IPVS 2012 KOREA

22nd International Pig Veterinary Society Congress

June 10 (Sunday) - June 13 (Wednesday), 2012
Jeju, Korea

Happy Pigs - Healthy People

PROCEEDINGS

VP-059

| Virology & Viral Diseases-INFLUENZA |

First detection and caracterization of pandemic H1N1 influenza virus and association with *Mycoplasma hyopneumoniae* in captive wild boar in Brazil

N Biondo[1], D Gava[2], NL Simon[2], CS Rocha[3], S Silveira[4], MF Schiochet[2], MAZ Mores[2], R Schaefer[2], JR Ciacci-Zanella[2], DESN Barcellos[1]

[1]*Veterinary Medicine School, Federal University of Rio Grande do Sul/UFRGS, Porto Alegre, Brazil,* [2]*Embrapa Swine And Poultry Research Center, Animal Health Laboratory, Concórdia, Brazil*

[3]*College of Biological Sciences, University of Contestado, Concordia -SC, Brazil,*

[4]*DTI, CNPq, Brasilia, Brazz.* janice@cnpsa.embrapa.br

Introduction

Swine influenza is an acute respiratory disease caused by Influenza A virus (IAV). Clinical signs include cough and sneezing as well as fever and reduced feed efficiency (9). The main lesions are pulmonary consolidation that might present interlobular edema, and microscopic lesions are characterized by bronchointersticial pneumonia (5, 8). In Brazil, the circulating subtypes in swine population are cH1N1, H3N2 and pH1N1 (10). In wild boars the subtypes H1N1 and H3N2 have been detected (6), but there are no reports of IAV infection in Brazilian wildlife, especially wild boars. The aim of this study was to investigate the involvement of IAV in pneumonia cases and its association with *M. hyopneumoniae* (Mhp) in captive wild boars.

Materials and Methods

Captive wild boars raised under two farms of semi-intensive production systems were sampled in this work. Sixty lungs with pneumonia-like gross lesions were collected in a slaughterhouse, and the lung lesion scoring was calculated (8).

Molecular assay: Viral RNA was extracted using MagMAX kit (Ambion). The RT-PCR was performed using specific primers for influenza matrix gene (4). The positive samples were tested by quantitative real time PCR (qPCR) for cH1N1 and pH1N1 subtypes (7). The RT-PCR products were gel-purified using BigDye XTerminator Purification Kit (Qiagen) and nucleotide sequences were determined using an ABI3130xI Genetic Analyzer. The consensus sequences were generated using v2.5 SeqScape (Applied Biosystems) and analyzed using BLAST (NCBI).

Viral Isolation (VI): The VI was performed in 9 days old SPF embrionated chicken eggs (ECE), by chorio-alantoic inoculation. After incubation, the chorio-alantoic fluid was collected and tested by haemagluttination test (HA).

Histopathology (HE) and Immunohistochemistry (IHC): All tissues were fixed in formalin, processed by HE and evaluated by IHC to IAV (11) and Mhp detection (2).

Results

The median pneumonia-like gross lesions score of analyzed samples was 28.6%. Diaphragmatic lobes consolidations were observed in 13 out of 60 (21.6%) lungs. Eleven out of 60 lungs (18.3%) were positive for IAV by RT-PCR. All 11 samples were IAV qPCR positive, with viral copies ranging from 4.58 to 6275 copies/uL. Seven out of 11 were pH1N1 qPCR positive, with viral copies ranging from 4.65 to 3863 copies/uL. Three samples were sequenced and showed gene M identity of 98 and 99% with pandemic A/H1N1/2009 influenza virus that has been circulating in humans. None of the samples had viral titers after inoculation in ECE. Microscopic lesions were characterized by bronchointersticial pneumonia and inflammatory exudates with predominance of mononuclear cells. Marked hyperplasia of bronchus associated lymphoid tissue (BALT) was also observed. The IHC was negative for IAV and positive in all 11 samples for Mhp.

Discussion and Conclusion

This is the first report of pH1N1 influenza virus infection in captive wild boars in Brazil. The pH1N1 virus was detected in pigs for the first time in Brazil in 2010 (10). The fact that intact viral particles have not been detected by isolation or antigen by IHC can be explained by the short course of the disease (6). The animals excrete large amount of virus during the acute phase (5-7 days post infection), which decreases over time. The antigen presence in bronchi occurs in greater quantities before the development of lesions and can be detected between 48-72 hours after infection (5). The association of IAV and Mhp is reported in domestic pigs (1, 3). Initial infection with Mhp before IAV inoculation increased influenza clinical signs and pathogenesis due to the H1N1 virus but did not modify significantly outcomes of H1N2 infection (1). These findings show that pH1N1 and Mhp circulate in Brazilian captive wild boars.

References

1. Deblanc C et al.: 2012, Vet Microbiol epub and print.
2. Ecco R et al.: 2009, Pesq Vet Bras 29: 461-468.
3. Fablet C et al.: 2012, Vet Microbiol epub and print.
4. Fouchier et al: 2000, J Clin Microbiol 38, 4096-4101.
5. Janke BH.: 2000, J S Healht Prod 8: 79-83.
6. Kaden VA et al.: 2008, Vet Microbiol 131: 123-132.
7. Lorusso et al. 2010. J Virol Methods 164(1-2): 83-87.
8. Madec F & Kolbisch M.: 1982, J R Porc 14: 405-412.
9. Olsen CW et al.: 2006, Disease of Swine 469-482.
10. Schaefer R et al.: 2011, Pesq Vet Bras 31: 761-767.
11. Vincent L et al.: 1997, J Vet Diag Invest 9: 191-195.

Acknowledgement

This work was funded by Embrapa Swine and Poultry, CNPq (578102/2008-0) and UFRGS.