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Development of a surveillance scheme for equine influenza in the UK and characterisation of viruses isolated in Europe, Dubai and the USA from 2010–2012

Alana L. Woodward [a], Adam S. Rash [a], Donna Blinman [a], Samantha Bowman [a], Thomas M. Chambers [b], Janet M. Daly [c], Armando Damiani [d], Sunitha Joseph [e], Nicola Lewis [f], John W. McCauley [g], Liz Medcalf [a], Jenny Mumford [h], J. Richard Newton [a], Ashish Tiwari [b], Neil A. Bryant [a], Debra M. Elton [a,*]

[a] Animal Health Trust, Lanwades Park, Kentford, Newmarket, Suffolk CB8 7UU, UK
[b] Gluck Equine Research Center, Department of Veterinary Science, University of Kentucky, Lexington, KY 40546, USA
[c] School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington, Leicestershire LE12 5RD, UK
[d] Institute of Virology, Freie Universitat Berlin, Philippstr. 13, 10115 Berlin, Germany
[e] Central Veterinary Research Laboratory, P.O. Box 597, Dubai, UAE
[f] Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ, UK
[g] MRC National Institute for Medical Research, Mill Hill, London, UK
[h] Cambridge Infectious Diseases Consortium, Department of Veterinary Medicine, University of Cambridge, Madingley Road, Cambridge CB3 0ES, UK

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ABSTRACT

Equine influenza viruses are a major cause of respiratory disease in horses worldwide and undergo antigenic drift. Several outbreaks of equine influenza occurred worldwide during 2010–2012, including in vaccinated animals, highlighting the importance of surveillance and virus characterisation. Virus isolates were characterised from more than 20 outbreaks over a 3-year period, including strains from the UK, Dubai, Germany and the USA. The haemagglutinin-1 (HA1) sequence of all isolates was determined and compared with OIE-recommended vaccine strains. Viruses from Florida clades 1 and 2 showed continued divergence from each other compared with 2009 isolates. The antigenic inter-relationships among viruses were determined using a haemagglutination-inhibition (HI) assay with ferret antisera and visualised using antigenic cartography. All European isolates belonged to Florida clade 2, all those from the USA belonged to Florida clade 1. Two subpopulations of clade 2 viruses were isolated, with either substitution A144V or I179V. Isolates from Dubai, obtained from horses shipped from Uruguay, belonged to Florida clade 1 and were similar to viruses isolated in the USA the previous year. The neuraminidase (NA) sequence of representative strains from 2007 and 2009 to 2012 was also determined and compared with that of earlier isolates dating back to 1963. Multiple changes were observed at the amino acid level and clear distinctions could be made between viruses belonging to Florida clade 1 and clade 2.

* Corresponding author. Centre for Preventive Medicine, Animal Health Trust, Lanwades Park, Kentford, Newmarket, Suffolk CB8 7UU, UK. Tel.: +44 1638 750659x1262; fax: +44 1638 555659.
E-mail address: debra.elton@aht.org.uk (D.M. Elton).

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1. Introduction

Equine influenza virus (EIV) is a major cause of respiratory disease in horses and spreads rapidly between naïve animals. Although rarely fatal in otherwise healthy horses, EIV can cause severe disruption to the racing and breeding industries. It can also cause more severe clinical signs in animals with concurrent disease, such as hyperadrenocorticism, or in those under physiological stress.

Influenza A viruses are subtyped according to their surface glycoproteins haemagglutinin (HA) and neuraminidase (NA). HA mediates virus entry, by binding to sialic acid receptors on the host cell surface and mediating fusion of viral and host membranes (Skehel and Wiley, 2000). NA is involved in virus release from infected cells by cleaving sialic acid, it may also play a role in virus entry by allowing the virus to penetrate the mucus layer of the respiratory tract (Seto and Rott, 1966; Matrosovich et al., 2004).

Two subtypes of influenza are known to have infected horses, H3N8 and H7N7. Equine H7N7 was first isolated in 1956, equine H3N8 emerged in 1963 and spread globally over the following two years. Between 1963 and the late 1970s both subtypes co-circulated in horses and reassortment occurred between them, indicating the occurrence of mixed infections (Ito et al., 1999). There have been isolated reports of seroconversions to H7N7 in unvaccinated animals, however virus of this subtype has not been isolated since 1979 and has been considered extinct for 20 years (Madić et al., 1996; Webster, 1993). During the 1980s the H3N8 subtype diverged into 2 sub-lineages, Eurasian and American (Daly et al., 1996). The American lineage has since been divided further into the Kentucky, South American and Florida sublineages (Lai et al., 2001). More recently, the Florida sub-lineage has diverged into two clades, based on HA sequence and antigenic differences (Bryant et al., 2009; Lewis et al., 2011). Between 2006 and 2009, Florida clade 2 was seen predominantly in Europe with occasional isolation of clade 1 strains in the UK and Ireland; in North America, recent isolates have all belonged to clade 1 (Gagnon et al., 2007; Damiani et al., 2008; Bryant et al., 2011; Gildea et al., 2012).

Both clades have caused large outbreaks of equine influenza in the last 10 years. Examples include the UK in 2003 (clade 2), Japan and Australia in 2007 (clade 1), India in 2009 (clade 2), Mongolia and China from 2008 to 2009 (clade 2) and most recently in several countries in South America during 2012 (clade 1) (Newton et al., 2006; Callinan, 2008; Ito et al., 2008; Yamanaka et al., 2008; Virmani et al., 2010; OIE-WAHID Interface).

Vaccination is an effective method of control for equine influenza, providing protection by the induction of antibodies to viral surface glycoproteins, particularly HA. The role of antibodies to NA is unclear for EIV, however antibodies to human influenza NA are thought to contribute to protective immunity and neutralising titres correlate with reduced virus shedding in small animal models (Murphy et al., 1972; Brett and Johansson, 2005). Like other influenza viruses, EIV undergoes antigenic drift and is able to evade antibody responses to divergent strains (Yates and Mumford, 2000). Vaccine strains for equine influenza therefore need to be updated regularly and a formal process of vaccine strain selection is in place, overseen by the World Organisation for Animal Health (OIE). Genetic, antigenic and epidemiological data are considered prior to recommending changes to vaccine strains; current OIE recommendations are to include a representative of both Florida clade 1 and clade 2 viruses. To date, genetic and antigenic characterisation for selection of vaccine strains has focussed solely on the HA glycoprotein and antigenic drift in NA has been largely ignored.

Our aim was to improve the monitoring of field strains of EIV. Here we describe the establishment of a surveillance programme for EIV in the UK, to encourage the submission of equine nasal swab samples. We present the haemagglutinin-1 (HA1) sequences and antigenic characterisation of recent field strains from the UK, Dubai, Germany and USA and compare them with current OIE vaccine strain recommendations. We show that the Florida clade 1 and clade 2 viruses have diverged further since the OIE recommendation to include both in commercial vaccines. We also show that multiple changes have occurred within the NA gene segment of equine influenza H3N8 viruses since 1963.

2. Methods

2.1. Sentinel practice scheme

An invitation letter was sent to 60 veterinary practices with equine practitioners within the UK. Further practices were recruited to the scheme by invitation following submission of samples to the diagnostic laboratories at the Animal Health Trust (AHT). Participants were offered free diagnostic testing for samples from equids with suspected influenza, either nasal swabs or paired serum samples. A telephone helpline and dedicated website (www.equiflunet.org.uk) were also made available. Sampling packs were sent to each contributing veterinary practice, including submission forms, virus transport medium and swabs. Newsletters were also distributed to keep participants informed of relevant information. All positive diagnoses were followed up by telephone contact to collect epidemiological data, including vaccination histories of affected animals.

2.2. Diagnostic testing for presence of EIV

Nasopharyngeal swabs were taken from horses showing signs of acute respiratory disease, or close contacts of affected animals. Swabs were placed in sterile tubes containing 5 mL virus transport medium (PBS containing 200 U/mL streptomycin, 150 U/mL penicillin, 5 μg/mL fungizone (Gibco) and 600 μg/mL tryptone phosphate broth). All equine nasopharyngeal swabs sent to the AHT from 2010 to 2011 were then assayed by an in house nucleoprotein (NP)-ELISA as described previously (Cook et al., 1988). Briefly, plates were coated with rabbit polyclonal serum against A/equine/Sussex/89 (H3N8), nasal swab extract was added to the plates and incubated for up to one hour. After washing, bound influenza antigen was detected by incubation with a monoclonal antibody to EIV nucleoprotein followed by anti-mouse peroxidase