared in this way is commercially non-viable. Industrial know-how is therefore the most important factor for the manufacturer and the profitability of his operation, and for the bank owner who expects the product quality to be similar to a freshly made product. Presently, virus particle recovery, expressed in micrograms of antigen, after production of the final vaccine product is about 70%, which signifies that 30% or more of the virus particles from the initial cultures are regularly lost during the manufacturing process.

The second technical disadvantage associated with antigen banks is the antigen losses which occur during storage at -130°C . At this ultra-low temperature, virus particles rupture or aggregate over time (3). These phenomena are not well documented: firstly, because stability seems to be strain-dependant and secondly, because the data are proprietary and not readily published by manufacturers (34). It is accepted and considered to be normal by manufacturers that 10% of the initial virus particles will be lost within the first five years of storage of highly purified antigens. A very limited number of studies have demonstrated that after 14 years of storage up to 40% of the antigen mass may be lost (3, 34). Such data clearly indicates that the storage duration for strategic reserves is limited and do not support a 'buy and store indefinitely' policy. Regular monitoring and quality control are necessary during the storage period.

The third technical disadvantage associated with antigen banks is that, as already mentioned, the list of antigens stored is predefined and, thus, the bank may not contain the appropriate antigens to respond to a particular epidemiological need. Like several other animal pathogens, the FMD virus has a range of diverse serotypes and a large number of strains within some of the serotypes to which

Table III
Differentiating infected from vaccinated animals (DIVA) system applied to cattle herds vaccinated against foot and mouth disease with vaccines produced with purified antigens from the European antigen bank

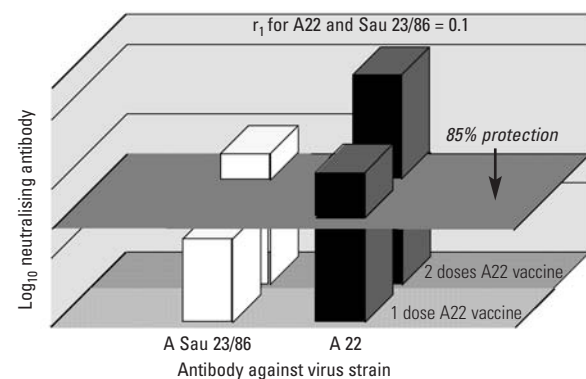
Cattle herds	Seropositivity to FMD virus	Seropositivity to FMD virus NSP
Infected/carriers	Yes (>2 years)	Yes (>2 years)
Multivaccinated and non- infected	Yes (>2 years)	No
Non-infected/Non-vaccinated	No	No

FMD: foot and mouth disease
NSP: non-specific proteins

there is limited cross-immunity. Consequently, there is a probability that the list of antigens retained in an antigen bank may not match or provide immunity against a new pathogen appearing in the field and may become obsolete over a ten year storage period depending on how much the epidemiological situation has changed.

For example, in 1996 a severe A22 related virus outbreak was observed in Albania, which rapidly contaminated a part of the former Yugoslav Republic of Macedonia. The only suitable type A antigen available in the EUFMD antigen bank at the time of the outbreak was the A22 Iraq 1964 virus which was ranked with a serological relationship of only 30% ($r_1=0.3$) with the newly emerged virus. Despite the low serological relationship, a joint decision was made by the EU Commission and the EUFMD to use the A22 Iraq vaccine against the A22 Albania-96 virus and to inject two doses at one month intervals to achieve the level of immunity necessary to stop the epizootic (a similar observation related to a Saudi outbreak is illustrated in Fig. 2).

Additionally, as demonstrated recently by the UK FMD crisis in 2001, viruses occurring in any region of the world are a potential threat to all other regions, no matter how far away from each they are, and consequently should also be considered for inclusion in national or regional antigen banks. Strain selection is a complex responsibility for manufacturers and bank owners. An antigen collection should strive to reflect the major strains involved in recent epidemiological situations and also the strains expected to be involved in potential epidemiological situations in the next five years.



EUFMD: European Commission for the Control of Foot-and-Mouth-Disease

Fig. 2
Low immunological relationship (10%) between the vaccine strain (A 22 Iraq 1964) and a field strain from Saudi Arabia (A Saudi 1986)

A22 Iraq strain is now present in the EUFMD antigen bank
 The second injection of vaccine A 22 Iraq 1964 boosted cross-reactive neutralising antibody levels against the A Saudi 1986 field strain above an expected protection level of 85% (white columns)
 Source: C.G. Schermbrucker (unpublished results)

However, this attempt is hampered because the standard sera produced by manufacturers from their vaccines are again proprietary and this prevents governments or international organisations from being able to constantly match the existing antigens against an evolving epidemiological situation.

The fourth technical disadvantage associated with antigen banks, from the point of view of governments and international organisations, is the vulnerability of the reserves. Even when properly stored and monitored carefully by owners or manufacturers, antigen reserves are vulnerable to terrorism, accidents, or other unpredictable destructive events. Strategic reserves are valuable assets and essential materials for governments and international organisations. Consequently, security should be guaranteed in all cases. One of the solutions to minimising risks associated with strategic reserves involves splitting the antigen reserves between two or more storage sites that are situated at a considerable distance from one another (30). Having more than one storage and adjacent formulation facility is also very convenient when different orders requesting different emergency vaccines are submitted at the same time.

Strategic reserves of vaccines and antigens: the European Union viewpoint in 2006

The current 27 Member States of the EU are home to numerous species that are susceptible to FMD, accounting for approximately 300 million domestic animals. The EU is a major producer and exporter of food of animal origin but also imports products of animal origin from a wide range of countries. Following the establishment of the European Single Market, a high animal health status has been maintained despite a number of serious setbacks due to major outbreaks in certain parts of the Community of infectious animal diseases, such as classical swine fever, foot and mouth disease and, more recently, highly pathogenic avian influenza and bluetongue.

The economic and social consequences of these epizootics together with epidemiological and climatic developments have increased consideration of the role of vaccination in controlling animal diseases of major importance to international trade.

Thanks to these developments vaccination against, for example, African horse sickness or bluetongue has never attracted major media attention and a flexible legislation has minimised the implications of such vaccination on trade.

The great success of a recent oral vaccination campaign against classical swine fever in wild boar in certain areas of the Community has stimulated the establishment of a limited reserve of vaccine against this disease. Recently, the Community adopted legislation on the purchase of additional quantities of a marker vaccine against classical swine fever and specified certain conditions on the use of such vaccines.

At the Agriculture Council convened on 21 December 2004, the European Health and Consumer Protection Commissioner, Markos Kyprianou, announced a new EU Animal Health Strategy to improve the prevention and control of animal disease in the EU. According to the strategy, the Commission plans to propose a Communication in 2007 setting out actions for 2007-2013. The Commission intends to develop a new and improved animal health strategy for the EU that will go beyond what has already been achieved with the existing animal health policy. The announcement concluded that animal disease outbreaks are costly and that there are also ethical issues related to the use of mass slaughter as a disease control method. Furthermore, there is growing concern about the potential impact of certain animal diseases on human health, e.g. a disease like avian influenza could lead to a worldwide pandemic. The new EU animal health strategy, therefore, aims to develop a policy on disease prevention, make emergency vaccination a more viable option, simplify the legislation, and make better use of financial resources. The existing EU animal health policy has undergone an external evaluation, the results of which were discussed at the Conference on Community Animal Health Policy on 7 November 2006 in Brussels (26).

With the recent enlargement of the EU, the Community now shares common borders with a geographical area in which certain major epidemic diseases are not yet eradicated. To stabilise and further improve the animal health situation in those countries neighbouring the EU require close cooperation between EU Member States and infected countries, when possible within the framework of international organisations or through regional agreements, as well as a constant high level of disease awareness and preparedness by the EU Member States, including the capacity for emergency vaccination.

Historically, Council Directive 85/511/EEC established Community measures for the control of FMD (9) (repealed by Directive 2003/83/EC) (15) and required Member States that practised vaccination to carry out vaccination programmes in a more systematic way and in combination with stamping out of infected herds and ring vaccination where necessary. Upon adopting Directive 90/423/EEC (11) (repealed by Directive 2003/83/EC) the Council decided to abandon prophylactic vaccination in eight of

the then twelve Member States that practised such vaccination in cattle and, in turn, made provisions for the use of vaccines in emergency situations and established Community reserves of concentrated inactivated antigen (CIA) of the FMD virus. The details on these reserves are contained in Council Decision 91/666/EEC (13) (last amended by Council Regulation (EC) No. 807/2003) (17). To ensure the quality of the vaccines formulated from the stored antigens, Council Decision 91/665/EEC (12) designated a Community Coordinating Institute and described its functions. However, for technical reasons this Institute was dissolved after the Decision expired on 31 December 1996.

Decision 91/666/EEC also outlined procurement procedures through public tender and provided through the veterinary fund regulated by Decision 90/424/EEC for the financing of the supply and storage of the antigen and the formulation and distribution of the vaccines formulated from such antigen (10) (amended by Directive 2003/99/EC) (16).

It is important to note that the arrangements for the Community antigen bank were not only made to ensure independence from manufacturers and a strategic distribution of relevant antigens but also with the prospect of slaughter of the vaccinated animals. Consequently, little attention was paid to acquiring a marketing authorisation for these vaccines as required for veterinary medicinal products administered to food producing animals in accordance with the Community code relating to veterinary medicinal products described in Directive 2001/82/EC (14) (amended by Directive 2004/28/EC) (18).

Legal aspects

At present the Community control measures for FMD are laid down in Council Directive 2003/85/EC (15) (amended by Decision 2006/552/EC) (25). The new Directive formulates a more prominent role for emergency vaccination in controlling FMD. The Directive distinguishes between 'suppressive vaccination' of animals that are intended to be destroyed following vaccination, and 'protective vaccination' of animals that are intended to be kept alive. In either context, emergency vaccination is incorporated in a stamping out policy applied to infected and suspected to be infected animal holdings and contact holdings and is followed by testing on vaccinated animals with subsequent slaughter of animals in holdings that had contact with the field virus. For the most part, the policy follows the recommendations for the re-establishment of FMD-free status without practicing vaccination (Article 2.2.10.7) and the surveillance guidelines (Appendix 3.8.7) in the OIE *Terrestrial Animal Health Code (Terrestrial Code)*.

The relevant provisions for the Community antigen reserves are contained in Articles 80 to 84 of the Directive and in Annex XIV. In order to facilitate the process of deciding whether or not to implement emergency vaccination, the Directive incorporated recommendations from the report of the European Commission's Scientific Committee on Animal Health and Welfare published in 1999 on the 'Strategy for emergency vaccination against foot and mouth disease (FMD)' (21).

The new legal framework places particular emphasis on marketing authorisation for the vaccines and requirements for the purity of the vaccines with regard to inducing antibodies against NSP. Such requirements are in line with the relevant recommendations in the OIE *Terrestrial Manual* (paragraph 4(c) of Chapter 2.1.1).

Following the recommendations of the report of the Scientific Committee on Animal Health and Animal Welfare in April 2003 on 'Diagnostic techniques and vaccines for foot and mouth disease, classical swine fever, avian influenza and some other important OIE List A diseases' (23), the Community supports the validation of appropriate tests for the detection of infected animals in vaccinated herds. It is worth mentioning that the European Parliament has been following the aforementioned recommendations with great interest and supports the development of tools that make emergency vaccination a viable disease control option.

Procurement of antigens

The following procedures are observed when there is an intention to purchase quantities and subtypes of FMD virus antigen:

- the Commission evaluates the recommendations for priority antigens issued at least once a year by the FAO EUFMD Research Group. However, following the designation of a Community Reference Laboratory in 2006 in accordance with Commission Decision 2006/393/EC (24), it will now be an integral part of the duties and functions of the laboratory to advise the Commission on the priority antigens that should be banked for possible emergencies;
- after obtaining the opinion of the Standing Committee on the Food Chain and Animal Health, which takes into account the estimated needs in accordance with the contingency plans of Member States, the Commission adopts a formal Decision on the purchase of antigens;
- following a public tender advertisement published in the 'S series' of the *Official Journal of the European Union*, a special commission selects the best offer and defends its choice to the Advisory Committee for Procurements and Contracts. However, in certain cases a negotiated

procedure is recommended when the antigens to be purchased may possibly be formulated together with existing stocks of the same strain, other relevant strains, or other relevant serotypes from the same manufacturer in order to provide a complete vaccination campaign, for example, that would be administered in a neighbouring third country;

- subsequently, two contracts are concluded between the Commission and the manufacturer of choice which include the conditions of supply and storage of antigen and the formulation, production, labelling, and delivery of the ready-to-use vaccines reconstituted from the antigens.

The Community purchased antigens in 1993, 1997, 1999, 2000, 2003, and 2006.

Designation, functions and duties of antigen banks

Over the last decade the application of the rules laid down in Decision 91/666/EEC has been modified to take into account technical developments, changes in the structure of the pharmaceutical industry, and production standards. While Directive 2003/85/EC repealed Decision 91/665/EEC and thereby abandoned the established concept of a Community Coordinating Institute as the quality checking institution for antigens stored in the bank, it maintained Decision 91/666/EEC until new provisions could be put in place.

Decision 91/666/EEC allows the Commission to designate premises as a Community antigen bank for the storage of CIA. Following inspection, two of the three designated institutions storing antigens for the Community were abandoned in 2005, thus, concentrating the antigens at two distinct sites of a single manufacturer to reduce the risks of damage to the antigen.

The relevant provisions for the functions and duties of the antigen bank are described in Annex I of Decision 91/666/EEC. In particular the bank shall:

- store the Community reserves of CIA of the FMD virus in such a way as to maintain the usefulness of the antigens for the production of a safe and potent vaccine for emergency use against FMD. In accordance with the European standards for 'Good Manufacturing Practice' this will include keeping adequate records of the conditions under which the antigen is stored, performing regular checks, and when necessary adjusting the temperature regime. The CIA shall be stored at -70°C or colder;
- deliver CIA to the place of formulation, bottling, and distribution of the vaccine at the request of a Member State when emergency vaccination is applied in accordance with Community rules or at the request of the Commission for use of the vaccines in the EU or a third country.

Although the provisions of Decision 91/666/EEC do not contradict Annex XIV to Directive 2003/85/EC, they should be replaced for legal clarity and in order to take into account the Position Paper on Requirements for Vaccines against Foot-and-Mouth Disease (8), adopted by the Committee for Medicinal Products for Veterinary Use (CVMP) on 16 June 2004, and Article 80(4) of Directive 2003/85/EC which requires that:

‘The conditions for the establishment and maintenance of Community reserves of antigen and authorised vaccines at the premises of preferably at least two manufacturing establishments shall be laid down in contracts concluded between the Commission and the manufacturing establishments. Such contracts shall include at least:

- a) conditions for supply of quantities and subtypes of concentrated inactivated antigen;
- b) conditions for secure storage of antigen and authorised vaccines;
- c) guarantees and conditions of rapid formulation, production, bottling, labelling and distribution of vaccines.’

Subtypes and quantities of antigen of the foot and mouth disease virus in the European Union antigen bank

Annex I to Decision 91/666/EEC, as amended by Decision 2001/181/EC (22), requires that the bank maintain antigens in quantities that are sufficient to carry out emergency vaccination, taking into account the estimated risk that the different subtypes present to Community livestock (at least 2 million doses of each subtype). Actual antigen stocks vary between 2 and 5 million doses for individual serotypes and strains depending on the estimated risks and the amounts required to formulate polyvalent vaccines.

The Chief Veterinary Officers of the Member States receive regular updates on the status of the bank in the framework of the Standing Committee on the Food Chain and Animal Health and the secretariat of the EUFMD is informed during the biannual meetings of the Executive Committee.

Technical requirements for the supply of concentrated inactivated antigens and vaccine formulation

Annex II to Decision 91/666/EEC specifies the technical requirements for the supply of CIA and its formulation into vaccines. These requirements are, when applicable, included in the appropriate contracts.

Technical requirements for supply and storage

The storage and supply of vaccines from CIA stored in the European antigen bank are subject to the following technical requirements:

a) The production of antigen and the preparation of finished vaccine shall be carried out in accordance with the principles and guidelines of good manufacturing practice for veterinary medicinal products as laid down in Commission Directive 91/412/EEC of 23 July 1991 (20).

b) In accordance with Article 65 and Annex XII of Directive 2003/85/EC, the establishment which supplies the CIA must comply with the ‘Security standards for FMD laboratories’ outlined in the report of the 30th Session of the FAO EUFMD (27), and the establishment producing the antigen must be included in the list of establishments authorised to handle live FMD virus in Annex XI (B) to the aforementioned Directive. This list was recently amended by Decision 2006/552/EC in order to take account of certain commercial developments in the sector.

c) Full details should be provided on the tests conducted by the producer on the seed virus, cells, and other materials used in the production process. Samples of each master seed virus must be made available for confirmatory testing of identity, purity, safety and potency.

d) The virus shall be propagated in cell cultures. Cells and other ingredients shall be tested to verify freedom from bacteria, fungi, mycoplasma and extraneous viruses. After culture, the virus shall be separated from the particulate matter by appropriate procedures. No seed virus, cell, or ingredient of animal origin shall be derived from animals infected or suspected to be infected with bovine spongiform encephalopathy (BSE). Account shall be taken of:

– the opinion of the EMEA on the potential risks associated with medicinal products in relation to BSE (16 April 1996) (19)

– the current guidelines administered by the CVMP and the Committee for Medicinal Products for Human Use (CHMP) described in the document entitled ‘Minimising the risk of transmission of agents causing spongiform encephalopathy via medicinal products’ (29). It must in particular be ensured that bovine tissue originating in countries affected by BSE is not used or is only used under particular conditions. Documentary evidence of the origin of bovine products shall be made available for confirmatory tests of identity and purity.

e) The antigen and the vaccine must comply with the requirements of the European Pharmacopoeia, particularly those concerning safety, innocuity and sterility.

f) The antigen and the vaccine must exceed the requirements of the European Pharmacopoeia with regard

to potency and should have an observed potency of 6 PD50 in cattle

g) Virus inactivation using cyclised binary ethyleneimine (BEI) or an equivalent method must be validated. The fluids from culture shall be transferred into sterile vessels within 24 h after the addition of the inactivating agent. After completion of the inactivation period, samples shall be removed to verify that inactivation was successful. The inactivation test must comply with the FMD vaccine monograph of the European Pharmacopoeia. For each batch of antigen the kinetics of inactivation must be followed and documented by the producer. The range of inactivation must be such that the entire batch is free from infective virus, and the safety margin should be in the range of about 3 log₁₀ (based on extrapolation).

h) Further processing must be carried out in a non-contaminated environment (FMD virus free). The antigen shall be concentrated and purified by a method that will result in a reduction of the original volume by at least 1/100th and preferably by 1/200th or greater. The purification procedure will be sufficient to ensure a long shelf life of the finished vaccine. The antigen content of the CIA shall be determined as 146 S particles. The manufacturer must specify the number of finished vaccine doses corresponding to the volume unit of CIA.

i) The CIA shall be supplied in containers suitable for storage above liquid nitrogen. Each container shall be labelled with the serotype, serial number, date of harvest and volume, and be sequentially numbered to indicate the order in which the containers were filled. The number of vaccine doses corresponding to the volume of concentrated material in the container shall be indicated.

j) The batch of CIA must be tested prior to delivery to the storage facilities for sterility, innocuity, and potency, in accordance with the European Pharmacopoeia. For these tests, samples of CIA must be formulated into the vaccine product by the manufacturer. Delivery of the batch of CIA to the storage facilities of the manufacturer will be authorized after completion of the tests.

k) Representative samples from the batches of CIA (one batch per subtype) must be made available in sufficient quantity by the contracted manufacturer together with complete information on the tests performed and a detailed description of the vaccine formulation protocol to ensure that potency testing can be performed in accordance with the European Pharmacopoeia each year during the five year storage period. Reformulation of the antigen into vaccine for testing will be carried out by the manufacturer who shall inform the Commission of the results of the tests performed. A batch could be considered unsatisfactory if the 146 S particle content is found to be significantly lower than at the time of the challenge test.

l) Each batch of CIA may be tested on behalf of the Commission by an independent institution at any time for 146 S particles and potency within the five year storage period and during the five years after the Contractor's warranty has ended. The testing shall take place on samples of vaccine reconstituted from stored CIA by the manufacturer. For this purpose the manufacturer shall arrange for sufficient representative samples of each batch at the time of delivery of the CIA to the storage facilities and reserve these samples for external testing.

m) The antigen provided by the producer should have an expected stability of at least five years.

Formulation of vaccines

The formulation and production of vaccines from the CIA stored in the European antigen bank are subject to the following requirements:

a) the guarantee provided by the manufacturer that the vaccine supplied fully complies with the European Pharmacopoeia;

b) supply of the vaccine within the following time limits:

– immediate supply, i.e. delivery of a minimum of 300,000 doses and a maximum of 2 million doses of finished vaccine per formulation site within four days following notice by the Commission;

– urgent supply, i.e. delivery of 1.5 million doses in oil emulsion and 5.5 million doses in aqueous formulation within a period of 5 to 14 days following notice by the Commission;

c) formulation of the vaccines according to the prescription of the producer. Vaccines for pigs will be formulated as oil emulsions. For cattle, vaccines adjuvanted with aluminium hydroxide, saponin or oil may be used;

d) disposal and replacement of any batches deposited in the antigen bank that are found to be unsatisfactory when reconstituted and tested. The cost of testing, disposal, and production of the replacement batch will be the responsibility of the producer;

e) delivery in bottles of suitable size (labelled in the language or languages of the country in which the vaccine is to be used) to predefined places as close as possible to the outbreak;

f) formulated vaccines must be stored at cool temperature conditions as specified in the European Pharmacopoeia. The shelf life should be at least four months, but is normally guaranteed by the contractor to be 24 months, subject to compliance with storage conditions.

Access to and operation of the European antigen bank

The use and operation of the antigen bank is embedded in the decision tree that is used when determining if vaccination should be implemented. Such a decision may only be taken by an affected Member State, except under particularly severe circumstances when the Commission may present a proposal to the Standing Committee on the Food Chain and Animal Health in order to protect wider Community interests.

According to the right of initiative for emergency vaccination within the framework of the approved contingency plans, all Member States have equal drawing rights from the bank independent of the existence of a supplementary national bank. In the case of an emergency, coordination between Members would have to be ensured through the Standing Committee on the Food Chain and Animal Health. For Member States that are members of the EUFMD, coordination between the member countries of that organisation is facilitated through annually updated inventories that are kept as classified information at the EUFMD headquarters and can be accessed by the Chief Veterinary Officers of the member countries.

In the case of emergency vaccination in Member States, the formulation of vaccines is triggered by a request by a Member State to the Commission, independent of whether the decision to vaccinate was initiated by the Member State or was based on a Commission Decision.

The Community has concluded agreements with various neighbouring and some distant countries on regulated and limited access to the bank in the case of an emergency. The Commission therefore welcomes the OIE initiative concerning the establishment of guidelines for international standards for vaccine banks, which are described in Chapter I.1.11 of the *Terrestrial Manual* (45).

The Commission is actively participating in OIE led discussions on cooperation between various antigen and vaccine banks in different regions of the world. However, differences in production standards and registration requirements as well as security aspects have impeded the establishment of a global network of antigen banks. To overcome these difficulties, the relevant services in the Commission actively participate in various FMD oriented programmes, such as in the Work Programme No. 4 on Vaccine Reserves, within the framework of the FMD/CSF Coordination Action (<http://www.fmd-and-csf-action.org/about/workplan/>).

The European antigen bank has been utilised in FMD control measures carried out in third countries: the Balkans in 1996, certain Maghreb states in 1999, the Far East in

2000, and Turkey in 2000 and 2006. When supplying vaccines to countries in the Far East and Turkey the established requirements for immediate supply were met. However, in certain cases it was observed that the timely delivery of the ready-to-use vaccines donated by the Community was delayed due to lack of coordination between different governmental bodies in the beneficiary country involved in the operation.

Testing of antigens

The results of a first round of external testing of antigen stock in the European antigen bank were published in 1996. The Community Coordinating Institute, which is no longer in operation, reported satisfactory results upon testing of four of the antigens in both cattle and pigs (4).

More recently, the Commission adopted Decision 2001/75/EC 'for safety and potency testing of foot-and-mouth disease vaccines and bluetongue vaccines', which included testing of FMD virus antigens banked since 1993. Potency testing carried out in cattle, in accordance with the requirements of the European Pharmacopoeia, confirmed that the tested antigen had a potency significantly above the required 6 PD50, despite the prolonged storage period.

Potency testing in pigs is not described in the European Pharmacopoeia and such testing was not included in the recent review of the FMD monograph of the European Pharmacopoeia due to known problems of overwhelming challenge conditions resulting from unprotected pigs re-challenging other protected vaccinates before isolation. In accordance with Decision 91/666/EEC, antigen must also be suitable for the preparation of oil emulsion vaccines for pigs, in which case 1/6 of the volume of a single dose must protect at least five out of ten pigs when challenged by intrapodal injection of 1,000 ID50. However, when an oil emulsion vaccine formulated from the same antigens was tested in accordance with the relevant guidelines described in the OIE *Terrestrial Manual*, the vaccine failed the test. This failure was most likely due to problems similar to those described previously in comparable tests conducted by Barteling *et al.*, 1996 (4).

Following the designation of a Community Reference Laboratory, plans have been drawn up to proceed with challenge testing in the upcoming years. However, it is important to recognise the difficulties associated with potency testing in the Member States and, thus, to encourage scientists and manufacturers to collaborate in developing suitable alternatives to replace animal experiments, such as seromonitoring of vaccinated animals or the employment of *in vitro* techniques as described by Ahl *et al.*, 1990 (2).

The use of tests for the detection of antibodies against non-structural proteins

With the modifications that were first introduced into the FMD chapter in the fourth edition of the OIE *Terrestrial Manual* and the adoption of amendments to the FMD chapter of the OIE *Terrestrial Code* in May 2002, emergency vaccination may become a more attractive option for controlling FMD.

Modern FMD vaccines should not induce antibodies against NSP if used for the purpose of emergency vaccination. Modifications to the FMD Monograph incorporating such purity requirements were not adopted by the European Pharmacopoeia but were supported by the European Commission and have been included in past procurement activities. Following the Position prepared by the Immunological Working Party of the European Medicines Agency and adopted by the CVMP, it is now up to the purchaser to request that the manufacturer provide substantiation of the claim that the vaccine produced is suitable for post-vaccination surveillance in accordance with OIE requirements.

With regard to the stocks currently maintained in the European antigen bank, guarantees have been provided by the manufacturer that any antigen purchased since 1996 will not induce the production of antibodies against NSP even after multiple administrations. This statement is supported by field findings where serosurveillance was carried out following emergency vaccination in third countries with vaccines supplied from the European antigen bank and through a challenge test requested by the Commission.

Security aspects of operating the European antigen bank

The risks of intentional introduction of FMD virus were discussed at a meeting with participants from the OIE, FAO, EUFMD and EC Commission at FAO Headquarters on 6 and 7 February 2002.

The Commission services shared the conclusions that even the worst case scenario of an intentional simultaneous multi-focal outbreak caused by more than one distinct serotype or strain of FMD virus would not be a feasible approach for bioterrorists if emergency vaccination was a viable option of disease control within the framework of national contingency plans.

Subsequently, certain recommendations from the aforementioned meeting have been taken into account in recent Commission legislative activities. In particular, future control measures for FMD should include

requirements for contingency plans against such scenarios and, in addition, classification of any information about the quantities and subtypes of CIA in the banks.

Conclusion

The experience gained from the use of antigen banks for the control of FMD outbreaks in countries that had remained free from disease for a long time prior to the outbreak shows that this strategic option works effectively in delivering large quantities of vaccine and controlling the spread of disease in fully susceptible populations. Antigen banks represent the best strategy against the lightning spread of FMD in unvaccinated livestock. The key requirement for the success of emergency vaccination is that experts must select the appropriate strains(s) to be stored in the bank and the appropriate strain to be utilised in emergency vaccination campaigns. If an appropriate strain is not available in the antigen bank then an effective vaccine cannot be reconstituted.

The costs of maintaining an updated antigen bank are very few compared to the cost of FMD epizootics in developed countries. The use of emergency vaccination avoids a potential resort to massive culling, which is costly and is usually associated with considerable public concerns regarding animal welfare.

The recent possibility of banking highly purified antigens consisting of ultra-low levels of FMD virus non-structural proteins offers emergency vaccine users the option to perform serological tests that allow differentiation of infected from vaccinated animals (DIVA strategy). The demonstration that the virus is no longer circulating in the livestock in areas in which the emergency vaccine was administered is a necessary step to regain official recognition by the OIE of FMD-free status (46).

Although, until now, antigen banks have mainly been under the management of FMD-free countries, they have been used successfully in a few infected countries through international collaborations. One of the next steps in the antigen bank programme should be the rapid expansion of this successful model to include antigen banks devoted to transboundary diseases. Attracting the interest of vaccine producers in supplying international antigen banks devoted to the main transboundary scourges is necessary in order to achieve this goal.

With the establishment of the Community antigen bank, the EU has developed an operational and effective system to respond to a possible FMD emergency. Such a response system is expensive and can never secure full protection. It therefore remains a primary objective of national authorities and international bodies to prevent the

introduction and spread of this disease into geographical regions that are disease free as well as the dissemination of new virus strains into endemically infected areas.

In order to improve the efficiency of the existing antigen bank, the authors, taking into account numerous discussions with experts from diagnostic, research, and vaccine production laboratories, as well as epidemiologists and administrators, believe that the following points should be urgently addressed:

- the development and validation of alternative potency testing methods to the currently prescribed challenge test in cattle. This is particularly important in light of the decreasing availability of suitable animal housing space and of animal welfare considerations;
- the development of rapid procedures for the determination of the degree of cross-protection between new field isolates and existing vaccines with the aim of replacing, when possible, the costly development of new

vaccines by modulating the composition and potency of currently available vaccines to achieve sufficient cross protection;

- a serious engagement of vaccine manufacturers to facilitate the above objectives and to adhere to minimum standards for the production of vaccines that would allow international cooperation between the banks in the case of an emergency and the exchange of vaccines in the case of shortages;
- compliance of OIE Member Countries with internationally agreed standards for disease notification and information exchange. Such compliance should include the involvement of reference laboratories and the exchange of suitable samples between laboratories for the rapid identification of the virus topotype and the antigenic relationship with existing vaccines, where necessary with the support of international animal health organisations.

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Banques d'antigène et de vaccins : prescriptions techniques, et rôle de la banque d'antigène de l'Union européenne dans la vaccination d'urgence contre la fièvre aphteuse

M. Lombard & A.-E. Füssel

Résumé

Les banques d'antigène et de vaccins constituent des stocks de matériel immunogène prêt à entrer dans une composition vaccinale (pour l'antigène en vrac) ou prêt à être utilisé (pour les vaccins) si cela s'avérait nécessaire pour les différentes parties contribuant à la banque. Ces stocks ont été mis en place (surtout dans les pays européens indemnes de fièvre aphteuse) afin de maîtriser les épisodes imprévus de fièvre aphteuse survenant après que l'application régulière de la vaccination ait été interdite, dans les années 1990. Pour diverses raisons, y compris le manque d'antigènes adéquats ou de tests discriminatoires à utiliser en cas de vaccination d'urgence, aucune banque de ce type n'a à ce jour été prévue pour contrôler les autres maladies transfrontalières, bien qu'au cours des dernières années des stocks de vaccins aient été constitués par la Communauté européenne pour étayer les mesures de lutte contre la fièvre catarrhale du mouton ou le peste porcine classique.

L'antigène du virus de la fièvre aphteuse stocké dans les banques l'est à très basse température (habituellement $-130\text{ }^{\circ}\text{C}$) afin de garantir une durée de conservation d'au moins cinq ans, par opposition aux deux années de conservation garanties par le stockage à $+4\text{ }^{\circ}\text{C}$. Un volume de 50 litres d'antigène concentré peut contenir jusqu'à 15 millions de doses pour application chez les bovins, en vertu des spécifications de puissance prescrites dans le *Manuel des tests de diagnostic et des vaccins pour les animaux terrestres* de l'OIE. Le choix de l'antigène/souches vaccinales à stocker dans la banque et la sélection des souches à utiliser en cas de vaccination d'urgence sont de la responsabilité des

especialistas de la fiebre aftosa. Los autores estudian el rol de los tests serológicos que permiten reconocer a los animales infectados dentro de una población vacunada, lo que es necesario para evaluar el estatus con respecto a la fiebre aftosa. Los autores resaltan también los beneficios y los inconvenientes técnicos de los bancos de antígeno y de vacunas en general. Para concluir, el artículo recuerda la experiencia de la Unión Europea (UE), que organiza, renueva y supervisa una importante banca de antígeno del virus de la fiebre aftosa desde 1993, así como el uso de esta banca europea dentro del marco de programas internacionales fuera de la UE.

Mots-clés

Banca de antígeno – Banca de vacuna – Fiebre aftosa – Método DIVA – Proteína no estructural – Selección de la cepa vacunal – Stock estratégico – Estrategia de profilaxis – Unión Europea – Vacunación de urgencia.



Bancos de antígenos y vacunas: requisitos técnicos y papel del banco europeo de antígenos en vacunaciones de emergencia contra la fiebre aftosa

M. Lombard & A.-E. Füssel

Resumen

Los bancos de antígenos y vacunas constituyen reservas de material inmunógeno listas para ser formuladas en forma de vacuna (antígenos a granel) o para uso inmediato (vacunas) en caso de necesidad de una de las partes interesadas en el banco. Esas reservas fueron instituidas (básicamente por países europeos libres de fiebre aftosa) con el fin de luchar contra episodios inesperados y graves de fiebre aftosa una vez prohibidas las vacunaciones sistemáticas a partir de los años noventa. Por varias razones, incluyendo la falta de antígenos adecuados o de pruebas discriminatorias que se pueden utilizar en caso de la vacunación de emergencia, tales bancos no han sido hasta ahora desarrollados para controlar otras enfermedades transfronterizas, aunque durante los últimos años la Comunidad Europea ha reservado bancos de vacunas para apoyar las medidas de control para lengua azul o peste porcina clásica.

Los antígenos del virus de la fiebre aftosa de esos bancos se almacenan a temperaturas muy bajas (en general -130°C) para garantizar un tiempo de conservación mínimo de cinco años, frente al año o dos años de vida que presentan las vacunas a $+4^{\circ}\text{C}$. Un volumen de 50 litros de antígenos a elevada concentración puede contener hasta 15 millones de dosis para ganado vacuno, según las especificaciones normativas sobre potencia farmacológica que figuran en el *Manual de pruebas de diagnóstico y vacunas para animales terrestres* de la OIE. Los especialistas sanitarios en fiebre aftosa tienen la responsabilidad de seleccionar tanto las cepas de origen de los antígenos y vacunas que se conservarán en un banco como las cepas apropiadas para vacunaciones de emergencia. Los autores examinan el uso de pruebas serológicas para distinguir en la población vacunada los animales infectados,

distinción indispensable para el reconocimiento del estatus de "libre de fiebre aftosa". Además, los autores destacan las ventajas y desventajas técnicas de los bancos de antígenos y de vacunas en general. Por último, presentan la experiencia de la Unión Europea (UE) a la hora de organizar, renovar y gestionar desde 1993 un banco de antígenos de la fiebre aftosa de un volumen considerable, y describen su empleo en actuaciones internacionales fuera del territorio de la UE.

Palabras clave

Banco de antígenos – Banco de vacunas – Estrategia de lucha – Fiebre aftosa – Método DIVA – Proteína no estructural – Reserva estratégica – Selección de cepas vacunales – Unión Europea – Vacunación de emergencia.



References

- Adamowicz Ph., Legrand B., Guerche J. & Prunet P. (1974). – Un nouveau procédé de concentration et purification de virus. Application au virus de fièvre aphteuse produit sur cellules BHK 21 pour l'obtention de vaccins hautement purifiés. *Bull. Off. Int. Epiz.*, **81**, 1125-1150.
- Ahl R., Haas B., Lorenz R.J. & Wittmann G. (1990) – Report of the 1990. Session of the Research Group of the Standing Technical Committee of EUFMD, Lindholm, Denmark. Food and Agriculture Organization, Rome, 51-60.
- Barnett P.V. & Statham R.J. (1998). – Report of the 1998 Session of the Research Group of the Standing Technical Committee of EUFMD, September, Aldershot, UK. Food and Agriculture Organization, Rome, Appendix 38, 272.
- Barteling S.J., Swam H., Anemaet D.A.J., Tuyn C. & Vreeswijk J. (1996). – Report of the 1996 Session of the Research Group of the Standing Technical Committee of EUFMD, 2-6 September, Hachamisha, Israel. Food and Agriculture Organization, Rome, Appendix 12, 85 pp.
- Bergman I.E., Mello P.A. de, Neitzert E., Beck E. & Gomez I. (1993). – Diagnosis of persistent aphthovirus infection and its differentiation from vaccination response in cattle by use of enzyme-linked immunoelectrotransfer blot analysis with bioengineered nonstructural viral antigens. *Am. J. vet. Res.*, **54** (6), 825-831.
- Berlinzani A., Brocchi E. & De Simone F. (1998). – Report of the 1998 Session of the Research Group of the Standing Technical Committee of EUFMD, September, Aldershot, UK. Food and Agriculture Organization, Rome, Appendix 22, 166 pp.
- Brocchi E., Sorensen K. & Mackay D. (2004). – The use of serology as part of the exit strategy to the 1996 epidemic of FMD in the Balkans. In Proc. Conference on Control of infectious animal diseases by vaccination (A. Schudel & M. Lombard, eds), 13-16 April, Buenos Aires. *Dev. Biol. (Basel)*, **119**, 283-292.
- Committee for Medicinal Products for Veterinary Use (2002). – Position paper on requirements for vaccines against foot-and-mouth-disease, EMEZ/CVMP/775/02-FINAL. European Medicines Agency, London, UK, 1-14. Available at: <http://www.emea.eu.int/pdfs/vet/press/pp/077502en.pdf>.
- Council of the European Union (1985). – Council Directive 85/511/EEC of 18 November 1985 establishing Community reserves of foot-and-mouth-disease. *Off. J. Eur. Communities*, **L 315**, 11.
- Council of the European Union (1990). – Council Decision 90/424/EEC of 26 June 1990 on expenditure in the veterinary field. *Off. J. Eur. Communities*, **L 224**, 19.
- Council of the European Union (1990). – Council Directive 90/423/EEC of 26 June 1990 establishing Community measures for the control of foot-and-mouth-disease. *Off. J. Eur. Communities*, **L 224**, 13.
- Council of the European Union (1991). – Council Decision 91/665/EEC of 11 December 1991 designating a Community Coordinating Institute for foot-and-mouth disease vaccines and laying down its functions. *Off. J. Eur. Communities*, **L 368**, 19.
- Council of the European Union (1991). – Council Decision 91/666/EEC of 11 December 1991 establishing Community reserves of foot-and-mouth-disease vaccines. *Off. J. Eur. Communities*, **L 368**, 21-25.
- Council of the European Union (2003). – Council Directive 2001/82/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to veterinary medicinal products. *Off. J. Eur. Union*, **L 311**, 1.
- Council of the European Union (2003). – Council Directive 2003/85/EC of 29 September 2003 establishing Community measures for the control of foot-and-mouth-disease repealing Directive 85/511/EEC and Decisions 89/531/EEC and 91/665/EEC and amending Directive 92/46/EEC. *Off. J. Eur. Union*, **L 306**, 1.

16. Council of the European Union (2003). – Council Directive 2003/99/EC of 17 November 2003 on the monitoring of zoonoses and zoonotic agents, amending Council Decision 90/424/EEC and repealing Council Directive 92/117/EEC. *Off. J. Eur. Union*, **L 325**, 31.
17. Council of the European Union (2003). – Council Regulation (EC) No. 807/2003 of 14 April 2003 adapting to Decision 1999/468/EC the provisions relating to committees which assist the Commission in the exercise of its implementing powers laid down in Council instruments adopted in accordance with the consultation procedure (unanimity). *Off. J. Eur. Union*, **L 122**, 36-62.
18. Council of the European Union (2004). – Council Directive 2004/28/EC of 31 March 2004 amending Directive 2001/82/EC on the Community code relating to veterinary medicinal products. *Off. J. Eur. Union*, **L 136**, 58.
19. European Agency for the Evaluation of Medicinal Products [now the European Medicines Agency] (1996). – Press Release: 11th Meeting of the committee for veterinary medicinal products, EMEA/CVMP/081/96. Available at: <http://www.emea.europa.eu/pdfs/vet/press/pr/008196en.pdf>.
20. European Commission (1991). – Commission Directive 91/412/EEC of 23 July 1991 laying down the principles and guidelines of good manufacturing practice for veterinary medicinal products. *Off. J. Eur. Communities*, **L 228**, 70.
21. European Commission (1999). – Strategy for emergency vaccination and foot-and-mouth-disease (FMD). In Report of the Scientific Committee on Animal Health and Welfare, adopted 10 March 1999. Available at: http://ec.europa.eu/food/fs/sc/scah/out22_en.pdf.
22. European Commission (2001). – Commission Decision 2001/181/EC of 22 February 2001 amending Annex I to Council Decision 91/666/EEC establishing Community reserves of foot-and-mouth disease vaccines and updating Decision 2000/112/EC with regard to distribution between antigen banks of antigen reserves. *Off. J. Eur. Communities*, **L 66**, 39.
23. European Commission (2003). – Diagnostic techniques and vaccines for foot-and-mouth disease, classical swine fever, avian influenza and some other important OIE list A diseases. In Report of the Scientific Committee on Animal Health and Welfare, adopted 24-25 April 2003. Available at: http://ec.europa.eu/food/fs/sc/scah/out93_en.pdf.
24. European Commission (2006). – Commission Decision 2006/393/EC of 31 May 2006 concerning the designation of the Community reference laboratory for foot-and-mouth disease. *Off. J. Eur. Union*, **L 152**, 31.
25. European Commission (2006). – Commission Decision 2006/552/EC of 3 August 2006 amending Annex XI to Council Directive 2003/85/EC as regards the list of laboratories authorised to handle live foot-and-mouth disease virus for vaccine production. *Off. J. Eur. Union*, **L 217**, 29.
26. European Commission (2006). – EU Animal Health Strategy (2007-2013). Available at: http://ec.europa.eu/food/animal/diseases/strategy/index_en.htm.
27. European Commission for the Control of Foot-and-Mouth Disease (1993). – Security Standards for FMD laboratories. Report of the 30th Session, 27-20 April, Food and Agriculture Organization, Rome, Appendix 6 (ii), 67-78.
28. European Directorate of the Quality of Medicines and Health Care (EDQM) (2002). – FMD (ruminants) vaccine (inactivated) monograph. In European Pharmacopoeia, 4th Ed. EDQM, Strasbourg, 2266 pp.
29. European Medicines Agency (EMA) (2004). – Draft: Note for guidance on guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products, amendments to sections 6.2 and 6.3, EMEA/410/01/rev.3 draft. Available at: <http://www.emea.europa.eu/pdfs/vet/regaffair/041001en.pdf>.
30. Füssel A.E. (2004). – The use of antigen and vaccine banks in case of emergency vaccination in the European Community. In Proc. Conference on Control of infectious animal diseases by vaccination (A. Schudel & M. Lombard, eds). 13-16 April, Buenos Aires. *Dev. Biol. (Basel)*, **119**, 307-315.
31. House J., Lombard M., House C., Dubourget P. & Mebus C. (1992). – Efficacy of an inactivated vaccine for African horse sickness serotype 4. In Bluetongue, African horse sickness and related orbiviruses: Proc. Second international symposium (T.E. Walton & B.I. Osburn, eds). CRC Press, Boca Raton, Florida, USA, 891-895.
32. House J., House C., Dubourget P. & Lombard M. (2003). – Protective immunity in cattle vaccinated with a commercial scale inactivated bivalent vesicular stomatitis vaccine. *Vaccine*, **21** (17-18), 1932-1937.
33. Lei J.C. & McKercher P.D. (1979). – Report of the 1979 Session of the Research Group of the Standing Technical Committee of EUFMD, 12-14 June, Lindholm, Denmark. Food and Agriculture Organization, Appendix B1, 24.
34. Lombard M., Dubourget P. & Schumacher C. (2003). – Strategic reserves, advantages and disadvantages: the manufacturer's experience. In Proc. International Symposium on Foot-and-Mouth Disease: Control Strategies (B. Dodet & M. Vicari, eds), 2-5 June 2002, Lyons. Editions scientifiques et médicales, Elsevier SAS, 221-231.
35. Lubroth J. (1998). – Report of the 1998 Session of the Research Group of the Standing Technical Committee of EUFMD, September, Aldershot, UK. Food and Agriculture Organization, Rome, Appendix 24, 182 pp.
36. Lubroth J. & Brown F. (1995). – Identification of native foot-and-mouth disease virus non-structural protein 2C as a serological indicator to differentiate infected from vaccinated livestock. *Res. vet. Sci.*, **59**, 70-78.

37. Mackay D.K.J., Forsyth M.A., Davies P.R., Berlinzani A., Belsham G.J., Flint M. & Ryan M.D. (1998). – Differentiating infection from vaccination in foot-and-mouth disease using a panel of recombinant, non-structural proteins in ELISA. *Vaccine*, **16** (5), 446-459.
38. Merial Animal Health Ltd, Virology Department of Federal University Rio de Janeiro & Pan American Foot-and-Mouth Disease Centre (PAHO/WHO) (2001). – Repeated administration of maximum payload emergency vaccines made from inactivated purified antigen concentrates do not induce significant titres of antibodies against non-structural proteins of foot-and-mouth disease virus. In Report of the 2001 Session of the Research Group of the Standing Technical Committee of EUFMD, 12-15 September, Island of Moen, Denmark. Food and Agriculture Organization, Rome, Appendix 18, 88-93.
39. Palma E.L. (2004). – A global virtual network for FMD in case of emergency. In Proc. Conference on Control of infectious animal diseases by vaccination (A. Schudel & M. Lombard, eds), 13-16 April, Buenos Aires. *Dev. Biol. (Basel)*, **119**, 317-331.
40. Park J.H., Park Y.J., Kim J.K., Oem J.K., Lee K.N., Kye S.J. & Joo Y.S. (2004). – Vaccination as a control measure during the outbreak of FMD in 2000 in Korea. In Proc. Conference on Control of infectious animal diseases by vaccination (A. Schudel & M. Lombard, eds), 13-16 April, Buenos Aires. *Dev. Biol. (Basel)*, **119**, 63-70.
41. Plumiers F.H. (2004) – Foot-and-mouth disease control using vaccination: the Dutch experience in 2001. In Proc. Conference on Control of infectious animal diseases by vaccination (A. Schudel & M. Lombard, eds), 13-16 April, Buenos Aires. *Dev. Biol. (Basel)*, **119**, 41-49.
42. Sanchez Vizcaíno J.M. (2004) – Control and eradication of african horse Sickness with vaccine. In Proc. Conference on Control of infectious animal diseases by vaccination (A. Schudel & M. Lombard, eds), 13-16 April, Buenos Aires. *Dev. Biol. (Basel)*, **119**, 255-258.
43. Sorensen K.J. & Naletoski I. (1998). – Report of the 1998 Session of the Research Group of the Standing Technical Committee of EUFMD, September, Aldershot, UK. Food and Agriculture Organization, Rome, Appendix 23, 176 pp.
44. World Organisation for Animal Health (OIE) (2004). – Foot and mouth disease, Chapter 2.1.1. In Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, 5th Ed. OIE, Paris, 124-125.
45. World Organisation for Animal Health (OIE) (2005). – Guidelines for international standards for vaccine banks. Chapter I.1.11. In Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (web version only). Available at: http://www.oie.int/eng/normes/mmanual/A_00018.htm (accessed on 21 March 2007).
46. World Organisation for Animal Health (OIE) (2006). – Foot and mouth disease, Chapter 2.2.10. In Terrestrial Animal Health Code, 15th Ed. OIE, Paris, 119-130.
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