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ISOLATION AND CHARACTERIZATION OF PANDEMIC H1N1 INFLUENZA VIRUSES FROM PIGS IN BRAZIL

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Introduction

Since the beginning of the influenza pandemic in humans, in April 2009, there were concerns about the possibility of the spillover of this novel influenza virus to swine populations worldwide. Several experimental studies demonstrated the susceptibility of pigs to the pandemic A/H1N1, providing a fast and efficient spread among pigs (1). Consequently, 2 years after the emergence of A/H1N1 in humans, 22 countries have notified to the OIE the presence of Pandemic A/H1N1 in pig herds. In Brazil, until recently, influenza infection in pigs was not considered a problem. Nevertheless, serologic studies conducted in recent years indicated the presence of antibodies against classical SIV H1N1 and H3N2 subtypes in pig herds in 10 Brazilian states (2, 3). However, a recent outbreak of respiratory disease in pigs in 2010, have indicated a possible circulation of a novel influenza virus in pigs.

Herein we describe an outbreak of SIV in pigs caused by the Pandemic influenza virus (A/H1N1/2009), in a pig farm maintained by Embrapa Swine and Poultry Research Center, during sampling of pigs as a part of an ongoing research project.

Materials and methods

On 30 January, 2010, a farm consisting of a 175-sow farrowing- nursery operation with 754 animals that ranged from newborn piglets to nursery pigs showed signs of respiratory disease consistent with SIV infection. Nearly 29% of the pigs were affected (5 sows and 213 nursery pigs) showing clinical signs of fever, cough and loss of appetite, which lasted about 10 days. No clinical signs were observed in piglets and no animal had died. Nasal swabs and lung tissue were collected from twelve infected pigs. Viral isolation was carried out in SPF embryonated chicken eggs and in MDCK cells. Lung tissues were processed for histopathologic examination and for immunohistochemical (IHC) analysis. Viral detection was done by IHC, immunocytochemistry test (ICC), RT-PCR and sequencing. The coding region of Hemagglutinin (HA) gene of influenza virus was amplified in a one-step RT-PCR (Qiagen) using a primer set for the pandemic H1N1/ HA gene (WHO, CDC, Atlanta). Primers for sequencing the M and NA genes were retrieved from Chan et al. (4). 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 references available in GenBank.

Results

All tested samples were positive for influenza A by RT-PCR. One sample was isolated in cells, confirmed by immunocytochemistry test, using as primary antibody an anti-influenza virus nucleoprotein (5) and by the amplification of the M gene by RT-PCR (6). Histopathologic lesions in lungs were characterized by necrotizing bronchiolitis with mild to moderate interstitial pneumonia. IHC analysis was positive for influenza A. The complete CDS of HA (1769bp) and partial M (897bp) and NA (603bp) genes were constructed with Sequence Scape software. Blast analysis showed 99% nucleotide identity of the HA, M and NA genes with the pandemic influenza virus (A/H1N1/2009) that have been circulating in humans.

Table 1. Blast analysis of Brazilian H1N1 isolate

Gene	Ident(%)	E - value	Virus designation	Access No.
HA	99	0.0	(A/Guandg/55/2009/H1N1)	HQ011423
M	99	0.0	(A/Kenya/0026/2009 H1N1)	HQ214452
NA	99	0.0	A/Guang/45/2009/H1N1)	HQ011420

Discussion

The present study described the first isolation of the Pandemic H1N1 influenza virus in Brazilian pigs. Even though previous serologic studies have indicated the circulation of SIV in Brazilian pigs, little is known about the genetic composition of SIV isolates. In Brazil, pig herds are not vaccinated against SIV, nor is there monitoring of SIV in this specie. Blast analysis showed that the Brazilian H1N1 SIV is closely related with pandemic influenza A (H1N1) viruses that have been circulating in humans.

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