Case report of equine influenza (EI) in Italy, in 2014

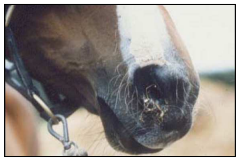
Gian Luca Autorino, Antonella Cersini, Raffaele Frontoso, Giuseppe Manna, Francesca Rosone, Maria Teresa Scicluna
National Reference Centre for Equine Diseases (CERME)

Istituto Zooprofillatico Sperimentale delle Regioni Lazio e Toscana "M. Aleandri", Via Appia Nuova 1411, Rome, Italy

Introduction In the last decade, outbreaks of equine influenza virus (EIV), in the European horse population, were due to the circulation of the H3N8 virus, American lineage and, more recently, apparently only clade 2 of the sublineage Florida. Although EIV is endemic in Italy, diagnostic requests in the course of clinical suspects of this infection are sporadic. Last Italian isolations of EIV were in 2005, with strains (Rome and Bari) presenting a high identity to A/eq/SouthAfrica/4/2003 (successively included in the sublineage Florida, clade 1). Like other influenza viruses, EIV undergoes antigenic shift/drift and is able to evade antibody response to divergent strains. For this, monitoring of circulating EIV is essential for the regular verification of the efficacy of the available vaccines.

Materials and Methods

• In January 2014, following the onset of respiratory distress at one of the stables in a racetrack in Rome, an investigation was conducted by the CERME. The episode was characterised by fever and dry coughing and involved eleven flu vaccinated foals introduced at the end of 2013;



• Preliminary tests were conducted on the nasal swabs of these animals, using a panel of Real Time (RT) PCRs for the detection of the most frequently occurring equine respiratory viruses: EIV, Equine Herpes Virus 1 and 4, Equine arteritis virus, Equine rhinitis A virus, Equine rhinitis B virus;

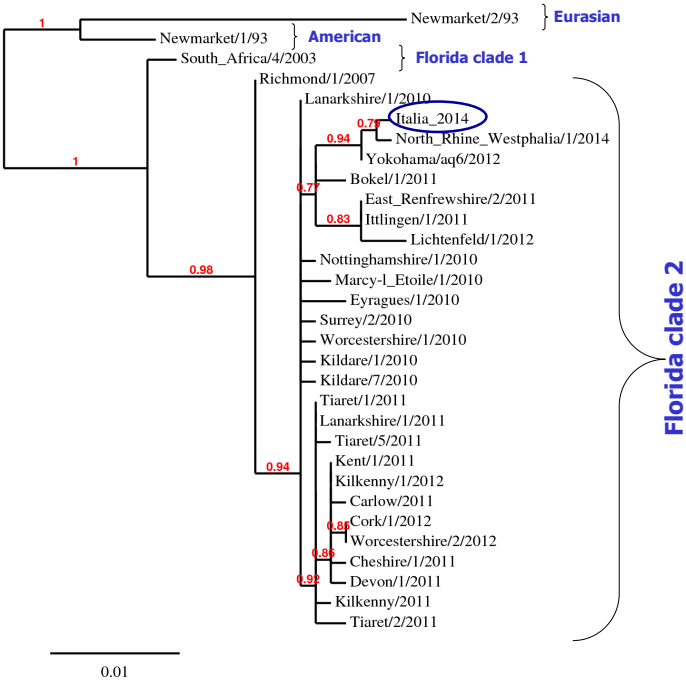
• The screening for EIV was carried out by PCR, targeting a region of the protein M gene of influenza virus type A (1);

• The PCR positive swabs were inoculated in chicken embryos eggs for viral amplification;

• Reverse transcription RT-PCR was performed as described by Woodward (2), on RNA extracted from the allantoic liquid or directly from the nasal swabs, employing a specific pair of primers for the haemagglutinin (HA1) region of segment IV of the viral genome;

• Analysis of H3N8 nucleotide sequences was conducted and a maximum likelihood tree was obtained using PhyML version 3, initially including 190 nucleotide sequences.The final phylogenetic tree was constructed with 31 nucleotide sequences representative of the latest EI outbreaks closest the strain isolated from the outbreak and with prototype strains of H3N8.

Figure 1: Phylogenetic tree (Bootstrap values, obtained after 100 replicates, are shown at major nodes). Phylogenetic groups are shown on the right and are labelled as appropriate.



Results

• Four of the 11 foals were found EIV positive in the screening RT-PCR and virus was isolated from only 2;

•The amplicon of 1080 bp showed a 99% identity with strain "North Rhine Westphalia" (GenBank acc.num. KJ538149), isolated at around the same time in Germany and the strain "Yokohama" (GenBank acc.num. AB761396.1), isolated in Japan, in 2012, both pertaining to clade 2;

• No sequence differences were observed within the amplified region of the HA1 gene, between the samples directly extracted from the swabs and those from the allantoic fluid of infected eggs;

• The strains isolated in the present outbreak (Italia 2014) showed 7 amino acid changes of HA1 when compared to clade 2 prototype strain A/eq/Richmond/1/2007 but no substitution when compared with strain North Rhine Westphalia 2014" (fig. 1).

Table 1: Vaccine strain composition and OIE recommendations

vaccine name	vaccine strains included	conform to OIE recommandations		
		1995	2004	2010
Duvaxyn IE	European H3N8 - Suffolk/89 American H3N8 - Newmarket/1/93 H7N7 - Prague/56	**yes**	no	no
Equip FT	European H3N8 - Borlänge/91 American H3N8 - Kentucky/98 H7N7 - Newmarket/77	**yes**	no	no
Equilis Prequenza	European H3N8 - Newmarket/2/93 Florida Clade 1 H3N8 – South Africa/4/0	no	**yes**	no
Proteqflu	European H3N8 - Newmarket/2/93 Florida Clade I H3N8 - Ohio/03	no	**yes**	no

Discussion and conclusions

• The strain Italia 2014 is the first evidence of circulation in Italy of clade 2 of sublineage Florida, American lineage H3N8.

• The present case study confirms what was reported in 2013, by the Panel of experts of the OIE, for the surveillance of EI and vaccine composition, relative to the exclusive circulation of clade 2 in Europe, in 2013.

• Owing to the young age of the animals involved in the episode, it was not possible to establish if vaccine inefficacy was due to the limited number of interventions or the vaccine virus composition.

• Starting from 2004, of the vaccines commercially available, only the composition of some has been updated with the recommended strains: A/eq/SouthAfrica/2003 and A/eq/Ohio/2003, both belonging to clade 1 but none with the presently circulating strains of clade 2, for which protection still needs to be verified (Table 1) (3).

• Surveillance for EIV is highly recommended especially for verifying the appropriateness of the vaccine virus composition.

• Our findings are in support of the updated OIE vaccine recommendations for 2014, of including both Florida clades 1 and 2.

• Further investigations are in progress to verify the antigenic significance of the 7 amino acid changes of HA1 of the current circulating strains to the clade 2 prototype strain A/eq/Richmond/1/2007.

Acknowledgements
The authors would like to thank Dr. Armando Damiani, Expert of the OIE Reference Laboratory for the scientific support

References

1. Spackman E. et al. (2002) - Development of a real-time reverse transcriptase PCR assay for type A influenza virus and the avian H5 and H7 hemagglutinin subtypes J.Clin. Microbiol. 40: 3256-60;
2. Woodward AL. et al., (2014) – 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. Vet. Mic. 169 113 –127
3. Adapted from http://www.equiflunet.org.uk/.