

Short Communications

Equine influenza viruses isolated during outbreaks in China in 2007 and 2008

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EQUINE influenza virus (EIV) belongs to the Orthomyxoviridae family of viruses and is represented by two subtypes, H7N7 and H3N8. The H3N8 subtype circulates in the equine population throughout most of the world (Timoney 1996, Webster 1998). The World Organisation for Animal Health (OIE) conducts a surveillance programme for EIV, coordinated by the OIE Reference Laboratory at the Animal Health Trust, Newmarket, UK. This constant surveillance provides information on currently isolated strains of EIV, which guides the composition of new vaccines (OIE 2006, 2008). In China, EIV was isolated from infected horses in 1989 (Webster and Guo 1991, Guo and others 1992) and 1994 (Shortridge and others 1995), but little is known about the more recent status of EIV in China.

In September 2007, an influenza-like respiratory infection of horses was observed in the Xinjiang Uygur Autonomous Region of China. During the following six months, the disease spread rapidly to other areas of China, infecting local equids. This short communication describes the genetic characteristics of EIV strains isolated during this outbreak of equine influenza and also reports on the epidemic status of EIV infections in China.

Nasopharyngeal swabs were collected from equids showing influenza-like signs in affected areas for identification of the pathogen according to the procedures described by the OIE (2004). Ten EIV isolates (Table 1) were recovered from horses, donkeys and Asian wild horses (*Equus przewalskii*) in different regions of China, mainly in provinces in the north of the country (Fig 1). All eight gene segments of the isolated influenza viruses were amplified by RT-PCR and sequenced (GenBank accession numbers EU794492 to EU794579); sequencing revealed that all 10 EIV sequences were of

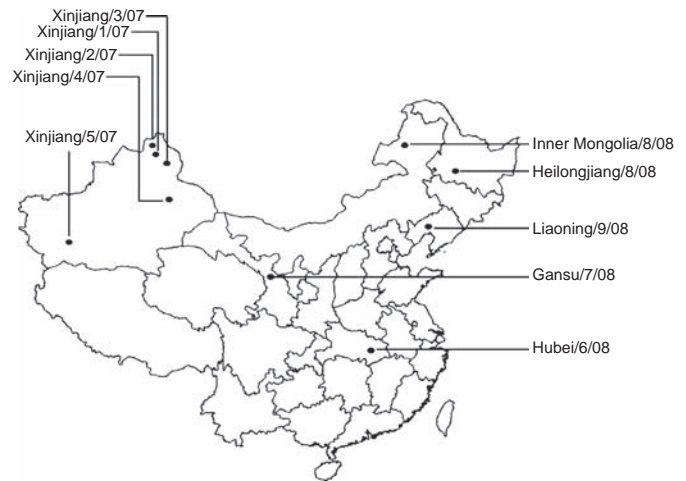


FIG 1: Map of China showing the locations of equids from which equine influenza viruses were isolated in 2007 and 2008

subtype H3N8. The EIV subtype H3N8 can be further classified into American and European lineages on the basis of differences in the following residues of the haemagglutinin (HA) gene: 93Val-Asp, 178Thr-Ile, 204Gln-Lys, 222Lys-Glu, 228Ile-Val, 276Arg-Lys, 288Pro-Leu and 291Thr-Ile (Daly and others 1996, Damiani and others 2008). The Chinese isolates described in the present study were all of the American lineage, based on homology at these residues. In contrast, the Chinese EIV strains identified in the 1994 outbreak (Shortridge and others 1995) were of the European lineage.

The epidemiological distribution of EIV infection in China was investigated by randomly sampling equine sera from seven provinces for anti-HA antibody in April and May 2008 (Table 2). Using the A/Eq/Xinjiang/3/2007 virus strain, haemagglutination inhibition assays were performed on the serum samples, and anti-EIV antibody was detected at frequencies ranging from 7.1 per cent in samples from Beijing to 76.2 per cent in samples from Inner Mongolia (Table 2). Sera collected from Beijing and Tianjin had significantly lower rates of seropositivity than samples from other provinces (Table 2). Given that the seven provinces sampled in the present study were located across the country and there were no strict controls on the movement of horses between provinces, it is likely that equine influenza had spread to numerous unsampled provinces. The sera samples were collected from unvaccinated horses, but some horses in Beijing and Tianjin had been vaccinated (Proteqflu; Merial) during May 2008. It is likely that this, and the strict control of horse circulation, prevented the spread of EIV in these areas. Further samples were taken from seropositive horses in the same areas in May and June, 2008, for attempts at virus isolation, but no viruses were identified. The authors therefore suspect that the epidemic of equine influenza may already have subsided at the time of sampling.

Previous studies have reported the isolation of EIV in China in 1989 and 1994. The 1989 strain was identified as a new sublineage of H3N8 (Webster and Guo 1991, Guo and others 1992) but has not re-emerged since. In contrast, the 1994 epidemic strains were identified as European-lineage viruses (Shortridge and others 1995). The 10 EIV strains isolated in 2007 and 2008 described here belong to the American lineage and exhibited an antigenic drift when compared with European lineage viruses (Daly and others 1996). The authors conclude that these viruses were recently introduced into the Chinese equine population. The lack of pre-existing immunity among Chinese equids to this antigenically drifted EIV, combined with the lack of an EIV vaccination programme in China,

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TABLE 1: Equine influenza virus (H3N8) isolates recovered from equids in China during 2007 and 2008

| Virus isolate | Host species | Town/province | Date |
|--------------------------------|------------------|---------------------------|---------------|
| A/equine/Xinjiang/1/2007 | Horse | Buerjin/Xinjiang | November 2007 |
| A/equine/Xinjiang/2/2007 | Horse | Habahe/Xinjiang | November 2007 |
| A/equine/Xinjiang/3/2007 | Horse | Fuyun/Xinjiang | November 2007 |
| A/equine/Xinjiang/4/2007 | Asian wild horse | Jimusaer/Xinjiang | December 2007 |
| A/equine/Xinjiang/5/2007 | Donkey | Hetian/Xinjiang | December 2007 |
| A/equine/Hubei/6/2008 | Horse | Wuhan/Hubei | January 2008 |
| A/equine/Gansu/7/2008 | Horse | Lanzhou/Gansu | February 2008 |
| A/equine/Inner Mongolia/8/2008 | Horse | Hulunbeier/Inner Mongolia | February 2008 |
| A/equine/Liaoning/9/2008 | Horse | Fuxin/Liaoning | April 2008 |
| A/equine/Heilongjiang/10/2008 | Horse | Shangzhi/Heilongjiang | April 2008 |

TABLE 2: Seroprevalence of equine influenza virus in seven provinces of China in 2008, determined by haemagglutination inhibition (HI) assay

| Location | Number of samples | Number (%) HI positive | Date |
|----------------|-------------------|------------------------|----------|
| Jilin | 50 | 30 (60.0) | April 22 |
| Hebei | 73 | 30 (41.1) | April 23 |
| Tianjin | 45 | 10 (22.2) | April 24 |
| Beijing | 70 | 5 (7.1) | April 25 |
| Heilongjiang | 15 | 4 (26.7) | April 29 |
| Inner Mongolia | 21 | 16 (76.2) | May 6 |
| Guangdong | 50 | 30 (60.0) | May 7 |
| Total | 324 | 125 (38.6) | |

were the likely causes for the outbreak that occurred during 2007 and 2008.

Due to the absence of an EIV vaccination programme and up-to-date biosecurity protocols in China (Daly and others 2004), further outbreaks of equine influenza in the future are inevitable. Therefore, the development of strict preventive measures is essential. It is also important to monitor the current epidemic state of equine influenza

in China, which will provide data essential for updating vaccines and preparing proper prevention protocols.

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