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Using epidemics to map H3 equine influenza virus determinants of antigenicity

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ABSTRACT

Equine influenza is a major cause of respiratory infections in horses and causes widespread epidemics, despite the availability of commercial vaccines. Antigenic drift within the haemagglutinin (HA) glycoprotein is thought to play a part in vaccination breakdown. Here, we carried out a detailed investigation of the 1989 UK outbreak, using reverse genetics and site-directed mutagenesis, to determine the individual contribution of amino acid substitutions within HA. Mutations at positions 159, 189 and 227 all altered antigenicity, as measured by haemagglutination-inhibition assays. We also compared HA sequences for epidemic and vaccine strains from four epidemics and found that at least 8 amino acid differences were present, affecting multiple antigenic sites. Substitutions within antigenic site B and at least one other were associated with each outbreak, we also identified changes in loop regions close to antigenic sites that have not previously been highlighted for human H3 influenza viruses.

Introduction

It is well known that mammalian influenza viruses undergo antigenic drift through acquisition of amino acid substitutions within the viral glycoproteins haemagglutinin (HA) and neuraminidase (NA), leading to the occurrence of disease epidemics. For this reason, vaccine strains to influenza viruses eventually become ineffective, unless they are updated regularly. The HA1 domain appears to be under the most selective pressure, consistent with its role in induction of neutralising antibodies (Nelson and Holmes, 2007). Substitutions in human H3 viruses have been associated with changes in charge, acquisition of glycosylation sites and also alteration of receptor binding avidity (Blackburne et al., 2008; Kobayashi and Suzuki, 2012; Lin et al., 2012). Changes in HA are often accompanied by substitutions in NA and it is believed that the activity of HA and NA should be balanced (Mitnaul et al., 2000; Kaverin et al., 1998; Baigent and McCauley, 2001). The rate of antigenic change differs between influenza viruses of different species: human H3N2 viruses appear to drift more rapidly than either swine H3N2 or equine H3N8 viruses, as assayed by haemagglutination inhibition (HI) with ferret antisera. This has been demonstrated by antigenic cartography, which indicated that human H3N2 viruses underwent multiple 'cluster jumps' between 1968 and 2003 (Smith et al., 2004) whereas equine and swine viruses evolved fewer antigenic clusters over a similar period of time (de Jong et al., 2007; Lewis et al., 2011).

Influenza virus of the H3N8 subtype was first reported in horses in 1963 and subsequently spread around the world, affecting the UK in 1965 (Rose, 1966). Early reports suggested that equine influenza virus (EIV) did not undergo antigenic drift (Burrows et al., 1981), however it is now clear that the virus acquires mutations in HA that lead to antigenic drift in much the same way as other influenza A viruses (Daniels et al., 1985; Oxburgh et al., 1993; Lewis et al., 2011). Multiple lineages and sub-lineages have evolved since 1963, including the divergence of the Eurasian and American viruses of the late 1980s (Daly et al., 1996), followed by further division of the American lineage into the Kentucky, Argentinian and Florida clade 1 and 2 (FC1, FC2) sublineages (Lai et al., 2001; Bryant et al., 2009). Following the initial pandemic wave in 1965, there have been three further country-wide outbreaks in the UK in 1979, 1989 and 2003. As a result of the 1979 outbreak, which caused substantial disruption to the racing industry, mandatory vaccination was introduced for racing Thoroughbreds in the UK. Other competitive bodies now require vaccination for competition and rules are laid down by the British Horseracing Authority and Federation Equestra Internationale (FEI).

In 1989 the UK epidemic affected recently vaccinated and unvaccinated animals alike, indicating that the causative strain had undergone significant antigenic drift from the existing vaccine strains (Livesay et al., 1993; Binns et al., 1993). A similar outbreak occurred in Hong Kong in 1992, also affecting vaccinated animals (Powell et al., 1995). The vaccines in use at the time included the EIV prototype H3N8 strain A/equine/Miami/63, but also more recent pre-divergence strains from

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1979 to 1981. In both the 1989 UK outbreak and the Hong Kong outbreak the viruses belonged to the Eurasian sub-lineage of EIV (Lai et al., 1994, 2001). Following the 1989 outbreak in the UK, a surveillance programme was established to monitor antigenic drift in EIV and make recommendations for suitable strains for vaccine use.

In 2003, a large-scale outbreak started in Newmarket, affecting racing Thoroughbreds despite recent vaccination (Newton et al., 2006). Again, this was suggestive of antigenic drift, although the use of simple killed vaccines was also implicated. Vaccines in use at the time contained Eurasian and American (Kentucky) strains from the 1990s. A further example of large-scale vaccination breakdown occurred in Japan in 2007, in which vaccines contained Eurasian and American (Argentinian) strains from 1993 and the outbreak virus belonged to FC1 (Ito et al., 2008). A consistent feature of these large scale outbreaks was that commercial vaccines all contained strains that were at least 10 years older than the outbreak strain.

Antigenic characterisation of influenza viruses for the purpose of vaccine strain selection has been carried out using haemagglutination-inhibition (HI) assays for human and equine influenza viruses for many years (WHO, 2002). Ferret antisera distinguish readily between EIV strains, whereas equine antisera are typically more cross-reactive (Hinshaw et al.,1983; Burrows and Denyer, 1982). Current criteria used for the selection of EIV vaccine strains also include comparison of amino acid sequences for HA1. More than four amino acid changes in two or more antigenic sites, based on those mapped for human H3N2 viruses and/or an 8-fold or greater difference in HI titre between vaccine and circulating virus strains are considered significant (Wiley and Skehel, 1987; Wilson and Cox, 1990). However, the equine H3 molecule remains poorly characterised in this respect and antigenic sites are typically extrapolated from human H3 viruses (Barbic et al., 2009; Ito et al., 2008). The five antigenic sites of human H3 are thought to represent antibody-binding sites and were originally mapped on the basis of sequence variation amongst field strains and generation of escape mutants, selected by passaging viruses in the presence of monoclonal antibodies or neutralising human antisera (reviewed by Wiley and Skehel, 1987; Wilson and Cox, 1990). To date, these methods have not been applied consistently to EIV.

There is no legal requirement for EIV vaccines to be updated and the process from recommendation of new strains by the OIE to the appearance of updated commercial vaccines on the market typically takes several years. It is therefore important that reliable predictive methods are developed so that suitable recommendations can be made in a timely fashion. One area of importance is mapping the regions of EIV H3 that are important for antigenicity, rather than relying on those mapped for human H3 viruses. In this context, we undertook a detailed antigenic comparison of vaccine and outbreak strains from the 1989 UK outbreak. Reverse genetics and site directed mutagenesis were applied to determine the effect of individual amino acid differences between the outbreak and vaccine strains, with the aim of adding to current knowledge of the significant amino acid changes for equine H3. The study was extended by comparison of HA sequences from outbreak and vaccine strains associated with other known epidemics of equine influenza, using data from 1979, 1989, 2003 and 2007.

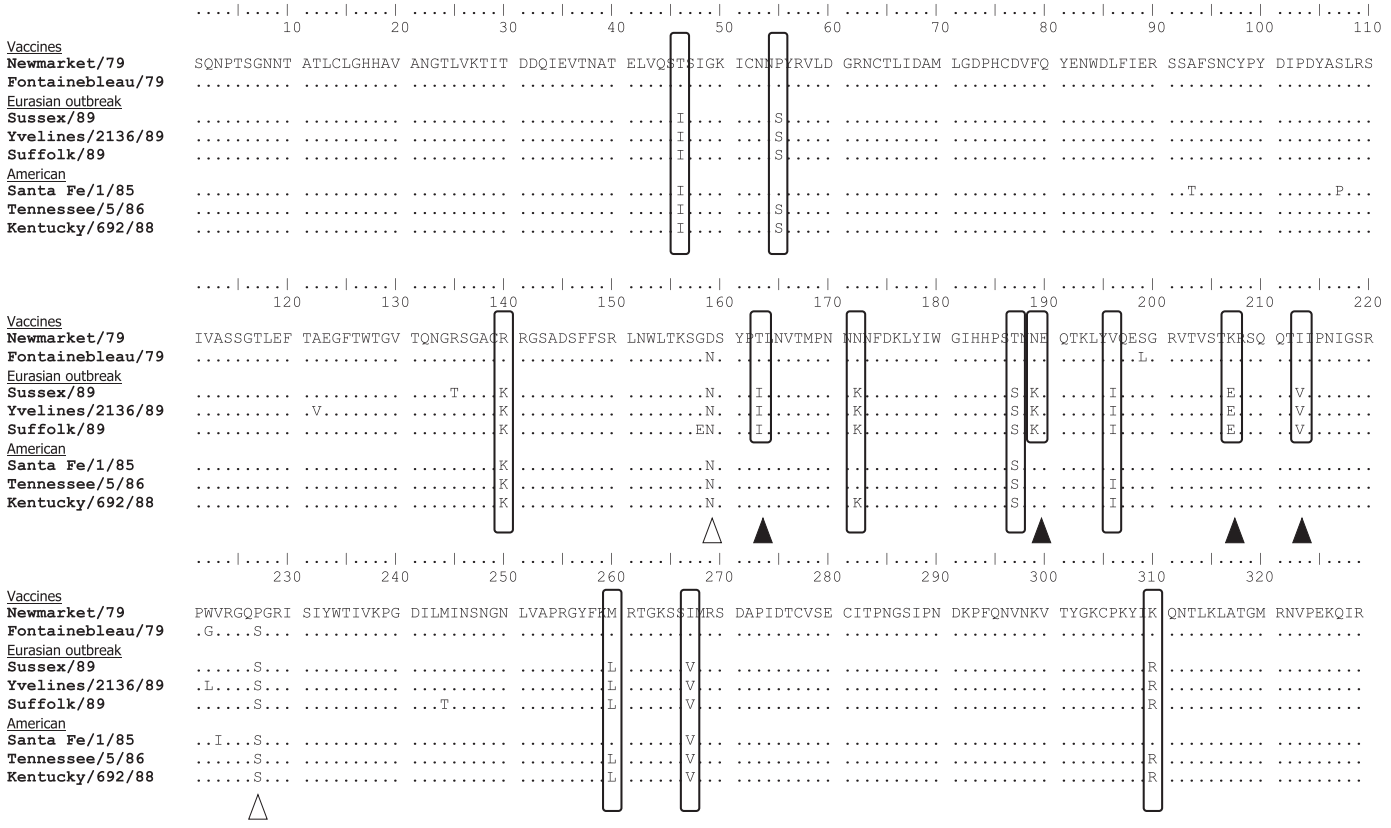

Fig. 1. Comparison of vaccine and outbreak EIV strains: 1989 UK outbreak. Derived amino acid sequences for HA1 were aligned for two vaccine strains and three isolates from the 1989 outbreak in Europe, three contemporary strains from the American (Kentucky) sub-lineage are included for reference. Amino acid substitutions between the 1979 vaccine strains and 1989 field strains are outlined in black. Substitutions unique to the Eurasian outbreak strains are indicated by black triangles, two further differences restricted to the Newmarket/79 vaccine strain are shown by open triangles. Residues are numbered from 1 to 329, starting with serine at the start of the predicted mature polypeptide for HA1. Genbank accession numbers are: Newmarket/79 – KJ643908, Fontainebleau/79 – KJ643904, Sussex/89 – KJ643906, Yvelines/2136/89 – BAA33940, Suffolk/89 – KJ643907, Santa Fe/1/85 – ACD85286, Tennessee/5/86 – ACA24656 and Kentucky/692/88 – ACA24579. Source of data and passage history are provided in Table S1.