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PROCEEDINGS



### Genetic characterization of influenza viruses isolated from pigs in Brazil in 2009-2011

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#### Introduction

Influenza A virus (IAV) infections are endemic diseases in pork producing countries around the world. As a result of an investigation of IAV infection in commercial herds in Southern Brazil, the first report of pandemic H1N1 (H1N1pdm) influenza virus in pigs was described (3). Since then, outbreaks of mild to moderate respiratory disease have been frequently observed in pig herds. The objective of this work was the molecular characterization of influenza viruses isolated from pigs during 2009-2011.

#### Materials and Methods

646 samples of nasal swab or lung tissue collected from pigs of various ages and raised in commercial herds in Southern Brazil were analyzed. Initially, biological samples were screened for influenza virus M gene by reverse transcription-PCR (2). Positive RT-PCR samples were submitted to virus isolation in SPF embryonated chicken eggs or in MDCK cells. Viral RNA was extracted from allantoic fluid or cell culture supernatant, reverse-transcribed and the nucleotide sequencing of influenza gene segments were performed. The sequencing reactions employed BigDye Terminator chemistry and the products were run on an Applied Biosystems 3130xl Genetic analyzer. Influenza virus gene sequences were assembled with SeqScape v2.5 software (Applied Biosystems). Phylogenetic analyses were performed using the Neighbor-Joining method in the MEGA 5.01 software based on nucleotide sequences.

#### Results

A total of 111 (17.18%) samples were positive to influenza A by RT-PCR and 46 (41.44%) viruses were isolated. Complete and partial HA, NA, M, NP, PB1, PB2 and PA gene segments of 25 influenza viruses were obtained. Based on the sequence analyses of HA, NA, M and PB1 genes, 16 influenza viruses showed a high identity (98-100%) with H1N1pdm influenza virus that has been circulated in humans and in pigs since 2009. Five influenza viruses were closely related to an American H3N8 equine influenza virus (EIV) and four virus isolates, based on the sequence analyses of HA, NA, M, NP, PB1, PB2 and PA genes, revealed to be a novel H1N2 influenza virus that had not been detected in pigs in Brazil before the recent influenza outbreak in pigs. Moreover, the sequence analyses of this new influenza virus revealed that it is a reassortant virus with the glycoprotein coding-genes (H1 and N2) of human origin

and the internal genes (M, NP, PB1, PB2 and PA) derived from H1N1pdm influenza virus.

#### Conclusions and Discussion

Although previous serologic studies have indicated the circulation of influenza viruses in pig herds in Brazil (1), outbreaks of IAV infection in pigs was not observed before 2009. A recent study emphasizes that antibodies against H1N1pdm were not detected in pig's sera before 2009 (4). Moreover, after the onset of H1N1pdm in pigs, outbreaks of IAV infection have been frequently observed in growing-finishing pigs. The emergence of a reassortant H1N2 influenza virus in 2011 brings concern whether this novel influenza virus subtype is going to be established in pig herds, and contributing to the emergence of new viruses.

This work emphasizes the importance of influenza surveillance in pigs in order to increase the very limited amount of information on the prevalence and evolution of influenza viruses in Brazilian pig populations.

#### References

1. Brentano et al.: 2002, Comunicado Técnico, Embrapa Suínos e Aves, Concórdia, p.1-6.
2. Fouchier et al.: 2000, J Clin Microbiol 38: 4096.
3. Schaefer et al.: 2011, Pesq Vet Bras 31 (9): 761.
4. Zanella et al.: 2011, Proc. 6<sup>th</sup> Intern Emerg Re-emerg Viral Dis: 261.

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