



Review

Estimating the protection afforded by foot-and-mouth disease vaccines in the laboratory



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ABSTRACT

Foot-and-mouth disease (FMD) vaccines must be carefully selected and their application closely monitored to optimise their effectiveness. This review covers serological techniques for FMD vaccine quality control, including potency testing, vaccine matching and post-vaccination monitoring. It also discusses alternative laboratory procedures, such as antigen quantification and nucleotide sequencing, and briefly compares the approaches for FMD with those for measuring protection against influenza virus, where humoral immunity is also important. Serology is widely used to predict the protection afforded by vaccines and has great practical utility but also limitations. Animals differ in their responses to vaccines and in the protective mechanisms that they develop. Antibodies have a variety of properties and tests differ in what they measure. Antibody-virus interactions may vary between virus serotypes and strains and protection may be affected by the vaccination regime and the nature and timing of field virus challenge. Finally, tests employing biological reagents are difficult to standardise, whilst cross-protection data needed for test calibration and validation are scarce. All of this is difficult to reconcile with the desire for simple and universal criteria and thresholds for evaluating vaccines and vaccination responses and means that oversimplification of test procedures and their interpretation can lead to poor predictions. A holistic approach is therefore recommended, considering multiple sources of field, experimental and laboratory data. New antibody avidity and isotype tests seem promising alternatives to evaluate cross-protective, post-vaccination serological responses, taking account of vaccine potency as well as match. After choosing appropriate serological tests or test combinations and cut-offs, results should be interpreted cautiously and in context. Since opportunities for experimental challenge studies of cross-protection are limited and the approaches incompletely reflect real life, more field studies are needed to quantify cross-protection and its correlation to *in vitro* measurements.

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1. Introduction

Foot-and-mouth disease (FMD) is caused by a highly contagious virus (FMDV) eradicated from parts of the world but still common in Africa and Asia [1]. FMDV infects cloven-hoofed, domestic and wildlife species, can be disseminated by a variety of means and causes widespread illness and economic loss due to the disease, costs of control and trade losses. The virus is in the genus *Aphthovirus*, family *Picornaviridae* and exists as multiple serotypes that do not cross-protect, with incomplete intra-serotype cross-protection between some strains [2–5]. Particular serotypes and strains predominate in different parts of the world, with seven global pools recognised. The epidemiology of the disease is characterised by cyclical patterns of increased incidence and spread, reflecting the waxing and waning of population immunity, virus evolution and transmission opportunities [6].

Vaccines are extensively used for prophylaxis and emergency response and can be highly effective in limiting disease and the spread of the infection [7]. However, many factors that can influence the success of vaccination must be carefully controlled [8,9]. The changing patterns of FMD occurrence and viral antigenic phenotypes necessitate ongoing surveillance and review of the protection afforded by vaccines, facilitated by an international network of reference laboratories (<https://www.foot-and-mouth.org/Ref-Lab-Network>) and leading to regional and in some cases national vaccine strain recommendations [10,11]. Methods, especially laboratory techniques, to measure and predict vaccine induced protection are the subject of this review, complementing publications focussing on vaccine strain selection [12,13].

2. FMD diversity and protective immunity

There are seven serotypes of FMDV: one unrecorded since 2004 (serotype C), three largely restricted to Africa (Southern African Territory serotypes 1, 2 and 3) and one to Asia (serotype Asia 1). In contrast, serotypes O and A are widely distributed; serotype O being the most common (~70% of recorded outbreaks) whilst serotype A exhibits greater antigenic diversity. There is continuous evolutionary diversification of the pool of circulating viruses with periodic emergence of new strains and replacement of old ones [14,15,3].

The immune response to FMDV infection involves innate and adaptive immunity [16], mostly studied in cattle, which develop a serotype-specific protection against disease that can be long-lasting [17]. Antibodies transferred through colostrum or passive immunization can prevent FMD [18]. Current commercial vaccines produced from inactivated, cell culture grown virus capsids provide serotype-specific and antibody-mediated protection, but the immune response is qualitatively and quantitatively different to that induced by infection [19]. Immunity and detectable antibodies decline after primary vaccination and must be boosted to sustain protection [20,21]. Anamnestic responses to emergency vaccination can rapidly induce protective immunity, even in animals with low levels of circulating antibody [8]. Long-lasting immunity may develop after multiple rounds of vaccination [22].

Protective antibodies are directed against the surface of the icosahedral FMDV capsid formed of hierarchically arranged and repeating viral subunits (see Fig. 1 and [23]). The target epitopes are often conformational, involving a three-dimensional interaction between different viral proteins, protomers or pentamers. The virus capsid attaches to target cells via a conserved receptor binding site, but other exposed amino acids are highly variable and act as immune decoys [24]. Antibody binding is focussed on antigenic sites, where amino acid changes are frequent and can substantially affect cross-protection between viruses [13].

3. Vaccine induced protection against FMD

Protection induced by vaccination can be measured by potency tests in which vaccinated animals are inoculated with a fixed, high dose of virulent virus by a defined route given at specified times after immunisation. Potency tests in cattle have two international standards [25] but tests in pigs are also used, for example, in China [26]. The ability of vaccination to prevent the virus from disseminating, to cause foot lesions, after intradermolingual (cattle) or intramuscular (pig) inoculation, is taken as the measure of protection with comparison to unvaccinated but challenged, control animals. A design in which all animals receive the dose of vaccine that will be used in the field (PGP design; protection against generalised podal infection) provides a probability of protection where 75% is the pass mark (12 out of 16 animals protected). Subdividing the vaccinated animals into smaller groups, given different vaccine doses (PD₅₀ design; 50% protective dose) provides a quantitative measure of vaccine potency and the minimum pass mark is 3PD₅₀. If all vaccinated animals are protected, then the limit of protection is not established, reducing discrimination between high potency vaccines as an indicator of cross-protection. The findings have high confidence intervals due to variation in the responses of individual animals and the limitation on numbers of animals used per test. The power of PD₅₀ tests can be improved by optimising the relationship between the vaccine doses tested and the desired protection threshold [27].

The protection afforded by vaccination depends upon the antigen dose and integrity, the formulation of the adjuvant, the antigenic match between the vaccine and challenge viruses and the route, weight and timing of the challenge [28,29,30]. Vaccination schedule, herd immunity and complementary control measures will also have a major impact on protection in the field [8,20,31] (Fig. 2).

While potency tests occur under well-defined and controlled conditions, the circumstances of field vaccination and challenge will be diverse. For example, vaccine antigen integrity (affected by expiry date and cold chain) and proficiency of administration will influence the effective antigen dose, whilst species, breed, prior immunity (including maternal immunity) and vaccination, as well as general nutritional and health status may affect the ability to respond to vaccination [32,33]. Additionally, field vaccines are often multivalent, containing antigens of different serotypes, and/or different strains of a given serotype. Potency tests have the advantage of being standardised but may not mimic the most

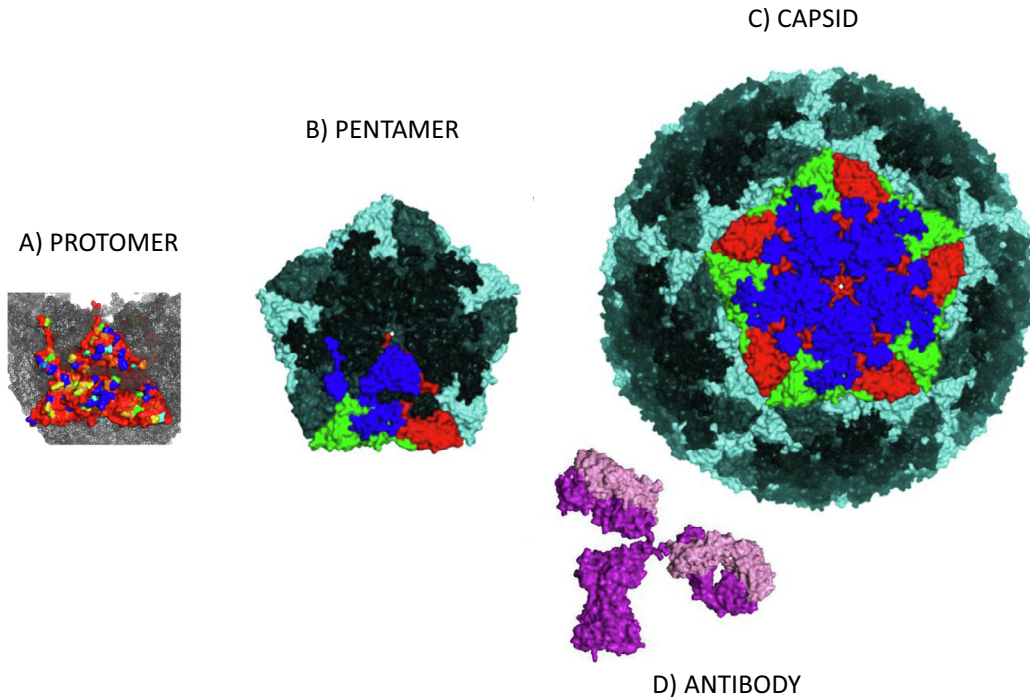


Fig. 1. Schematic for relationships between protomer, pentamer, capsid and antibody. (A) Protomer surface-exposed amino acids coloured to show variability (based on 150 Serotype O FMDV capsids) from blue (most variable) to red (least variable). (B) & (C) Protomer/pentamer surface-exposed amino acids coloured according to protein of origin (VP1, blue; VP2, green; VP3, red). (D). Antibody immunoglobulin (IgG) to scale with the virus, coloured pink (light chains) and purple (heavy chains). Images compiled from Borley D (2012) [23] with permission. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

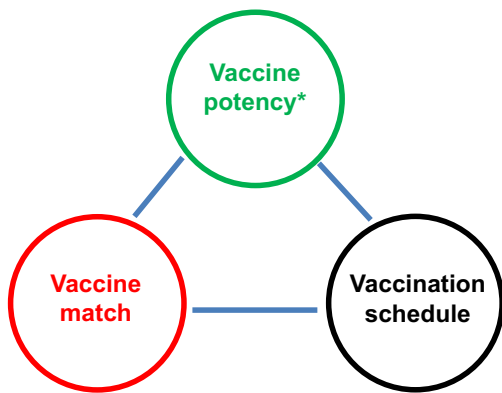


Fig. 2. Efficacy triad of potency, match and schedule. *Potency = strength of elicited protective immunity, principally determined by antigen dose and properties (intrinsic immunogenicity and integrity) and adjuvant.

common routes of natural infection, namely inhalation (in the case of ruminants) or ingestion (in the case of pigs) of lower challenge doses of field virus. The tests measure protection from disease, whereas blocking transmission may be a key objective of some vaccination programmes. Studies to determine the protective effect of FMD vaccines in the field are rare and published accounts have been limited by being retrospective rather than prospective [34,35].

The amount of anti-capsid antibody present at the point of challenge is correlated to clinical protection [28,36]. However, individual animals vary in both the level of antibody induced by vaccination and that required for protection. Pay (1984) [8] reported a standard deviation of 0.4 for the log virus neutralisation test (VNT) antibody response to primary vaccination of cattle, equating to 95% of responses being between 1:20–1:720 for a mean of 1:120. There is also a grey area, in which low levels of antibody may or may not be

protective, whilst some animals may be protected without detectable antibodies [37]. Animals with low levels of total antibodies but high IgG1 or strong interferon gamma responses to *in vitro* antigen restimulation could be protected [38,39].

4. FMD cross-protection

Potency tests required for registration and batch release testing of vaccines [25] are usually based on homologous challenge, in which vaccinated animals are inoculated with a virulent version of the same virus incorporated into the vaccine. In contrast, heterologous challenge tests are uncommon and carried out for research or commissioned on an *ad hoc* basis to verify *in-vitro* antigenic matching test results, usually to predict the protection afforded against an emerging threat.

Cross-protection between strains of serotype A and its correlation with serology was studied in [40]. As expected, the pre-challenge antibody titres to the challenge virus, measured by VNT, were a better predictor of protection (up to 70% sensitivity at 87% specificity) than those against the homologous vaccine strain. The study also illustrated the inter-relationship between vaccine potency and antigenic match, in that high potency vaccines (some ≥ 32 PD₅₀) eliciting strong homologous antibody responses could provide cross-protection against heterologous challenge strains despite a poor antigenic match (Fig. 2). Re-vaccination and combining multiple vaccine strains can also broaden the antibody response, improving the likelihood of cross-protective immunity [41–43].

5. Applications of serological tests in evaluating FMD vaccines and vaccination

Serological tests that measure anti-virus capsid antibodies (SP tests for structural protein antibodies) can be used to assess

protection afforded by vaccines (or prior infection). Other antibodies, produced after infection and directed against viral non-structural proteins (NSP tests), can be used to quantify infection in vaccinated populations as an indicator of vaccination impact [44] but are not considered in this review.

SP tests are used as indirect measures of vaccine potency and match in order to address a number of practical questions about vaccines and vaccination ([9,25]; Table 1).

One-way antigenic relationship tests (r_1 or matching tests) are used by international reference laboratories and vaccine manufacturers to assess the cross-reactive potential of viruses in existing or candidate vaccines against identified virus threats [12,25]. An advantage is that the testing laboratory does not need access to virus or antiserum from a particular vaccine batch, because it is the antigenic suitability of the vaccine strain that is being measured. However, the test does not take account of vaccine potency or vaccination schedule that will also have a major influence on the protection afforded (Fig. 2). Once a batch of vaccine, dose and schedule has been provisionally selected, a better predictor of protection is the strength of the antibody reactivity induced against the field virus for which protection is sought [40], an approach so far mainly used to assess population immunity after vaccination.

6. Traditional serological tests

The two main tests that have been used to measure SP FMD antibodies, as indicators of immunity, are ELISA, principally the liquid phase blocking ELISA (LPBE), and the VNT (Table 2).

Table 1
Use of serological tests to evaluate vaccine match or efficacy.

	Vaccine match	Indirect potency
Test	One-way antigenic relationship (r_1) test (VNT or LPBE)	Homologous or heterologous SP serology (VNT, LPBE, Avidity ELISA, Isotype ELISA)
What is measured	How cross-reactive are vaccine strain antibodies with other viruses	Amount of antibody to homologous or heterologous virus (different tests measure different antibodies)
Uses	Conditional selection of a vaccine strain, with final efficacy dependent upon formulation and vaccination schedule	Indirect potency of a vaccine (e.g. for batch release) or population immunity for post-vaccination monitoring
Advantages	Speed and simplicity compared to <i>in vivo</i> tests. Batch independence.	Speed and simplicity compared to <i>in vivo</i> tests. Heterologous tests take account of match.
Issues	Poor reproducibility (worse for VNT); vaccine strain and BVS availability; poorly predicts protection on its own (re-vaccination, higher dose or multiple strains in a vaccine can broaden antibody specificities).	Reproducibility; brand and batch specific (unless batches are consistent); only post-purchase monitoring unless batch release sera available; field virus antigen preparation (for avidity/isotyping)
Definitive alternatives	Combined results of homologous and heterologous potency based on the same vaccine batch	Respective homologous and heterologous potency tests

NB: VNT, virus neutralisation test; LPBE, liquid phase blocking ELISA; BVS, bovine vaccinal serum.

Table 2
Comparison between serological tests.

Feature	VNT	LPBE	SPCE	Avidity and isotype ELISAs
Status	Currently used	Currently used	Not validated for predicting protection	Novel methods
Measures	Virus neutralising antibodies	Virus binding antibodies	Virus binding antibodies	Quality of virus binding antibodies
Biohazard	Use of live virus	Inactivated or live virus	Inactivated or live virus	Inactivated virus
Reproducibility	Lower	Higher	Higher	Higher
Easy to change test virus	Yes	Needs specific reagents	Not possible in commercial assays.	Moderate. Virus needs to be purified
Volume of vaccine serum required	Large	Small	Small	Small
Correlation to protection	Better	Worse	Not established	Better, more assessments required
Speed	Slow	Fast	Fast	Fast
Availability	In house method	Limited commercial availability	Widely used commercial and in-house methods	In house method

NB: VNT, virus neutralisation test; LPBE, liquid phase blocking ELISA; SPCE, solid phase competition ELISA.

Extensive studies have correlated potency test outcomes with pre-challenge antibody titres measured using the VNT and LPBE [45–47]. The threshold for protection is higher in LPBE than VNT and with either test, the threshold can differ between virus serotypes and strains. The correlation between VNT titres and protection may be laboratory dependent, due to a lack of inter-laboratory reproducibility [45,48].

Commercially available, solid phase competition ELISAs (SPCE) have become alternatives to the LPBE for SP serology. However, for use in vaccine evaluation, their correlation with protection has to be validated by either cross-calibration to gold standard methods, or ideally by testing sera from potency tests where the vaccinated animals have known protection test outcomes. There is, as yet, little data on their power to predict protection [49]. Another limitation, is that whereas the test reagents (viruses in the case of VNT and virus antigens and detecting antibodies in the case of LPBE) can be matched to the vaccine or field viruses for VNT and LPBE, the antigens and blocking antibodies are fixed and usually of unknown antigenic relevance (beyond serotype) in commercial assays.

7. Measuring antigenic relationships

When used for vaccine matching, one-way antigenic relationship (r_1) tests employing VNT must be repeated to verify the consistency of the result [50] whereas ELISA can be more consistent [51]. For both tests, use of pooled, medium to high titre sera reduces the inter-animal and inter-assay variation [52]. VNT r_1 results do not correlate consistently with those determined by

ELISA [53]. Furthermore, the amino acid changes in the capsid that have been identified as altering seroreactivity also differ in some cases for the two tests [53]. Variability may also be observed between r_1 values obtained when different virus isolates from related outbreaks are matched to the same vaccine(s) (e.g. O Egypt 2016 viruses matched against O Manisa vaccine; <http://www.wrlfmd.org/country-reports/country-reports-2016>). Finally, antigenic relationship tests are notoriously difficult to validate. The gold standard against which they can be judged is the combined result of a homologous and heterologous potency test, both using the same vaccine batch, but such data sets are rarely available. Low potency test precision due to constraints on the numbers of animals used [54] may be mitigated by using individual animal results to get a clearer picture, before combining them into the PD_{50} result. However, this cannot be used comparatively, as the individual animals cannot be challenged with both the homologous and heterologous strains.

8. Measuring other properties of antibodies

Alternative serological ELISA tests have been developed in Argentina [38,55] that measure FMD antibody avidity and IgG isotypes (Table 2). The tests react vaccine antisera with purified antigens prepared from each field virus against which protection is to be measured. In the avidity test, the amount of bound antibody is compared with and without urea treatment, whilst in the isotype test, the amount of bound IgG1 and IgG2 is measured, with total IgG1 or IgG1:IgG2 ratio as the indicator of protection. As no comparison is made to the reaction of the antisera against the vaccine antigen, there is no requirement to hold stocks of vaccine virus, which have restricted availability due to their proprietary nature. It appears that IgG class and avidity correlate better with protection than total FMDV antibody (LPBE) or *in vitro* neutralising antibody (VNT) [56]. As ELISAs, these tests also have a good repeatability and can utilise inactivated virus antigen. A practical complication is the requirement to prepare purified antigens for each test virus from high titre virus stocks, although alternative easier purification methods are being developed, for example using size exclusion chromatography (see below). The tests are yet to become routine in international reference laboratories but are in the process of being transferred and evaluated in different parts

of the world and against different serotypes and strains to verify and agree thresholds indicative of protection.

9. Difficulties with antigenic relationship tests

The lack of reproducibility of serological tests could be partly addressed by repetition, if time and cost were not issues, but overcoming the uncertainty of r_1 matching test results, as predictors of vaccine induced protection, requires additional information on vaccine potency and schedule.

Problems of reproducibility have multiple origins, some already discussed. The reagents used to undertake the tests are very difficult to standardise and different cell culture systems may be used. Vaccine antisera are fundamental to one-way relationship, avidity and isotype tests but contain antibodies which may differ qualitatively and quantitatively according to the vaccine used to raise them, the conditions of vaccination, the timing of blood collection and both between and within species differences in the immune response of immunised animals. Cell culture amplification of the field virus stocks required for testing may select for *in vitro* replication leading to fixation of mutations in capsid surface amino acids that can alter the antigenic signatures of the viruses [57].

Different tests measure different aspects of antibody–virus interaction. The VNT measures antibodies that block virus replication in cell cultures but, *in vivo*, other arms of the immune system may have a synergistic effect, such as antibody dependent phagocytosis and cytotoxicity. In contrast, the LPBE measures total antibody binding, which may include cross-reactive but non-protective antibodies to dissociated (internal) capsid components, especially if degraded vaccines or test antigens are used. The avidity and isotyping ELISAs provide specificity for the strength of reaction or the type of antibody involved, reactive to the whole 146S viral particle.

Finally, although a well-matched vaccine will be superior to a poorly matched one, if all other factors are the same (Fig. 3), because of the importance of potency and vaccination schedule, poorly matched vaccines can sometimes protect in the field, and well-matched ones fail to do so.

In practice, the difficulties of vaccine matching can be mitigated by testing multiple field isolates repeatedly to reliably discern patterns of reactivity and by considering other indicators from the laboratory, the field and experimental challenges [12]. An example is

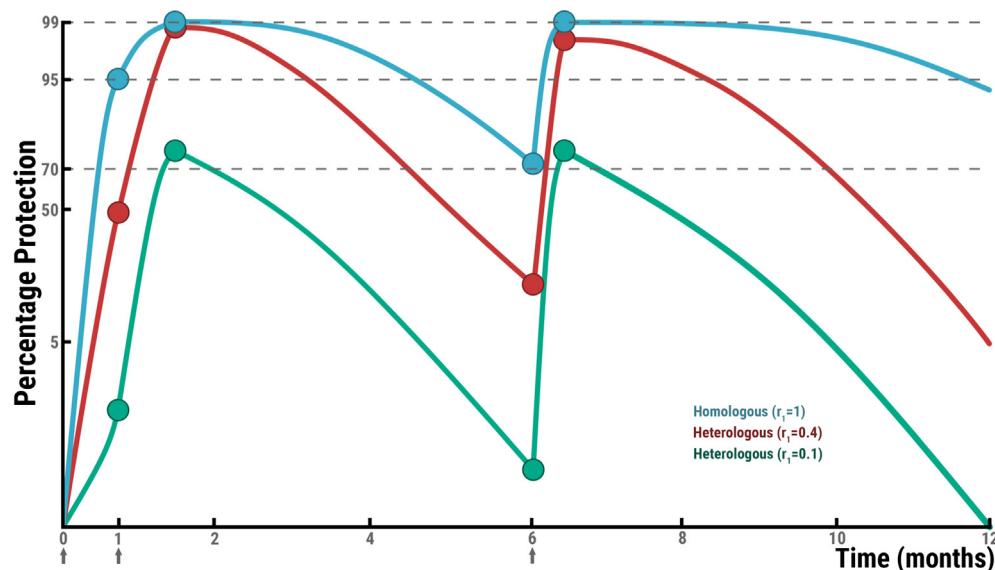


Fig. 3. Protection windows conferred by homologous and heterologous FMD vaccines. Arrows show vaccination time points. Schematic representation, from Pay (1984) [8], based on a figure compiled by Tim Doel, redrawn by Antonello Di Nardo. The profiles of the antibody responses will be affected by vaccine potency.

provided by the characterisation of emerging genotype A/VII viruses from central Asia where concerns over poor matches against vaccines, licensed for use in Europe, were backed up by identification of amino acid changes at critical antigenic sites [58] and were sufficient to justify cross-challenge tests of the cross-protection deficit [59]. In selecting vaccines for antigen banks, matching data can be combined with information on threat prioritisation (http://www.ipcinfo.org/fileadmin/user_upload/eufmd/Open_Session2016/Pragmatist_McLaws.pdf).

10. Establishing antibody thresholds for measuring protection

As discussed, the correlations between serology and protection differ between tests, vaccine seed strains, adjuvants and outbreak viruses and are affected by antigenic relationships, as well as animal-to-animal variation and assay variability. Additionally, animals with weak immunity may still be primed to respond rapidly to boosting by emergency vaccination [8]. This creates difficulties in setting meaningful threshold levels of antibody for protection for vaccine batch acceptance and for surveys of population immunity in the field. Ideally, a relevant potency test will have been conducted so that the relationship between serology and protection can be empirically established, even if the timing and strength of challenge may not be the same as that to be predicted in the field. A degree of compromise and uncertainty will, therefore, always remain. If the vaccine virus is antigenically close to the field virus against which protection is sought, then a homologous potency test can provide the necessary sera and associated protection data for establishing this relationship and choosing a cut-off. If the only available vaccines have an incomplete antigenic match, then a heterologous potency test is more appropriate for calibration of the serology.

As sera from homologous potency tests are not always available for test calibration, whilst heterologous potency tests are rarely performed, alternative compromises are often required:

1. In the absence of any specific potency test reagents or information, test thresholds can be set based on averages determined previously from homologous potency tests for the serotype and test in question [e.g. 45, 47]. However, due to the above-mentioned variables, the accuracy of these estimates may vary. If the test to be used is different from the one for which the threshold has been published, then some form of cross-calibration will be required.
2. If only a relevant homologous test has been done when a heterologous one would have been indicated, then the threshold established from homologous potency testing can be used with the field virus substituted for the vaccine strain in the SP test.
3. Instead of monitoring population immunity in terms of protection, checks that the vaccination has been done properly can use, as threshold, the level of antibody in animals known to have been correctly vaccinated at the appropriate time prior to sampling. Any SP test can be used for this approach. A small-scale field study of vaccinated animals can provide the necessary sera [9]. It may be impractical to collect samples over multiple vaccination cycles to determine appropriate cut-offs for older animals, but checking population immunity in young animals that have received fewer vaccinations is usually more important.

11. Estimating vaccine potency from antigen content

The efficacy of inactivated vaccines is strongly influenced by the quantity of intact FMDV particles (“capsids” or “virions”) and up to

a certain threshold, increases will strengthen the immune response and the elicited protection. For the linear part of the relationship, a tenfold increase in antigen content results in an approximately threefold increase in VNT titre [60]. Therefore, manufacturers standardise the amounts of antigen to help achieve consistent potency of different production batches of vaccine. The optimum antigen payload differs between serotypes. Traditionally, the particle content has been determined spectrophotometrically after size separation by sucrose density gradient (SDG) centrifugation [61]. However, SDG is cumbersome and difficult to standardise, so simpler alternatives have been developed based on size exclusion chromatography [62], double antibody sandwich ELISAs using conformation dependent antibodies [63] and the thermofluor release assay [64]. Nevertheless, like other indirect approaches to estimate potency, the measured dose of each vaccine must be correlated to protection derived from live virus challenge tests [65]. Methods to measure the content of intact antigen in formulated vaccine would be extremely useful.

12. Sequence-based approaches to vaccine strain selection

Nowadays, it is easier to sequence the RNA genome of FMDV viruses than to develop and employ serological techniques. Therefore, in principle, it should be possible to study the deduced amino acid sequence differences of antibody binding sites on FMDV virus capsid surfaces and thereby predict antigenic relatedness and cross-protection between FMDV viruses. The former has been done (initially in [66]), but correlations with cross-protection are complicated by the aforementioned shortage of cross-protection challenge studies. Moreover, variable dominance of antigenic sites may complicate efforts to predict cross-protection from capsid gene sequences [67], requiring more complex models that can predict changing immunodominance as well as identifying the direct effects of identified epitope changes.

Multiple studies have correlated surface changes on FMDV virions to altered antigenic phenotypes as measured by serology, starting with monoclonal antibody escape mutants and later, making comparisons between vaccines and panels of field viruses (reviewed by [13]). This has identified many epitopes and antigenic sites, with similarities and differences found between serotypes (e.g. [68]). In some cases, the impact on antigenic phenotype of substitutions in predicted sites has been validated using reverse genetics [69,53]. To identify antigenic relationships and their predictors, linear mixed effects models, and more recently, more sophisticated Bayesian models [70], were developed to account for variation in pairwise cross-neutralization titres using only viral sequences and structural data. Identifying substitutions in surface-exposed structural proteins that correlate with loss of cross-reactivity is a basis for predicting both the best vaccine match for any single virus and the breadth of coverage of new vaccine candidates from their capsid sequences, where it was found to correlate strongly with gold-standard VNT r_1 values [66]. However, predictive power for cross-protection is impossible to compare given the shortage of challenge studies. More resources dedicated to post-vaccination outbreak monitoring and serosurveillance in endemic countries, where natural challenge experiments regularly occur, would make such comparisons more feasible.

13. Serology as a measure of protection for influenza

Antibody mediated protection is a significant component of other viral diseases. For influenza, in particular, there are many similarities to FMDV in the approaches and challenges for defining vaccine induced protection.

An inactivated vaccine is used for human seasonal influenza, the efficacy of which can be reduced if the incorporated vaccine strain is mismatched to the field virus against which protection is needed. As for FMDV, propagating the influenza virus can alter antigenicity, in this case through egg-adaptive changes in the influenza virus haemagglutinin protein [71]. For influenza vaccines, antigenic distance is determined using the haemagglutination inhibition (HI) assay or various neutralisation assays, where the titre of antisera to the vaccine candidate is compared between the field and vaccine strain (equivalent to the r_1 value used for FMDV vaccine matching). A 4-fold drop in titre between vaccine and field strains is considered to be a substantial antigenic difference that may indicate a new vaccine seed strain is needed [72]. However, the raw heterologous titre is taken into account in this process, with lower titres increasing the importance of drop, as we have discussed above with respect to high potency FMDV vaccines. As for FMDV, the serological assays used to assess the antibody responses to influenza vaccines are hard to standardise [73]. Like FMDV's LPBE, the HI assay is quicker and more reproducible than neutralisation assays, but most current H3N2 field viruses do not agglutinate red blood cells. To improve matters, more optimal use of and better understanding of the data produced by neutralization assays has been recommended, along with increased standardization of the tests themselves to make them more comparable between centres [71]. However, neutralization assays are time consuming to carry out, reducing throughput, and so they are mainly used where they are the only option – for H3N2 viruses.

The data obtained from the antigenic characterization of viruses using HI and neutralization assays, as well as the serological reactivity of pre- and post-vaccination human sera and epidemiological and clinical information, are combined with extensive genetic sequencing data to select vaccines. These sequence data are used alongside much more extensive records of known epitopes on the haemagglutinin glycoproteins than are available for FMDV, to identify substitutions of particular concern. Similar computational

techniques to FMDV have been applied to influenza [74]: antigenic cartography was the first computational technique developed to help to predict vaccine match (originally for influenza, [75], and then later for FMDV, [76]) by reducing the “noise” caused by variable serum quality and potency; the FMDV sequence-based predictors described above have also been translated to influenza, and have been refined to identify epitope changes and predict vaccine match from sequence and serology data very efficiently for the much larger datasets influenza provides [77,78]. Models can also be used to combine haemagglutinin serological and mutation data [79]. A qualitatively different class of model has emerged recently for influenza, however, which directly predicts clade “fitness”, or likely future survivorship, given the current prevalence and immunological history (inferred from past prevalence, [80]). This model is now being used to predict effectiveness of candidate vaccines in the next flu season, based on the clades predicted to be circulating. Indeed, all three of these classes of models (cartographic, epitope-change and fitness-based) are now being used in reports to formal vaccine composition meetings (VCMs) as an aid to decision making, though the primary focus remains on the unmodeled serology and sequence data. Indeed, the only non-serologic test that has been accepted by regulatory authorities as a surrogate for a vaccine preventable disease is also lab-based – the IFNg Eli-Spot that was used to support licensure of the live-attenuated influenza vaccine (FluMistTM) [73].

In contrast to FMDV, seasonal influenza vaccine selection happens formally at 6-monthly VCMs coordinated by the World Health Organization that determine the vaccine for one whole hemisphere at a time (<https://www.who.int/influenza/vaccines/virus/en/>). As with FMDV, other factors are taken into consideration when making recommendations regarding influenza vaccine composition including the immunogenicity of a selected strain to develop adequate humoral immunity, and its growth potential for use in vaccine production.

Serology is also used to predict the strength and duration of protection induced by inactivated rabies virus vaccines, with some

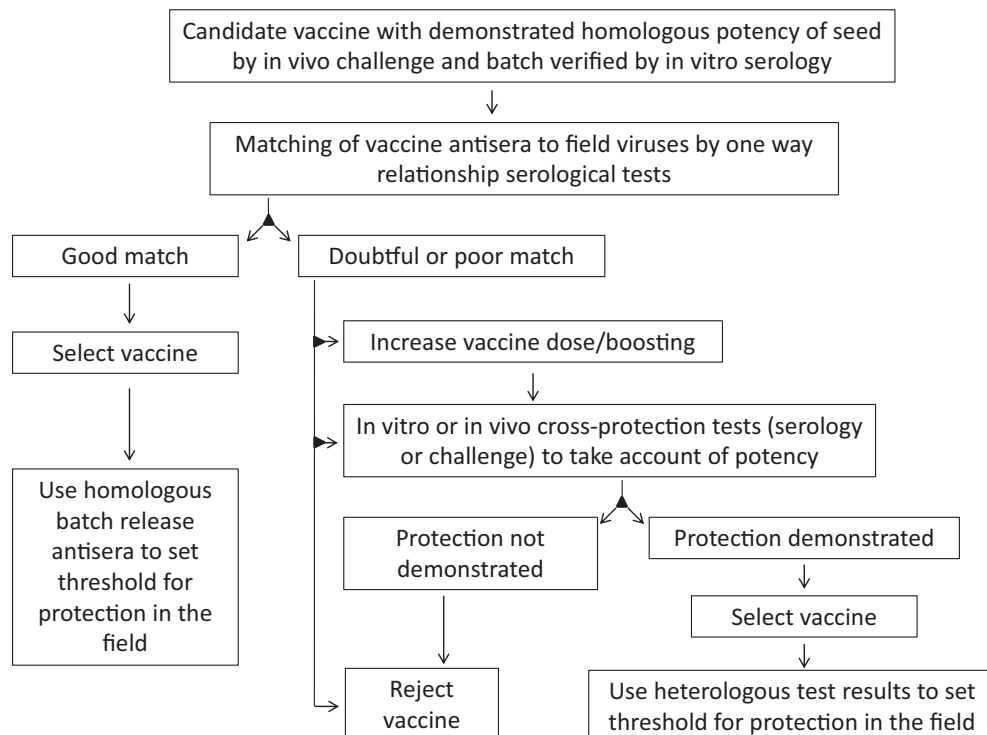


Fig. 4. Use of challenge tests and serology for FMD vaccine selection and monitoring. Arrows represent decisions, and triangles represent decision alternatives.

similar challenges for test standardisation and for use and interpretation of *in vivo* tests [81–84].

14. Conclusions

Serology is widely used to assess FMD vaccines and vaccination. Antigenic suitability of vaccines makes use of matching tests that assess the serological response to the vaccine strain against field isolates. At product registration, full potency tests with homologous live virus challenge are required, but serology can be used for subsequent potency checks of batch-to-batch vaccine consistency. Finally, vaccinated populations are screened using serology to find out if vaccination has been done properly, whether enough animals have been protected and how long immunity has persisted. A combination of different serological techniques that measure heterologous titres as well as matching may be needed in vaccine selection and post vaccination monitoring and more validation is required for new commercial assays and especially for the promising avidity and isotype specific assays that have mostly been evaluated in South America, where the range of FMD virus strains is limited.

The challenge with all of these procedures is that the results can be influenced by many situation-specific variables, not all of which are easy to control. A systematic approach is therefore required that analyses the evidence for the assumptions made at each step. Moreover, confidence in the results is only possible if the appropriate test combinations and calibrations are performed (Fig. 4) with analysis of a sufficient number of animals, field viruses and tests. Many similar issues are confronted with use of serology for monitoring influenza and rabies vaccines and vaccination.

Ultimately, it is impossible to validate measures of cross-protection without actual cross-protection data. Experimental challenge studies with dangerous pathogens in large animals are not easy to perform, but as more countries with endemic FMD move towards control using vaccination, we should take advantage of the opportunities afforded to study the effectiveness of FMD vaccination in the field, where vaccinated animals are invariably challenged by heterologous field viruses, by funding suitable studies to better understand existing and new *in vitro* correlates of cross-protection alongside vaccination campaigns.

Declaration of Competing Interest

The authors have no conflicts of interest.

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