



# IPVS2022

26<sup>th</sup> international pig veterinary society congress - rio de janeiro - brazil



June  
21<sup>st</sup>-24<sup>th</sup>

## Proceedings IPVS2022

RIO DE JANEIRO/RJ, BRAZIL

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## Human-to-swine spillover and onward transmission of H1N1pdm09 in Brazil

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### Introduction

Human-to-swine influenza A virus (IAV) spillover events occur with regular frequency (1). A subset of 2009 H1N1 pandemic viruses (pdm09) continue to be transmitted from humans to swine and evolve within susceptible pigs (2). Additionally, despite a swine-origin, the H1N1pdm09 lineage is now the predominant H1 human seasonal virus, and sustained transmission of the lineage in swine likely poses a public health risk if swine-adapted viruses with H1N1pdm09 genes have antigenically drifted from human seasonal H1 vaccines (3). The objective of the present study was to identify and characterize human-to-swine H1N1pdm09 spillovers in the Brazilian swine herd.

### Materials and Methods

Nasal swabs and lung samples were collected from pigs between 2010 and 2020 and tested for IAV/M gene by RT-qPCR (4). Positive samples were submitted for viral isolation in SPF embryonated chicken eggs or in MDCK cells, and sequencing. Seventy H1 hemagglutinin (HA) and 55 N1 neuraminidase (NA) gene sequences were generated for this study. BLASTn was used to identify the 1000 most similar HA and NA sequences to the Brazilian swine isolates. In addition, 2008-2009 human seasonal sequences, vaccine isolates, and H1N1pdm09 sequences identified as ancestral to the pandemic (5) were included in the dataset. Nucleotide alignments were generated with MUSCLE v3.8.4 software after excluding duplicate sequences. H1pdm09 and N1pdm09 final alignments included, respectively, 5,151 and 4,805 sequences.

A maximum likelihood tree was inferred following automatic nucleotide substitution model selection for each gene using IQ-TREE v1.6.1. Statistical support for the inferred tree was evaluated using the rapid bootstrap algorithm with 1,000 pseudoreplicate datasets.

To estimate the time of the most recent common ancestor (tMRCA) of unique human-to-swine spillovers (2 sequences or more), we subsampled the larger human and swine HA data using TARDiS. This algorithm generated H1pdm09 and N1pdm09 datasets to 49 and 50 sequences, respectively, optimizing genetic diversity and temporal coverage. Each new alignment was evaluated in TempEst v1.5.3 to quantify temporal signal. Sequences from each spillover clade were combined individually with the subsampled datasets. These alignments were analyzed in BEAST v1.10.4 under a relaxed lognormal molecular clock and a non-parametric Bayesian

Skyline demographic model. The MCMC chain was run for  $4 \times 10^8$  chain steps and convergence was evaluated in TRACER v1.6 after excluding an initial 10% burn-in.

### Results

Phylogenetic analyses identified multiple human-to-swine introductions of the H1N1pdm09 lineage in Brazil swine herds. However, not all these spillovers demonstrated sustained onward transmission. Our analyses suggest 8 genetic clades in which the virus maintained sustained transmission in Brazilian swine, in addition to 9 events of self-limited infections in which the virus was introduced into pigs but did not persist. The N1pdm09 results were generally concordant with the HA data and demonstrated 7 sustained spillover events and 8 events of dead-end infections.

Phylodynamic analysis indicates that the H1N1pdm09 in Brazilian pigs evolved from human seasonal viruses that were transmitted between 2007 and 2018. Most of these introductions occurred between 2007 and 2011 and only a few were detected after 2016.

### Conclusions and Discussion

Human-to-swine transmission of the H1N1pdm09 virus has been reported globally (2). The H1pdm09 and N1pdm09 gene phylogenetic analysis conducted in this study show that these segments were repeatedly introduced into swine populations in Brazil since 2007. Approximately half of these introductions resulted in self-limited transmission with consequent extinction. However, several clades showed sustained onward transmission over multiple years, suggesting that the virus was continuously transmitted between pigs. The H1N1pdm09 lineage is shared with humans, consequently, the evolution of this lineage within swine poses a potential risk to public health. Thus, determining how interspecies transmission of the H1N1pdm09 affected genetic diversity across the genome, reassortment patterns, and antigenic phenotype is a critical future research direction (6).

### Acknowledgments

Embrapa (project 22.16.05.004.02) and ARS/USDA (contract 58-5030-0-055-F).

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