Genetic and antigenic diversity of contemporary influenza A virus in swine in Brazil

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Introduction

Respiratory infections caused by influenza A virus (IAV) became frequent in Brazilian swine herds following the introduction of H1N1pdm in 2009. IAV is commonly detected in swine showing mild to severe acute respiratory disease, as the only pathogen, or in association with other viral or bacterial agents (1). Previous investigation revealed substantial genetic diversity of IAVs detected in pigs. H3N2 and H1N2 swine IAV from Brazil were characterized to be most closely related to human seasonal influenza viruses that circulated during the late 1990s and early 2000s. Viral diversity increased after reassortment with co-circulating H1N1pdm09 virus internal genes (2). The objective of the present study was to continue influenza monitoring in pigs to characterize contemporary genetic and antigenic evolution of this important respiratory pathogen.

Materials and Methods

Nasal swabs and lung samples collected from commercial swine in herds located in seven Brazilian states were sent to a veterinary diagnostic laboratory for screening of respiratory agents involved in the porcine respiratory disease complex. IAV positive samples by RT-PCR were submitted for virus isolation in SPF chicken eggs and/or MDCK cells, and genetic sequencing. RNA was extracted from pig samples and the eight gene segments were amplified by RT-PCR using PathAmp FluA reagents. DNA libraries were prepared and submitted for sequencing using Ion Torrent system. Influenza genomes were assembled using Newbler v.2.9.

H1 and H3 hemagglutinin (HA) gene alignments were generated for these sequences alongside a random sample of global human and swine IAVs downloaded from the Influenza Research Database. For each alignment, a maximum likelihood phylogeny was inferred, and statistically supported clades of viruses that demonstrated onward transmission in Brazil were identified. An HA1 amino acid consensus was determined for each clade, and representative strains were identified for antigenic characterization. The representative H1 and H3 IAV were tested by hemagglutination inhibition (HI) using a panel of swine sera against global swine and human seasonal IAV, with newly generated monovalent swine anti-sera against Brazilian swine IAV (3).

Results

From 2009-18, a total of 1010 samples were collected from nursery pigs from farms located in southern, midwestern, and southeastern Brazil. Four hundred and twenty-three (423) samples were positive for IAV by RT-PCR. Ninety-nine samples were isolated and submitted for sequencing, 41 H1N1, 30 H1N2, and 28 H3N2. 38 H1N1 genes were related to the 2009 pandemic (H1N1pdm09) but were derived from at least 9 human-to-swine transmission events from 2009 to present, and included a clade (n=23) of viruses that demonstrated sustained transmission in Brazilian swine. 24 H1N2 and 3 H1N1 viruses were detected and these fell within three distinct human-to-swine transmission events prior to 2009, and were similar to human seasonal H1 viruses circulating between 2000 and 2005. The 28 H3N2 viruses comprised three statistically supported clades, all derived from a single human-to-swine transmission event in the mid-1990s with subsequent genetic evolution. Of the 27 H1N2 strains that we were able to whole-genome sequence, six H1N2 viruses had seven gene segments derived from H1N1pdm, and one segment (NA gene) derived from human-seasonal H3N2 virus. The remaining IAV genomes sequenced so far had H1N1pdm internal genes.

Antigenic maps were generated from the HI data. Antigenic distances (1 AU equals a 2-fold loss in HI titer) demonstrated significant variability among IAV within each clade, and at least 4 AU distance from putative human-seasonal precursor viruses to representative circulating swine strains.

Conclusions and Discussion

These data demonstrated the role and importance of human-to-swine transmission in the evolution and diversity of swine IAV in Brazil. Five co-circulating clades of viruses were identified within three subtypes. Antigenic characterization of representative isolates suggested that Brazilian swine IAV are regionally unique and swine vaccines may have limited efficacy. The swine IAV also demonstrated antigenic divergence from human seasonal strains and therefore may also pose a zoonotic risk.

Acknowledgments

Embrapa (project 22.16.05.004.00).

References

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