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DIVERSITY OF INFLUENZA A VIRUSES IN SWINE IN BRAZIL

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Introduction

Influenza A viruses (IAVs) circulating in swine present important economic concerns for the swine industry and a pandemic threat for humans. Since 2009, transmission of pandemic H1N1 (H1N1pdm) virus from humans to pigs has been documented in numerous countries (1, 2, 3, 4), including Latin American countries, as Argentina, Brazil, Colombia and Mexico, owing to human-to-swine transmission events that have occurred since then (5, 6).

Although Brazil hosts one of the largest swine populations in the world, there has been little evidence prior to 2009 of IAV circulation in Brazilian swine herds. Following the detection of H1N1pdm in pigs in Brazil in 2009 (7, 8), surveillance efforts increased.

Here, a phylogenetic analysis of newly sequenced influenza viruses from Brazil's swine herds provides evidence of multiple influenza A viruses of human seasonal virus origin circulating in swine.

Materials and methods

Sampling: A total of 1881 nasal swabs and 89 lung tissue samples were collected from swine from 131 pig farms located in seven Brazilian states during 2009-2012. Fifty-nine nasal swabs (3.13%) and 58 lung samples (65.16%) were positive to IAV by RT-qPCR. Virus isolation was performed into MDCK cells or into SPF embryonated chicken eggs and 41 IAVs were obtained.

Sequencing: Whole-genome sequencing was performed using an ABI 3130xl and Illumina MiSeq. The obtained sequences were assembled using Phread/Phrap softwares (<http://www.phrap.org>). In total, sequence data was obtained for 35 IAVs. However, only 16 IAVs whole-genome sequence data had sufficient quality to be included in the analysis.

Phylogenetic analysis: Five H1N2, four H3N2 and seven H1N1pdm IAVs were analyzed. Nucleotide alignments were generated for five data sets: 'H1s' (human seasonal virus-like), 'H1p' (pandemic virus-like), H3, N1p (pandemic virus-like) and N2, including other related human and swine viruses, collected globally, as background.

Divergence times: A time-scaled Bayesian approach was employed to estimate the timing of the human-to-swine transmission events associated with the H1s, H3, and N2 segments occurred.

Results

The four H3N2 viruses from Brazilian swine are monophyletic (100% bootstrap) and closely related to

human seasonal viruses from the late 1990s. The five H1N2 viruses are also monophyletic (64% bootstrap) on the H1 tree, and are closely related to seasonal H1N2 viruses that circulated in humans during 2001-2003.

Five Brazilian swine viruses of the H1N2 and H3N2 subtypes belong to one N2 clade (97% bootstrap) and two H1N2 belong to a second N2 clade (100% bootstrap). Both clades are closely related to human seasonal H3N2 viruses from the late 1990s.

Therefore, Brazilian swine viruses of the H1N2 subtype that contain H1 segments related to human seasonal H1N2 viruses have acquired a different N2 of human H3N2 origin via two different reassortment events.

The seven viruses of human pandemic H1N1 origin also were not monophyletic on the H1 or N1 tree, indicating that these viruses are the result of multiple separate human-to-swine introductions of the H1N1pdm virus, rather than clonal expansion of a single introduced lineage.

Discussion

Here, a phylogenetic analysis of newly sequenced influenza viruses from Brazil's swine herds provides evidence that multiple influenza A viruses of human seasonal virus origin have been circulating in swine for more than a decade. These H3N2 and H1N2 swine viruses have not been detected in any other countries to date. The co-circulation of multiple genetically diverse influenza virus lineages of the H1N1, H1N2, and H3N2 subtypes introduces new challenges for the control of influenza in Brazil's swine herds, including design of cross-protective vaccines.

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