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Genomic analysis on an equine influenza virus strain from a Brazilian outbreak in 2012

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BACKGROUND

The equine influenza virus (EIV) is responsible for one of the most important respiratory diseases in horses, mules and donkeys.

OBJECTIVES

Analyze the genome of an equine influenza virus from a Brazilian outbreak, 2012.

MATERIALS AND METHODS

Three H3N8 virus strains (A/equine/Sao Paulo/1.19/2012, A/equine/Sao Paulo/16.19/2012 and A/equine/Sao Paulo/24.19/2012) were isolated from horses with respiratory disease in the State of São Paulo during the Brazilian outbreak in 2012 [1]. Viral RNA was extracted from the allantoic fluid and cDNA synthesized [2]. Amplification and sequencing [3] were applied to the HA gene of the three strains, while the eight genes of A/equine/Sao Paulo/1.19/2012 were sequenced and analyzed. Specific primers were applied [4] for amplification and sequencing. Polymerase (PA, PB1 and PB2) internal primers were designed for sequencing so as to allow complete codon analysis. The confidence levels of all the sequences were evaluated using the FinchTV™ software. The eight genes from different EIVs available in the GenBank and EpiFlu (GISAID) databases were compared with the Sao Paulo/1.19/2012 strain. The nt neighbor-joining trees were constructed using Mega 5.0.5 [5], by means of the Maximum Composite Likelihood substitution model and Bootstrap method for phylogeny test, with 1,000 repetitions [6].

RESULTS

The nucleotide sequences were deposited in GenBank (accession numbers: KJ955628, KM032366 - KM032369 and KM190933 - KM190935, KT460192 and KT460193) . All eight genes clustered in the Florida Clade 1 sublineage. (Fig 1).

DISCUSSION

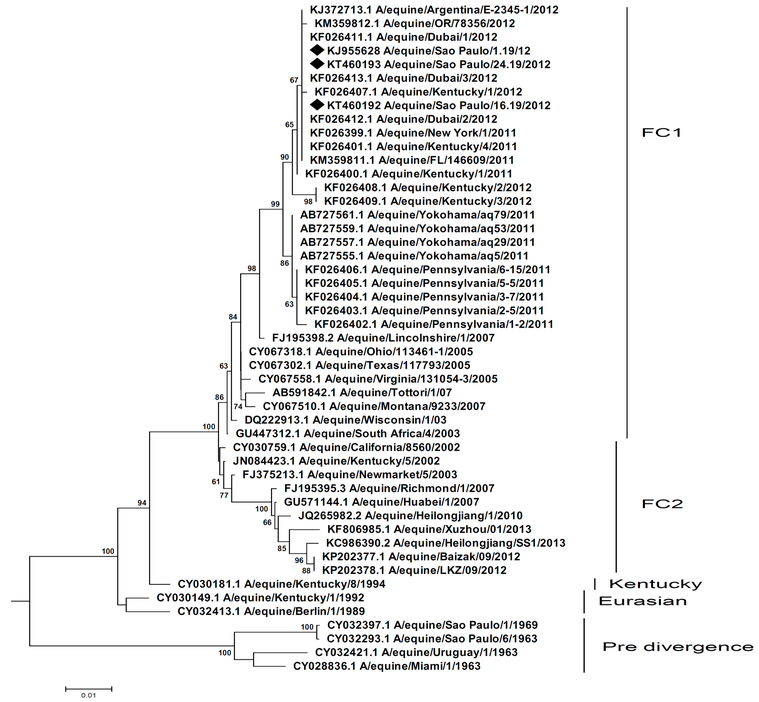
The hemagglutinin was most identical to the Rio Grande do Sul/2012, Dubai/2012, Argentina/2012 and Uruguay/2012 strains and to some North American/2011-2012 strains

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Fig. 1 - Neighbor-joining nucleotide tree, maximum composite likelihood model, with 1000 bootstrap repetitions of 49 HA1 subunit sequences of H3N8 EIVs. FC1 - Florida Clade 1, FC2 - Florida Clade 2, American, Eurasian and Pre-divergence. The viruses characterized in this study are indicated by diamonds (◆). The analysis was based on the nucleotides HA1 (1– 987).



CONCLUSION

This study provides genomic information about EIV Brazilian strains from State of São Paulo.

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REFERENCES

1. Villalobos, E.M.C., et al. Isolation, sequencing and phylogenetic analysis of equine influenza virus causing the 2012 outbreak in São Paulo, Brazil. In: 24th Congresso Brasileiro de Virologia, 2013.
2. Favaro, P. F. et al. Evolution of equine influenza viruses (H3N8) during a Brazilian outbreak, 2015. Brazilian Journal of Microbiology, v. 16, p. 31368, 2017.
3. Gildea, S., et al. The molecular epidemiology of equine influenza in Ireland from 2007–2010 and its international significance. *Equine Vet. J.* 44, 4, 1-6, 2012.
4. Hoffmann, E., et al. Universal primer set for the full-length amplification of all influenza A viruses. *Ach. Virol.* 146, 12, 2275-2289, 2001.
5. Tamura, K., et al. Mega 5: molecular molecular evolutionary genetics analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony methods. *Mol. Biol. Evol.* 28, 10, 2731-2739, 2011.
6. Felsenstein, J., 1984: Phylogenies and the comparative method. *Am. Nat*., 125, 1-15.