Equine influenza – surveillance and control

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Equine influenza virus (EIV) is considered the most important respiratory virus of horses because it is highly contagious and has the potential to disrupt major equestrian events. Equine influenza (EI) can be controlled by vaccination but it has been demonstrated repeatedly in the field that antigenic drift impacts on vaccine efficacy. EI surveillance maintains awareness of emergence and international spread of antigenic variants. It not only serves as an early warning system for horse owners, trainers and veterinary clinicians but is fundamental to influenza control programmes based on vaccination. Data on outbreaks of EI and strain characterisation is reviewed annually by an Expert Surveillance Panel (ESP) including representatives from OIE and WHO. This panel makes recommendations on the need to update vaccines based on analysis of evidence of disease in well vaccinated horses, antigenic changes, genetic changes and when possible, experimental challenge data. However, the disparity in the level of surveillance and virus collection in different countries results in potentially biased information about the relative prevalence of different viruses. There is a need for increased surveillance on a global level and a greater awareness of the benefits of updating the vaccines. The vaccine companies have traditionally been slow to respond to the ESP recommendations. Veterinary clinicians have a major role to play in purchasing vaccines with epidemiologically relevant strains and promoting their benefits to their clients.

Keywords Equine, influenza, surveillance.

Equine influenza (EI) caused by equine influenza virus (EIV), an Orthomyxovirus, is usually a self-limiting disease characterised by pyrexia, coughing and nasal discharge.[1] The mortality rate associated with EI is very low, unless disease is exacerbated by secondary bacterial infection or continued work of the horse. EIV is less pathogenic than the ubiquitous equine herpesviruses (EHV1 and EHV4), yet it is considered the most important respiratory virus of horses. This is because it is highly contagious and has the potential to disrupt major equestrian events and cause significant economic loss.[2] The equine population is highly mobile, and horses travel long distances by road and air for competition and breeding purposes.[3] When an infected horse is introduced into a susceptible population virus spread can be explosive.[2] The incubation period can be <24 hours in naïve horses, and the continuous coughing, which is a major feature of the disease, serves to release large quantities of virus into the environment. In a partially immune population, seronegative horses are usually the index cases.[4] They amplify virus and serve as a source of infection to their cohorts. Large outbreaks are often associated with the congregation of horses at equestrian events. Their dispersal after the event can lead to widespread dissemination of virus. This was illustrated in Ireland in 1989 and in Australia in 2007.[1,5] In Ireland, the first cases diagnosed during the 1989 epizootic were seronegative horses at the Royal Dublin Show (RDS) in August. In the week after the show, positive samples were submitted to the laboratory from horses that had returned to their home premises located throughout the four provinces of Ireland. Further spread in the non-Thoroughbred population was facilitated by the congregation of horses at a second major international show held in the south of the country 1 week after the Dublin show. Some horses returned home from both these shows to mixed yards, and the virus spread rapidly to the Thoroughbred population. Race meetings took place all over Ireland in early autumn, and influenza spread in the racehorse population. In the Australian outbreak in 2007, the initial spread of the virus in the general horse population was linked to a "one-day event" at Maitland, in New South Wales. The virus then spread to the Thoroughbred population, and by December, it was estimated that over 75 000 horses had been infected. In the Japanese outbreak, in the same year, the reverse situation pertained, i.e. the initial outbreaks were in racehorses and the virus then spread to the non-Thoroughbred population.[6]

EI was first recognised in 1956, and vaccination was introduced in the late 1960s in Europe and North America. The majority of EI vaccines are adjuvant-inactivated virus

or subunit vaccines, and antibodies against the virus haemagglutinin (HA) in these vaccines correlate with protection.[7,8,9] Adjuvants such as carbomer and improved antigenic presentation systems such as immune-stimulating complexes (ISCOMs) have increased the effectiveness of these conventional vaccines, which stimulate a protective but short-lived immunity.[10] Live attenuated vaccines such as the cold-adapted virus and recombinant vaccines such as the pox-based vaccines have also been developed.[11,12] These vaccines aim to stimulate an immune response more closely resembling that induced by natural infection, but their ability to do this is as yet, largely unproven. Neither of these vaccines induces a sterile immunity, which is desirable for international travel. The pox-based vaccine was used in the control of EI in a major outbreak in South Africa (2003) and during the Australian outbreak in 2007.[5] The canarypox vector in the vaccine expresses only the HA genes enabling the differentiation of infected horses from vaccinated horses (DIVA) using an ELISA to detect antibody to the EI nucleoprotein. Technology applied to human vaccines has recently been adapted to EI. A live attenuated virus with an altered NS1 protein engineered by reverse genetics has been shown to decrease clinical signs and virus shedding in experimental challenge studies in horses.[13,14] This vaccine, which is not yet commercially available, has the potential to be readily updated by the insertion of the HA of a new strain.

The majority of EI vaccines contain two subtypes, H7N7 and H3N8, although H7N7 viruses have not been isolated for almost three decades and are considered extinct.[15] These equine viruses are more genetically stable than human influenza viruses, but the antigenic drift of the H3N8 viruses impacts on vaccine efficacy. This has been demonstrated repeatedly in the field. Since the introduction of mandatory vaccination in the UK in 1981, there have only been two large outbreaks of EI involving vaccinated racehorses, in 1989 and in 2003; in both instances, the vaccine strains had been isolated 10 years earlier.[16,17] In 1989, vaccinated horses in Ireland succumbed to infection, and it was demonstrated that there were 16 amino acid differences in the HA of the field virus and the vaccinal strains.[18] This was analogous to the situation in the UK where the importance of antigenic and genetic drift was supported by experimental challenge studies in ponies, confirming that vaccine mismatch reduced protection against infection and virus shedding.[19] In the field, higher levels of antibody were required to protect horses against heterologous strains.[20] Mathematical modelling studies have also suggested that epidemics are more likely to occur when the vaccines have not been updated.[21]

Vaccine efficacy is of importance to all countries irrespective of their disease status. EI has been reported worldwide with the exception of a small number of island countries, including New Zealand and Iceland. Australia

experienced its first incursion in 2007, but the virus is now considered to have been eradicated.[5] EI is endemic in Europe and America.[1] Other parts of the world such as Japan, South Africa, India and Hong Kong suffer occasional incursions, but the disease is not endemic.[1] Vaccination plays a major role in protecting equine populations against EI on all continents. In endemic countries, the economic losses caused by EI can be minimised by vaccination of highly mobile horses, and many racing authorities and equestrian bodies have mandatory vaccination policies that serve as an insurance for business continuity. Non-endemic countries rely on vaccination of imported horses and quarantine to prevent an incursion. The majority of these countries also permit or require vaccination of their indigenous horse population to reduce the impact of an incursion. However, other countries such as Australia and New Zealand only permit vaccination of indigenous horses under restricted circumstances and rely primarily on quarantine and the vaccinal status of imports to protect their susceptible populations. Unfortunately, vaccinated horses can be subclinically infected and shed virus. Many countries including South Africa (1986, 2003), India (1987), Hong Kong (1992), Dubai (1995) and Australia (2007) have experienced EI epizootics related to the importation of such horses.[3,5,22] This is less likely to occur if vaccines are updated with epidemiologically relevant strains as the ability to prevent virus shedding correlates with the antigenic relatedness between the vaccine and the challenge virus.[19] When EI was diagnosed in Australia a 72- hour nationwide "horse standstill" was imposed prior to the introduction of zoning, vaccination and eradication at an estimated cost of over a billion Australian dollars.[5] The horses that introduced the virus into the quarantine facility in Sydney had been vaccinated with products containing outdated strains, i.e they had not been updated in line with the 2004 recommendations to contain a virus of the Florida sublineage (http://www.equineinfluenzainquiry.gov.au).

EI surveillance and strain characterisation are fundamental to influenza control programmes based on vaccination. Vaccine strains must be representative of those in circulation. It is only through surveillance that vaccine companies learn which viruses are relevant. Surveillance also serves as an early warning system for horse owners, trainers and veterinary clinicians, facilitating the implementation of appropriate prophylactic and control measures. Horses frequently receive a booster vaccination following notification of increased influenza activity. In a country where horses are vaccinated against EI, the sector that is not initially affected has an advantage. In Ireland, the diagnosis of influenza in the UK in July 1989 followed by events at the RDS served as an early warning. The widespread vaccination of racehorses after the initial diagnosis of EI in the non-Thoroughbreds is likely to have inhibited the amplification of
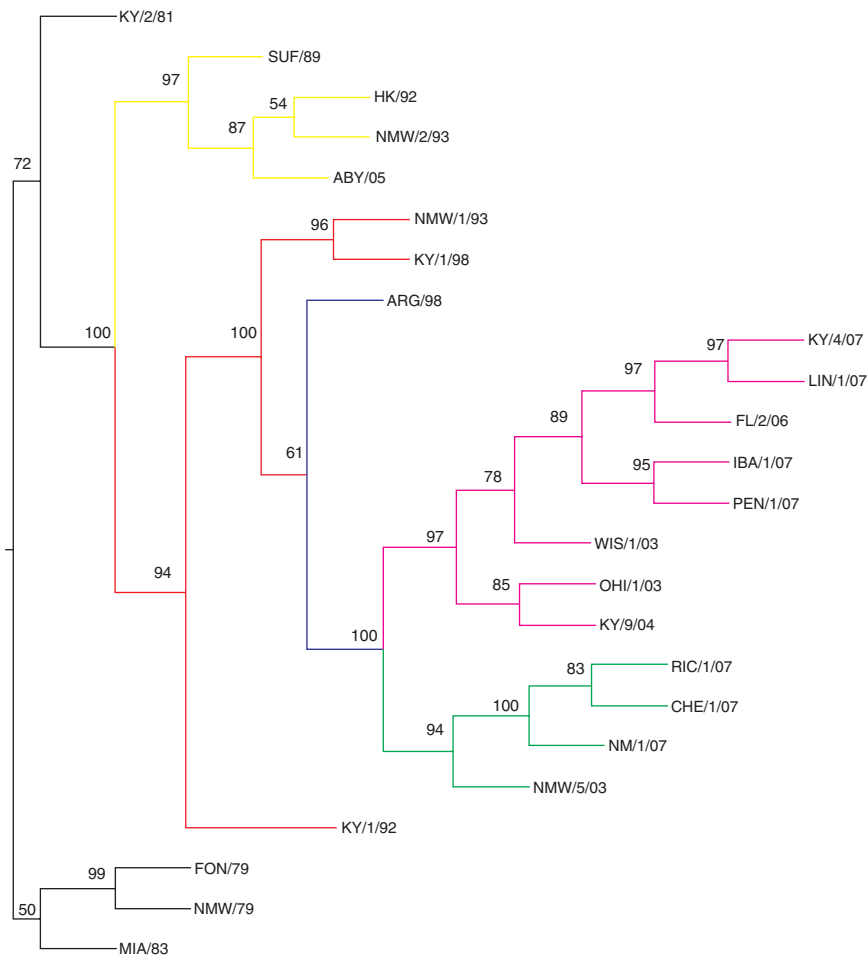
Figure 1. Phylogenetic Tree of HA1 nucleotide sequences (see Appendix 1). Phylogenetic analysis of the HA1 nucleotide sequences encoded by equine influenza virus, subtype H3N8. Bootstrap values obtained after 100 replicates are shown at the major nodes. Phylogenetic groups are shown by continuous bars on the right and are labelled as appropriate. Black = pre-divergent; Yellow = Eurasian; Red = American; Blue = Argentina sublineage; Purple = Florida sublineage Clade 1; Green = Florida sublineage Clade 2. A summary of virus included in the phylogenetic tree above is shown in the table below.

virus and the severity of the disease in this population. Although some non-Thoroughbred equestrian activities were cancelled, no race meetings were cancelled in Ireland as a result of equine influenza in 1989. This contrasts with the situation in Hong Kong in 1992, where an outbreak that commenced in racehorses at the Royal Hong Kong Jockey Club's racing facility led to the postponement of racing for a month.[23]

EI surveillance serves to reduce the economic impact of the disease by maintaining awareness of emergence and international spread of antigenic variants. It is also useful when investigating the source of a virus, for example the virus that caused the 2007 outbreak in Australia was virtually identical to the virus that caused the 2007 outbreak in Japan, which in turn was closely related to viruses isolated at that time in North America (http://www.equineinfluenza-ainquiry.gov.au). The current EIV strains are believed to be

of avian origin, and international surveillance is essential for the timely identification of a novel virus in the equine population. An outbreak of EI in north-west China in 1989 was caused by an avian virus (A/Equine/Jilin/89).[24] It was reported that over 13 000 horses were affected and that the mortality rate was up to 35%. However, the virus did not persist and failed to spread beyond China. It appeared that when the virus crossed the species barrier to horses, it lost its ability to replicate in ducks. More recently, avian H5N1 has been associated with respiratory disease in donkeys in Egypt.[25] If in the future an avian influenza virus adapted to horses and started to spread efficiently, early detection and virus characterisation would be extremely useful in facilitating the development of an effective vaccine.

Surveillance of EI is not only important to identify changes that could impact on equine health but also those that have implications for interspecies transmission. EI has

been associated with outbreaks of respiratory disease in dogs primarily in North America[26] and has been isolated from pigs in China.[27] Although there is currently little evidence of zoonotic infection with EI, all influenza viruses have the potential to jump the species barrier, to reassort and to evolve into a public health threat.

A formal equine influenza surveillance programme has been in place since 1995. The OIE reference laboratories and other laboratories around the world collect data on outbreaks of EI and strain characterisation, which is reviewed annually by an Expert Surveillance Panel (ESP) including representatives from OIE and WHO.[9] This panel makes recommendations on the need to update vaccines, which then are published in the OIE Bulletin. The criteria for updating EI vaccines are similar to those for human influenza vaccines and are based on analysis of evidence of disease in well-vaccinated horses, antigenic changes, genetic changes and when possible, experimental challenge data. The genetic analysis is currently based on the sequencing of the HA gene. The antigenic analysis is based on detection of changes in the HA based on haemagglutination inhibition (HI) tests carried out with ferret and horse antisera. Horse antisera are cross-reactive, but ferrets mount a more strain-specific response.[28] More recently, antigenic cartography has been used to visualise and assist with the analysis of these data.[29]

As the majority of EI epizootics have been caused by H3N8 viruses, there have been several changes in the H3N8 vaccine strains since the 1960s. The original vaccines contained one or more virus relating to the prototype virus A/equine/Miami/63. After severe outbreaks in both vaccinated and unvaccinated horses in 1979–1981 in North America and Europe, an "additional prototype virus" such as A/equine/Fontainbleu/1/79 or A/equine/Kentucky/1/81 was included. After the outbreaks in 1989, many vaccines were updated to include an A/equine/Suffolk/89 like virus.[30] Subsequent phylogenetic analysis indicated that the H3N8 viruses, which had been evolving as a single lineage,[31] had diverged into what were designated a Eurasian and an American lineage based on their initial geographical distribution.[32] In 1995, the ESP recommended that the vaccines be updated to include a representative of each lineage and that the ancient viruses such as A/equine/Miami/63 be removed.[33] As the American lineage predominated and spread internationally, three sublineages emerged, the Argentina, Kentucky and Florida.[34] The Florida sublineage has more recently diverged into two Clades; Clade 1 includes the viruses A/equine/South Africa/4/2003, A/equine/Sydney/2007 and A/equine/Ibaraki/2007 responsible for the major epizootics in South Africa, Australia and Japan and Clade 2 includes A/equine/Newmarket/03 and other viruses that have been circulating in Europe since 2003. Clade 2 viruses were responsible for recent outbreaks in Mongolia, China and India. In 2004, the ESP recommended that the representative of the American lineage in the vaccines be updated to an A/equine/South Africa/4/2003-like virus. In 2009, the paucity of isolates of the Eurasian lineage isolated over a 5- year period led the ESP to state that it no longer supported the inclusion in vaccines of a virus of that lineage.[35] On review of the data collected during 2009 and the continuing evolution of the Florida sublineage, the ESP recommended in 2010 that vaccines for the international market contain both a Clade 1 and Clade 2 virus of that sublineage. The genetic relation-

Table 1. Characterisation of equine influenza isolates by haemagglutination inhibition using reference ferret antisera

Reference virus	N/1/93 (Am)	N/2/93 (Eu)	Ken/98 (Am)	N/5/03 (FC2)	SA/4/03 (FC1)
A/eq/Newmarket/1/93	**128**	8	128	81	20
A/eq/Newmarket/2/93	40	**81**	32	20	8
A/eq/Kentucky/98	256	8	**256**	128	32
A/eq/Newmarket/5/03	91	8	91	**362**	91
A/eq/South-Africa/4/03	16	<8	256	81	**406**
Florida clade 1					
A/eq/Lincolnshire/1/07	16	<8	<8	64	256
A/eq/Florida/2/06	8	<8	16	45	256
A/eq/Kentucky/4/07	11	<8	32	91	512
Florida clade 2					
A/eq/Richmond/1/07	64	<8	128	256	64
A/eq/Cheshire/1/07	128	<8	128	724	128
A/eq/Newmarket/1/07	64	<8	64	128	32

Homologous titres are shown in bold. N/1/93-A/eq/Newmarket/1/93, N/2/93-A/eq/Newmarket/2/93, Ken/98-A/eq/Kentucky/98, N/5/03-A/eq/Newmarket/5/03, SA/4/03-A/eq/South-Africa/4/03. Am, American Lineage; Eu, Eurasian Lineage; FC2, Florida sublineage clade 2; FC1, Florida sublineage clade 1.

ships of these viruses are illustrated in Figure 1, and their antigenic relationships are summarised in Table 1.[36]

As the last confirmed outbreak owing to infection with H7N7 was in 1979, the ESP no longer recommend that a virus of this subtype be included in the vaccines.

There are many problems encountered with EI surveillance, exacerbated by a lack of funding in some countries. It can be difficult to obtain samples as horse owners frequently do not perceive a benefit in acquiring a confirmatory diagnosis for a self-limiting respiratory disease. Also, while the introduction of the highly sensitive RT-PCR for EI in diagnostic laboratories[37] has revolutionised the diagnosis of this disease, there is frequently a failure to submit positive sample material to an OIE reference laboratory for virus characterisation. In many instances, virus isolation and characterisation are never performed, but some laboratories retain the sample material for their own investigation. OIE reference laboratories have a role to play in assisting such laboratories with the technical training and supply of reagents to enable them to characterise viruses in a timely manner. Currently, the disparity in the level of surveillance and virus collection in different countries results in potentially biased information about the relative prevalence of different viruses. There is a need for increased surveillance on a global level and a greater awareness of the benefits of updating the vaccines. The vaccine companies have traditionally been slow to respond to the ESP recommendations. Regulatory authorities need to facilitate the updating of EI vaccines by simplifying and harmonising the licensing procedures. Veterinary clinicians have a major role to play in purchasing updated vaccines and promoting their benefits to their clients. There is a significant financial investment required to update vaccine strains, but little incentive to do so if there is no demand in the marketplace. Finally, in 2008, the Australian Quarantine and Inspection Service (AQIS) revised their requirements for importation stating that horses must be vaccinated with a vaccine containing the strains recommended by the ESP prior to importation (http://www.aqis.gov.au). This has heightened awareness of the role of epidemiologically relevant vaccine strains in countries that export horses to Australia. Influenza control would benefit if regulatory bodies within the horse industry insisted on the use of updated vaccines.

Acknowledgements

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Appendix 1. Equine influenza virus isolate, lineage, abbreviation and accession number

Location	Lineage	Virus name	Abbreviation	HA1
Miami, USA	Pre-div	A/eq/Miami/63	MIA/63	M29257
Fontainebleau, France	Pre-div	A/eq/Fontainebleu/79	FON/79	CY032405
Newmarket, UK	Pre-div	A/eq/Newmarket/79	NWM/79	D30677
Kentucky, USA	Pre-div	A/eq/Kentucky/2/81	KY/2/81	CY028820
Suffolk, UK	Eu	A/eq/Suffolk/89	SUF/89	X68437
Hong Kong	Eu	A/eq/HongKong/92	HK/92	L27597
Kentucky, USA	Am	A/eq/Kentucky/1/92	KY/1/92	CY030149
Newmarket, UK	Am	A/eq/NewMarket/1/93	NWM/1/93	X85088
Newmarket, UK	Eu	A/eq/Newmarket/2/93	NWM/2/93	X85089
Kentucky, USA	Am	A/eq/Kentucky/1/98	KY/1/98	AF197241
Ohio, USA	FC1	A/eq/Ohio/1/03	OHI/1/03	DQ124192
Wisconsin, USA	FC1	A/eq/Wisconsin/1/03	WIS/1/03	DQ222913
Newmarket, UK	FC2	A/eq/NewMarket/5/03	NWM/5/03	FJ375213
Kentucky, USA	FC1	A/eq/Kentucky/9/04	KY/9/04	FJ195451
Aboyne, Scotland	Eu	A/eq/Aboyne/05	ABY/05	EF541442
Florida, USA	FC1	A/eq/Florida/2/06	FL/2/06	FJ195403
Richmond, UK	FC2	A/eq/Richmond/1/07	RIC/1/07	FJ195395
Ibaraki, Japan	FC1	A/eq/Ibaraki/1/07	IBA/1/07	AB360549
Pennsylvania, USA	FC1	A/eq/Pennsylvania/1/07	PEN/1/07	FJ195406
Lincolnshire, UK	FC1	A/eq/Lincolnshire/1/07	LIN/1/07	FJ195398
Kentucky, USA	FC1	A/eq/Kentucky/4/07	KY/4/07	FJ195404
Cheshire, UK	FC2	A/eq/Cheshire/1/07	CHE/1/07	FJ195410
Newmarket, UK	FC2	A/eq/Newmarket/1/07	NM/1/07	FJ195397

Pre-div, Pre-diversion of Equine influenza H3N8 virus; Am, American Lineage; Eu, Eurasian Lineage; FC1, Florida sublineage clade 1 (A/eq/Wisconsin/03-like); FC2, Florida sublineage clade 2 (A/eq/Newmarket/5/03-like); HA1, Haemagglutinin sequence accession numbers.