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## Introductions of pre-2009 human-origin seasonal influenza A viruses in Brazilian swine herds

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### Introduction

Swine H1 viruses are classified into three major genetic lineages: 1A, 1B and 1C. The 1B lineage resulted from repeated introductions of human seasonal H1 influenza A viruses (IAVs) into swine that occurred independently in Europe and America, giving rise to 1B.1 and 1B.2 virus lineages, respectively (1). IAV subtypes H1N1, H1N2 and H3N2 are endemic in Brazilian herds. Previous characterization of swIAV H1N2 from Brazil revealed that the most closely related human seasonal IAV circulated during the early 2000s (2). Since 2009 outbreaks of respiratory infections associated with H1N1pdm IAV have become frequent in Brazilian swine (3) and viral diversity increased after reassortment with co-circulating H1N1pdm virus internal genes (2). The objective of the present study was to identify and characterize 1B swine IAV isolated from pigs in Brazil.

### Materials and Methods

Forty-nine IAVs of subtypes H1N2 and H1N1, previously isolated from swine, were selected for genetic characterization. Pig samples were collected between 2011 and 2020 from commercial farms located in six Brazilian states, distributed in three geographic regions. Viral RNAs were extracted and prepared for sequencing using the Ion Torrent platform. Nucleotide alignments were generated using a dataset of H1 hemagglutinin (HA) sequences of 1B lineage comprising: (a) Brazilian swIAVs sequenced for this study (n=49), (b) Brazilian swIAVs sequenced and published previously (n=6), and (c) related human and swine HA genes collected globally (n=3916). Alignment was generated using MAFFT v7.490 with default options followed by manual correction and curation using the program AliView v1.28. The final data set included a total of 3,971 H1 sequences collected globally from 1933 to 2020. A maximum-likelihood phylogenetic tree was inferred using IQ-TREE v2.1.3 following the automatic best-fit model selection process. Statistical support was assessed using ultrafast bootstrap (UFboot) and single branch tests (SH-aLRT) with 2000 replicates. The estimated time periods of human-to-swine transmission were inferred using TreeTime v.0.8.5.

### Results

The H1 dataset included 56 Brazilian swIAV sequences with 49 H1N2, six H1N1, one H1Nx and two human variants (H1N2v) of swine origin. Phylogenetic analysis demonstrated three strongly-supported (UFboot  $\geq$  95% and/or SH-aLRT  $\geq$  80%) clades composed exclusively

of Brazilian swine HA genes characterizing three separated introductions of the H1 segment from human seasonal influenza virus origin. New clade designations within the 1B lineage are proposed: 1B.2.3, 1B.2.4 (Lopes *et al.*, 2022, *unpublished*) and 1B.2.6 (this study). Clade 1B.2.3 included 19 sequences (12 H1N2, 5 H1N1, 1 H1Nx and 1 H1N2v) collected in southern, midwestern and southeastern Brazil during 2011 to 2020. Clade 1B.2.3 was closely related to human seasonal H1N1 viruses isolated during the 2006-07 human influenza season. The estimated time of human-to-swine transmission was 2003.6-2007.6. Clade 1B.2.4 comprised 34 H1N2 IAVs collected in southern and southeastern Brazil from 2011 to 2020 that diverged into two statistically supported clades. One clade comprised 24 sequences (including a H1N2v), and the second clade had 10 H1N2 swIAVs. Clade 1B.2.4 was closely related to a human seasonal H1N2 virus collected in the early 2000s. The estimated time of transmission of the human H1N2 virus into swine was 2001.4-2001.8. The newly identified clade 1B.2.6 contained six sequences (5 H1N2 and 1 H1N1) collected during 2019 in Minas Gerais state (Southeastern region) and was closely related to human seasonal H1N1 viruses from the late 1980s. The estimated time of human-to-swine transmission was approximately 1986.2-2007.5.

### Conclusions and Discussion

These data demonstrate the occurrence of three separate introductions of human seasonal H1 IAVs into Brazilian swine herds leading to three different co-circulating lineages of H1N1 and H1N2 swIAVs specific to Brazil. The long branch length of clade 1B.2.6 likely represents the lack of sampling of swIAVs and only allows for time of origin of the human-to-swine transmission event to be estimated broadly between 1986.2 and 2007.5. Future analysis of additional gene segments may indicate additional introductions and reassortment between human seasonal viruses and endemic swine IAV in Brazilian pigs. These results reinforce the significant contribution of human-to-swine IAV transmission to the genetic diversity of IAV in swine and reiterate the importance of surveillance in pigs.

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### References

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