associated with their comparatively higher codon biasness, low CAI, low mutation pressure and very less adaption of tRNA pool of equine cells. Hence, the study increased our understanding of factors involved in viral adaptation, evolution, and fitness towards their hosts.

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Molecular evolution of H3N8 equine influenza virus in Argentina

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Since 1963, Equine influenza virus (EIV) H3N8 subtype has been responsible for numerous outbreaks of respiratory disease in horses worldwide. The aim of this work was to study the molecular characteristics of H3N8 EIV detected in Argentina, estimate their diversification and infer the potential source of infections. Nucleotide and deduced amino acid (aa) sequences for the complete HA gene of the EIV detected in Argentina between 1985 and 2012 were analyzed along with sequences obtained from the Influenza Research database. Maximum Likelihood (ML) and Bayesian phylogenetic trees were built up. Bayesian coalescent analyses were carried out to estimate the time of the most recent common ancestors (tMRCA) and the dynamic population growth. The phylogenetic trees demonstrate that H3N8 EIV detected in Argentina grouped in 4 well-supported monophyletic clades: Group VIII (G.VIII), South American clade 1 (SA1) and 2 (SA2), and Florida clade 1 (FC1). The viruses circulating in Argentina in 1985 (n=2), together with a Chilean strain, formed G.VIII (tMRCA~1984). The EIVs detected during the period 1993-1996 (n=5) grouped in the SA1 (tMRCA~1992), composed only by Argentinian strains, and their most probable origin was a Kentucky 1992 strain. The strains circulating in the period 1997-2005 (n=8) grouped in the SA2 (tMRCA~1997), formed a monophyletic group together with a 2006 Chilean strain, and were closely related to California 1997 and Kentucky 1994 strains, feasible ancestors of this group. The viruses detected in 2012 (n=6) grouped within the FC1 (tMRCA~2011) and formed a cluster with the viruses detected in Florida in 2011. The demographic reconstruction showed a remarkable increase in viral diversity in 2006, a slight decrease between 2007 and 2009, and an abrupt decrease since 2009. Compared with A/eq/Fontainebleau/79 (pre-divergent prototype strain), Argentinean strains belonging to G.VIII have 9 aa substitutions: T46I, A93T, R140K, T182S, G222W, V223I, I267V, I187B and L199S, these last two situated at the antigenic site B. In comparison with A/eq/Newmarket/1/93 (American lineage reference strain), the SA 1 Argentinean viruses carried 4 aa substitutions: N132K, Q189N, Q190E and E193K, the last 3 at the antigenic site B, and the SA2 strains showed only one aa substitution at the antigenic site D (I214V). The possible ancestors for SA1 and SA2 also possess the same aa substitutions at the antigenic sites B and D, respectively. EIV Argentinean strains detected in 2012 belonging to FC1 contained 5 aa substitutions in comparison with A/eq/Ohio/01/03 (FC1 reference strain): G7D, R62K, D104N, A138S and V223I. In addition, two different subpopulations can be recognized among them, one with a substitution in K-14A and the other one in K-14T and M70V, this last (M70V) being present only in Argentinean strains. Except for K-14A and M70V, the strain considered to be the origin of the group (A/eq/Florida/146609/11) has the same aa substitutions. The obtained results allow us to hypothesize that the outbreaks of equine influenza in Argentina were due to 4 introductions of virus, presumably from North America. The rapid increase in the relative genetic diversity in 2006 could be due to the co-circulation of different lineages, while the abrupt decline observed after 2009 could be related to a subsequent reduction in transmission of EIV H3N8 connected to the incorporation of Florida Clade 2 strains in vaccines.

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The contribution of specific haemagglutinin mutations to equine influenza vaccine breakdown

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In 1979, influenza caused a major epidemic amongst horses across Europe including Newmarket, UK. Subsequently, vaccines were produced using multiple outbreak strains including A/equine/Newmarket/79 and A/equine/Fontainebleau/1/79 (both subtype H3N8) which prevented further UK outbreaks until 1989 when a new antigenic drift variant emerged. The A/equine/Sussex/89 (H3N8) strain came from one of the affected regions in the UK where both unvaccinated and, notably, vaccinated horses were affected. The accumulation of mutations within important antigenic epitopes of the virus surface glycoprotein haemagglutinin (HA) can lead to a decrease in the efficiency of antibody recognition. To study this phenomenon in relation to the outbreak, three mutations, previously shown to have a pronounced effect on recognition by ferret sera, have been incorporated into equine influenza pseudotyped lentiviruses (PVs). PVs provide a flexible platform for virological mutagenesis studies and antibody screening assays. The PVs were generated via co-transfection of HEK293T/17 cells with four plasmids expressing the equine influenza HA surface glycoproteins, HIV gag-pol, firefly luciferase reporter gene and TMPRSS2 endoprotease (to cleave the HA which is necessary for viral infectivity). The resulting viruses were harvested and pseudotype virus antibody neutralization assays (PVNAs) against Newmarket/79-specific sera (to mimic the pre- and post-outbreak situation) were performed. Neutralizing antibodies provide protection and the PVNA is a sensitive technique to measure such protective antibody responses compared to other traditional assays (HI and SRH) that measure antibody inhibited surface glycoprotein binding. The PVNA highlighted specific single amino acid mutations in the putative major epitope sites that altered the ability of the sera to neutralize the PVs, indicating their importance in vaccine protection.

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Assessment of antigenic difference of equine influenza virus by challenge study in horses

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Vaccination with inactivated vaccines is the primary means of control of equine influenza which is caused by the infection with equine influenza viruses of the H3N8 subtype. Many outbreaks have been reported among vaccinated horses primarily due to the antigenic mismatch between the vaccine strains and the field strains. We previously reported that the horse antiserum raised to