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Review
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EPIDEMIOLOGY OF EQUINE INFLUENZA VIRUSES: PATHOGENICITY AND TRANSMISSIBILITY

EPIDEMIOLOGIJA VIRUSA INFLUENCE KONJA: PATOGENOST I PRENOSIVOST

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Summary

A large-scale outbreak caused by equine influenza virus of the H3N8 subtype has occurred in each decade since an H3N8 virus was first isolated from horses in 1963. Each epidemic, and some minor outbreaks, has influenced equine influenza surveillance and vaccination policies in the UK and elsewhere. The latest widespread outbreak of equine influenza occurred in 2003. The possible reasons for infection of horses despite intensive vaccination are currently being investigated and may shed new light on the epidemiology of equine influenza.

Key words: Equine influenza epidemiology; Vaccine strain selection

HISTORIC EPIDEMIOLOGY OF EQUINE INFLUENZA

Influenza virus was first isolated from horses in Czechoslovakia in 1956 during a widespread epidemic of respiratory disease among horses in Eastern Europe [1], this was the prototype strain for the H7N7 subtype of equine influenza virus A/eq/Prague/56 (H7N7). The H7N7 subtype is still represented in vaccines in many countries although H7N7 viruses may only persist at low levels in some parts of the world [2-4] and the last published isolation of an H7N7 virus was in Egypt in 1989 [5].

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Virus of the H3N8 subtype was first isolated in 1963. The prototype virus, A/eq/Miami/63, was introduced into the equine population of Florida with the importation of horses from Argentina [6], and has continued to cause outbreaks of disease in Europe and North America since. Vaccines against equine influenza were introduced shortly after this epidemic.

Antigenic variants can give rise to large-scale disease epidemics such as occurred in 1979 to 1981 in Europe and North America. As a consequence of the 1979 epidemic, annual vaccination for racehorses and competition horses became mandatory in several European countries and representative strains from these epidemics were subsequently incorporated into vaccines. Following implementation of a mandatory vaccination policy in 1981, equine influenza was not diagnosed in Ireland and the UK for almost a decade. In 1989, an extensive epidemic of equine influenza was experienced in Europe. At this time, vaccines contained prototype strains from earlier epidemics (i.e. Miami/63, Fontainebleau/79 or Kentucky/81). Antigenic and genetic analysis of a virus isolated in the UK (A/eq/Suffolk/89) demonstrated that it differed significantly from the prototype strains (A/eq/Fontainebleau/79 and A/eq/Kentucky/81) of the previous European epidemic [7]. A vaccination and challenge study in ponies demonstrated that the ability of a vaccine to reduce virus excretion was directly correlated with the antigenic relatedness of the vaccine strain (Miami/63, Fontainebleau/79, Kentucky/81 or Suffolk/89) with the Sussex/89 challenge virus [8]. The Suffolk/89 vaccine was most effective and Miami/63 least effective. Thus it was confirmed that equine influenza vaccines might not provide adequate protection if they contain outdated vaccine strains that have become irrelevant as a result of antigenic drift. A meeting of World Health Organization (WHO) and Office International des Epizooties (OIE) experts in 1993 recommended that a modern variant should be incorporated into vaccines and, in order to assist with future choices of vaccine strain, the surveillance efforts should be intensified [9].

Phylogenetic analysis of the HA molecule demonstrated that since around 1986, H3N8 viruses have diverged into 2 distinct evolutionary lineages, designated 'American' and 'European' on the basis of the geographic origins of virus strains comprising the different lineages [10]. Although the geographic distinction has become less apparent,

Table 1. Landmarks in the epidemiology of equine influenza A H3N8 viruses

Event	Significance
1963 epidemic	First isolation of H3N8 virus from horses.
1979 epidemic	Mandatory vaccination policies introduced.
1989 epidemic	Antigenic drift demonstrated to be responsible for vaccine failure.
1993 outbreak	Two geographically distinct co-circulating lineages of H3N8 virus identified.

particularly due to the isolation of American lineage viruses in Europe, 2 distinct lineages of H3N8 viruses continue to co-circulate. The functional significance of this phylogenetic dichotomy has been examined in studies of virus antigenicity using polyclonal antisera [10] and monoclonal antibodies [11]. Additional vaccination and challenge studies demonstrated that differences observed between H3N8 viruses isolated in the same year (1993) but from different lineages (American versus European) are sufficient to compromise cross-protection [12]. Field data from outbreaks in Newmarket supported the experimental data [12].

A European rapid licensing system for influenza vaccines containing updated strains has been developed on the basis that accurate standardisation of in vitro vaccine potency tests allows a reduction in animal testing of the final product, such that delays in new products reaching the market can be avoided [13]. The OIE Reference Laboratory at Newmarket co-ordinates an ongoing surveillance programme by OIE and WHO reference laboratories aimed at providing information on suitable strains for incorporation in vaccines. Following a meeting of WHO/OIE experts in 1995, [14] an Expert Surveillance Panel was formed and it was recommended that vaccines should contain one H3N8 virus representative of the American lineage (e.g. Newmarket/1/93 or Kentucky/94) and a representative of the European lineage (e.g. Newmarket/2/93). The Expert Surveillance Panel meets annually and its recommendations are published in the OIE bulletin. Human influenza vaccines are reviewed on an annual basis and in most years at least one of the 3 components is updated. However, antigenic drift occurs at a much lower rate in equine influenza viruses than in human influenza viruses and it was anticipated that the equine vaccines would not need to be updated so frequently. Indeed, the recommendation made in 1995 remained unchanged for almost 10 years.

Table 2. Equine influenza outbreaks associated with international movement of horses

Year	Country affected	Source of infection
1986	South Africa	North America
1987	India	Europe
1992	Hong Kong	UK/Ireland
1995	Dubai	North America
1998	Philippines	North America

INTERNATIONAL MOVEMENT OF HORSES

Some of the most devastating epidemics of equine influenza have occurred when the virus has been introduced into areas previously free of the disease (Table 2). In South Africa in 1986, race meetings were cancelled for over 2 months because of an influenza

epidemic [15]. A year later, during an influenza epidemic in India, over 27,000 equidae were affected and several hundred animals died [16]. In 1992, an outbreak of equine influenza affected the majority of horses in Hong Kong, despite vaccination, and severely disrupted racing [17]. There is evidence to suggest that these epidemics resulted from the importation of infected horses from Europe [18]. Recent outbreaks in Dubai, [19] the Philippines and Puerto Rico caused by importation of infected horses from America further highlight the ease with which equine influenza can be introduced into susceptible populations as a result of international movement of horses. The Code Commission of the OIE recommends that importing countries that are free of 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 prior to travel.

RECENT EPIDEMIOLOGY OF EQUINE INFLUENZA

Efforts directed at international surveillance for equine influenza have substantially increased in the past 10 years. The International Collating Centre (ICC) receives reports of episodes of infectious disease from nominated veterinary contacts in participating countries on a quarterly basis and provides regular reports to subscribers around the world. Although limited, the data reported by the ICC are the only data available for non-notifiable diseases such as equine influenza and provide a global perspective. During the period 1995 to date, some European countries reported the laboratory diagnosis of influenza on an almost annual basis, as did the USA, suggesting that the virus is endemic in these regions. Only Australia, New Zealand and Iceland are known to have remained entirely free from equine influenza.

From 1993 to 2002, outbreaks of equine influenza were frequent but limited in extent in the UK. However, a more widespread outbreak of equine influenza occurred in 2003, with related viruses isolated initially in the UK and subsequently in France, Ireland, Italy and Croatia [20]. Between March and May 2003, equine influenza virus infection was confirmed as the cause of respiratory disease among both vaccinated and non-vaccinated horses of different breeds and types in at least 12 locations in the UK [21]. In the largest outbreak, 21 Thoroughbred training yards in Newmarket, comprising more than 1300 racehorses, were variously affected with horses showing signs of coughing and nasal discharge during a 9-week period. Many of the infected horses had been vaccinated within the previous 3 months with a vaccine that contained representatives from both the European (A/eq/Newmarket/2/93) and American (A/eq/Newmarket/1/93) H3N8 influenza virus lineages. Antigenic and genetic characterisation of viruses from Newmarket and elsewhere indicated that they were all closely related to representatives of a sub-lineage of American viruses (e.g. Kentucky/5/02). This was the first time that this

virus sub-lineage had been isolated in the UK. It is not yet clear why disease occurred so extensively in the recently vaccinated racehorses in Newmarket as single radial haemolysis (SRH) antibody levels in acute sera appeared adequate and on the basis of current criteria there did not appear to be significant antigenic differences between the infecting virus and A/eq/Newmarket/1/93, the American lineage virus representative in the most widely used vaccine, to explain the vaccine failure. However, there was evidence for significantly fewer infections among younger (2-year-old) horses than older animals, despite similar high levels of antibody. This is counterintuitive as older animals would be expected to accumulate greater resistance to infection than younger animals that have not been vaccinated as many times. The finding is consistent with a qualitative rather than quantitative difference in the immunity conveyed by vaccination, and the vaccine histories of these animals are being investigated.

It was also observed during the 2003 outbreak in the UK that although clinical signs were milder among vaccinated horses, there was some evidence that the virus was more pathogenic in other strains such as those responsible for localised outbreaks in 1993. Relatively severe clinical signs were observed in some unvaccinated horses with coughing being a particularly pronounced feature. This observation was confirmed when unvaccinated seronegative ponies were experimentally infected with a representative strain from the Newmarket 2003 outbreak (J Daly, unpublished observations). Indeed, there were two independent cases of unvaccinated horses with confirmed equine influenza virus infection that developed neurological signs, one of which was euthanased as a result [22]. It has previously been recognised that disease severity differs between strains of equine influenza; experimental infection with a strain representative of the 1989 epidemic (Sussex/89) induces more severe clinical signs than infection with either of the strains isolated during a limited outbreak in Newmarket in 1993. Watrang et al. (2003) [23] demonstrated that the severity of clinical signs correlated with induction of cytokines. It is possible that the ability of a strain to cause more widespread epidemics as opposed to localised outbreaks is related to its pathogenicity.

In December of 2003, an equine influenza outbreak occurred in South Africa. Having had no evidence of equine influenza infection in the country for several years, the requirement for mandatory vaccination of competition horses had been lifted. The outbreak spread from the Cape Town quarantine station, which had received a shipment of horses from several different countries including the UK and the USA (A. Guthrie, personal communication). Genetic characterisation of the virus suggested that the virus arrived with infected horses from the USA, the HA1 being identical in sequence to the US isolate Ohio/03 (T Chambers, personal communication). The South African 2003 virus was distinguishable from the UK 2003 prototype virus by 2 amino acid substitutions in the HA1 (Fig. 1), which had a significant impact on the antigenicity of the virus. In

light of the extensive spread of the more recent American lineage viruses and the antigenic and genetic differences observed in the most recent American lineage strains, the recommendation of the Expert Surveillance Panel in 2004 was to update the American lineage component of vaccines from Newmarket/1/93 or Kentucky/04 to a South Africa/4/03-like virus.

In the past, epidemics of the H3N8 subtype of equine influenza have informed vaccine policy decisions. The widespread outbreak of equine influenza among well-vaccinated Thoroughbred horses in Newmarket during 2003 confounded current thinking on the causes of equine influenza outbreaks. In addition to further examination of the antigenic relationship between the Newmarket 2003 strains and the American lineage vaccine strain, research is also being directed at examining levels of strain-specific immunity in acute sera obtained during the outbreak in Newmarket, and virulence of the Newmarket 2003 isolates.

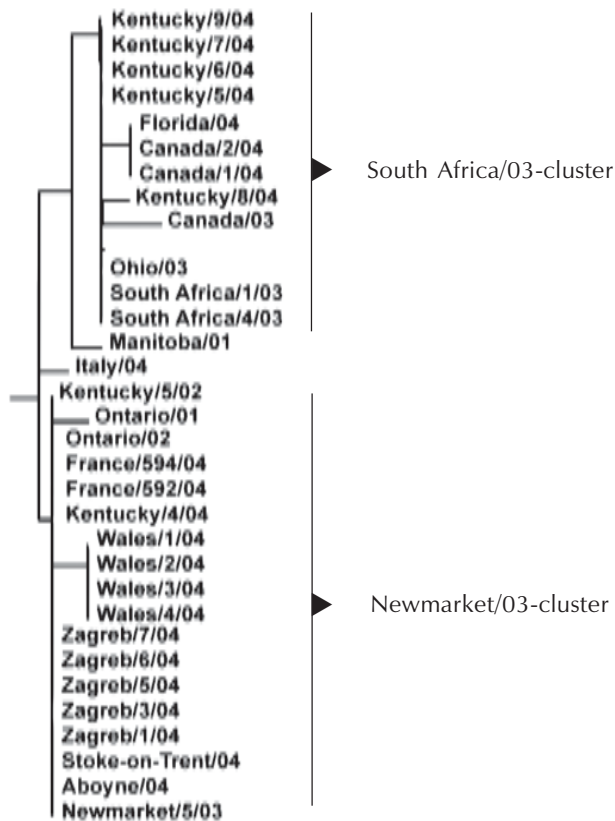


Fig. 1. Detail from phylogeny of HA1 of American lineage H3N8 isolates showing recent isolates

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Sažetak

Podtip H3N8 virusa influence konja uzrokovao je velik broj epizootija svakog desetljeća otkako je 1963. godine prvi put bio izdvojen iz konja. Svaka epizootija, a i neke manje pojave, utjecale su na mjere nadzora i cijepljenja u Velikoj Britaniji i drugdje. Posljednja velika epizootija influence konja zabilježena je 2003. Mogući razlozi prijemljivosti konja usprkos stalnog cijepljenja još se istražuju i mogli bi dati novo svjetlo na epidemiologiju influence konja.

Ključne riječi: Epizootiologija influence konja; Odabir cjepnog soja