Dr. Sendi is an attending physician and lecturer in infectious diseases at Bern University Hospital and University of Bern, Switzerland. His research interests are group B Streptococcus in nonpregnant adults and infections of the locomotor apparatus.

References

address correspondence: Barbara Spellerberg, Institute of Medical Microbiology and Hygiene, University of Ulm, Albert-Einstein-Allee 11, 89081 Ulm, Germany; email: barbara.spellerberg@uniklinik-ulm.de

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

Paola Cristina Resende, Priscila Silva Born, Aline Rocha Matos, Fernando Couto Motta, Braulia Costa Caetano, Maria do Carmo Debur, Irina Nastassja Riediger, David Brown, Marilda M. Siqueira

Author affiliations: Oswaldo Cruz Institute, Rio de Janeiro, Brazil (P.C. Resende, P.S. Born, A.R. Matos, F.C. Motta, B.C. Caetano, D. Brown, M.M. Siqueira); Central Laboratory of the State of Parana, Curitiba, Brazil (M.C. Debur, I.N. Riediger)

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We report the characterization of a novel reassortant influenza A(H1N2) virus not previously reported in humans. Recovered from a 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

Address for correspondence: Barbara Spellerberg, Institute of Medical Microbiology and Hygiene, University of Ulm, Albert-Einstein-Allee 11, 89081 Ulm, Germany; email: barbara.spellerberg@uniklinik-ulm.de

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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).

Table. Highest degree of gene identity of the influenza A/Parana/720/2015 (H1N2v) subtype strain identified from a patient in Brazil with other swine and human strains*

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

* 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.

Acknowledgments
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Dr. Resende is a postdoctoral researcher at the Laboratory of Respiratory Viruses at Oswaldo Cruz Institute, FIOCRUZ, Rio de Janeiro, Brazil, National Influenza Center for the World Health Organization. Her research interests are reconstructing the phylogenetic profile of influenza viruses and on identifying genomic viral polymorphisms associated with an increase in virulence and antiviral drug resistance.

References

Avian Pox in Native Captive Psittacines, Brazil, 2015


Author affiliation: Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil

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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.

A vian 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 hyperplasic 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...
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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.
PB1

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

A/Parana/720/2015
H1N2v

PB2
PB1
PA
HA
NP
NA
MP
NS

Human seasonal H3N2 lineage
Avian – North American lineage

H1N1pdm09 PB1

H3 Seasonal PB1
H1N1 vaccine strains
Swine and avian H1N2 strains
Brazil swine H1N2 cases
Brazil human H1N2v case
Human H1N2 cases

Human seasonal H1 lineages
Classical swine H1N1 – North American lineage

Human seasonal H1 lineages
Human Seasonal H1 lineages (only swine)

Classical Swine H1 lineages
Swine H1 Eurasian lineage
Avian H1 lineage

Swine seasonal H3 lineages
Avian – North American lineage
Human seasonal H3N2 lineage
NP

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

H1N1pdm09 NP

Human seasonal H3N2 lineage
Avian – North American lineage
Classical swine H1N1 – North American lineage

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

Human seasonal N2 lineage

Classical swine H1N1 – North American lineage

Avian – North American lineage