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Genomic analysis of influenza a virus from captive wild boars in Brazil reveals a human-like H1N2 influenza virus

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Introduction

Influenza is an acute respiratory disease that affects human and several animal species worldwide (3). In Brazil, H1N1, H3N2 and 2009 pandemic H1N1 (A(H1N1)pdm09) influenza A viruses (IAVs) circulate in domestic swine herds(4, 5). Whereas wild boars are susceptible to IAV infection, the close contact with humans andother animal species brings concern about the infection of captive wild boars with IAVs. Here we describe the histopathological, virological and genomic analyses of lungs from captive wild boars presenting lung consolidation, suggestive of IAV infection.

Materials and Methods

Lung samples from 60 captive wild boars presenting gross lesions of consolidation were collected at slaughter.The sampleswere screened by RT-PCR (1) andquantitative real-time PCR (qRRT-PCR) (2)for IAV detection.Additionally, virus isolation (VI)was performed inembryonated chicken eggs and the chorioallantoic fluids were tested by the hemagglutination test.One virus sample (GenBankaccession nº: KF572613–KF572620)wasfully sequenced using Illumina'sgenome analyzer platform (MiSeq). The obtained sequences were assembled using the Newbler Assembler V.2.9 andphylogenetic analysis was performed using the Neighbor-Joining method in the MEGA 5.2 software (6). For pathological analysis, lung samplespositive to IAV by qRRT-PCR were fixed in formalin and processed by HE and immunohistochemistry (IHC) (7).

Results

Eleven out of 60 lungs (18.3%) were positive to IAV by RT-PCR and seven out of eleven were also positive for A(H1N1)pdm09 by qRRT-PCR, with viral load ranging from 4.65 to 3863copies/uL. No IAV was isolated on ECE, eitherembryo death was observed.Chronic diffuse bronchopneumonia was observed in all samples and IHC analysis was negative for influenza A antigen. The genomic analyses revealed that the HA and NA genes clustered with IAVs of the human lineage and the six internal genes were derived from the H1N1pdm09 IAV. The analysis of the H1 gene showed that it grouped with H1-δ cluster and is closely related to seasonal human influenza viruses from 2002-2003. The N2 gene grouped to seasonally human H3N2 viruses from the late 1990s. The six remaining internal genes (PB2, PB1, PA, NP, M and NS) were almost identical (99-100%) to A(H1N1)pdm09 viruses.This is a novel reassortant H1N2 IAV carrying genes derived from human H1N2, H3N2 and A(H1N1)pdm09 influenza viruses.

Conclusions and Discussion

This is the first report of a H1N2 influenza virus infection in captive wild boars in Brazil.Although IAV infection is endemic in commercial pig herds,outbreaks of clinical swine influenza were only reported after the introduction of A(H1N1)pdm09 influenza virus in pigs (4, 5). Moreover, there is little information about IAV infection in captive wild boars and about the genetic composition of IAVs. The human-like H1 influenza virus was first detected in pigs in Canada in 2004 and it has been recognized as the dominant genotype in the U.S (3).Added to this, theviruses belonging to the δ-cluster were shown to be paired either with an N1 or an N2 gene of human lineage (3). The histological lesions observed suggest that the IAV infection occurred earlier and it could explain the negative results in IHC and VI. In summary, a reassortant human-like H1N2IAV circulates in captive wild boar populations in Southern Brazil. Prevention and control of the transmission of IAVs, among captive wild boars, should be considered to minimize their impact in both swine production system and public health.

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