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Whole-Genome Characterization of a Novel Human Influenza A(H1N2) Virus Variant, Brazil

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DOI: <http://dx.doi.org/10.3201/eid2301.161122>

We report the characterization of a novel reassortant influenza A(H1N2) virus not previously reported in humans. Recovered from a pig farm worker in southeast Brazil who had influenza-like illness, this virus is a triple reassortant containing gene segments from subtypes H1N2 (hemagglutinin), H3N2 (neuraminidase), and pandemic H1N1 (remaining genes).

Influenza A(H1N2) viruses have been described in human, avian, and especially swine populations over many years (1,2). In contrast to the widespread circulation of seasonal H1N1 and H3N2 viruses, subtype H1N2 has been observed only sporadically in humans (1,3–7). Human H1N2 infections were reported during 1988–89 from sporadic cases over the winter in China (3). In 2000, another H1N2 subtype strain emerged in the human population and became widespread in Europe, with sporadic cases reported in the Middle East, Asia, Africa, and the Americas during 2001–2003 (1,4). In Brazil, this H1N2 subtype strain was detected in humans in the southeast region during the winter of 2002 and in the northern region at the beginning of 2003 (5). This 2000–2003 H1N2 subtype strain had a genetic origin similar to the 1988–1989 H1N2 strain from China, both reassortants between human seasonal H1N1 and H3N2 subtype lineages (3,4).

In contrast, sporadic cases of zoonotic human infections with swine-origin H1N2 subtype variants (H1N2v) have also been described (6,7). In Brazil, the passive monitoring of influenza A viruses in pigs has taken place since 2009 (8). Recently, a phylogenetic study revealed that H1N2 subtype viruses have circulated undetected in swine herds in Brazil for more than a decade, and reassortments may have occurred (9). These viruses seem to be reassortants originating from an ancestor virus introduced to

pigs from humans in the late 1990s and early 2000s and remained as a relic from a now-extinct human-host hemagglutinin lineage. However, after the emergence in humans of influenza A(H1N1)pdm09 in 2009, reassortment events lead to H1N2 viruses acquiring internal genes segments from the pandemic strain (9).

Even though these H1N2 subtype strains from Brazil circulating in the swine population, they have not been detected in humans. We report detection and characterization of a variant H1N2 subtype strain (H1N2v) with a genomic origin not previously reported in humans.

This virus was identified from a nasopharyngeal aspirate collected on November 26, 2015, from a 16-year-old girl from a rural area in Castro City, Paraná, in the southern region of Brazil. Castro has ≈67,000 inhabitants and is a major livestock hub for dairy cattle, poultry, and pigs. The patient did not present any risk factors for influenza but showed development of an influenza-like illness with an onset of symptoms (fever, cough, sore throat, chest pain, and myalgia) on November 23, 2015. The follow-up local investigation reported that she had been working at a swine farm and confirmed direct patient contact with pigs. She had not received a prior influenza vaccine or antiviral treatment, and her clinical recovery was uneventful.

The virus sample was sent to the Central Laboratory of the State of Parana, where an influenza A virus strain was detected. This strain could not be further subtyped using the influenza real-time reverse transcription PCR protocol recommended by the World Health Organization.

Genomic Sanger sequencing of the strain was conducted at the National Influenza Center, IOC, FIOCRUZ, Rio de Janeiro, Brazil. BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) was performed for each gene segment sequenced and revealed strong identity with an H1N2 virus subtype genome detected in swine in Santa Catarina, a state in southern Brazil, in 2011 (Table). The human viruses with closest identity to the H1N2v virus detected in this study were a 2003 H1N2 subtype human lineage (hemagglutinin gene), a 1998 H3N2 subtype human seasonal lineage (neuraminidase gene), and A(H1N1)pdm09 subtype lineage for all other genes. This virus isolate was designated as influenza A/Parana/720/2015(H1N2v); the genome sequence is available in the GISAID database (<http://platform.gisaid.org>; submission no. EPI_ISL_223342).

We conducted phylogenetic reconstructions of each gene segment by the maximum-likelihood method using a dataset with all H1N2 subtype sequences and some representative sequences for H1N1 and H3N2 subtypes available in influenza genetic databases (online Technical Appendix, <http://wwwnc.cdc.gov/EID/article/23/1/16-1122-Techapp1.pdf>). Our analysis revealed that each gene segment of A/Parana/720/2015(H1N2v) had the same phylogenetic profile as recent swine H1N2 subtype sequences

Table. Highest degree of gene identity of the influenza A/Parana/720/2015 (H1N2)v subtype strain identified from a patient in Brazil with other swine and human strains*

Gene segment	BLAST hits†	% Identity
PB2	A/swine/Brazil/185-11-7/2011(H1N2)	98
	A/San Diego/INS194/2009(H1N1)	98
PB1	A/swine/Brazil/185-11-7/2011(H1N2)	99
	A/Singapore/GN285/2009(H1N1)	98
PA	A/swine/Brazil/185-11-7/2011(H1N2)	98
	A/Texas/67/2009(H1N1)	98
HA	A/swine/Brazil/185-11-7/2011(H1N2)	97
	A/New York/487/2003(H1N2)	95
NP	A/swine/Brazil/185-11-7/2011(H1N2)	99
	A/Tennessee/F1057c56/2010(H1N1)	98
NA	A/swine/Brazil/185-11-7/2011(H1N2)	97
	A/Malaysia/17392/1998(H3N2)	93
M	A/swine/Brazil/185-11-7/2011(H1N2)	99
	A/Mexico City/INER16/2009(H1N1)	99
NS	A/swine/Brazil/185-11-7/2011(H1N2)	98
	A/Singapore/TT454/2010(H1N1)	98

*Sequence submitted to GISAID (<http://platform.gisaid.org>; submission no. EPI_ISL_223342). HA, hemagglutinin gene; M, matrix gene; NA, neuraminidase gene; NP, nucleoprotein gene; NS, nonstructural protein gene; PB2, polymerase basic 2 gene; PB1, polymerase basic gene; PA, polymerase acid gene.

†Sequence with major identity on BLAST.

from Brazil. This supports the BLAST findings and suggests a recent swine–human infection by the H1N2v strain. Because similar swine strains have been identified in pigs ≈300 km distant from where the human case occurred (9), the virus is likely to have been circulating in pigs in Castro City. The H1N2v subtype we report contained the S31N marker in the matrix 2 protein, which confers resistance to the adamantane antiviral class, similar to A(H1N1)pdm09 viruses (10).

To date, no further H1N2 subtype human cases have been detected in Brazil; however, influenza virus strains from this region and period are under investigation to confirm whether more H1N2v subtype cases may have occurred. This report highlights the need for influenza surveillance in humans and animals, as well as in their interface, especially during influenza season when transmission is high. To ensure early detection, surveillance should focus on geographic areas when influenza A viruses subtypes co-circulate and where human–animal contact is frequent.

Acknowledgments

We thank the National Council for Scientific and Technological Development (CNPq) for financial support to conduct this research, and thank the influenza group from Department of Molecular Biology of Central Laboratory of the State of Parana, Irina Riediger, Maria do Carmo Debur, Alix Mazzetto, and Mayra Presibella, for the initial analysis and collection of epidemiological data. We gratefully acknowledge the influenza program team at the Brazilian Ministry of Health, especially Dr. Sergio Nishioka and Walquiria Almeida.

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Avian Pox in Native Captive Psittacines, Brazil, 2015

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DOI: <http://dx.doi.org/10.3201/eid2301.161133>

To investigate an outbreak of avian pox in psittacines in a conservation facility, we examined 94 birds of 10 psittacine species, including sick and healthy birds. We found psittacine pox virus in 23 of 27 sick birds and 4 of 67 healthy birds. Further characterization is needed for these isolates.

Avian pox is caused by avipoxvirus. Infections occur worldwide in domestic and wild avian species (1), are suggested to be host family- or order-specific, and are modulated by habitat and ecologic niche (2). Avipoxviruses have been described in Brazilian *Amazona* spp. and *Pionus* spp. parrots with severe diphtheritic upper digestive lesions, experimentally causing the formation of cutaneous lesions in chickens; chicken and parrot strains will not provide cross protection (3). The presumptive diagnosis, based on typical pocklike skin lesions of papular or nodular hyperplastic and hypertrophic skin foci or upper digestive diphtheritic form in severe cases (1), may be confirmed by detection of avipoxvirus DNA by PCR (4).

In June 2015, an outbreak of avian pox occurred among 10 species of native Brazilian psittacines (n = 94) maintained in a conservation facility. In addition to the typical pocklike nodular skin lesions, the psittacines had weight loss and reduced activity; 3 died. The outbreak lasted 3 months; remission of lesions occurred within ≈3 weeks in each bird.

Skin scrapings were collected from the cutaneous lesions of affected birds (online Technical Appendix Figure 1, <http://wwwnc.cdc.gov/EID/article/23/1/16-1133-Techapp1.pdf>), and conjunctiva and cloaca swabs were collected from all 94 psittacines showing cutaneous lesions (27 birds) or not (67 birds). Skin samples treated with an antibacterial–antimycotic drug mixture (Gibco; Thermo Fisher Scientific, Waltham, MA, USA) were inoculated onto the chorioallantoic membrane (CAM) of 10-day-old specific pathogen-free chicken embryos and typical pocklike CAM lesions were obtained (online Technical Appendix Figure 2). Cutaneous samples and the CAM of inoculated

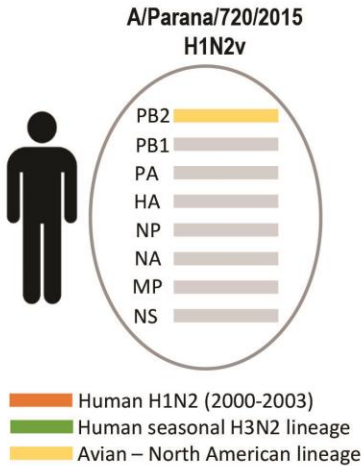
Whole-Genome Characterization of a Novel Human Influenza A(H1N2) Virus Variant, Brazil

Technical Appendix

Technical Appendix Figure (following pages). Maximum-likelihood (ML) trees of each gene segment (PB2, polymerase basic 2 gene; PB1, polymerase basic gene; PA, polymerase acid gene; HA, hemagglutinin gene; NP, nucleoprotein gene; NA, neuraminidase gene, M, matrix gene; NS, non-structural protein gene) were performed to reconstruct the phylogenetic profile of influenza A/Parana/720/2015 (H1N2)v (Figure, red). The dataset used includes H1N1 (orange) and H3N2 (blue) seasonal and H1N1pdm09 (pink) vaccine strains and all H1N2 whole genomes available in GenBank or the Global Initiative on Sharing All Influenza Data. The H1N2 subtype strains were classified as Brazilian swine H1N2 (green), worldwide swine and avian (black), human H1N2 and swine-origin H1N2v strains (purple). The Hasegawa-Kishino-Yano + gamma distribution among the sites was the best-fitting nucleotide substitution model and was used to reconstruct the trees. ML reliability of branches was evaluated using an approximate likelihood ratio test; the interior support branch cutoff value was ≥ 0.9 .

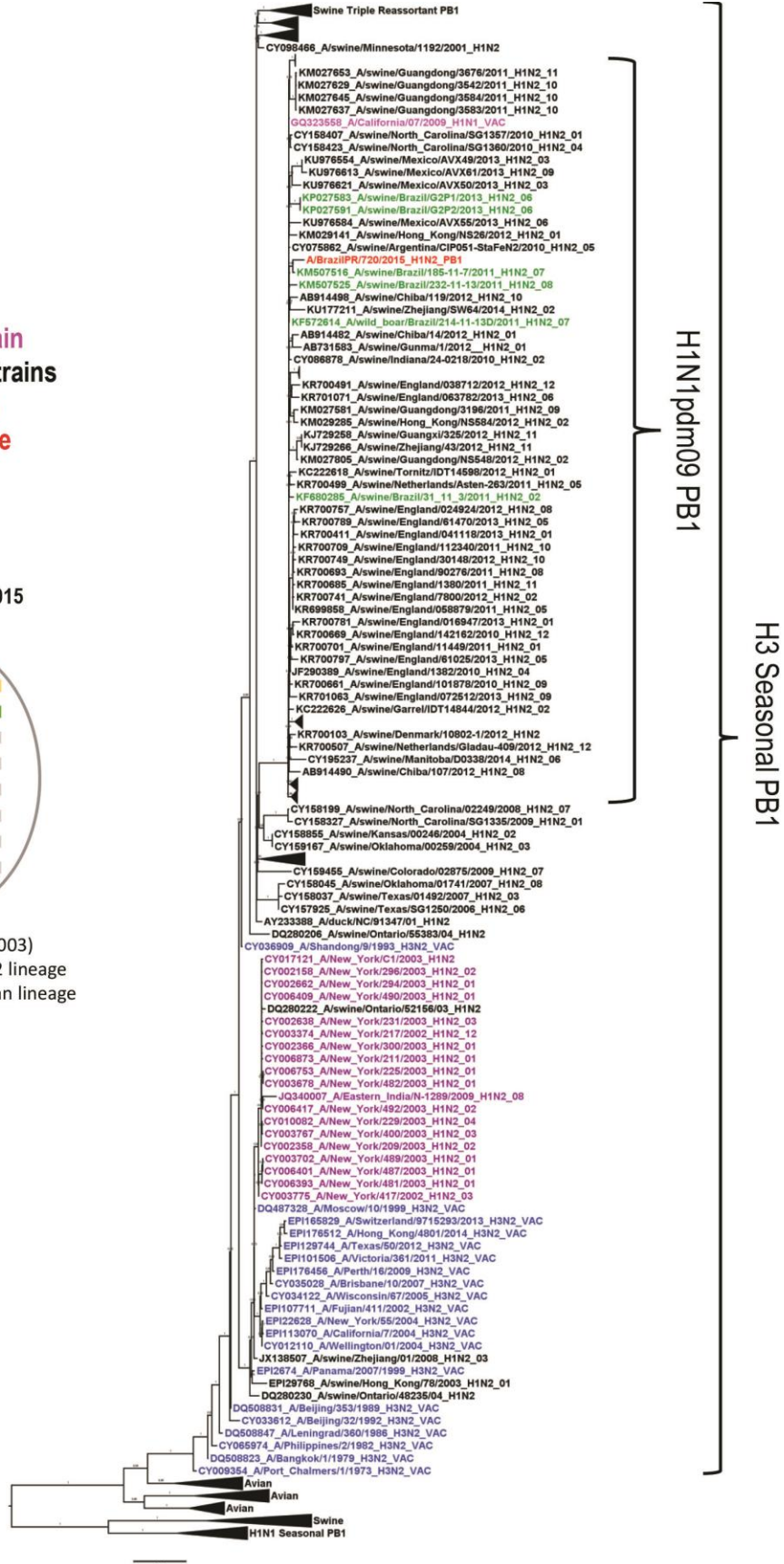
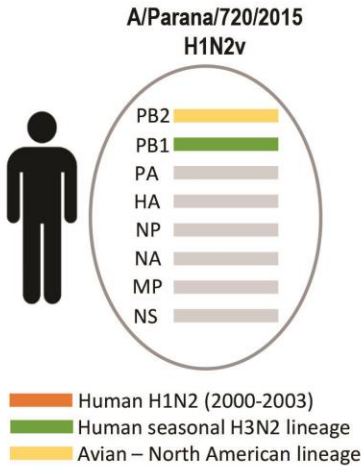
PB2

H1N1pdm09 vaccine strain
 Swine and avian H1N2 strains
 Brazil swine H1N2 cases
 Brazil human H1N2v case



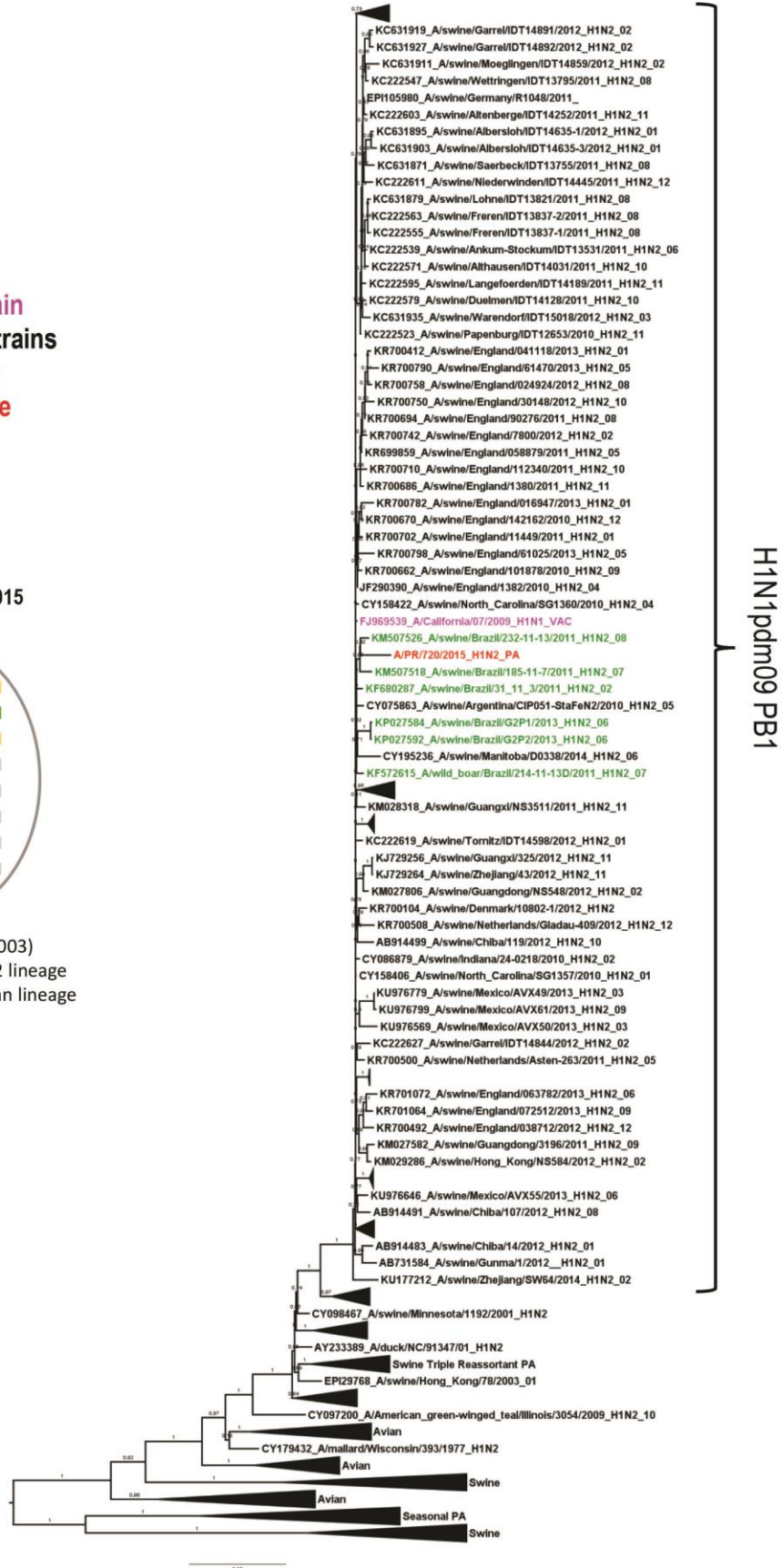
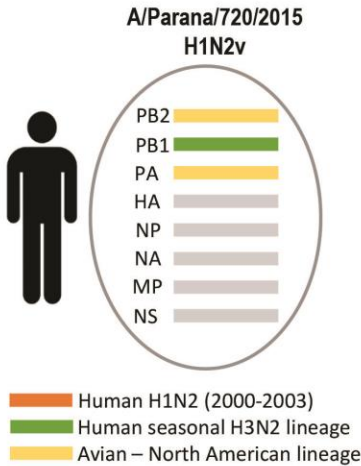
PB1

- H3N2 vaccine strains
- H1N1pdm09 vaccine strain
- Swine and avian H1N2 strains
- Brazil swine H1N2 cases
- Brazil human H1N2v case
- Human H1N2 cases



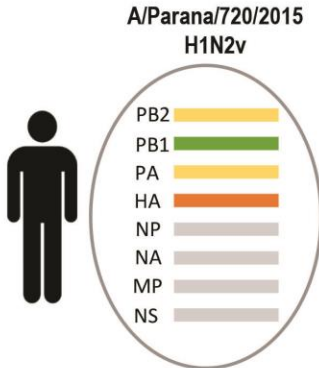
PA

H1N1pdm09 vaccine strain
 Swine and avian H1N2 strains
 Brazil swine H1N2 cases
 Brazil human H1N2v case

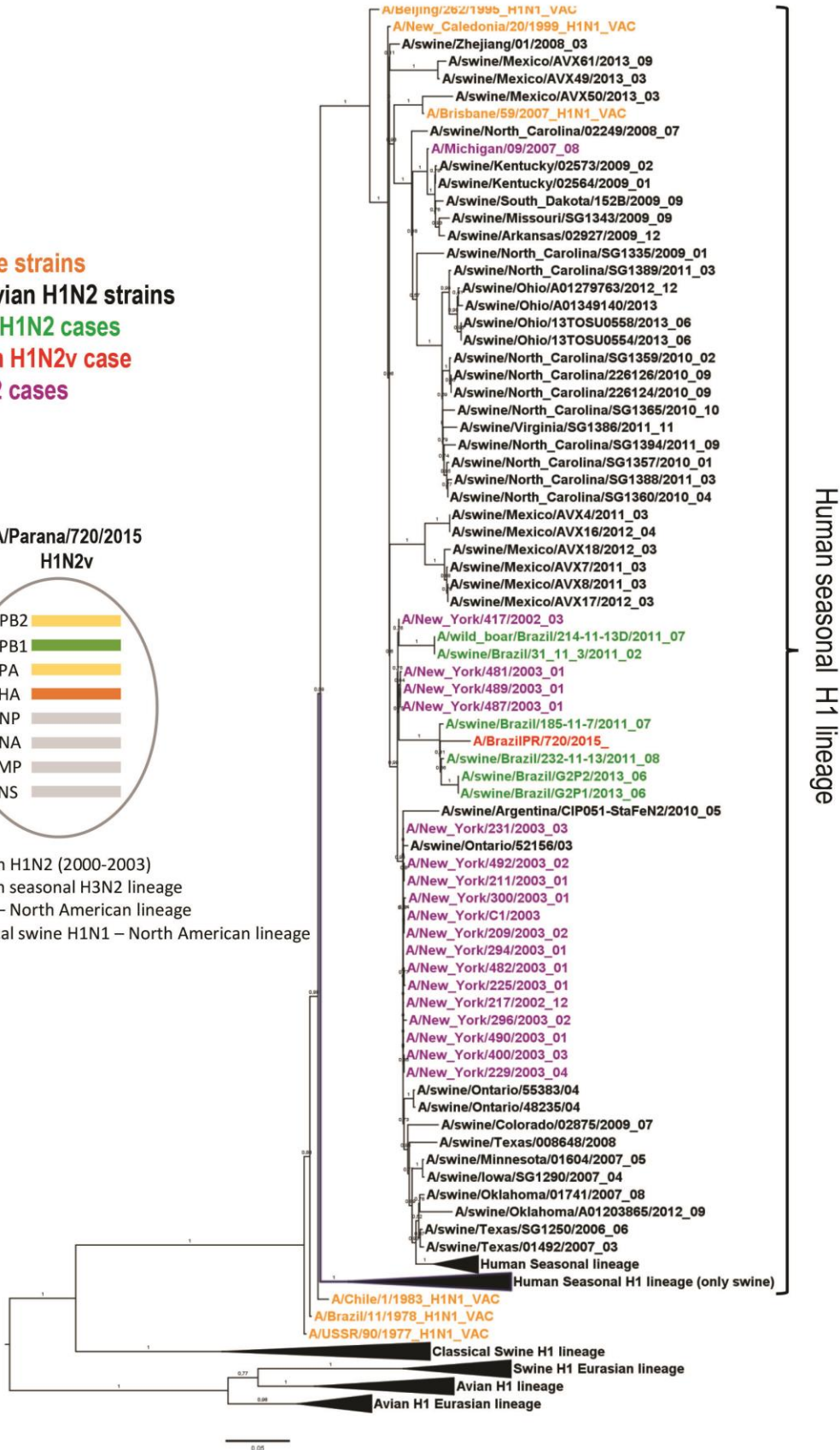


HA

H1N1 vaccine strains
 Swine and avian H1N2 strains
 Brazil swine H1N2 cases
 Brazil human H1N2v case
 Human H1N2 cases

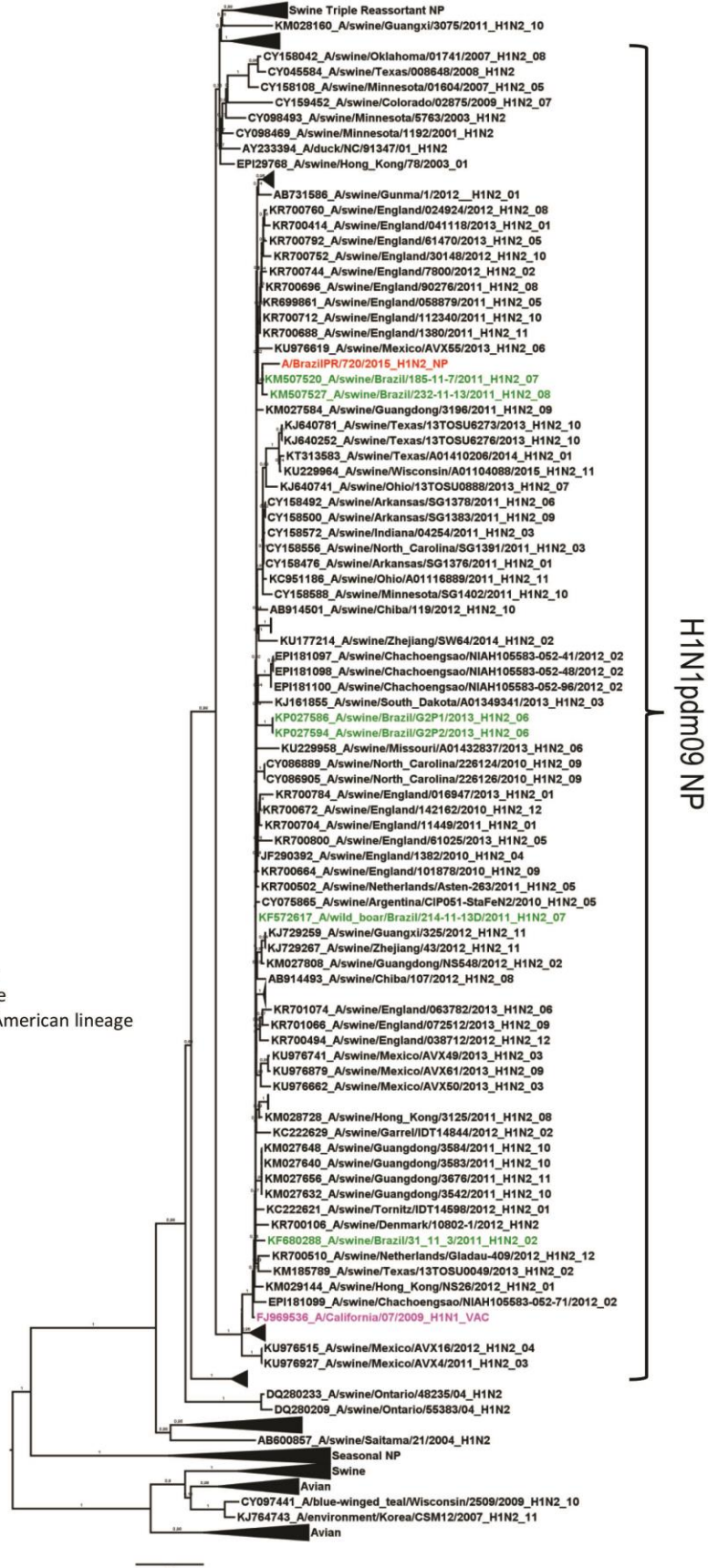
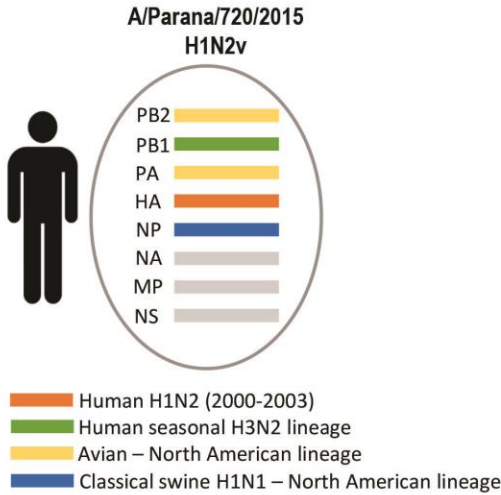


Human H1N2 (2000-2003)
 Human seasonal H3N2 lineage
 Avian – North American lineage
 Classical swine H1N1 – North American lineage



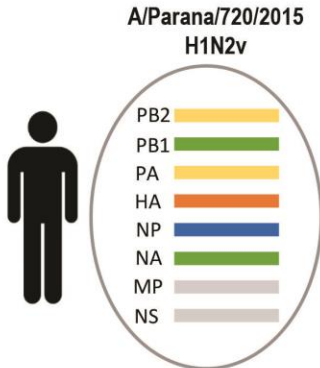
NP

H1N1pdm09 vaccine strain
 Swine and avian H1N2 strains
 Brazil swine H1N2 cases
 Brazil human H1N2v case

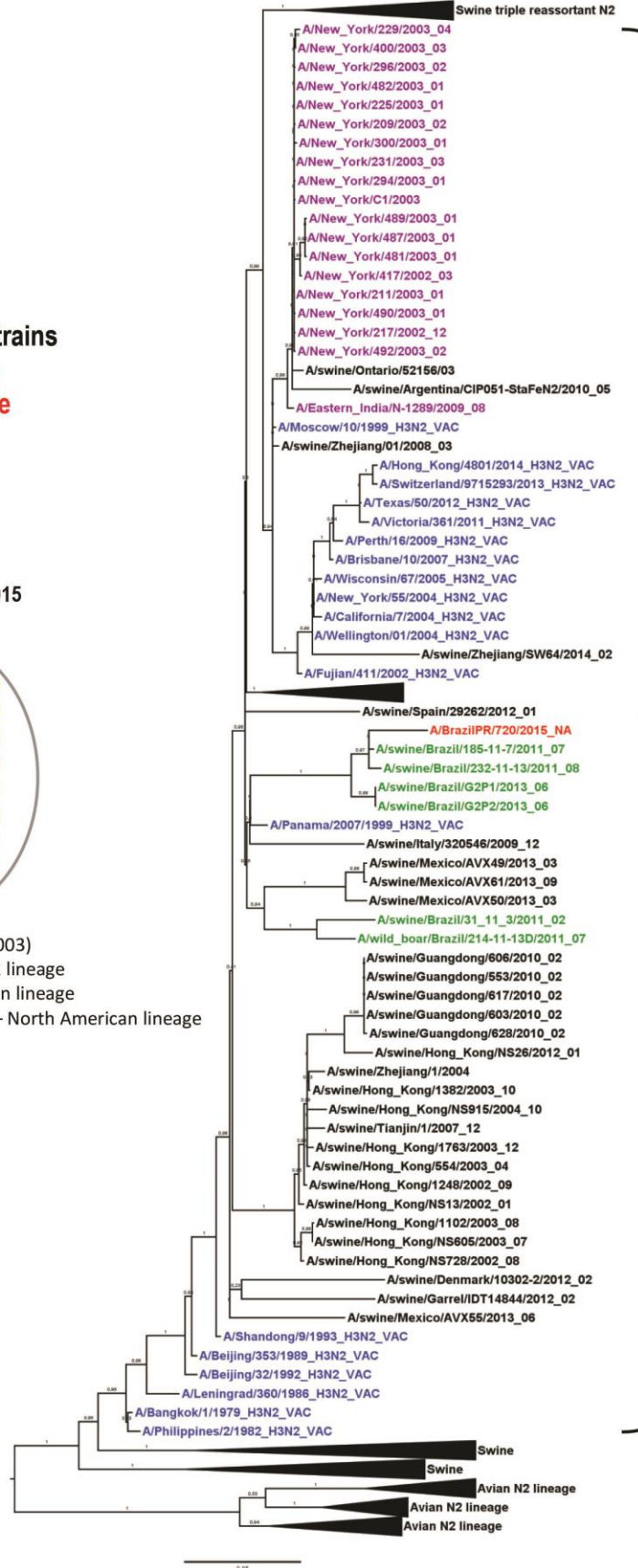


NA

H3N2 vaccine strains
 Swine and avian H1N2 strains
 Brazil swine H1N2 cases
 Brazil human H1N2v case
 Human H1N2 cases



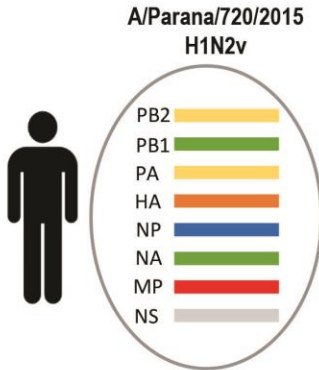
Human H1N2 (2000-2003)
 Human seasonal H3N2 lineage
 Avian – North American lineage
 Classical swine H1N1 – North American lineage



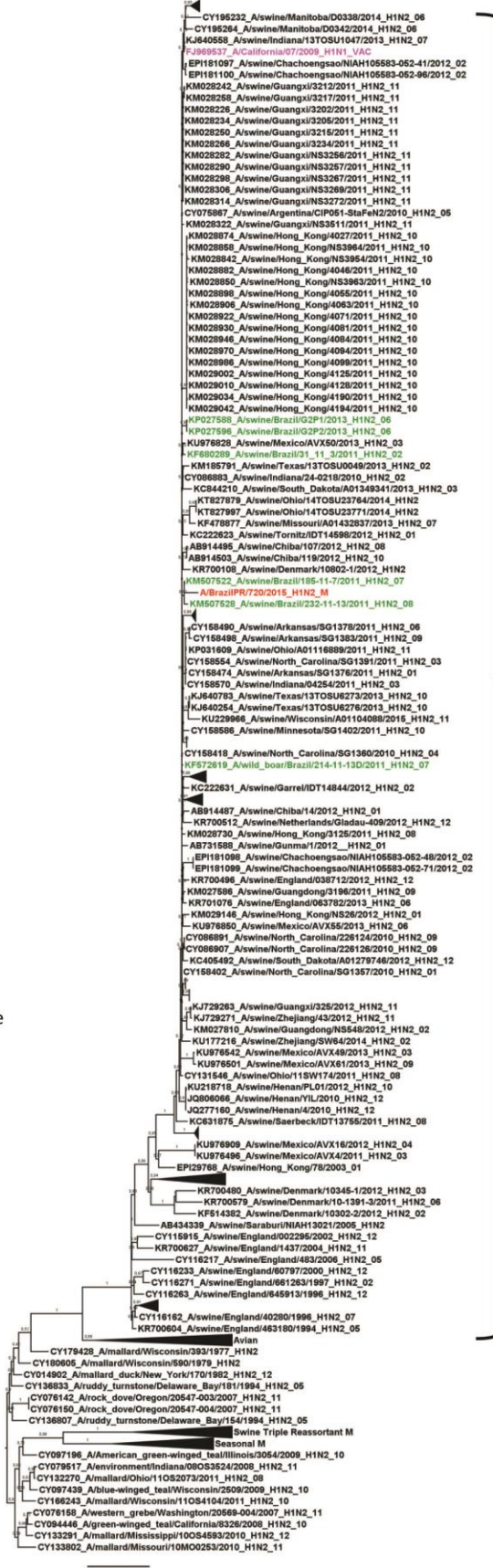
Human seasonal N2 lineage

MP

H1N1pdm09 vaccine strain
 Swine and avian H1N2 strains
 Brazil swine H1N2 cases
 Brazil human H1N2v case



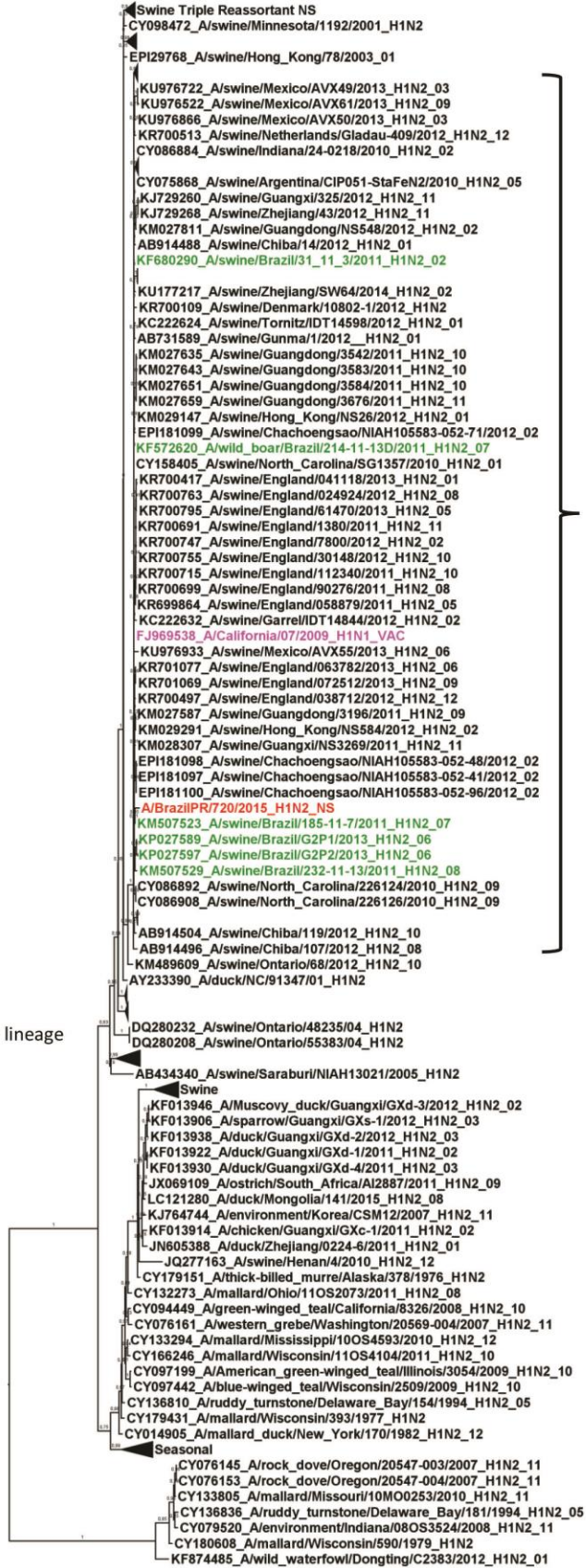
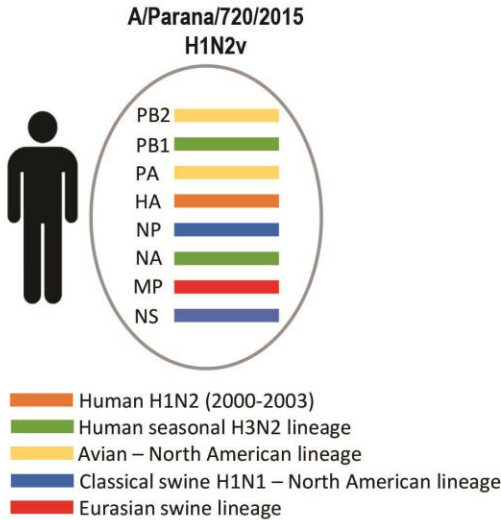
- Human H1N2 (2000-2003)
- Human seasonal H3N2 lineage
- Avian – North American lineage
- Classical swine H1N1 – North American lineage
- Eurasian swine lineage



H1N1pdm09 M

NS

H1N1pdm09 vaccine strain
 Swine and avian H1N2 strains
 Brazil swine H1N2 cases
 Brazil human H1N2v case



H1N1pdm09 M