Vaccines and viral antigenic diversity

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Summary
Antigenic diversity among ribonucleic acid (RNA) viruses occurs as a result of rapid mutation during replication and recombination/reassortment between genetic material of related strains during co-infections. Variants which have a selective advantage in terms of ability to spread or to avoid host immunity become established within populations. Examples of antigenically diverse viruses include influenza, foot and mouth disease (FMD) and bluetongue (BT). Effective vaccination against such viruses requires surveillance programmes to monitor circulating serotypes and their evolution to ensure that vaccine strains match field viruses. A formal vaccine strain selection scheme for equine influenza has been established under the auspices of the World Organisation for Animal Health (OIE) based on an international surveillance programme. A regulatory framework has been put in place to allow rapid updating of vaccine strains without the need to provide full registration data for licensing the updated vaccine. While there is extensive surveillance of FMD worldwide and antigenic and genetic characterisation of isolates, there is no formal vaccine strain selection system. A coordinated international effort has been initiated to agree harmonised approaches to virus characterisation which is aimed at providing the basis for an internationally agreed vaccine matching system for FMD supported by the OIE. The emergence and spread of BT in Europe have resulted in an intensification of vaccine evaluation in terms of safety and efficacy, particularly cross-protection within and between serotypes. The most important requirement for producing vaccines against viruses displaying antigenic diversity is a method of measuring antigenic distances between strains and developing an understanding of how these distances relate to cross-protection. Antigenic cartography, a new computational method of quantifying antigenic distances between strains has been applied to human and equine influenza to examine the significance of viral evolution in relation to vaccine strains. This method is highly applicable to other important pathogens displaying antigenic diversity, such as FMD.

Keywords

Introduction
Understanding the genetic diversity of viral pathogens and how it is modulated by host immunity, transmission bottlenecks, epidemic dynamics and population structures is essential for the development of effective control measures (26). Ribonucleic acid (RNA) viruses with their short replication times, particular propensity to mutate during replication, and other strategies for diversification, are a particular challenge (27). The best-known example of antigenic diversity of a virus and its importance for vaccines is that of human influenza for which there is in-depth knowledge of virus serotypes, their evolution and their significance for vaccine efficacy. The global
surveillance and monitoring of human influenza and emergence of new viruses from animal reservoirs are embedded in the Global Influenza Programme of the World Health Organization (WHO) (67) and the basic requirements for effective surveillance, outbreak response and updating of vaccine strains are well established. The development of the programme has required the coordination of a network of reference laboratories, an annual strain review mechanism, acceptance of recommendations on vaccine strains by national authorities, internationally accepted standards for vaccines and an updating mechanism that can respond rapidly to changing epidemiological conditions. This article focuses on diseases of veterinary species which have similar requirements and reviews progress in understanding pathogen diversity and in establishing systems to identify appropriate vaccine strains in response to changing epidemiological situations.

There are a number of viral diseases affecting animals which are antigenically diverse and require similar approaches to control by vaccination. Probably the most studied in relation to vaccine strain selection are (i) influenza, and in particular equine influenza (43), and (ii) foot and mouth disease (FMD) (69). Both diseases are caused by viruses demonstrating a high degree of antigenic diversity and evolution. Additionally, there are other veterinary viruses which, although they do not show the same degree of antigenic evolution, do display multiple serotypes. Such viruses include, for example, the orbiviruses, bluetongue (BT) and African horse sickness (AHS), where serotype identification is important for appropriate vaccine strategies.

**Mechanisms producing viral diversity**

During replication of viruses, mistakes occur in the process of producing copies of viral nucleic acid which are known as mutations. Viruses containing ribonucleic acid (RNA) generate a higher rate of mutation than viruses containing deoxyribonucleic acid (DNA) because there is no effective proof-reading mechanism in the replication strategies employed by RNA viruses (20). As a result, ‘clouds’ of mutants or quasi species are generated during infection, however, many fail to transmit, a phenomenon known as transmission bottle-necks.

If random mutations have some selective advantage in terms of viral fitness (ability to replicate within the host and transmit and spread in a population) or avoidance of the immune response (ability to avoid neutralisation by antibody generated by earlier related strains) then these mutations may become fixed in the population of progeny viruses (7). These processes are well recognised in a number of RNA viruses such as influenza, FMD and BT.

**Genetic and antigenic drift**

The progressive accumulation of random genetic mutations is known as genetic drift which may or may not result in changes in amino acid sequence of viral proteins. If the genetic code for amino acid changes then this results in altered antigenic characteristics and is known as antigenic drift. There are a number of factors which drive the selection of antigenic variants and in some populations antigenic variants co-exist while in others emerging variants replace earlier viruses. These processes are known as viral evolution and understanding its basis and predicting likely trends are an important aspect of controlling virus diseases (7).

**Antigenic diversity arising from recombination and reassortment**

Genetic and associated antigenic changes can also occur as a result of deletions and genetic rearrangements caused by nucleic acid splicing and recombination events, as has been reported for foot and mouth disease virus (FMDV). In influenza virus infections for example, a key event arising from the segmented genome is the reassortment of genes during mixed infections of the same cells with two different viruses. This is an important mechanism for the emergence of new influenza viruses and has been reported for human, avian and swine influenzas (11, 37). The process of generating a new influenza virus with a unique combination of surface glycoproteins by reassortment is called antigenic shift. This process has also been reported in BT virus (BTV). Although virus virulence is not being considered in detail here, it is noteworthy that reassortment of surface glycoproteins from one virus on a novel background of internal genes from another virus can significantly alter the pathogenicity of influenza viruses.

**Selection and survival of variants**

Key factors affecting the selection of variants relate to the virus, the host immune response and the population size and structure. For example, viruses with a high infectivity have a selective advantage as they are more successful in transmission. Viruses with altered antigenic sites, particularly those involved in virus–cell attachment, may be capable of avoiding neutralising antibody present in a population as a result of previous infection. This phenomenon of immune selection of variants is particularly important for infections where immunity is not lifelong, where there is a high rate of mixing within host populations and where animals are exposed to repeated infections by closely related viruses.

Selection and survival of variant viruses is also affected by host population structure and size, which is well illustrated by considering influenza of different species. Viral
evolution in human influenza has been extensively studied and within the subtypes, one strain largely replaces another on a global scale, as viruses replicate in a partially immune population creating the need to escape the immune response.

A similar pattern is seen in equine influenza, although there is not such a strong effect due to lower population densities and lower rates of infection. Additionally, there may be little mixing among different populations, which encourages evolution of discreet co-existing lineages under potentially different selection pressures.

By comparison, influenza in pigs and domestic poultry held in isolated environments is, in general, reliant on the rapid introduction and constant availability of young immunologically naïve hosts within the breeding and farming structures. There are few opportunities for re-infection as stock is slaughtered at a young age. Maintenance of infection in such naïve populations and absence of partially immune older animals does not provide the same driving forces for immune selection and lack of mixing between farms encourages development of multiple lineages. For example, swine influenza, while showing antigenic diversity with multiple strains co-existing, shows less immune-driven evolution.

Thus, antigenic variability is driven not only by the ability of the viruses to mutate and their ability to transmit between hosts, but also by the opportunities for survival that present themselves as a result of the immune environment and population size and structure.

Equine influenza: addressing issues of antigenic diversity in relation to vaccines

Background to vaccination with equine influenza

In 1956 the H7N7 subtype of equine influenza was first isolated in Prague and the prototype was designated as A/equine1/Prague/56. Within a 7 year period a second equine influenza virus of the H3N8 subtype was isolated from horses in Florida and was designated A/equine2/Miami/63. Both subtypes caused major epidemics and in the mid 1960s vaccination against equine influenza was introduced. The early vaccines contained the prototype strains of the H7N7 and H3N8 subtypes grown in eggs, inactivated and combined with an oil adjuvant. The early products were not widely accepted as they were highly reactogenic, but as acceptable adjuvants were found vaccination became the accepted means of control, particularly in performance animals such as race horses.

In well-vaccinated populations vaccine breakdown attributable to H7N7 viruses was rare or non-existent, however, repeated infections with the H3N8 subtype have been reported over a long period. Much research has been undertaken to establish the contribution of vaccine potency and antigenic variation to this observed vaccine failure (43, 44).

Virus structure and variability

Influenza viruses are single-stranded RNA viruses with segmented genomes comprised of 8 segments (genes) coding for structural components of the virus particle and non-structural components important for replication within host cells. The two most important structural proteins demonstrating genetic and antigenic variation which are relevant to protection and vaccination are the envelope glycoproteins, the haemagglutinin (HA) and the neuraminidase (NA). Of these, the HA is particularly important as it mediates virus attachment to the host cell and antibody induced against the HA neutralises virus infectivity. The ability of the virus to evolve in terms of the antigenic character of the HA (antigenic drift) is crucial for avoidance of population immunity and immunity derived from inactivated vaccines, which is largely reliant on antibody to HA. The NA is involved in elution of virus from cells and the spread of infection between cells, but although the NA is known to vary, there is little information on the impact of its antigenic drift on vaccine efficacy.

Antigenic and genetic variation of equine influenza viruses

As with other influenza A viruses, both subtypes of equine influenza exhibit genetic and antigenic variation. The evolution of the HA gene has been well studied because of its importance in relation to virus neutralisation and protection. Attention has been focused on the A/equine2 (H3N8) virus as this has been the predominant strain circulating since the 1960s and more importantly because there have been repeated reports of vaccine breakdown in the field. The majority of studies on the antigenic character of the HA and its relationship to viral neutralisation have been conducted using haemagglutination inhibition (HI) tests, exploiting the fact that influenza viruses naturally agglutinate erythrocytes and that antibody inhibiting agglutination is a measure of virus neutralisation (VN). Much antigenic analysis of influenza viruses has relied on the use of ferret sera as this species is susceptible to infection with influenza and provides strain specific antisera which can discriminate between strains in HI tests.
In 1983, Hinshaw reported that there had been major antigenic drift in viruses isolated between 1979 and 1981 as compared with the prototype virus Miami/63 (31). However, they also recognised, based on antigenic analysis with ferret sera in HI tests, that some viruses similar to the prototype Miami/63 virus were co-circulating with the more recent variants. On the basis of this data they recommended that additional strains (Fontainebleau/79 or Kentucky/81) should be included in vaccines, which at the time contained only the prototype H3N8 virus, Miami/63. At that time surveillance and virus collection was sporadic and there was no certainty that the strains selected as vaccine strains were representative of the predominant strains circulating.

Subsequent genetic analysis (32) based on sequencing the HA gene of a larger panel of viruses from around the world, revealed that the H3/Equine/2 HA gene was evolving essentially as a single lineage, however, antigenic analysis revealed that the resultant changes to the amino acid sequence gave rise to viruses which were both similar to and distinct from the prototype H3N8 virus, Miami/63. It was noted that the pattern of evolution was similar to that seen in human influenza and it was proposed that it was driven by immunological pressure, i.e. the existing immunity to historical viruses present in the older population. This study did not demonstrate that not only was the degree of antigenic drift important, i.e. the number of mutations which had arisen and become fixed in the HA molecule, but their identity and location was also important because genetically distant viruses could nevertheless react in a similar way in HI tests.

In both these studies antigenic differences between prototype and recent strains were measured using HI tests and post infection ferret or rabbit sera or monoclonal antibodies. Where fourfold differences in reactivity of sera with different virus strains could be detected it was concluded that the viruses were significantly different in terms of antigenicity, which may have implications for vaccines. At that time no attempt was made to assess the significance of such antigenic differences for vaccine efficacy in the target species. The significance of fourfold differences in HI tests was assumed to have immunological relevance based on experience with human influenza viruses.

The conclusion that the antigenic drift might compromise vaccine efficacy was not accepted by others. Burrows et al (13) concluded that the antigenic differences detected between the prototype strain Miami/63 and the new variants Fontainebleau/79 and Kentucky/81 (demonstrated using ferret sera and monoclonal antibodies) were unlikely to be important because post vaccination sera from horses vaccinated with Miami/63 was highly cross-reactive with the recent 1979 isolates (13). This lead to a debate about the relevance of antigenic differences detected using post infection and post vaccination sera from laboratory animals as compared to sera from target species (43) and hindered progress in the understanding of significance of antigenic variation in equine influenza viruses in relation to vaccine efficacy.

Genetic and antigenic drift has been periodically reported from a number of different countries (34, 52). However, a particularly important observation was made in a joint study by OIE Reference Laboratories in the United Kingdom (UK) and in the United States of America (USA). These laboratories examined viruses from 1963 to 1994 and revealed that genetic and antigenic variants were co-circulating as a result of a divergence in the single lineage of the H3N8 viruses (originally described by Kawaoaka et al. (32)) into two sublineages representing isolates originating from the Americas on the one hand and viruses from Europe and Asia on the other (15). However, these lineages did not remain geographically separate and in the early 1990s American-like viruses were identified in Europe probably reflecting the significant traffic of horses from the USA to Europe for racing (Fig. 1).

At that time vaccines manufactured in America contained American isolates and most vaccines manufactured in Europe contained European viruses. Thus, horses vaccinated with European viruses were reliant on cross-protection when exposed to viruses from the American lineage and vice versa.

The two sublineages of the H3N8 viruses have continued to evolve and sequencing has revealed the appearance of a number of clades (subgroups) within the lineages, some of which have geographic origins, e.g. the South American branch of the American sublineage (34).

**Evidence of antigenic drift affecting vaccine efficacy in the field**

Vaccine breakdown has been reported during a number of outbreaks of influenza A/equine/2 over many years, but this had been largely attributed to poor vaccine efficacy, or vaccination schedules which did not accommodate the short duration of immunity provided by the early inactivated vaccines. In 1976 (68) and 1979 (12) vaccinated horses became infected, but those horses which succumbed to infection had low or undetectable antibody at the time of exposure. Thus, at this stage there was no firm evidence for antigenic drift being the explicit cause of vaccine failure.

In contrast, in 1989 a major epidemic of equine influenza A/equine/2 occurred in the UK and elsewhere and first cases were identified in regularly vaccinated army horses with high levels of antibody prior to infection (36). Although the infection was generally mild in well
vaccinated horses it spread rapidly through populations indicating that levels of virus shedding were significant even in the absence of severe clinical signs. At the time of the outbreak, available vaccines contained the prototype strain Miami/63 and a strain from the 1979-1981 epidemic such as Fontainebleau/79, Kentucky/81, Brentwood/79 or Borlange/79.

In the intervening ten years between 1979 and 1989 there had been a major improvement in vaccine potency as a result of the introduction of challenge models in the target species to assess vaccine efficacy and establishment of acceptability thresholds for vaccines in terms of antigen content (measured as µg HA) and levels of antibody (measured by Single Radial Haemolysis [SRH]) that are consistent with protection. As a result many of the European vaccines available at that time had demonstrable efficacy against homologous strains as judged by HA content, serological responses generated and protection against challenge infection (46, 47). These observations further supported the conclusion that significant antigenic drift had occurred in 1989.

Significance of antigenic variation measured by haemagglutination inhibition tests in relation to vaccine efficacy in the target species

As knowledge of the genetic and antigenic diversity and evolution of equine influenza grew and field observations suggested antigenic drift may have played a role in vaccine breakdown, it became essential to establish the significance of antigenic variability as measured by HI tests with ferret sera for vaccine efficacy in the target species.

A series of four viruses spanning a period of 26 years (Miami/63, Fontainebleau/79, Kentucky/81 and Suffolk/89) were examined in a cross-protection study in ponies in which groups of ten ponies were vaccinated with two doses of inactivated vaccines prepared from each strain containing equivalent HA content and challenged with a recent isolate Sussex/89 (43). Protection was measured in terms of serological responses, virus excretion and clinical signs following challenge. The key findings from this study were that vaccines derived from both recent and historic viruses provided equally effective clinical protection in terms of reduction in pyrexia and coughing in vaccinates as compared to unvaccinated controls. In contrast, the ability of vaccines to protect against infection and suppress virus excretion following challenge was directly related to the antigenic relatedness of the challenge and vaccine viruses, with the Miami/63 vaccine allowing significantly more virus excretion than the Suffolk/89 virus most closely related to the challenge virus Sussex/89 (Table I). This difference in protection could not be attributed to differences in potency because similar levels of HI antibody to the challenge virus (Sussex/89) were stimulated by the Miami/63 and the Suffolk/89 vaccines (Table II). Furthermore, SRH antibody levels to Sussex/89 were higher in the Miami/63 vaccine group than in the Suffolk/89 vaccine group (Table III).
This study was the first to demonstrate that antigenic differences between equine influenza strains detected by HI tests with ferret antisera were significant for vaccine efficacy in the target species, particularly with respect to protection against infection and virus excretion. However, it also demonstrated that vaccines containing viruses ill-matched to epidemiologically relevant strains provided a degree of clinical protection which could mask infection while allowing copious amounts of virus to be excreted. These data supported the conclusion that for the control of influenza at the herd level it is important that vaccines contain virus strains which match currently circulating strains in order to minimise virus shedding.

These observations also raised the question of geographic variation and its importance for vaccine strain selection. While the majority of vaccines available in the USA, Europe and centres of thoroughbred racing around the world are produced by large multinational companies, other vaccines are made locally for specific populations, for example in South America, Japan, Eastern Europe and India. It became important to explore whether antigenic differences between viruses of different locations are likely to affect vaccine efficacy.

Competition animals travel extensively and internationally and it is likely that such horses are exposed to viruses from different locations. While originally it was held that equine influenza evolved as a single lineage, the observations made in the early 1990s revealed that the A/equine/2 lineage diverged into American and Eurasian sublineages. Subsequent to that observation a further sublineage of the American-like viruses has been recognised as originating from South America (34) (Fig. 2). It is central to international control of equine influenza to understand the significance of the antigenic differences between these subpopulations (or clades) for vaccine efficacy.

With this objective in mind a series of vaccination and challenge studies in the target species have been performed to examine cross-protection between strains arising from the American and Eurasian lineages. The prototype viruses Newmarket/2/93 (Eurasian) and Newmarket/1/93 (American) were selected and used in cross-protection studies in horses (16, 82). As with the study to examine the significance of temporal antigenic drift, it was found that the vaccines containing viruses from the two lineages provided a significant degree of cross-protection against each other in terms of suppression of clinical signs such as coughing and pyrexia. Interestingly it was also found that the American lineage virus protected equally well against the European virus as against the homologous American virus in terms of infection and reduction in virus excretion (82). In contrast, the European lineage virus vaccine was not as effective in protecting against infection and virus excretion when challenged with the American lineage virus as compared with the protection afforded against a homologous challenge (16).

While the differences between the protection observed using the different vaccines were subtle under experimental conditions in limited groups of ponies, it has been demonstrated, using mathematical models, that the likely impact of such variations in suppression of virus excretion on immunity in a population is significant (53) (Fig. 3). Furthermore, field observations have supported this conclusion. In a limited outbreak of the European lineage virus, it was found that horses vaccinated with a product containing a European virus and with SRH antibody levels above the protective threshold were protected against infection (50). In contrast, in a similar
outbreak caused by an American lineage virus, horses vaccinated with a European virus vaccine were not protected even when antibody levels were above the protective threshold (49) (Fig. 4). Thus, predicting the likely efficacy of a vaccine is based not only on potency but also suitability of the vaccine strains in the field.

Fig. 2
Phylogenetic tree of the American lineage of H3N8 equine influenza viruses showing South American clade

Annual review of vaccine strains and criteria for changing strains

While cross-protection studies in the target species are the ultimate test of the significance of antigenic drift, it is not practical to base vaccine strain selection on such studies because of the difficulty of accessing influenza-free ponies, and the cost and time required to undertake large animal experimentation. This holds true for many virus vaccines. Therefore, in order to identify a reliable predictor of significant antigenic drift, there has been considerable effort to examine the relationship between protection in the target species, protection in hamsters as a small animal model and antigenic differences discriminated by HI tests using ferret, horse and hamster sera (16).

As already mentioned, ferrets produce highly strain-specific sera following infection with influenza strains, whereas horse sera are more cross-reactive. However, analysing the reactivity of post infection ferret sera in HI tests remains a useful way to compare the antigenic differences between strains and it provides an indication of cross-protection (Fig. 5).

Surveillance and equine influenza expert surveillance panel

As there is considerable international traffic of Equidae, it is important to conduct surveillance on a global scale and there are continuing efforts to collect viruses from around the world for sequencing and antigenic analysis. While the numbers of viruses screened are low by comparison with human influenza, surveillance has provided a picture of the evolution of equine H3N8 strains and the importance of inadequately vaccinated animals in the transmission of viruses globally. Based on the WHO model for surveillance,
analysis of viruses and vaccine strain selection, an Equine Influenza Expert Surveillance Panel has been set up under the auspices of the OIE to review on an annual basis outbreaks of equine influenza, vaccine performance, antigenic and genetic character of new virus isolates and to take decisions on the need to update vaccine strains. The panel includes experts from the WHO collaborating laboratories at the National Institute of Medical Research and the National Institute for Biological Standardisation and Control in London, the three OIE Equine Influenza Reference Laboratories in Germany, the UK and the USA and other experts involved in equine influenza surveillance. Their conclusions are reported annually by the OIE.

**Developing criteria**

Originally, the criteria that were applied to decisions about the need to change vaccine strains were based on those used for human influenza and included vaccine breakdown in the field, fourfold differences detected in HI tests with ferret sera between vaccine strains and predominant field isolates, discrimination between vaccine and field viruses by post-vaccinal equine sera and genetic sequence of the HA1 molecule. Additionally, these criteria have been judged against cross-protection studies in horses and hamsters in order to validate their relevance to the criteria applied to equine influenza viruses. It has become clear that post-vaccinal horse sera are generally unable to discriminate between viruses unless there are major antigenic differences, therefore this test has become less important in the decision-making processes. Decisions to change vaccine strains are normally conservative and are only recommended when there are measurable antigenic differences as a result of significant genetic mutations between vaccine and predominant field strains and evidence of vaccine breakdown. For example, European lineage viruses which can be discriminated from vaccine strains based on fourfold differences with ferret sera but which have not established and spread in the equine population have not warranted a recommendation to change vaccine strains.

To date, strain differences identified with ferret sera appear to correlate well with limited cross-protection studies conducted in horses, however, patterns of cross reactivity between panels of ferret sera and viruses are complex and difficult to interpret by eye. Recent advances in computational methods are revolutionising the way such data can be analysed and a method known as antigenic cartography has been applied to historical data from human influenza and equine influenza (65).
The majority of equine influenza vaccines are inactivated whole virus (46) or viral subunits (47) combined with an adjuvant. The immune response of the horse to vaccination is relatively short-lived and multiple doses are required to maintain complete protection against infection, although a degree of clinical protection is provided with fewer doses.

The basis of vaccine potency for inactivated vaccines is well understood and relates to the amount of immunologically active HA contained in the vaccine and the efficacy of the adjuvant in enhancing circulating antibody to HA (80). Many studies in immunologically naïve horses have demonstrated a direct relationship between µg HA in vaccines (79) and antibody responses in horses measured using an SRH test (44). Furthermore, the level of SRH antibody stimulated is indicative of the level of protection acquired against challenge infections in vaccinated horses, with 150 mm² being identified as the threshold for protection, provided that the vaccine contains a virus antigenically similar to that being used to test the vaccine by challenge infection (44). Furthermore, this threshold for protection against experimental infection is valid for a field situation (50). Therefore, the efficacy of a vaccine in a field situation can be predicted based on accurate measurement of immunologically active HA in the vaccine, SRH antibody stimulated by the HA in combination with adjuvant and protection against challenge infection; however, the predictions will only be accurate if the virus used as a standard for the single radial diffusion (SRD), or as antigen in the single radial haemolysis (SRH), or as challenge virus for experimental infection, is antigenically indistinguishable from the vaccine strain.

The requirements of the European Pharmacopoeia for licensing equine influenza vaccines are described in Monograph No. 249 and utilise these relationships. Testing requires measurement of vaccine antigen, antibody responses in horses, and challenge infection studies with at least one virus included in the vaccine (23).

Vaccine strains are recommended by the Equine Influenza Expert Surveillance Panel and are published by the OIE. Currently, it is recommended that vaccines should contain representatives of the Eurasian and American sublineages of the H3N8 virus. Inclusion of H7N7 virus is no longer recommended on the basis that such a virus has not been isolated for more than 20 years. Viruses originating between 1989 and 1993 are still accepted for the European lineage, however, recent antigenic drift and field outbreaks caused by American lineage viruses have lead to a recommendation that vaccine viruses should be updated to representatives from 2003 such as South Africa/2003. The selection of virus strain is not prescriptive but selected strains must be shown to be antigenically similar to those recommended.

Fast track licensing system

Once new recommendations are made it is highly desirable that vaccines are updated as quickly as possible and to this end a fast track licensing system has been developed for updating vaccine viruses in Europe. These Guidelines, which have been developed by the Immunological Working Group of the European Medicines Evaluation Agency (22), recognise the well-established relationship between µg immunologically active HA in the vaccine, levels of SRH antibody generated in the target species and protection against challenge infection. They operate on the principle that if a vaccine has been licensed according to the European Pharmacopoeia standards, which also use these relationships in their requirements for potency and efficacy testing, and that in the process of updating a vaccine strain no other parameter of the vaccine is changed, then manufacturers are only required to demonstrate safety and the ability of the final product to generate protective levels of antibody in the target species against the new strain. This obviates the need for challenge studies and generation of duration data, significantly reducing the testing required to license the updated vaccine.

International considerations for vaccine strain selection and standardisation of licensing procedures

The majority of vaccines are made in the USA or Europe, and efforts are ongoing to harmonise licensing procedures between the European Pharmacopoeia and the USDA.
A series of WHO/OIE consultations have been held to work towards international harmonisation of vaccine standards (45). In recent years, challenge tests have been accepted by the USDA as useful for efficacy and a document is now under review to provide a fast track licensing system for updating strains for vaccines produced in the USA (70).

**Influenza of other species**

The same basic principles apply for influenza of other species, but control processes other than vaccination may be more suitable. The relevance of antigenic diversity has been examined for swine influenza vaccines (72) and good cross-protection has been demonstrated between divergent strains. This has been attributed to the use of very potent adjuvants in swine vaccines which may compensate for antigenic differences. Thus, to date, vaccine strain selection has not become an important issue for swine influenza.

With the recent outbreaks of H5 and H7 avian influenza there is an increasing interest in vaccination as a method of control to avoid massive slaughter of infected flocks. The main aspect of genetic variation studied in avian influenza has been the switch to highly pathogenic virus from viruses with low pathogenicity of the same serotype. However, there have been some recent reports of antigenic drift occurring under the immune pressure of vaccination (35). Therefore, it is likely that if vaccination becomes widely used to protect poultry against avian influenza more attention to strain evolution and vaccine strain selection will be required.

**Foot and mouth disease virus**

**Introduction**

The potential impact of antigenic diversity on the control of FMD is well recognised, however, the task of setting up adequate response systems is enormous and needs to take account of the number of serotypes and subtypes, host range, political and socio-economic constraints. Since the 2001 outbreak of FMD in Europe caused by serotype O, there have been renewed efforts to improve the procedures in place for surveillance of FMD on an international scale. These include collection and submission of viruses to reference laboratories, and development of the scientific and technical approaches to examining antigenic diversity among FMDV strains and assessment of its relevance for vaccine strain selection. These issues have been examined in a number of reviews on FMDV vaccines (17, 18) and vaccine strain selection (54) and are addressed in the most recent foot and mouth disease chapter in the OIE Terrestrial Manual (Chapter 2.1.1.). The importance of having an early warning system for emergence of variant strains is well recognised. The ability to rapidly analyse new viruses and measure their antigenic relatedness to existing vaccine strains is crucial to providing effective vaccines used in rapid response control programmes and for laying down new viruses in vaccine banks.

**Genetic and antigenic variability**

The molecular basis of antigenic variation in FMDV has been extensively studied and it is well known that FMDV exhibits a high degree of genetic and antigenic variation (21). As with other RNA viruses such as influenza, this high level of variation is attributable to the error-prone replication of viral RNA and the lack of a proof-reading mechanism associated with the viral replicase (20, 66). Thus, mutations are constantly being produced in progeny viruses and subsequently selected for or against as the virus is transmitted within a population, depending on whether the mutations are beneficial for virus survival (29, 38). There are 7 serotypes of FMDV known as O, A and C (historically regarded as European types), Asia-1, and SAT 1, 2 and 3 (from the South African Territories) (6, 75). Within each serotype there are varying degrees of diversity with subtypes recognised in some serotypes. There is a particularly high diversity among SAT 1 and 2 viruses which has been ascribed to generation of variants in persistently infected buffalo (75). Antibody generated by infection or vaccination against one serotype fails to cross-protect against all other types. Furthermore, antigenic differences within a serotype may be so great that there is little or no cross-protection between strains of the same serotype (3).

During infection some mutations are selected under the influence of immune pressure (10), while others become fixed even in the absence of immune pressure (19, 64). This viral evolution can occur in distinct populations of susceptible animals in separate geographic locations (Fig. 6) (77), resulting in the maintenance and evolution of distinct lineages within an FMDV virus serotype (40, 75). These so-called topotypes are an important feature of FMDV as they may have significantly different antigenic characteristics which could impact on vaccine efficacy (Fig. 7) (60).

**Virus structure and antigenic sites**

Foot and mouth disease virus is a small non-enveloped positive-stranded RNA virus belonging to the Picornaviridae family. The single-stranded RNA is comprised of a large open reading frame (ORF) encoding a single polypeptide which undergoes proteolytic cleavage to form non-structural proteins involved in virus replication and four structural proteins (VP1, VP2, VP3, VP4) which...
are incorporated into the virus capsid. VP1, VP2 and VP3 are exposed on the viral surface and carry major antigenic sites. An important cell attachment site with a conserved structure is located between variable regions on the highly immunogenic loop of VP1 which protrudes from the capsid surface. This region is capable of eliciting neutralising antibody and its variable nature leads to both intra- and inter-typic antigenic variation (73). Some other epitopes (or antigenic sites) are dependent on the tertiary structure of the virus particle (41) and are only present in the intact virus known as the 146S particle (named on the basis of its sedimentation coefficient). Additionally, different FMDV types are able to attach to different cell types using a range of cellular receptors (25) and different host species may preferentially recognise different antigenic sites (1). Thus, the virus epitopes involved in attachment to cells and virus neutralisation are complex.

Foot and mouth disease virus vaccines and vaccine banks

Foot and mouth disease virus vaccines are generally purified inactivated whole-virus particles combined with adjuvants (their production and use is reviewed by Doel [17, 18] and Ahl et al [2]). During the manufacturing process the antigenic content of the vaccine is measured as the amount of 146S particles. Following inactivation and combination with adjuvant, potency is measured in terms of ability to generate virus neutralising antibody, with the ultimate test of efficacy being challenge infection of vaccinated cattle with a challenge virus homologous with the vaccine virus. While there is some data on the relationship of antigenic content, antibody responses and protection against infection, it has not been possible to describe these relationships for all the serotypes and
subtypes within them. There are international standards for potency recommended by the OIE and these relate to normal routinely used vaccines. Vaccines are manufactured and supplied by local laboratories around the world as well as by multinational companies, and depending on the source of vaccine there is more or less adherence to recommended standards. Efficacy under field conditions is highly variable depending on quality and potency of vaccines, strain matching tests and species infected. However, modern vaccines properly standardised are reported to be efficacious (5).

As well as vaccines designed for routine use there is a requirement for stockpiles of emergency vaccines in vaccine banks maintained in disease-free countries such as those in Western Europe, North America and Australasia (4, 24). The vaccines are stored as a safeguard against incursions of disease against which the population will have no immunity. Since it is not possible to predict which serotypes may cause an outbreak, it is desirable for vaccine banks to store a full spectrum of serotypes and subtypes to respond to any potential eventuality. These vaccines are stored as virus concentrate over liquid nitrogen and in an

![Phylogenetic tree of SAT 1 viruses isolated in East Africa between 1971 and 2000](image_url)

**Fig. 7**

Phylogenetic tree of SAT 1 viruses isolated in East Africa between 1971 and 2000

*Source: W Vosloo*
emergency are diluted to concentrations higher than normal vaccines as the aim is to arrest spread of infection with a single dose. Understanding the impact of strain diversity between vaccine strains and field strains is very important for predicting the likely contribution of emergency vaccination strategies to the eradication of the infection. There is also an important interplay between vaccine potency and strain diversity, as highly potent vaccines containing a heterologous strain may be as effective in control as a well matched vaccine strain in a low potency vaccine and at present there is little data to inform governments of the best vaccines to select in a crisis.

**Vaccine strains**

Vaccine strains are selected on the basis of a number of characteristics, but good growth characteristics and the ability to elicit an antibody response which is broadly cross-reactive within a subtype are the most important (17). This is a major challenge for vaccine manufacturers globally, but particularly for the providers of vaccine banks which hold a range of vaccines or vaccine concentrates to enable disease-free countries to respond to incursions of FMDV with vaccination programmes.

**Epidemiology of foot and mouth disease and the use of vaccines**

Inactivated vaccines are in routine use in some regions where FMDV is endemic and the virus types included in the vaccines reflect those which are prevalent in the region. In general the SAT1, SAT2 and SAT3 types have been restricted to sub-Saharan Africa, with only occasional incursions into North Africa and the Middle East. Serotypes O, A and C have also been reported from the African continent (Fig. 8). In South America there have been intensive efforts to eradicate FMD through a vaccination policy, but type O and A viruses continue to be isolated (3), and there has been a need in recent years to modify vaccine strains in response to a variant virus of the A serotype. In Asia there are large unmonitored reservoirs and types O, A and Asia 1 are endemic in some regions (62). In South Eastern Europe types O and A, and occasionally Asia 1, have also been reported in recent years (61). Of particular note is the dramatic spread of type O (pan-Asian lineage), which was first reported from Northern India, but spread east to Taipei China and west to the Middle East and the Balkans. Eventually it was shipped to South Africa in 2000 and reached Europe in 2001.

The ability for this virus to spread rapidly through populations and to be transported in the form of contaminated products is a clear indication of the importance of horizon scanning as part of a preparedness policy (59). It is essential to maintain an awareness of current virus types and the strains within those types which are circulating and this is a major challenge given the diversity of strains even within one continent (Fig. 8) (76). A cornerstone of effective vaccination programmes to control and eradicate the disease in endemic areas and to prevent incursions into normally disease-free areas is the use of vaccines containing strains that are well matched to the outbreak strains. The huge logistical problems to achieving this on a global scale are reviewed by Paton et al. (54). In some regions such as South America there are well coordinated surveillance programmes and vaccine strain selection systems, whereas in other regions there is little attempt to monitor circulating strains or submit viruses to national or reference laboratories for characterisation.

**Initial characterisation and selection for vaccine matching tests**

Isolates collected from around the world are submitted to OIE reference laboratories and the Food and Agriculture Organization (FAO) World Reference Laboratory for foot and mouth disease (Institute of Animal Health [IAH], Pirbright, UK) for identification, genetic analysis and serological typing. Identification is normally achieved by enzyme-linked immunosorbent assay (ELISA) with a panel of type specific antisera. Sequencing of part of the VP1 gene allows comparison with other viruses already typed and submitted to the database (33). This comparison very often allows the origin of the outbreak strain to be located as virus topotypes can be identified in this way. As an example there are at least 8 topotypes of serotype O. This data can give an indication of whether the virus strain submitted has been isolated before or whether it is unusual and warrants vaccine matching tests, given the high mutation rate and consequent variable nature of FMDV, where possible several isolates from the same outbreak are characterised and submitted for vaccine matching.

**Foot and mouth disease virus international surveillance and virus typing**

In parallel with genetic studies cross-neutralisation tests with reference sera that have been prepared to previously characterised viruses are conducted to examine the cross-reactivity between outbreak strains and the available vaccine strains. ELISA tests are also used to examine antigenic relationships. The purpose of this exercise is to identify the virus type and ascertain whether the isolates are closely related to currently held vaccine strains of the relevant type or are antigenically distinct. As already mentioned, it is important, particularly for procurers of vaccines, to appreciate that within a single type there may
be a wide spectrum of strains, some of which barely cross-react and therefore would not cross-protect.

**Laboratory tests used internationally to characterise viruses and match them to vaccine strains**

As a result of the independent regional efforts to address the problem of vaccine strain selection and disparate approaches used by local and multinational vaccine manufacturers, a number of tests have been developed for comparing field isolates with vaccine strains. These include the calculation of R values (relationship values) from serological cross-reaction studies using VN, complement fixation and ELISA tests to compare the reactivity of outbreak and vaccine strains with antisera to vaccine virus (54). Additionally, in South America this approach of comparing vaccine and field viruses serologically has been refined by using sera from vaccinated cattle which were subsequently challenged, thus allowing a prediction of protection to be made based on the serological cross-reactivity (54). The major drawback with all these tests is that there has been little standardisation or harmonisation.
of techniques and reagents or provision of international reagents for standardisation. Furthermore, there is only very limited data from cross-protection studies using emerging viruses as challenge viruses against heterologous vaccine strains. Thus, interpretation of such data in terms of vaccine efficacy in a field situation remains uncertain.

Future initiatives

It is clear that with increasing international trade and travel, FMDV has the means by which to spread rapidly around the world. It is essential, particularly for the disease-free regions, to maintain effective horizon scanning so that they are prepared for the emergence of new strains.

To date, there has been no internationally coordinated programme of collection and review of FMDV isolates as is conducted for equine influenza. However, the laboratory at the IAH, Pirbright, which is the FAO World Reference Laboratory for foot and mouth disease, has characterised many viruses from around the world. Other laboratories have played similar roles at a regional level. It has been recognised that to respond to the challenges of the strain diversity of FMDV these resources need to be pooled (54).

Following the 2001 outbreak in Europe a ‘coordinated action’ has been funded by the European Union to enable OIE reference laboratories round the world to create a network of information and reagents in order to harmonise approaches to virus characterisation and comparison with vaccine strains. This will bring together expertise from the UK, South America, Russia and sub-Saharan Africa and provide an opportunity for international harmonisation. The aims are to develop standardised methods of best practice; collect, characterise and archive viruses which represent FMDVs global diversity; exchange reagents and information to facilitate efficient vaccine matching and to report annually to the OIE and FAO.

Orbiviruses: bluetongue and African horse sickness

Structure and variability

Bluetongue and AHS viruses are members of the Orbivirus genus in the family Reoviridae. They are arthropod-borne (Culicoides sp.) viral diseases of ruminants and equidae respectively (14, 74). Orbiviruses have double-stranded RNA segmented genomes and as such have the potential for displaying broad antigenic diversity; as evidenced by the 24 serotypes of BT and 9 serotypes of AHS. As expected, the replication of the RNA genome of orbiviruses is also prone to errors due to lack of a proof-reading polymerase. Diversity is also generated by gene segment swapping during mixed infections (27). However, the rate of evolution in arthropod-borne viruses is lower than in single-host pathogens such as equine influenza and it is hypothesised that it is limited by the alternating host replication cycles (Culicoides sp. and ruminants) which demand a compromise in fitness levels to enable the virus to replicate in both vertebrate and invertebrate cells (78).

The 10 genome segments code for seven structural proteins (VP1-7) and three non-structural proteins (NS1-3). VP2 is the major component of the outer capsid and the main antigen responsible for cell attachment and virus neutralisation, although VP5, another component of the capsid, also plays a minor role. There is some cross-protection between serotypes within each virus and this is attributed both to a degree of cross-neutralisation between serotypes with similar VP2 antigenic structures and also to cell-mediated immunity driven by the less variable internal antigens.

The gene segments evolve independently of one another by genetic drift in a host-specific fashion generating quasispecies populations in both ruminants and insects. It has also been shown that random mutations occurring in vertebrate cells may become fixed when ingested by Culicoides sp. (9). Thus, there are many complex opportunities for genetic and antigenic diversity.

The genetic diversity of BT has been exploited for epidemiological studies. Analysis of genes coding for the conserved VP3 or the NS3 proteins can be used for geographic typing and tracing (9, 27) whereas the VP2 gene segregates strains according to serotype (8). Nevertheless, in a recent investigation of BT in the Mediterranean Basin complete sequence analysis of the VP2 gene has proved very useful in identifying topotypes within a serotype and in tracing sources of infection (56).

Vaccines and antigenic diversity

Currently, most available vaccines for BT and AHS are classical attenuated vaccines developed by passaging viruses in embryonated eggs (BT) or mice (AHS) and are produced in tissue culture (74). These attenuated vaccine strains are not without risk and their main use has been to control the diseases in sub-Saharan Africa, therefore, knowledge of the impact of viral diversity on vaccine efficacy is limited. The low levels of cross-reactivity between serotypes have been exploited for vaccination against both BT and AHS. Thus, it is not necessary to include all serotypes in live vaccines in order to provide relatively broad protection against a range of serotypes (14, 74). In general, the success of this strategy has been assessed from field rather than experimental studies. The current inactivated vaccine contains serotypes 2 and 4 and...
cross-protection against other serotypes has not been reported.

The recent outbreaks of BT, serotypes 1, 2, 4, 8, 9 and 16, in the Mediterranean Basin (28) have focused attention on genetic and antigenic diversity of BT (56) and how it may relate to vaccine efficacy in the field. The use of the live attenuated BT vaccines in Europe and subsequent spread of the vaccine virus has also revealed the potential safety issues relating to live vaccines. The recent spread of BT serotype 8 in northern Europe (42) further focuses attention on appropriate vaccine strategies to respond to changing the epidemiological situation in Europe. Historically there has been much research to develop subunit vaccines as alternative vaccine candidates to both BT and AHS (58, 57) and to explore common antigens between serotypes. However, if inactivated vaccine strategies are pursued, antigenic diversity within and between serotypes will have much greater importance.

While it is recognised that VP2 is highly variable across and within serotypes, it is also recognised that the VP2 genes retain common regions across serotypes which may explain the degree of cross-reactivity observed between some serotypes. Similar observations have been made for AHS (55). The challenge is to assess how important the observed diversity is in terms of neutralisation and protection in the target species. To date, there have been few studies to examine this question. However, it was observed that there was a high homology at the molecular level between Italian isolates and the vaccine strain for BTV-2 (51), which was consistent with observed protection in the field (28, 63). In contrast, there was low genetic homology between the BTV-9 isolated in Italy and the vaccine strain, although cross-protection was demonstrated in a challenge study (G. Savini, unpublished findings). Interestingly, when amino acid sequences, as opposed to nucleotide sequences, were compared there was a higher degree of homology between the two BTV-9 strains. Thus, it appears that important epitopes relating to cell attachment may have been preserved in spite of the propensity for the virus to diversify genetically (56). Also, the observed protection may be in part due to the fact that live attenuated vaccines generate neutralising antibody to a number of surface epitopes on other viral proteins as well as elicit cell-mediated immunity.

Clearly, with the increasing importance of BT (and potentially AHS) in the changing global climatic conditions, there is a need to increase our understanding of vaccine efficacy against intra- and inter-typic variants of these viruses. This will require more cross-protection studies in the target species and analysis of protection in relation to antigenic characteristics.

Summary and conclusions

This article refers to the antigenic diversity of three different types of RNA viruses and briefly reviews its potential significance for different vaccination strategies. Although the genetic basis of virulence has not been addressed in this chapter it is crucial to the understanding of vaccine efficacy given that the immunity provided by vaccines can be overcome if infections are rapid within host or create high virus doses and spread rapidly through populations.

There are obviously many more viruses displaying similar characteristics which are generating intensive research efforts to examine antigenic diversity in relation to control. The appearance of bat lyssaviruses in Europe has initiated efforts to understand the antigenic significance of different lineages with respect to vaccination (48). Similarly, the explosion of infectious bursal disease infections in poultry has created huge interest in this avian birnavirus, where it is essential to understand the relative contribution of changes in virulence and antigenicity to the epidemiology of the disease (30, 71).

Ribonucleic acid viruses will remain an enormous challenge in disease control as new variant viruses emerge. However, prospects of responding more effectively are increasing. Collaborations between virologists, computational experts and mathematicians are opening up exciting new opportunities for monitoring viral diversity and predicting likely changes. As genome sequencing becomes a routine and rapid technique it becomes easier to track large numbers of viruses and assess genetic distances between isolates, and, consequently, compiling large databases becomes possible. As genetic data accumulates in parallel with antigenic data it is becoming possible to identify amino acid changes which are silent and those which have significant antigenic impact. Such studies are already ongoing for influenza and where profound changes in antigenicity of the HA have been associated with single amino acid substitutions, the causal nature of the observations are being examined using reverse genetics.

The development of microarray-based identification of antigenic variants of FMD virus provides prospects for speeding up the analysis of antigenic variation among large numbers of strains and, eventually, of vaccine strain selection (39).

To date, antigenic analysis of FMD viruses has relied on examination of R values based on VN tests or ELISA, and analysis of influenza has been based on the examination of cross HI data. The development of a sophisticated computational method called antigenic cartography (65) for measuring antigenic distances between strains has provided a step change in the way epidemiological data for
human influenza is reviewed annually and vaccine strains selected. This approach can provide a multidimensional image of the antigenic distances between viruses, how they cluster and the direction of their evolution. It has great potential for other viruses requiring this process of review and selection. It can be applied to historical data of serological reactions between viruses and sera used to compare strains. When linked with challenge data demonstrating protection by vaccines, as is possible for equine influenza, antigenic cartography is providing real insight into the important antigenic changes affecting cross-protection.

Les vaccins et la variabilité antigénique des virus

J.A. Mumford

Résumé
La variabilité antigénique des virus à acide ribonucléique (ARN) est le résultat de la mutation rapide qui intervient lors de la réplication et de la recombinaison/réassortiment de matériel génétique de souches apparentées, pendant une co-infection. Les souches variantes bénéficiant d’un avantage sélectif en termes de capacité de se propager ou de contourner l’immunité de l’hôte s’établissent au sein des populations. Le virus de l’influenza, le virus de la fièvre aphteuse et le virus de la fièvre catarrhale du mouton sont des exemples de virus présentant une variation antigénique. Pour être efficaces contre ces virus, les stratégies de vaccination doivent s’accompagner de programmes de surveillance visant à détecter les sérotypes en circulation et à retracer leur évolution afin d’assurer un parfait appariement entre les souches vaccinales et les souches sauvages. Sous les auspices de l’Organisation mondiale de la santé animale (OIE), un dispositif de sélection de souches vaccinales du virus de la grippe équine a été mis en place, fondé sur un programme international de surveillance. Un cadre réglementaire autorise désormais la réactualisation rapide des souches vaccinales sans qu’il soit nécessaire de fournir toutes les données d’enregistrement de ces vaccins réactualisés. La fièvre aphteuse fait l’objet d’une surveillance rigoureuse partout dans le monde, recourant à la caractérisation antigénique et génétique des isolats, mais il n’existe aucun système formel de sélection des souches vaccinales. Une initiative a été entreprise à l’échelle internationale pour harmoniser les méthodes de caractérisation des virus, dans le but d’établir la base d’un futur système d’appariement des vaccins vis-à-vis de la fièvre aphteuse, accepté sur le plan international et soutenu par l’OIE. En raison de l’émergence et de la propagation de la fièvre catarrhale du mouton en Europe, l’évaluation de l’innocuité et de l’efficacité de vaccins contre cette maladie a été intensifiée, notamment en ce qui concerne la protection croisée vis-à-vis de chaque sérotype et entre sérotypes. Le principal critère pour produire des vaccins dirigés contre des virus présentant une variabilité antigénique est de disposer d’une méthode permettant de mesurer la distance antigénique entre les souches et de mieux appréhender les relations entre ces distances et les mécanismes de protection croisée. Une nouvelle méthode de modélisation informatique permettant de chiffrer la distance entre souches, appelée cartographie antigénique, a été appliquée aux virus de la grippe humaine et équine dans le but d’élucider l’évolution de ces
Vacunas y variabilidad antigénica de los virus

J.A. Mumford

Resumen
Las rápidas mutaciones originadas por la replicación y recombinación/reordenamiento de material genético de cepas afines en infecciones simultáneas provocan la variabilidad antigénica de los virus ARN. Aquellas variantes cuya ventaja selectiva les permite propagarse, o evitar la inmunidad del huésped, se establecen en las poblaciones. Entre los virus que presentan variabilidad antigénica pueden mencionarse los responsables de la influenza, la fiebre aftosa y la lengua azul. Para que la vacunación contra esos virus sea eficaz es preciso recurrir también a programas de vigilancia de los serotipos circulantes y su evolución a fin de asegurarse de que las cepas vacunales neutralizan a los virus de campo. Se ha establecido un sistema oficial de selección de cepas vacunales contra la influenza equina, bajo los auspicios de la Organización Mundial de Sanidad Animal (OIE), basado en un programa de vigilancia internacional. Ese marco reglamentario permite actualizar rápidamente las cepas vacunales sin necesidad de presentar todos los datos para obtener la autorización de comercialización de la vacuna actualizada. Si bien la fiebre aftosa es objeto de una estrecha vigilancia en todo el mundo, caracterizándose los antígenos y genes de las muestras, aún no se dispone de un sistema oficial de selección de cepas vacunales. Con el apoyo de la OIE, se ha dado inicio a una iniciativa internacional conjunta para armonizar los métodos de caracterización de virus y echar los cimientos de un sistema de comparación de cepas vacunales contra la fiebre aftosa aceptado internacionalmente. La aparición y propagación de la lengua azul en Europa condujeron a intensificar la evaluación de la inocuidad y eficacia de las vacunas, en particular, la protección cruzada contra cada serotipo, y entre ellos. La condición más importante para producir vacunas contra virus que muestran variabilidad antigénica consiste en recurrir a un método de medida de las distancias antigénicas entre cepas y comprender la relación entre esas distancias y la protección cruzada. La cartografía antigénica, un nuevo método informático para medir las distancias antigénicas entre cepas, se ha aplicado a los virus de la influenza humana y equina con objeto de estudiar la importancia de su evolución en relación con las cepas vacunales. Este método puede aplicarse muy fácilmente a otros importantes agentes patógenos que presentan variabilidad antigénica, como el virus de la fiebre aftosa.

Palabras clave
References


