Selection of foot and mouth disease vaccine strains – a review


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Summary
The choice of the most appropriate strains of foot and mouth disease (FMD) virus vaccines to use in FMD control programmes and to store in vaccine antigen reserves is based on the matching of representative field isolates from outbreaks around the world to available vaccine strains. However, those involved in FMD control at a national level do not always give this work a high priority, while in countries without effective control of FMD there is little incentive to collect samples or to overcome the constraints on submission to international reference laboratories. In the short term, specific initiatives for targeted collection can provide samples on a periodic basis, but a long-term solution requires the development of FMD control measures. This must be underpinned by the strengthening of local Veterinary Services and laboratories, and by demand-driven provision of sufficient amounts of high-quality vaccine. Difficulties may be increased by commercial constraints on disclosure of the strains used for vaccine production and on the supply of reagents needed for matching tests. Vaccine matching tests are mainly based on in vitro methods – such as virus neutralisation, enzyme-linked immunosorbent assay with polyclonal antibodies and complement fixation – and are performed in a relatively small number of laboratories around the world. In addition to the difficulties of gathering representative field and vaccine strains, neither the reagents nor the methods used for vaccine matching are fully harmonised. Consequently, there is no strict equivalence in the results obtained. Alternative approaches using monoclonal antibody panels and/or viral capsid gene sequencing are being developed and could complement the currently employed serological tests. However, there is limited in vivo cross-protection information, more of which is essential for future validation of the vaccine matching methods. In response to the funding and leadership deficit for vaccine strain selection, a network of World Organisation for Animal Health (OIE) and Food and Agriculture Organization FMD reference laboratories has been established; this gives these laboratories the potential to strengthen the coordination of their work and reporting and thereby improve recommendations on vaccine strain selection.

Keywords
Foot and mouth disease – R value – Sampling – Vaccine matching.
Introduction

Foot and mouth disease (FMD) severely constrains international trade in animals and animal products. Western and central Europe, North America, parts of South America, South Africa, Australasia and some island regions of Asia are currently recognised as being free of FMD, but the disease is still widespread in many countries in Africa, Asia and the Middle East (Fig. 1). Foot and mouth disease is highly contagious, affects all cloven-hoofed animals, and is caused by a virus that exists as antigenically diverse serotypes and intra-typic variants (subtypes); thus infection or vaccination with one serotype of FMD virus (FMDV) does not cross-protect against other serotypes and may also fail to protect fully against other subtypes of the same serotype (4, 5, 24).

The incentive to control FMD varies, since losses due to the disease are more serious in herds of intensively managed and high-productivity animals and in exporting countries. Those parts of the world where FMD occurs can be divided into regions with active control schemes and those where practical steps to control the disease are either not taken or have little impact on overall levels of FMDV circulation.

FMD vaccines are supplied by either local manufacturers or international companies. The vaccines are prepared by large-scale growth of FMDV in cell cultures, followed by inactivation with an aziridine such as binary ethyleneimine. Thereafter, the inactivated viral materials can be formulated with adjuvants into ready-to-use vaccine, or stored as antigen concentrates over liquid nitrogen for many years (19).

Vaccination is widely used to control, eradicate and prevent FMD (16), although reliable figures are not available for current vaccine usage worldwide. Emergency vaccination may be employed in the event of an exotic FMDV serotype being introduced, and to enable this to be implemented rapidly reserves of FMD vaccine have been established, usually in the form of frozen antigens that can be formulated into vaccine at short notice. Such antigen reserves or vaccine banks are mainly held by FMD-free countries or groups of countries, stored either in public laboratories or by private companies (14, 15, 25, 32). Countries that maintain vaccine production plants for systematic vaccination locally could also supply vaccines for use in other regions in an emergency.

Multinational vaccine banks exist in Europe and North America, while there may be 20 or more national banks worldwide. Most of these banks are supplied with vaccine antigens by a small number of international companies (28). The various vaccine antigen banks hold vaccine strains judged to be suitable for protecting against the most likely incursions of disease, and the representatives of the national animal health services who manage these banks should know whether viruses from new outbreaks are matched by their vaccine strains. The degree of match could also be used to help model the value of emergency vaccination in different outbreak scenarios. Since the 2000/2001 outbreaks that affected disease-free regions in East Asia, South America, South Africa and Europe, there has been renewed interest in FMD vaccine antigen reserves.

To select the most appropriate strains of FMDV for use as vaccines to control disease in endemic areas and for
incorporation into vaccine reserves, it is necessary to monitor the current variety of prevailing FMDVs and to check the suitability of currently used or stored vaccine strains. This paper reviews the worldwide collection and antigenic characterisation of FMDV field isolates, and the prospects for an improved system of virus strain surveillance and vaccine updating.

Principles of vaccine selection

Estimating the cross-protection afforded by an FMD vaccine made from a particular virus strain against an antigenically related but not identical field virus is currently not an exact science, and a set of complementary approaches is usually used. The two most important vaccine-related determinants of the protection that a vaccine will afford are:

- how well it can stimulate a strong immunity (potency)
- how closely related it is to the field virus against which protection is sought (antigenic match).

Potency is influenced by the antigen quantity and quality, the antigenicity of the strain, the adjuvant and the formulation of the vaccine. The incorporation of more than one subtype in a vaccine and the use of booster immunisations may augment potency as well as broadening the antigenic spectrum of coverage. In vivo potency tests are usually conducted to verify the quality of vaccine batches and, because a standardised approach is required, a homologous virus challenge is normally employed. Potency can also be estimated from the magnitude of the serological response without challenge. The main in vitro indicator is the concentration of whole viral capsids after sucrose density centrifugation of unformulated vaccine antigen, although the relationship between antigen concentration and potency may be strain dependent and non-linear (8, 9).

In contrast to the routine use of homologous challenge studies to monitor potency, animal challenge is rarely used to evaluate cross-protection between a vaccine and particular field viruses (1, 6, 24) as this requires the use of unique challenge viruses and results of the procedure may not be available in time to inform a decision on vaccine selection and use. Instead, antigenic matching is usually estimated indirectly, by in vitro analysis of the antibody response to vaccination and by comparing the cross-reactivity of sera collected from vaccinated animals against the vaccine and field virus. The availability of reagents for matching to particular vaccines may limit the choice of what is possible, and such methods tend to ignore the effect of potency. There is also a lack of certainty on how the results of in vitro matching tests actually correlate to in vivo cross-protection.

Vaccine matching tests

The starting point for selecting the most appropriate vaccine strain to use against a particular field virus is to determine the latter’s serotype. This is normally established by an enzyme-linked immunosorbent assay (ELISA) or complement fixation test (CFT) using type-specific serological reagents, although methods based on monoclonal antibodies (mAbs) or genetic typing may also be used. If very large numbers of viruses have been isolated and serotyped, it may be neither feasible nor necessary to match them all to all of the available vaccines; rather, ways must be found for selecting a representative sample of field isolates and vaccine strains for analysis. At least two and preferably more isolates should be evaluated from any outbreak or antigenic or phylogenetic cluster; inconsistent results should be followed up to determine whether the inconsistencies are due to genuine differences or to an artefact of testing. Representative isolates can also be selected on the basis of the history accompanying the isolates; for example whether they were obtained from different species and locations, or at different times. Field evidence for an apparent lack of vaccine-induced protection may point to the use of an inadequately matched vaccine, but can also result from failures in vaccine quality or delivery.

Meanwhile, in the laboratory, the most useful screening methods for characterising FMD viruses are partial genetic sequencing and/or antigenic profiling, which can reveal the emergence of new strains for which vaccine matching may be required, or conversely indicate that an isolate is similar to one for which vaccine matching information is already available (24). However, care must be taken when extrapolating between nucleotide or deduced amino acid differences and antigenic homology, since the impacts of specific amino acid changes on antigenicity are mostly not well determined.

Once the virus has been serotyped and selected for vaccine matching, use can be made of in vitro serological methods which compare the ability of sera collected from vaccinated animals to react with the homologous vaccine virus and candidate field isolates (OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals – new edition in preparation) (18). In vitro neutralisation may be more relevant to in vivo protection than other measures of virus–antibody interaction, although non-neutralising antibodies may also be protective (11, 20). The advantages of ELISA over a virus neutralisation test (VNT) are that the test is rapid, can use inactivated antigens and requires smaller volumes of post-vaccination sera, which are often only available in limited quantities.

The most thorough serological evaluation of cross-protection is provided by the expected percentage of
protection (EPP) method (3), which estimates the likelihood that cattle would be protected against a challenge of 10,000 infective doses after a single or boosted vaccination. This requires the vaccine in question to have been extensively tested in hundreds of cattle by previous challenge tests with homologous virus, enabling tables of correlation between serological response and protection to be deduced by logistic regression. A panel of sera is also needed from at least 16 cattle, collected after vaccination and after re-vaccination. For the matching test, this panel of sera is tested for its ability to react with the homologous FMD vaccine strain and the FMDV field isolate to be matched using VNT or ELISA. The EPP is then determined from the serological titre obtained for each individual serum, by reference to the predetermined tables of correlation between serological titres and clinical protection. The mean EPP is then calculated from the EPP for each individual serum. An EPP of less than 75% when sera from a group of 16 re-vaccinated animals are used, or less than 70% when sera from a group of 30 re-vaccinated animals are used, is an indication that the vaccines will give inadequate protection against the field strain (26).

This method has been widely used by the Pan American Foot and Mouth Disease Center (PANAFTOSA: Centro Panamericano de Fiebre Aflosa), in South America, where the harmonised use of a restricted selection of vaccine strains makes it feasible to generate the potency data and serum panels that are necessary. However, the method depends upon the availability of these data and sera, and this precludes the wider application of the method to encompass all vaccine strains.

A simpler approach is to calculate relationship values (‘r’ values) between FMD viruses using pools of antisera prepared against each vaccine strain to be matched (so-called bovine vaccinal serum [BVS]); the antigenic similarity between vaccines and field isolates is estimated from their comparative reactivity with the appropriate serum pool. If VNT is used (30), the neutralising titres of the BVS against 100 TCID₅₀ (tissue culture infecting dose) of the homologous vaccine strain and the same dose of a field isolate are compared to determine how antigenically similar the field virus is to the vaccine strain. In the case of neutralisation, one-way comparative values (‘r,’ values) greater than 0.3 indicate that the field isolate is sufficiently similar to the vaccine strain for the vaccine to be likely to confer protection against challenge with the field isolate (29). Conversely, values less than 0.3 suggest that the field isolate is so different from the vaccine strain that the vaccine is unlikely to protect. In these cases, either the field isolate should be examined against alternative vaccine strains or, occasionally, it will be necessary to adapt a suitable field isolate to become a new vaccine strain.

Alternatively, serological cross-reactivity can be estimated using a two-step ELISA procedure for vaccine matching (18). First, the field isolate is grown and then titrated against rabbit and guinea-pig immune sera specific to each of the vaccine strains by means of ELISA. The rabbit and guinea-pig sera against which the field isolate has the highest titre gives an indication of the reference strain with which it has the highest affinity. Second, BVS from selected vaccine strains is titrated against the field isolate, with the homologous vaccine strain serving as a control, and the r value is calculated. For r values derived by ELISA, the following guidelines are used for interpretation (13):

- 0.4 to 1.0 indicates a close relationship between field isolate and vaccine strain
- 0.2 to 0.39 indicates that the field isolate is antigenically related to the vaccine strain and that the vaccine strain might be suitable for use if no closer match can be found, provided that a potent vaccine is used and preferably that animals are immunised more than once
- < 0.2 indicates that the field isolate is only distantly related to the vaccine strain, which is therefore unlikely to protect against challenge with the field isolate.

Complement fixation can also be used as the read-out of serological reactivity, using guinea-pig hyperimmune sera against epidemiologically relevant strains, although this method is now largely restricted to South America. For r values derived for CFT, values equal to or higher than 0.25 indicate that the field isolate is related to the vaccine strain (3).

Problems with vaccine matching tests

Many variables can affect the results obtained from in vitro serological matching tests. There may be uncertainty over the provenance and harmonisation of the vaccine strains. These are supplied to reference laboratories by vaccine manufacturers from their master seed stocks so that matching tests can be performed. As valuable proprietary assets, they may be provided only on condition that they are not analysed, since the manufacturer may wish to prevent the reference laboratory from establishing the origin of the strain, in case such information reaches vaccine-producing competitors.

Different manufacturers may have different versions of the same vaccine strain, which may be collected by one or more reference laboratories. At the Institute for Animal Health (IAH) at the Pirbright Laboratory in the United Kingdom, differences have been observed between the cross-reactivity of vaccine strains with the same name, and in one recent case, where it was possible to analyse the viruses concerned, a deletion was found in a critical antigen binding site of the viral capsid of one of the vaccine strains (Valarcher et al., unpublished results). One possible
step that could be taken would be to require vaccine companies to sequence and antigenically characterise their own strains, and at least verify to their own satisfaction that the names given are appropriate.

The next problem of standardisation concerns the antisera raised against the vaccine viruses for use in serological matching tests. These include the sera collected from vaccinated target species (usually BVS) whose cross-reactivity is to be measured, but also rabbit antigen capture antibodies used in ELISA, and guinea-pig hyperimmune sera used in CFT and as detector ligands for ELISA. In some countries, as with the vaccine strains, the BVS may be supplied to reference laboratories by vaccine companies, and different laboratories may receive different BVS for the same vaccine strains from different sources. Although there may be local agreements concerning the preparation of BVS between a particular vaccine manufacturer and a reference laboratory, there is no universal standard and therefore the results obtained by one reference laboratory may not be equivalent to those obtained by another. Differences may arise with respect to the number of animals used in BVS preparation, the dose and purity of antigen given, the adjuvant used, the exact time after vaccination at which BVS has been collected and the acceptable titre range against the homologous virus. Some inconsistencies may be smoothed out by the fact that the reactivity of these sera to field isolates is always related to that against the vaccine strain in question. However, experience has shown that different BVS prepared against the same vaccine strain can give markedly different matching results (Paton, unpublished results). Reference laboratories may also use different cell culture systems and different vaccine matching tests. Finally some tests have intrinsic variability that makes them poorly repeatable, so that multiple repetitions may be needed for full reliability (31).

An approach used in South America to overcome some of these limitations encompasses a regional network of National Laboratory Services coordinated by PANAFTOSA that selects and characterises the official regional vaccine strains. These strains are sent to the National Reference Laboratories for further distribution to vaccine manufacturers and for use in official batch control. In addition BVS, rabbit and guinea-pig antisera are produced with unified protocols by PANAFTOSA and by the National Laboratory Services.

The role of reference laboratories

To ensure that vaccines and antigen banks cover the global diversity of circulating FMDV as far as possible requires the continuous collection and characterisation of representative viruses from outbreaks occurring in different parts of the world. A thorough knowledge of the vaccine strains available worldwide, combined with relevant vaccine matching assessments, is also necessary. Laboratories in many countries with FMD control or eradication programmes perform virus characterisation of locally obtained FMDV isolates and match them to the vaccine strains prepared locally or regionally. Examples are to be found in India, the People’s Republic of China, Turkey, South Africa and Argentina. However, in many countries with endemic FMD there are no laboratories able to do more than basic characterisation such as serotyping.

FMD reference laboratories have been established to characterise FMDV isolates in support of regional control or eradication programmes in the Americas, western and central Europe and Southeast Asia (10). Such laboratories have been established in South America at the PANAFTOSA/Pan American Health Organization/WHO laboratory in Rio de Janeiro, Brazil, in the former Soviet Union at the All Russian Research Institute for Animal Health (ARRIAH), and most recently in Southeast Asia at the laboratory at Pakchong in Thailand. The reference laboratories of ARIAHL and PANAFTOSA are recognised by the OIE, which has also recognised FMD reference laboratories in Botswana (the Regional Reference Laboratory for Sub-Saharan Africa [RRL SSA]) and at the IAH-Pirbright in England. In the case of the RRL SSA and Africa, the laboratory was created prior to the establishment of a regionally coordinated disease control programme for FMD, while the IAH-Pirbright was established as a reference centre at a time when FMD eradication was still ongoing in Europe. Although the mandates for OIE reference laboratories include the characterisation of field isolates of viruses, the mandates do not specify the regional or global coverage of each reference laboratory. The Food and Agriculture Organization (FAO) has also designated the Pirbright Laboratory as a world reference laboratory (WRL) for FMD, and recognised PANAFTOSA, the Plum Island Animal Disease Center of the United States of America, and the Onderstepoort Veterinary Institute in South Africa as reference laboratories for FMD Figure 2 shows the countries from which FMDV samples or isolates have been submitted to different OIE reference laboratories in the last five years, while Table 1 shows the type of characterisation performed at these laboratories.

The laboratory at PANAFTOSA provides an example of how vaccine matching is used to determine whether a range of locally harmonised vaccine strains continues to be appropriate for regional use. National laboratories in South America perform typing by CFT and ELISA and subtyping by CFT, using panels of hyperimmune sera against epidemiologically relevant strains. Further characterisation (sequencing, mAb reactivity and vaccine matching) is performed either locally or in PANAFTOSA. Vaccine
matching studies to determine whether the official vaccine strains continue to be appropriate for regional use are mainly performed against the official vaccine strains used in the region, because historically the epidemiology of the disease never showed exotic strains. The recommended approach takes into consideration the \( r \) value by CFT (guinea-pig hyperimmune sera), and the expected protection level of the field strain against a panel of vaccinated and re-vaccinated cattle sera. Strains with \( r \geq 0.25 \) and 70% EPP against a panel of bovine revaccinated sera (from 30 cattle) are considered as belonging to the vaccine subtype, and therefore not requiring changes in vaccine composition. In some cases, representative field isolates are also tested for reactivity with BVS by VNT.

IAH: Institute for Animal Health (England)
PANAFTOSA: Centro Panamericano de Fiebre Aftosa (South America)
ARRIAH: All Russian Research Institute for Animal Health
RRL SSA: Regional Reference Laboratory for Sub-Saharan Africa

Fig. 2
Countries from which foot and mouth disease virus samples or isolates have been submitted to different reference laboratories between 2000 and 2005 (including those countries in transition)
Secondary transfers between the Pan American Foot-and-Mouth Center, the All Russian Research Institute for Animal Health and the Institute for Animal Health-Pirbright also occurred but are not shown

Table 1
Vaccine matching at different OIE reference laboratories

<table>
<thead>
<tr>
<th>Reference laboratory</th>
<th>Mandated by</th>
<th>Average number of samples received annually in last ten years (number of countries in brackets)</th>
<th>Number of field isolates sequenced per annum</th>
<th>Number of vaccine strains routinely matched to field isolates</th>
<th>Vaccine matching tests in routine use</th>
</tr>
</thead>
<tbody>
<tr>
<td>IAH-Pirbright</td>
<td>OIE, FAO</td>
<td>526 (31)</td>
<td>180</td>
<td>~30 (^{a})</td>
<td>( r ) values by liquid phase blocking ELISA and by VNT</td>
</tr>
<tr>
<td>PANAFTOSA</td>
<td>OIE, FAO, RIMSA</td>
<td>46 (9)</td>
<td>30</td>
<td>3</td>
<td>( r ) values by CFT and VNT, and EPP</td>
</tr>
<tr>
<td>ARRIAH</td>
<td>OIE</td>
<td>50 (5)</td>
<td>6</td>
<td>9</td>
<td>( r ) values by liquid phase blocking ELISA and by VNT</td>
</tr>
<tr>
<td>RRL SSA</td>
<td>OIE</td>
<td>50 (6)</td>
<td>35</td>
<td>7</td>
<td>( r ) values by VNT</td>
</tr>
</tbody>
</table>

\(^a\) ~70 vaccine strains and matching bovine vaccinal serum held
IAH: Institute for Animal Health
OIE: World Organisation for Animal Health
CFT: complement fixation test
ELISA: enzyme-linked immunosorbent assay
EPP: expected percentage of protection
FAO: Food and Agriculture Organization
In contrast, the Pirbright WRL has a mandate to receive and characterise FMDV isolates globally for the benefit of both endemic and disease-free regions. An attempt is therefore made to match representative field isolates received at the WRL to vaccine strains used locally as well as to those made by international vaccine companies and held in vaccine antigen reserves (13). The normal procedure is for all submitted viruses to be serotyped by ELISA, and this is followed by the sequencing of the complete VP1 gene. Representative viruses (based on sequencing subtype) are matched by ELISA to a selection of vaccine strains, including those most likely to be used and most likely to be effective. The two-step procedure by ELISA described above is carried out routinely on a selection of field isolates, and representative field isolates are also periodically tested for reactivity with BVS by VNT.

Reference laboratory services are usually provided without charge to the submitter of samples on condition that any results can be made available to the OIE, FAO and, on request, to national disease control authorities and vaccine manufacturers. Periodic reports from reference laboratories currently provide only summary information on the detailed characterisation of field isolates.

Collection and submission of field samples

International transport of specimens for FMD diagnosis has been constrained by the fact that such samples were classified as dangerous goods by the International Air Transport Association. Compliance with the rules for carriage by air was therefore difficult and costly, and restrictions often created unpredictable delays in transit, during which the virus present in samples could become inactivated. Preservative solutions can be added to samples to maintain the integrity of the nucleic acids for genome sequencing, even after prolonged storage without cooling. However, current methods, other than the addition of glycerol, make recovery of the infectious virus difficult (17). Since March 2005, however, diagnostic specimens have been categorised separately from dangerous pathogens, making their carriage subject to fewer restrictions.

Not all countries are willing to send samples to international reference laboratories; this may reflect a preference to have testing done in their own national laboratories or a lack of openness about disease reporting. However, reference laboratories can usually obtain representative virus isolates from outbreaks that represent new incursions into previously disease-free countries or regions. The same is true for those parts of the world with active control schemes, where outbreaks are investigated and samples or isolates are likely to be available, along with field information on vaccine efficacy. In contrast, due to the lack of resources or of incentives to carry out investigations, information and samples are rarely available from countries without control measures, although these countries may pose a significant threat of international disease spread.

Specific recommendations are difficult to make about the numbers of samples that should be collected and analysed from regions with endemic infection, since the optimal number will depend heavily on the prevailing circumstances and resource constraints. Guidelines on the intensity of sampling should be set regionally, within a defined FMD control programme that takes account of local conditions, and the relevant reference laboratory should take a lead in such decisions. Within ecosystems, it is important to ensure that there are no unexplored gaps where unknown variants may lurk undetected. This should be given priority in the first year or two of any investigations.

The key to obtaining samples for FMDV characterisation is to provide incentives for those who submit the samples. Those countries that feel the threat to their economies of FMD, which may spread to them from affected regions or countries where FMD is not treated as a priority, should commit themselves to supporting the implementation of eradication programmes in those regions and countries. Financial support for the costs of international sample shipment would be beneficial in some parts of the world that lack FMD control schemes or adequately resourced Veterinary Services. Sample collection could be funded by the international community in selected areas where a high degree of FMDV genetic diversity is consistently observed (such as parts of Africa and Asia), in order to detect new variants at an early stage.

In the long term however, FMD surveillance can only be sustained by the development of control schemes, supported by an adequate supply of FMD vaccines and the will to find out whether or not particular vaccines are the most appropriate that could be made available. This approach has been very successful in Europe and South America, and is beginning to be applied in other regions, such as Southeast Asia, leading to significantly better understanding of the virus types that are present. The programme in Southeast Asia is supported by a regional reference laboratory established at Pakchong in Thailand.

Another obvious way to provide incentives for those who submit samples is to improve the efficiency of the characterisation service provided by reference laboratories, for example by reducing turnaround times and making results more widely available.
Future approaches to vaccine matching

Foot and mouth disease viruses involved in outbreaks, and those with the potential to cause outbreaks, need to be properly characterised and matched with available vaccine strains, and the information must be made available to those who need it. This will require improvements at several different levels: structural arrangements, financing of activities, and technical issues relating to the methods employed. As far as the technical difficulties are concerned, there is the added problem that different approaches are entrenched in different regions of the world, limiting the prospect of developing unified methodologies in the near future. An obvious first step towards resolving this problem would be to work on establishing the equivalence of results.

Clearly, laboratory-based services will only improve significantly if there is demand for them from those with the responsibility for controlling FMD in different parts of the world who are also prepared, one way or another, to pay for the services. In some regions it is quite obvious that the present overriding concern in the selection of FMD vaccines is price, with quality frequently a secondary consideration. While such attitudes prevail, little progress will be made. For this reason, increasing the understanding and awareness of the issue among regional and national officials involved in FMD control is essential, and international agencies such as the OIE and FAO obviously have a primary role in this.

Several structural difficulties confront the provision of characterisation and matching services. There is a lack of consensus on whether vaccine matching should be dispersed or concentrated in a small number of centres. If vaccines and BVS could be made more widely available and test approaches standardised, then more matching could be done locally at the point of virus isolation. Otherwise, one or more centres should be established for such studies and representative viruses exchanged between them. There is at present no universally recognised coordination body for FMDV characterisation work, and so leadership at the international level is lacking. This encourages different regions of the world to go their own ways. A coordinating body is also needed to liaise with vaccine manufacturers and vaccine banks. Finally, the laboratories charged with carrying out the matching work need to be adequately financed. This could possibly be done through contributions from international agencies, vaccine manufacturers and vaccine banks. However, if reference laboratory functions are combined with commercial vaccine manufacture, this may lead to suspicion – whether justified or not – that the reference laboratory will manage the information it disseminates for its own commercial advantage.

In response to these needs, a ‘coordination action’ has been funded by the sixth framework programme of the European Union (EU). One of the objectives is to encourage reference laboratories to interact more closely in the control of both FMD and classical swine fever, and as a first step in addressing some of these issues, a network of OIE/FAO Reference Laboratories for FMD has been established.

| Constraints                                                                 | Solutions                                                                 |
|                                                                            |                                                                          |
| Impediments to field isolate and vaccine strain characterisation:          | Establish secure funding                                                 |
| – lack of foot and mouth disease control programmes                        | Develop regional control programmes with strong focus on high-quality vaccine provision |
| – insufficient attention to vaccine quality                                 | Establish and maintain local reference laboratories                      |
| – lack of incentive and resources to submit samples                        | Subsidise collection and local characterisation of samples               |
| – lack of samples (field and vaccine strains)                              | Establish agreements with vaccine manufacturers for making vaccines available while protecting commercial interests |
| Lack of equivalence between vaccine strain selection methods               | Facilitate sample transport                                              |
| – low availability of reagents (especially characterised antisera to vaccine strains) | Encourage disease reporting                                              |
| – uncertain correlation between methods                                     | Strengthen referral service of reference laboratories                   |
| – poor reproducibility of some tests                                       | Develop agreements on intellectual property rights in relation to exchange of virus isolates |

Table II
Constraints on current vaccine selection processes and some possible solutions

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established. This network aims to gather, generate, analyse and make available laboratory information on the international occurrence and spread of FMD, and to provide recommendations on vaccine strain selection for implementing control programmes and for maintaining vaccine antigen reserves. The network will seek to:

a) develop processes based on best practices to achieve equivalence in FMD laboratory outputs

b) collect, characterise (antigenically and genetically), archive and safeguard FMDVs that represent the global diversity of strains

c) agree a memorandum of understanding for exchange of materials and information, and if necessary a materials/information transfer agreement

d) develop a web-based tool for the network to share and make available laboratory information, including vaccine matching results, in as close to real time as possible

c) provide a unified annual network report to the OIE/FAO.

Initially, the network will consist of the four OIE/FAO Reference Laboratories for FMD (IAH-Pirbright, PANAFTOSA, ARRIAH and RRL SSA). Whether or not the network will be able to fulfill these aims will obviously depend critically on the provision of adequate financial support.

An overview of the constraints on current vaccine selection processes and some possible solutions are provided in Table II. The network of FMD reference laboratories should work to improve the equivalence of their matching results by agreeing procedures for BVS production and quality control and for exchange of relevant biological reagents. In those cases where vaccine seeds are not made available to the official laboratories, a dialogue with vaccine manufacturers is needed to establish the best compromise for making vaccines and BVS available while protecting commercial interests. Dependence on vaccine companies for BVS could be avoided if funding was available to enable reference laboratories to produce their own reagents.

A more standardised approach to vaccine matching in different laboratories would improve the comparability of their data, and interlaboratory comparative testing could verify this. A first step might be to organise an interlaboratory comparison of test outcomes, with or without use of the same sets of predistributed diagnostic reagents, vaccine strains and BVS; the FAO-sponsored FMD serology standardisation exercises could serve as a model for this type of activity (27). More information is also needed on what vaccines are available and who is doing matching for them, so that information gaps can be filled.

In the case of vaccine banks, there is also an opportunity for more joint actions. For example, different vaccine banks may carry out their own limited and necessarily imperfect selection tests, but much could be gained by pooling resources internationally. Such cooperation would assist in validating the methods that are used, and thereby reduce the need for duplication of efforts and strengthen the procedures for strain selection and the optimal provision of effective vaccines.

In the future, technological developments may also provide opportunities for more reliable in vitro vaccine matching. Monoclonal antibodies have proved useful for recognising and localising antigenic differences between FMDVs (12, 21, 23), and altered mAb reactivity patterns can identify new viral variants that need to be checked for vaccine matching (2). Use of mAb panels could offer a way of avoiding some of the difficulties that arise from the use of inherently variable batches of BVS for vaccine matching. In theory at least, mAb panels could be prepared that recognise the most important capsid antigen epitopes on particular vaccine strains, and field isolates could then be examined to see which of these epitopes are shared. However, this requires a suite of well-characterised mAbs that are representative of all important epitopes on all important vaccines. Cooperation should therefore be encouraged to develop international panels of mAbs for strain typing, pooling the fragmented collections held by different laboratories worldwide. Further research is also needed to determine the importance and interaction of different epitopes in protection, and therefore the mAb-based approach to vaccine matching cannot yet be fully realised.

Historically, it was easier to carry out serological vaccine matching tests than to sequence viral genes. This is no longer the case, and it would be highly desirable to be able to predict vaccine matches from objective comparisons of the capsid gene sequences of vaccine strains and field isolates. The difficulty lies in identifying which amino acid differences between a vaccine and a field isolate are significant. The capsid structure is known for several FMDV serotypes (O, A, C and South African Territories 1) and a number of key immunogenic sites have been identified (reviewed by Mateu [22]); for example, five humoral antigenic sites have been identified on the surface of the FMDV O capsid (7). Therefore, the likely significance of changes can be evaluated to some extent, although reliable molecular modelling methods of protein structure and interactions are still under development. If confidence in r value calculations could be established, then correlations could be sought between specific amino acid changes and r value alterations.
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Sélection des souches de vaccin contre la fièvre aphteuse – un aperçu


Resumen

Le choix des souches du virus de la fièvre aphteuse convenant le mieux à la fabrication de vaccins pour les programmes de prophylaxie de la fièvre aphteuse et à la constitution de banques d’antigènes repose sur l’appariement d’isolats représentatifs des divers foyers de par le monde et des souches disponibles de vaccin. Malheureusement, les responsables du contrôle de la fièvre aphteuse au niveau national n’accordent pas toujours à ce travail la priorité qu’il faudrait, tandis que dans les pays où la prophylaxie n’est guère efficace il existe peu d’incitations à réaliser des prélèvements et à surmonter les contraintes de leur envoi aux laboratoires de référence internationaux. À court terme, des initiatives spécifiques peuvent être prises pour la collecte ciblée et périodique de prélèvements, mais la recherche d’une solution durable exige de concevoir des mesures de prophylaxie proprement dites. Celles-ci impliquent nécessairement le renforcement des Services vétérinaires et des laboratoires locaux ainsi que la fourniture de vaccins de bonne qualité et en quantités suffisantes pour répondre à la demande. Les contraintes commerciales pesant sur la divulgation des souches utilisées pour la fabrication de vaccin et sur la fourniture de réactifs pour les tests d’appariement risquent d’accroître encore les difficultés. Les tests d’appariement des vaccins, qui recourent essentiellement à des méthodes in vitro telles que la neutralisation virale, le dosage immuno-enzymatique utilisant des anticorps polyclonaux ou encore la fixation du complément, sont réalisés dans un nombre relativement restreint de laboratoires dans le monde. Outre les difficultés d’obtenir des souches représentatives du virus en circulation et vaccinales, ni les réactifs ni les méthodes d’appariement des vaccins ne sont totalement harmonisés. En conséquence, l’équivalence absolue des résultats obtenus n’est pas garantie. Des approches alternatives recourant à des panels d’anticorps monoclonaux et/ou au séquençage dans le gène de la capsid virale sont en cours de mise au point et pourraient compléter les épreuves sérologiques actuelles. Toutefois, il n’existe actuellement que peu d’informations sur la protection croisée in vivo, et il faudra y remédier pour valider les futures méthodes d’appariement des vaccins. Afin de répondre au déficit d’orientation et de financement en matière de sélection de souches vaccinales, l’Organisation mondiale de la santé animale (OIE) et l’Organisation des Nations Unies pour l’alimentation et l’agriculture ont constitué un réseau de laboratoires de référence afin de doter ces derniers des capacités de coordonner et de publier leurs travaux et, partant, d’améliorer les recommandations applicables à la sélection des souches vaccinales.

Mots-clés

Appariement de souches vaccinales – Fièvre aphteuse – Prélèvement – Valeur r.

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Mots-clés

Appariement de souches vaccinales – Fièvre aphteuse – Prélèvement – Valeur r.
Estudio de la selección de cepas vacunales contra la fiebre aftosa


Resumen
La elección de las cepas vacunales más apropiadas para los programas de control de la fiebre aftosa y la creación de reservas de antígenos destinados a la fabricación de vacunas se basa en la comparación de muestras de campo, procedentes de brotes declarados en distintas partes del mundo, con las cepas vacunales disponibles. Sin embargo, en los países que participan en la lucha contra la fiebre aftosa, no siempre se concede una elevada prioridad a esa tarea. Y en aquellos que carecen de un control eficaz de la enfermedad, poco se incita a recoger muestras y respetar las precauciones necesarias para enviarlas a los laboratorios de referencia internacionales. A corto plazo, para disponer periódicamente de muestras, se podría recurrir a iniciativas específicas de muestreo selectivo. Pero para alcanzar una solución a largo plazo será preciso establecer medidas de control de la fiebre aftosa. La aplicación de esas medidas necesita, por un lado, el refuerzo de los Servicios Veterinarios y laboratorios locales y, por otro, el suministro de cantidades suficientes de vacunas de muy buena calidad. Las dificultades pueden multiplicarse debido a las limitaciones de la información, impuestas por razones comerciales, sobre las cepas empleadas para fabricar las vacunas, o del suministro de reactivos para las pruebas de comparación. Esas pruebas, tales como la neutralización viral, la inmunabsorción enzimática con anticuerpos policlonados y la fijación del complemento, se basan fundamentalmente en métodos in vitro y sólo se realizan en un número relativamente reducido de laboratorios mundiales. A la dificultad de reunir cepas de campo y vacunales representativas, se suma otro problema: ni los reactivos, ni los métodos de comparación de las cepas para vacunas han sido totalmente armonizados. Por consiguiente, los resultados obtenidos no son estrictamente equivalentes. Actualmente se están desarrollando técnicas alternativas, que emplean paneles de anticuerpos monoclonales y/o recurren a la secuenciación de la cápside del virus y que podrían complementar las pruebas serológicas existentes. Sin embargo, la información disponible sobre la protección cruzada in vivo, de fundamental importancia para la validación futura de los métodos de comparación de vacunas, es escasa. Para contrarrestar la ausencia de financiación e iniciativa destinada a la selección de cepas vacunales se ha establecido una red compuesta por los laboratorios de referencia de la Organización Mundial de Sanidad Animal (OIE) y la Organización de las Naciones Unidas para la Agricultura y la Alimentación (FAO), merced a la cual, esos laboratorios podrán trabajar y presentar informes en estrecha colaboración y, de ese modo, mejorar las recomendaciones sobre la selección de las cepas para vacunas.

Palabras clave
Comparación de vacunas – Fiebre aftosa – Muestreo – Valor r.
References


