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DETECTION OF A HORSE-DERIVED H3N8 INFLUENZA VIRUS IN PIGS IN BRAZIL

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Introduction

Swine influenza virus (SIV) is a commonly isolated pathogen of the porcine respiratory disease system. SIV causes an acute, infectious, respiratory disease with the occurrence of outbreaks in non immune herds. The infection has worldwide distribution in pig producing countries and is caused by various SIV subtypes with genetic makeup from multiple origins, including avian and human viruses (1). Although the disease is well characterized in many countries, little is still known in Brazil of the infection of pig herds with SIV.

The objective of the present work was to characterize a novel H3N8 SIV strain isolated from pig lungs in Brazil, in 2008.

Materials and methods

A total of 70 lung tissue samples from finishing pigs showing pulmonary lesions resembling SIV infection were collected at slaughter, from 2 herds located in Toledo city, Parana State, southern Brazil. Viral isolation was carried out in SPF embryonated chicken eggs. Viral detection was done by RT-PCR and sequencing. Primers for sequencing the M and NA genes were retrieved from Chan et al. (2). Primers for the HA gene were provided by the Avian Influenza Reference Laboratory, NVSL, APHIS, USDA. The sequencing reactions employed BigDye Terminator chemistry and the products were run on an Applied Biosystems 3130xl Genetic analyzer. Consensus sequence was generated using the SeqScape v2.5 software (Applied Biosystems). NCBI BLAST analysis was conducted to identify related reference viruses available in GenBank. A phylogenetic tree of HA gene segment was constructed using the neighbor-joining method in the MEGA 5.01 software based on nucleotide sequences.

Results

Five out of seventy (7.1%) samples were positive for influenza A by RT-PCR. Two samples were isolated in chicken eggs, confirmed by the HA test and the amplification of the influenza M gene by RT-PCR (3). Partial CDS of HA (727bp), M (938bp) and NA (960bp) were subjected to Blast analysis. The Brazilian SIV isolates were closely related (Table 1) to an American H3N8 equine influenza virus (EIV). Phylogenetic analysis (Mega 5.01 software, Neighbor joining, number differences model with 500 bootstraps) of the HA gene (nt918-1604) of the H3N8 SIV isolates with GenBank sequences from equine, avian and swine viruses from the Americas and Eurasia indicated that the Brazilian swine H3N8 virus grouped with a prototype H3N8 EIV, A/eq/Miami/1/1963/H3N8 (CY028836) and the only other available H3N8 sequences of equine viruses from Brazil (A/eq/SP/6/1963, CY032293; A/eq/SP/1/1969, CY032397) and Uruguay (A/eq/Uruguay/1/1963, M24718). The Brazilian SIV isolates did not clustered with avian or avian-derived H3N8 EIV, neither with H3N2 SIVs. The only other reported horse-derived H3N8 SIV isolated from pigs in China (4) is more closely related with later European H3N8 EIV from the 1990s.

Table 1. Blast analysis of Brazilian H3N8 isolates

Gene	Ident. (%)	E - value	Virus designation	Access No
HA	98.49	0.0	A/eq/Miami/1/1963	CY028836
M	100.0	0.0	A/eq/Miami/1/1963	CY028837
NA	100.0	0.0	A/equ/Swit./2225/1979	CY033491

Discussion

The present study showed the first isolation of a horse-derived H3N8 influenza virus in pigs in Brazil. Phylogenetic analyses indicated that the HA gene of the Brazilian H3N8 SIV isolates were more similar to the old American H3N8 EIV than to European H3N8 EIV. In Brazil, serologic studies in the last years detected antibodies against influenza subtype H3N8 in horses. However, the only described case of influenza infection of horses was from two outbreaks of EIV, in Sao Paulo in 1963 and in Sao Paulo and Rio de Janeiro in 1969 (5). Further virological studies in pig populations must to be performed in order to verify if the present case is a sporadic isolation or if H3N8 influenza viruses are established in pigs. Sequencing analysis of all eight viral segments from the Brazilian H3N8 SIV should provide a better understanding of possible epidemiology and origins of these viruses.

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