# Current perspectives on control of equine influenza

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**Review article**

# Current perspectives on control of equine influenza

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**Abstract** – Influenza A viruses of the H3N8 subtype are a major cause of respiratory disease in horses. Subclinical infection with virus shedding can occur in vaccinated horses, particularly where there is a mismatch between the vaccine strains and the virus strains circulating in the field. Such infections contribute to the spread of the disease. Rapid diagnostic techniques are available for detection of virus antigen and can be used as an aid in control programmes. Improvements have been made to methods of standardising inactivated virus vaccines, and a direct relationship between vaccine potency measured by single radial diffusion and vaccine-induced antibody measured by single radial haemolysis has been demonstrated. Improved adjuvants and antigenic presentation systems extend the duration of immunity induced by inactivated virus vaccines, but high levels of antibody are required for protection against field infection. In addition to circulating antibody, infection with influenza virus stimulates mucosal and cellular immunity; unlike immunity to inactivated virus vaccines, infection-induced immunity is not dependent on the presence of circulating antibody to HA. Live attenuated or vectored equine influenza vaccines, which may better mimic the immunity generated by influenza infection than inactivated virus vaccines, are now available. Mathematical modelling based upon experimental and field data has been applied to examine issues relating to vaccine efficacy at the population level. A vaccine strain selection system has been implemented and a more global approach to the surveillance of equine influenza is being developed.

**equine influenza / epidemiology / vaccine strain selection / surveillance**

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## 1. INTRODUCTION

Management procedures aimed at limiting the severity of disease and the spread of infection, whether on a local or international basis, require sensitive diagnostic techniques for rapid detection of clinical and subclinical infection. Equine influenza vaccines were first developed in the 1960s [4], and are used widely for control of equine influenza however, in spite of intensive vaccination programmes in some groups, equine influenza infections remain a serious problem. The H3N8 component of inactivated vaccines has been the subject of intense investigation with a view to identifying the reasons for vaccine breakdown against this subtype. Research has focussed on vaccine potency, adjuvants, vaccination schedules and antigenic drift. During the last decade, progress has been made in all these areas of investigation, providing new approaches to the control of equine influenza.

## 2. EPIDEMIOLOGY

Equine influenza was first recognised in 1956, when influenza was recovered during a widespread epidemic of respiratory disease among horses in Eastern Europe [58]. The virus (A/eq/Prague/56), which has an H7 haemagglutinin (HA) and an N7 neuraminidase (NA), was designated as the prototype equine influenza virus, historically referred to as equine subtype 1. The last confirmed outbreak caused by an H7N7 subtype virus was in 1979; however H7-specific antibody has been reported in horses believed to be unvaccinated, suggesting that the virus may still circulate in a subclinical form.

In 1963, an equine influenza virus of a different antigenic subtype (H3N8), originally designated as equine subtype 2, caused a major epidemic in the USA [64]. The prototype virus, A/eq/Miami/63, was introduced into the equine population of Florida with the importation of horses from Argentina [57]. Field evidence suggested that regular vaccination provided protection against H7N7 infections, but that the H3N8 component of the vaccine was less effective [53]. For example, in January 1976 a localised outbreak of H3N8 occurred in Thoroughbred horses in Newmarket (UK) at a time when many animals had recently been vaccinated [59]. Clinical influenza affected unvaccinated and some vaccinated horses, with the severity of disease corresponding with the period since vaccination. Stables in which over 75% of horses were vaccinated were not affected seriously [59]. Between 1978 and 1981, widespread epidemics of H3N8 viruses were reported in Europe and North America with infections occurring in vaccinated as well as unvaccinated horses [7, 28, 30, 52, 62]. In Britain in 1979, influenza was confined to unvaccinated horses during the first six months of the year, but spread to vaccinated Thoroughbreds in June 1979, providing clear evidence that the vaccines did not provide immunity against field infection for the full year between "booster doses" [6]. Racing was affected, and this led to the subsequent introduction of mandatory vaccination in the UK and Ireland in 1981.

In 1989, there was again a major epidemic of influenza H3N8 in Europe affecting not only unvaccinated but also large numbers of vaccinated horses [33]. This represented the first major outbreak in Britain since 1979. Outbreaks of equine influenza have occurred sporadically in Europe and on the American continent since the 1989 epidemic.

In the last 15 years, there have also been a number of serious outbreaks of H3N8 influenza in populations with no previous history of the disease. In 1986 and 1987, the infection was introduced into South Africa and India, respectively. The source of these outbreaks could be traced to the transportation of infected horses by air from areas where influenza was endemic. Inadequate quarantine at the port of entry allowed the introduction of infected horses into the local

susceptible populations with subsequent explosive spread of disease and some mortality. Analysis of the HA genes of the South African and Indian viruses have confirmed their close relationship to viruses circulating in the USA and Europe at the time. In 1989, an influenza epidemic was reported in horses in China with morbidity rates as high as 80% and mortality rates reaching 20% in some herds. Fatal cases were always associated with bacterial infection [21]. The origin of this outbreak was not traced to the importation of equidae and indeed the antigenic characteristics of this virus appear markedly different from other equine H3N8 isolates [22]. On the basis of sequence information, it was proposed that this virus was derived from an avian source and as such represented a new interspecies transmission event [65]. Although this avian-derived virus successfully transmitted to horses and lost its ability to infect ducks, it did not spread beyond China and did not persist in the local horse population beyond 1990 [23]. Further outbreaks in Hong Kong in 1992 [54], Dubai in 1995 [66], and the Philippines in 1997 highlighted the ease with which equine influenza outbreaks can be introduced into susceptible populations as a result of international movement of horses.

## 3. VACCINE POTENCY

Currently, the principal markers for resistance to and recovery from influenza virus infection are circulating antibodies specific for the HA and NA glycoproteins [1]. These glycoproteins are the principle determinants for cell entry in infection (HA) and for exit from the cell after virus replication (NA). Progress in assessing the protective efficacy of early vaccines was hampered by a lack of reliable methods to measure the HA content of vaccines and the host's antibody response to the HA. Additionally, there was no reproducible challenge method in horses for assessing the protection provided by vaccination. The HA content of vaccines was measured in chick cell agglutination (CCA) units and antibody responses to the HA were measured by the haemagglutination inhibition (HI) test. In some instances these methods are both still used. Early attempts to analyse the relationship between vaccine-induced antibody and protection against infection were confused by technical problems, and HI titres ranging from 8 to 128 were quoted as being protective [5, 31, 55, 59]. Improved methods of measuring vaccine potency, antibody responses and protection against infection have since been developed, facilitating progress in vaccine standardisation and design. A reliable in vitro potency test, the single radial immunodiffusion (SRD) test, has been introduced for measurement of immunologically active HA in equine influenza vaccines and has been evaluated in an international collaborative study [68]. A further international collaborative study demonstrated that the single radial haemolysis (SRH) assay is more reproducible than the HI test for measuring antibody to HA [38]. Furthermore, there is a direct relationship between vaccine potency, in terms of microgrammes of HA, and antibody to HA stimulated by inactivated vaccines as measured by SRH [41, 67].

Vaccine evaluation by experimental challenge infection of horses was slow to progress because of difficulties encountered in reproducing clinical disease [8, 31, 35, 56]. These difficulties have been overcome by using nebulised aerosols. This delivery system mimics a natural infection by producing infectious droplets (diameter < 5 mm) capable of reaching the upper and lower airways (D. Hannant, unpublished data) and avoids a concentration of challenge inoculum at the site of sampling. Using this challenge method, a series of experiments to measure the protection afforded by inactivated virus vaccines with a variety of adjuvants and antigen presentation systems have been performed. A number of experiments have used the SRD test to standardise inactivated vaccines, the SRH test to measure antibody responses in the horse and challenge infections

to assess protection from infection and disease. These studies have determined the relationships between vaccine potency, circulating antibody to HA and protection against infection and disease. Levels of antibody required for virological protection against challenge with an antigenically similar virus were between 120 to 154 $mm^2$, with evidence that a higher threshold was required for protection with increasing doses of nebulised virus [39]. The influenza epidemic in South Africa in 1986 provided a rare opportunity to examine vaccine efficacy in the field in a population where no natural immunity exists. From pre-infection antibody levels it was possible to estimate that an SRH value of around 160 $mm^2$ was consistent with a 90% protection rate based on the proportion of horses that seroconverted when exposed to infection [39].

The majority of current equine influenza vaccines contain inactivated whole virus (with adjuvants, which include oil, alhydrogel or carbomer) or subunit vaccines (ISCOMs or micelles combined with Quil A). It was found that antibody responses stimulated by vaccines containing aluminium phosphate or hydroxide were more durable than those induced by aqueous vaccines of equivalent antigenic content. Antibody nevertheless declined to low levels by 16 to 20 weeks after the second and third dose of vaccine. In contrast, the incorporation of a polymer adjuvant was found to stimulate antibody that remained at a high level for at least six months after the third dose of vaccine [43]. Similarly, vaccination with three doses of ISCOMs containing 15 mg HA resulted in the level of SRH antibody persisting at around 70 $mm^2$ for 15 months following the third dose [42].

The historical lack of standardisation of vaccines from different sources, and the undemanding standards of some licensing authorities, has resulted in the use of products with inadequate potency in terms of ability to stimulate antibody to the HA. Morley et al. [37] described a large double-blind field trial using a commercial killed vaccine that failed to demonstrate a significant difference in the rate of disease between vaccinated and unvaccinated animals in the face of a naturally occurring outbreak of disease in a population of horses stabled at a racetrack. The situation is improving with the establishment of European Pharmacopoeia international reference preparations to standardise serological tests for potency evaluation of vaccines, and the introduction of federal regulations on equine influenza vaccines in Europe [17] and, more recently, in the USA (9CFR parts 112 and 113).

## 4. NATURAL IMMUNITY AND LIVE VACCINES

Immunity provided by inactivated influenza virus vaccines, is dependent on high levels of circulating antibody to HA and, in the absence of such antibody, vaccinated horses are susceptible to infection. In contrast, infection with influenza induces long-term immunity independent of circulating antibody against HA. For example, ponies with low or undetectable anti-HA antibodies were clinically and virologically protected from challenge infection more than one year after natural infection [26]. This suggests an important difference in the immune response following infection compared with vaccination using inactivated virus. Additional components of the immune response that may be involved are the cellular immune and mucosal antibody responses local to the site of infection.

Cellular immune responses to influenza are well defined in man. The key cell-mediated immune response is the development of MHC class I restricted $CD8^+$ cytotoxic T lymphocytes (CTL), which are usually detectable within 3 to 4 days after infection. $CD8^+$ CTL lyse virus-infected host cells [70]. The epitopes recognised by CTL on the HA, nucleoprotein (NP), matrix (M1) and polymerase PB2 proteins are more highly conserved than those involved in humoral immunity. MHC class II-restricted $CD4^+$ T helper cells facilitate both humoral

and cellular immune responses and can exert cytolytic effects, though to a lesser extent than $CD8^+$ CTL. Whereas antibodies reduce virus load and restrict re-infection, cellular immune mechanisms probably play a more important role in clearance of virus during the convalescent period [12, 36]. Less is known about cellular immune responses in horses. Experimental infection of ponies with influenza induces a genetically restricted, antigen-specific CTL response that persists for at least six months [25]. Generation of CTL in this case probably occurs through endogenous antigen processing followed by peptide presentation via MHC class I molecules. In contrast, inactivated virus vaccines fail to stimulate a significant CTL response because the antigens undergo exogenous processing and presentation via MHC class II.

Equine influenza virus infection has been demonstrated to generate virus-specific mucosal IgA and serum IgGa and IgGb responses, whereas an inactivated virus vaccine induced only a serum IgG(T) response [44].

The qualitative differences between the immune responses that follow infection or vaccination with inactivated virus suggest that improvements can be made in vaccine design. Ideally, vaccines should induce broadly reactive, local and systemic, antibody and cellular immune responses, establish memory and consequently generate a rapid anamnestic response upon field exposure to equine influenza virus. The incidence of free and cell-associated virus is thereby reduced and recovery enhanced. Live attenuated and live, vectored equine influenza vaccines that should more closely mimic natural infection are available. The Merial vaccine PROTEQ Flu is a live recombinant vaccine that uses canarypox as the vector to express the HA genes of equine influenza viruses. The recombinant virus undergoes an abortive infection in mammalian cells so that no progeny viruses are made but the expressed viral antigens are processed endogenously and presented as peptides via MHC class I by the host cell in the same manner as occurs in natural infection but without associated infection risks. There is a wealth of evidence for canarypox vaccines inducing cellular immune responses to human immunodeficiency virus in man [18, 20], but this has yet to be demonstrated for the PROTEQ Flu vaccine.

A cold-adapted, temperature-sensitive, modified-live virus equine influenza vaccine (FluAvert IN Vaccine), which is delivered intranasally, is now licensed for sale in the USA. The safety and efficacy of the vaccine has been demonstrated in experimental studies, however the vaccine does not provide sterile immunity [10, 34, 60, 71]. No correlation was found between the concentration of serum antibody induced by vaccination and protection against infection, though an anamnestic response was demonstrated at seven days post infection [61]. Although there is evidence to show that primed animals will develop a serological response [71], it appears that the use of serum antibody response as a measure of live virus mucosal vaccines in naïve animals is inappropriate. Our ability to measure alternative correlates of immunity has lagged behind the development of these alternative vaccination strategies.

Induction of a cellular immune response to a conserved protein such as NP may potentially provide protection when the viral strains incorporated in the vaccine do not match circulating strains. Such cross-reactive immunity may even extend to partial protection against infection with a virus of a different subtype (heterosubtypic immunity). Infection of mice with a human influenza A virus of one subtype can induce partial protection against infection with virus of a different subtype [47], and a similar study in pigs suggested that $CD8^+$ T lymphocytes have a role in this heterosubtypic immunity [27]. Generation of such cross-reactive immunity in the horse could be advantageous in the event of a new subtype of influenza A virus emerging (or re-emerging) in the horse population.

## 5. OPTIMISING VACCINATION SCHEDULES

The early vaccination schedules for inactivated virus vaccines required two primary doses 4 to 6 weeks apart followed by annual booster doses. The current minimum requirements imposed for competition animals by the Federation Equines International are a primary course of two doses 4 to 6 weeks apart and a booster six months later followed by annual boosters. Mathematical models validated against experimental and field data have demonstrated that vaccination dramatically reduces both the incidence and size of epidemics, with larger outbreaks of equine influenza being exceptional amongst groups of vaccinated animals [19]. Thus the vaccination policy ensures a sufficient level of herd immunity to prevent large-scale outbreaks that are likely to lead to cancellation of race meetings and other equestrian events. However it is questionable whether the preliminary programme of three doses followed by annual vaccination provides sufficient immunity to protect young horses from the disease or individual training yards from small outbreaks of influenza. The short-lived immunity provided by inactivated vaccines has been acknowledged for some years, and it is apparent from various studies [13, 61, 63] that vaccination in accordance with the minimum requirements of Jockey Club rules and the vaccine manufacturer's recommendations leaves horses with low antibody titres for several months between their second and third vaccination. Newton et al. [46] found that SRH antibody levels in yearling Thoroughbreds on studs in Newmarket declined below a protective level within four months of a booster vaccination. Importantly, this also coincided with the autumn sales, a recognised risk period for transmission of influenza in young Thoroughbreds [45]. Later observations in yearlings entering training yards in Newmarket confirmed that antibody levels at this time were influenced by both time elapsed since the last vaccination and the total number of vaccines that had been previously administered [46]. Cullinane et al. [13] demonstrated that an additional 6-monthly booster would benefit horses that may be at high risk during this interval. Intensive vaccination regimes, involving booster doses every 30 to 60 days, have been practised in the USA. However, little is known about the potential adverse effects of administering a potent vaccine too frequently, which may attenuate the immune response. Using a stochastic model to assess the risk of an outbreak occurring in a Thoroughbred population in a typical flat racing training yard, Park et al. [51] suggested that increasing the frequency of vaccination in horses aged 2-years and upwards to include six monthly boosters would offer a significant increase in protection over annual vaccination.

Timing of the first vaccination may be critical to the subsequent development of antibody. Although it is recognised that maternal antibody generally inhibits the development of neonatal antibody synthesis, it has often been assumed that these antibodies have decayed to an insignificant level by 3 to 4 months. The temptation is to vaccinate elite stock prior to the loss of maternal antibodies to avoid any window of susceptibility. Foals born to mares vaccinated during the gestation period have high levels of maternal antibody within two days of birth [13, 61, 63]. In contrast to Liu et al. [32], who reported that maternal antibody persisted for only a short period, several authors [13, 61, 63] found that the majority of foals they tested had detectable (HI) antibody titres at three months of age but these had virtually disappeared at six months. Cullinane et al. [13] suggested that not only does vaccination in the face of maternal antibody interfere with the development of active immunity but that repeat vaccination in the face of maternal antibodies may induce tolerance. On the basis of their findings, they recommended that mares should be vaccinated against equine influenza in the last 6 to 4 weeks of pregnancy to ensure the transfer of protective levels of antibody in

the colostrum, and that foals should not be vaccinated until their maternal antibodies have waned (i.e. not until six months of age or they are seronegative).

## 6. VACCINE STRAIN SELECTION

Surveillance of antigenic drift is a cornerstone of influenza control programmes based on vaccination. As with other RNA viruses, influenza virus replication is highly error-prone, therefore newly synthesised viral genes have a high frequency of mutation. Many of these mutations are either inconsequential or detrimental to the virus, but mutations affecting the antigenic sites of the HA (and NA) can lead to the virus not being recognisable by pre-existing antibodies generated by infection or vaccination with an earlier strain, a process known as "antigenic drift". The formulation of human influenza vaccines is reviewed on an annual basis and in most years is changed to reflect the virus strains most representative of those in worldwide circulation.

Historically, antigenic drift in equine H3N8 viruses has been examined in HI tests employing post infection or post vaccination sera prepared in a number of different species. Conclusions about the antigenic relatedness of equine H3N8 viruses and the significance of observed differences with respect to the immunity induced have varied. For example, Hinshaw et al. [28] concluded than the majority of viruses isolated between 1979 and 1981 were substantially different from the prototype virus, Miami/63 included in the vaccine when compared using post infection ferret sera in HI assays, and that representatives of the new variant should be included in the vaccines. On the other hand, Burrows et al. [6, 7] concluded that the minor antigenic drift that they detected in viruses isolated between 1963 and 1979 did not justify a change in vaccine strains because post vaccination sera from horses immunised with Miami/63 virus were highly cross-reactive in HI tests with viruses from 1979. This conclusion did not take into account the findings of Haaheim and Schild [24] that strain-specific antibody is more effective than cross-reactive antibody in conferring protection.

Horse sera are relatively cross-reactive, particularly when taken from repeatedly vaccinated animals whereas ferrets develop a more strain-specific antibody response [39].

During the 1989 outbreak of influenza in the UK, only horses with very high levels of vaccine-induced antibody were protected against infection, raising the possibility that there had been significant antigenic changes in the 1989 isolate that prevented its neutralisation by antibody stimulated by vaccines containing Miami/63, Fontainebleau/79 or Kentucky/81. Sequencing of the HA1 gene and antigenic analysis using monoclonal antibodies suggested that there were significant differences between a representative 1989 strain and the vaccine strains in current use at the time [2]. The hypothesis was tested by vaccinating groups of ponies with monovalent vaccines containing either of the vaccine strains or a 1989 strain and experimentally challenging them with a 1989 virus [15]. Although all vaccines provided clinical protection, vaccine efficacy in terms of ability to eliminate virus excretion correlated directly with the degree of antigenic relatedness between vaccine and challenge strain. Following a meeting of OIE and WHO experts on newly emerging strains of equine influenza, it was recommended that equine influenza vaccines be updated to include a 1989 isolate, and that efforts be made to increase surveillance and virus characterisation [40].

Phylogenetic analysis of HA sequences revealed that equine H3N8 viruses, which had been evolving as a single lineage [29], apparently diverged into two distinct lineages during the mid-1980s [14] and, to date, both lineages continue to co-circulate independently (Fig. 1). Viruses in one lineage were predominantly isolated from horses in Europe, with the exception of one virus isolated in Canada in 1990, whereas

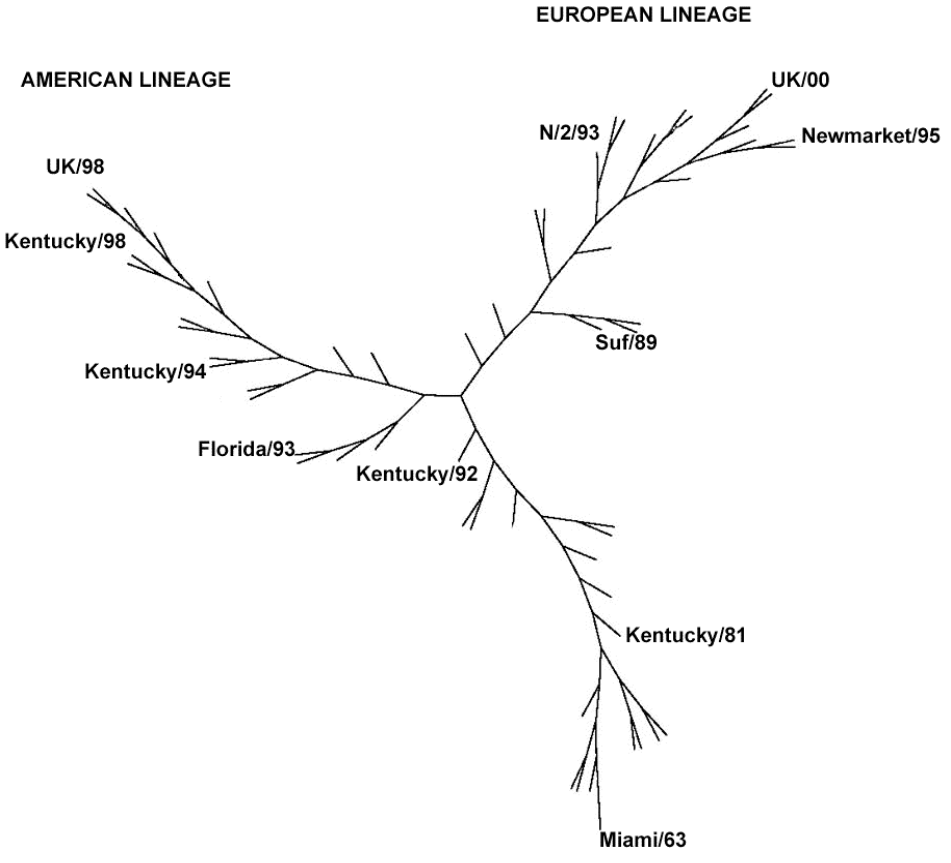


**Figure 1.** Phylogenetic tree constructed from equine influenza H3 HA1 amino acid sequences using parsimony method.

viruses in the other lineage were predominantly from horses on the American continent. It was apparent, however, that American lineage viruses had been introduced into Europe on at least one occasion. The genetic divergence of American and European lineage viruses was reflected in their antigenic reactivity, raising the question of the potential importance of geographical variations in antigenic character for vaccine efficacy. Further vaccination and experimental challenge studies in ponies suggested that vaccines containing virus from the American lineage may not be as effective in protecting against infection as the homologous vaccine against challenge with virus from the European lineage [69]. Field observations have supported the hypothesis that antigenic differences between viruses of the American and European lineages are sufficient to adversely affect vaccine efficacy. During an outbreak caused by a European lineage virus in vaccinated Thoroughbred horses in the UK in 1995, horses with antibody levels of more than around 140 $mm^2$ were protected against infection [46]. However,

during an outbreak caused by an American lineage virus in 1998, when the vaccines used contained only European lineage viruses, a quarter of horses with antibody levels higher than 140 $mm^2$ became infected [45].

The co-circulation of antigenic variants means that it is important to base the selection of new vaccine strains on knowledge of the dominant virus circulating in the field. Following a further consultation of OIE and WHO experts in 1995, a more formal surveillance system was established for equine influenza [48]. An international panel of experts including representatives from OIE and WHO influenza reference laboratories reviews data collected on outbreaks of influenza, vaccine performance in the field, and antigenic and genetic characteristics of new virus isolates annually. The expert surveillance panel make recommendations on the need to update vaccine strains, which are published in the OIE Bulletin. The criteria used for deciding on the need to update equine influenza vaccine strains are based largely on those used for human influenza vaccine strain selection, i.e. detection of changes in the HA as characterised by HI tests using ferret and horse antisera, genetic sequencing of the HA1 gene and vaccine breakdown in the field. Improved surveillance in the field, standardisation of the potency of vaccines and the introduction of a vaccine strain selection system has enabled the development of a fast-track licensing system for vaccines containing updated strains [16].

## 7. DIAGNOSIS

We have demonstrated that vaccinated horses are often only partially immune to influenza (particularly if vaccine strains are a poor match for circulating viruses) and may shed virus in the absence of clinical signs. Such animals present a significant risk for the spread of infection. Thus our ability to diagnose both clinical and sub clinical infections in partially immune animals is critical in attempts to control equine influenza.

For many years the diagnosis of equine influenza has relied on culture of virus in embryonated hens' eggs (and more recently Madin-Darby canine kidney cells) and measurement of antibody responses to the HA. Although a useful epidemiological tool, serological diagnosis of equine influenza tends to be retrospective because a convalescent sample taken around two weeks after an acute sample is required for a definitive diagnosis. This is because infection-induced antibody detected in an acute sample cannot be distinguished from vaccine-induced antibody.

An ELISA to detect antibody to the non-structural protein NS1 has been developed [3, 50]. As this protein is produced during an infection but is not incorporated into inactivated whole virus vaccines, it theoretically enables differentiation of antibody responses to infection from responses to vaccination with a traditional vaccine. With the introduction of live attenuated equine influenza vaccines, the potential usefulness of this test for confirmation of infection in vaccinated animals will probably be considerably reduced. However, the current trend towards genetically engineered vaccines may facilitate the development of DIVA (differentiation of infected from vaccinated animals) vaccines in which a specific gene encoding a highly immunogenic protein is modified or removed.

Detection of the presence of infectious virus by culture of virus in nasal secretions can take a minimum of 2 or 3 days, and if multiple passages are required confirmation of diagnosis is delayed further. A number of alternative assays based upon the use of a monoclonal antibody to detect nucleoprotein in nasal swab abstract provide a diagnosis within 24 h. An equine influenza-specific ELISA has been described [11]. When used in parallel with virus isolation during the 1989 equine influenza epidemic in Britain, the ELISA enhanced the virus detection rate by 44% [33]. Kits for

detection of human influenza are commercially available, and, because of the high degree of conservation of the nucleoprotein among influenza A viruses, one of these, the Directigen Flu-A assay, has been shown to be applicable to the diagnosis of equine influenza [9]. These direct detection methods are useful in the application of control measures, as they can be used as a basis for isolating horses excreting virus in order to reduce infection pressure and for a decision on curtailing exercise, which may exacerbate disease. They are also a useful adjunct to virus isolation, which remains essential for characterising new viruses and to provide future vaccine strains, as they permit virus isolation efforts to be focussed on samples known to be positive for equine influenza.

## 8. INTERNATIONAL CONTROL

The ever-increasing international movement of horses for competition and breeding purposes presents a challenge with regard to the control of equine influenza. Several explosive outbreaks of equine influenza attributable to the introduction of infected animals into susceptible indigenous populations have been described during the last 20 years [54, 66]. Due to economic and competitive issues, it is desirable for the disruption to training programmes caused by quarantine to be kept to a minimum when horses are moved. There is, therefore, a reliance on surveillance of influenza in the population that animals are leaving and on the effectiveness of vaccines to prevent viral shedding. When these measures fail, and subclinically infected horses shedding virus are transported, the short quarantine periods that are often used fail to prevent introduction of infection.

Regulations relating to the movement of animals based on the use of improved diagnostic techniques and vaccination policies that recognise the limitations of current products are now in place. The Code Commission of the OIE recommends that importing countries that are free of equine influenza should require that all horses travelling from endemic areas are fully vaccinated and have received their last booster dose within 2 to 8 weeks of travel [49]. A simple additional measure that can be implemented is the screening of antibody using the SRH assay, which can identify potentially susceptible animals that require revaccination to boost their antibody levels before travelling. The advent of more rapid diagnostic tests for equine influenza means that animals can be screened for viral shedding while still in quarantine at their destination before being released into potentially susceptible local populations.

## 9. CONCLUSION

There are still important goals to be met in the control of equine influenza. These include increased surveillance, virus recovery and characterisation from large equine populations in the Americas and Far East, and international harmonisation of vaccine standards and licensing procedures. However, many of the activities are now in place to provide vaccine manufacturers with the necessary information for production of effective vaccines containing epidemiologically relevant strains, and the development of rapid diagnostic assays has increased our ability to monitor equine influenza activity worldwide and avoid transmission of infection via movement of horses from areas where the infection is active.

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## REFERENCES


[1] Askonas B.A., McMichael A.J., Webster R.G., The antibody response to influenza virus, in: Beare A.S. (Ed.), Basic and Applied Influenza Research, CRC Press, Boca Raton, Florida, 1982, pp. 164–181.

[2] Binns M.M., Daly J.M., Chirnside E.D., Mumford J.A., Wood J.M., Richards C.M., Daniels R.S., Genetic and antigenic analysis of an equine influenza H3 isolate from the 1989 epidemic, Arch. Virol. 130 (1993) 33–43.

[3] Birch-Machin I., Rowan A., Pick J., Mumford J., Binns M., Expression of the nonstructural protein NS1 of equine influenza A virus: detection of Anti-NS1 antibody in post infection equine sera, J. Virol. Methods 65 (1997) 255–263.

[4] Bryans J., Control of equine influenza, in: Bryans J.T. (Ed.), Proc. 1st Int. Conference on Equine Infectious Diseases, Stresa, Italy, 1966, pp. 157–165.

[5] Bryans J.T., The antibody response of horses to two inactivated oil adjuvanted equine influenza virus vaccines, in: International symposium on influenza vaccines for men and horses, Paris, France, 1973, pp. 311–317.

[6] Burrows R., Denyer M., Antigenic properties of some equine influenza viruses, Arch. Virol. 73 (1982) 15–24.

[7] Burrows R., Denyer M., Goodridge D., Hamilton F., Field and laboratory studies of equine influenza viruses isolated in 1979, Vet. Rec. 109 (1981) 353–356.

[8] Cameron T.P., Alford R.H., Kasel J.A., Harvey E.W., Byrne R.J., Knight V., Experimental equine influenza in Chincoteague ponies, Proc. Soc. Exp. Biol. Med. 124 (1967) 510–515.

[9] Chambers T.M., Shortridge K.F., Li P.H., Powell D.G., Watkins K.L., Rapid diagnosis of equine influenza by the Directigen FLU-A enzyme immunoassay, Vet. Rec. 135 (1994) 275–279.

[10] Chambers T.M., Holland R.E., Tudor L.R., Townsend H.G.G., Cook A., Bogdan J., Lunn D.P., Hussey S., Whitaker-Dowling P., Youngner J.S., Sebring R.W., Penner S.J., Stiegler G.l., A new modified live equine influenza virus vaccine: phenotypic stability, restricted spread and efficacy against heterologous virus challenge, Equine Vet. J. 33 (2001) 630–636.

[11] Cook R.F., Sinclair R., Mumford J.A., Detection of influenza nucleoprotein antigen in nasal secretions from horses infected with A/equine influenza (H3N8) viruses, J. Virol. Methods 20 (1988) 1–12.

[12] Couch R.B., Kasel J.A., Immunity to influenza in man, Annu. Rev. Microbiol. 37 (1983) 529–549.

[13] Cullinane A., Weld J., Osborne M., Nelly M., McBride C., Walsh C., Field studies on equine influenza vaccination regimes in Thoroughbred foals and yearlings, Vet. J. 161 (2001) 174–185.

[14] Daly J.M., Lai A.C.K., Binns M.M., Chambers T.M., Barrandeguy M., Mumford J.A., Antigenic and genetic evolution of equine H3N8 influenza A viruses, J. Gen. Virol. 77 (1996) 661–671.

[15] Daly J.M., Yates P.J., Browse G., Swann Z., Newton J.R., Jessett D., Davis-Poynter N., Mumford J.A., Comparison of hamster and pony challenge models for evaluation of effect of antigenic drift on cross-protection afforded by equine influenza vaccines, Equine Vet. J. 35 (2003) 458–462.

[16] EMEA/CVMP/112/98, Notes for guidance: harmonisation of requirements for equine influenza vaccines-specific requirements for substitution or addition of a strain or strains, 1998.

[17] European Pharmacopoeia, Monograph on equine influenza vaccines, 3rd ed., 1998.

[18] Evans T.G., Kallas E.G., Campbell M., Andrews J.D., Schwartz D., Keefer M., Caudrelier P., Evaluation of canarypox-induced CD8(+) responses following immunization be measuring the effector population IFNgamma production, Immunol. Lett. 77 (2001) 7–15.

[19] Glass K., Wood J.L.N., Mumford J.A., Jessett D., Grenfell B.T., Modelling equine influenza 1: a stochastic model of within-yard epidemics, Epidemiol. Infect. 128 (2002) 491–502.

[20] Gorse G.J., Patel G.B., Belshe R.B., HIV type 1 vaccine-induced T cell memory and cytotoxic T lymphocyte responses in HIV type 1-uninfected volunteers, AIDS Res. Hum. Retroviruses 17 (2001) 1175–1189.

[21] Guo Y., Wang M., Zheng S., Wang P., Ji W., Chen Q., Aetiologic study on an influenza-like epidemic in horses in China, Acta Virol. 35 (1991) 190–195.

[22] Guo Y., Wang M., Kawaoka Y., Gorman O., Ito T., Saito T., Webster R.G., Characterization of a new avian-like influenza A virus from horses in China, Virology 188 (1992) 245–255.

[23] Guo Y., Wang M., Zhang G-S., Li W.-K., Kawaoka Y., Webster R.G., Seroepidemiological and molecular evidence for the presence of two H3N8 equine influenza viruses in China in 1993–1994, J. Gen. Virol. 76 (1995) 2009–2014.

[24] Haaheim L.R., Schild G.C., Antibodies to the strain-specific and cross-reactive determinants of the haemagglutinin of influenza H3N2 viruses. 2. Antiviral activities of the antibodies in biological systems, Acta Pathol. Microbiol. Scand. B 88 (1980) 335–340.

[25] Hannant D., Mumford J.A., Cell mediated immune responses in ponies following infection with equine influenza virus (H3N8): the influence of induction culture conditions on the properties of cytotoxic effector cells, Vet. Immunol. Immunopathol. 21 (1989) 327–337.

[26] Hannant D., Mumford J.A., Jessett D.M., Duration of circulating antibody and immunity following infection with equine influenza virus, Vet. Rec. 122 (1988) 125–128.

[27] Heinen P.P., de Boer-Luijtze E.A., Bianchi A.T.J., Respiratory and systemic humoral and cellular immune responses of pigs to a heterosubtypic influenza A virus infection, J. Gen. Virol. 82 (2001) 2697–2707.

[28] Hinshaw V.S., Naeve C.W., Webster R.G., Douglas A., Skehel J.J., Bryans J., Analysis of antigenic variation in equine 2 influenza A viruses, Bull. World Health Organ. 61 (1983) 153–158.

[29] Kawaoka Y., Bean W.J., Webster R.G., Evolution of the hemagglutinin of equine H3 influenza viruses, Virology 169 (1989) 283–292.

[30] Klingeborn B., Rockborn G., Dinter Z., Significant antigenic drift within the influenza equi 2 subtype in Sweden, Vet. Rec. 106 (1980) 363–364.

[31] Kumanomido T., Akiyama Y., Immunoeffect of serum and nasal antibody against experimental inoculation with influenza A-Equi-2 virus, Exp. Rep. Equine Health Lab. 12 (1975) 44–52.

[32] Liu I.K.M., Pascoe D.R., Chang L.W.S., Zee Y.C., Duration of maternally derived antibodies against equine influenza in newborn foals, Am. J. Vet. Res. 46 (1985) 2078–2080.

[33] Livesay G.J., O'Neill T., Hannant D., Yadav M.P., Mumford J.A., The outbreak of equine influenza (H3N8) in the United Kingdom in 1989: diagnostic use of an antigen capture ELISA, Vet. Rec. 133 (1993) 515–519.

[34] Lunn D., Hussey S., Sebring R., Rushlow K., Radecki S., Whitaker-Dowling P., Youngner J., Chambers T., Holland R., Horohov D., Safety, efficacy, and immunogenicity of a modified-live equine influenza virus vaccine in ponies after induction of exercise-induced immunosuppression, J. Am. Vet. Med. Assoc. 218 (2001) 900–906.

[35] Martens J.G., Development and evaluation of aerosol delivery of antivirals for the treatment of equine virus induced respiratory infections, Diss. Abstr. Int. 47 (1986) 69.

[36] McMichael A.J., Gotch F.M., Cullen P., Askonas B.A., Webster R.G., Cytotoxic T cell immunity to influenza, N. Engl. J. Med. 309 (1983) 13–17.

[37] Morley P.S., Townsend H.G.G., Bogdan J.R., Haines D.M., Efficacy of a commercial vaccine for preventing disease caused by influenza virus infection in horses, J. Am. Vet. Med. Assoc. 215 (1999) 61–64.

[38] Mumford J., Collaborative study for the establishment of three European Pharmacopoeia Biological Reference Preparations for equine influenza horse antiserum, Pharmeuropa (2000) 7–21.

[39] Mumford J., Progress in the control of equine influenza, in: Plowright W., Rossdale P.D., Wade J.F. (Eds.), Proc. 6th Int. Conference on Equine infectious diseases, Cambridge, 1992, pp. 207–218.

[40] Mumford J.A., Wood J., Conference report on WHO/OIE meeting: Consultation on newly emerging strains of equine influenza, Vaccine 11 (1993) 1172–1175.

[41] Mumford J.A., Wood J.M., Folkers C., Schild G.C., Protection against experimental infection with influenza virus A/equine/Miami/63(H3N8) provided by inactivated whole virus vaccines containing homologous virus, Epidemiol. Infect. 100 (1988) 501–510.

[42] Mumford J.A., Jessett D.M., Rollinson E.A., Hannant D., Draper M.E., Duration of protective efficacy of equine influenza immunostimulating complex/tetanus vaccines, Vet. Rec. 134 (1994) 158–162.

[43] Mumford J.A., Wilson H., Hannant D., Jessett D.M., Antigenicity and immunogenicity of equine influenza vaccines containing a Carbomer adjuvant, Epidemiol. Infect. 112 (1994) 421–437.

[44] Nelson K.M., Schram B.R., McGrergor M.W., Sheoran A.S., Olsen C.W., Lunn D.P., Local and systemic isotype-specific antibody responses to equine influenza virus infection versus conventional vaccination, Vaccine 16 (1998) 1306–1313.

[45] Newton J.R., Verheyen K., Wood J.L.N., Yates P.J., Mumford J.A., Equine influenza in the United Kingdom in 1998, Vet. Rec. 145 (1999) 449–452.

[46] Newton J.R., Lakhani K.H., Wood J.L.N., Baker D.J., Risk factors for equine influenza serum antibody titres in young Thoroughbred racehorses given an inactivated vaccine, Prev. Vet. Med. 46 (2000) 129–141.

[47] Nguyen H.H., Moldeveanu Z., Novak M.J., van Ginkel F.W., Ban E., Kiyono H., McGhee J.R., Mestecky J., Heterosubtypic immunity to lethal influenza A virus infection is associated with virus-specific $CD8^+$ cytotoxic T lymphocyte responses induced in mucosa-associated tissues, Virology 254 (1999) 50–60.

[48] OIE, Conclusions and recommendations from the consultation meeting of OIE and WHO experts on equine influenza, Newmarket, United Kingdom, September 18–19, 1995, OIE Bull. 108 (1996) 482–484.

[49] OIE Code, International Animal Health Code, 7th ed., Office International des Épizooties, Paris, France, 1998.

[50] Ozaki H., Sugiura T., Sugita S., Imagawa H., Kida H., Detection of antibodies to the non-structural protein (NS1) of influenza A virus allows distinction between vaccinated and infected horses, Vet. Microbiol. 82 (2001) 111–119.

[51] Park A.W., Wood J.L.N., Newton J.R., Daly J., Mumford J.A., Grenfell B.T., Optimising vaccination strategies in equine influenza, Vaccine 21 (2003) 2862–2870.

[52] Plateau E., Crucière C., Virat J., Benazet P., Grippe équine : isolement, caractérisation et étude sérologique dans divers foyers au cours de l'épizootie 1978-1979, Bull. Acad. Vet. Fr. 52 (1979) 189–194.

[53] Powell D.G., Burrows R., Spooner P., Mumford J., Thomson G., Field observations on influenza vaccination among horses in Britain, 1971–1976, Dev. Biol. Stand. 39 (1977) 347–352.

[54] Powell D.G., Watkins K.L., Li P.H., Shortridge K.F., Outbreak of equine influenza among horses in Hong Kong during 1992, Vet. Rec. 136 (1995) 531–536.

[55] Rouse B.T., Ditchfield J.B., The response of ponies to Myxovirus influenzae A-Equi-2 III. The protective effect of serum and nasal antibody against experimental challenge, Res. Vet. Sci. 11 (1970) 503–507.

[56] Rouse B.T., Ditchfield W.J.B., The response of ponies to Myxovirus influenzae A-equi 2 I. Serum and nasal antibody titres following exposure, Can. J. Comp. Med. 34 (1970) 1–6.

[57] Scholtens R.G., Steele J.H., U.S. epizootic of equine influenza, 1963: epizootiology, Public Health Rep. Washington 79 (1964) 393–398.

[58] Sovinová O., Tumová B., Pouska F., Nemec J., Isolation of a virus causing respiratory disease in horses, Acta Virol. 2 (1958) 51–61.

[59] Thomson G.R., Spooner P.R., Powell D.G., The outbreak of equine influenza in England: January 1976, Vet. Rec. 100 (1977) 465–468.

[60] Townsend H.G.G., Penner S.J., Watts T.C., Cook A., Bogdan J., Haines D.M., Griffin S., Chambers T., Holland R.E., Whitaker-Dowling P., Youngner J.S., Sebring R.W., Efficacy of a cold-adapted, intranasal equine influenza vaccine: challenge trials, Equine Vet. J. 33 (2001) 637–643.

[61] Van Maanen C., Bruin G., de Boer-Luijtze E., Smolders G., de Boer G.F., Interference of maternal antibodies with the immune response of foals after vaccination against equine influenza, Vet. Q. 14 (1992) 13–17.

[62] Van Oirschot J.T., Masurel N., Huffels A.D.N.H., Anker W.J.J., Equine influenza in the Netherlands during the winter of 1978–1979; antigenic drift of the A-equi 2 virus, Vet. Q. 3 (1981) 81–84.

[63] Van Oirschot J.T., Bruin G., de Boer-Luytze E., Smolders G., Maternal antibodies against equine influenza virus in foals and their interference with vaccination, Zentralbl. Veterinarmed. B 38 (1991) 391–396.

[64] Waddell G.H., Teigland M.B., Sigel M.M., A new influenza virus associated with equine respiratory disease, J. Am. Vet. Med. Assoc. 143 (1963) 587–590.

[65] Webster R.G., Yuanji G., New influenza virus in horses, Nature 351 (1991) 527.

[66] Wernery R., Yates P.J., Wernery U., Mumford J.A., An equine influenza outbreak in a polo club in Dubai, United Arab Emirates in 1995/96, in: Wernery U., Wade J.F., Mumford J.A., Kaaden O.-R. (Eds.), Proc. 8th Int. Conference on Equine Infections Diseases, Dubai, 1998, pp. 342–346.

[67] Wood J.M., Mumford J., Folkers C., Scott A.M., Schild G.C., Studies with inactivated equine influenza vaccine: I. Serological responses of ponies to graded doses of vaccine, J. Hyg. (Lond.) 90 (1983) 371–384.

[68] Wood J.M., Schild G.C., Folkers C., Mumford J., Newman R.W., The standardization of inactivated equine influenza vaccines by single-radial immunodiffusion, J. Biol. Stand. 11 (1983) 133–136.

[69] Yates P., Mumford J.A., Equine influenza vaccine efficacy: the significance of antigenic variation, Vet. Microbiol. 74 (2000) 173–177.

[70] Yewdell J.W., Hackett C.J., The specificity and function of T lymphocytes induced by influenza A viruses, in: Krug R. (Ed.), The influenza viruses, Plenum Press, New York/London, 1989, pp. 361–429.

[71] Youngner J.S., Whitaker-Dowling P., Chambers T.M., Rushlow K.E., Sebring R., Derivation and characterization of a live attenuated equine influenza vaccine virus, Am. J. Vet. Res. 62 (2001) 1290–1294.