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Short Communication

Evidence of reassortment of pandemic H1N1 influenza virus in swine in Argentina: are we facing the expansion of potential epicenters of influenza emergence?

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In this report, we describe the occurrence of two novel swine influenza viruses (SIVs) in pigs in Argentina. These viruses are the result of two independent reassortment events between the H1N1 pandemic influenza virus (H1N1pdm) and human-like SIVs, showing the constant evolution of influenza viruses at the human–swine interface and the potential health risk of H1N1pdm as it appears to be maintained in the swine population. It must be noted that because of the lack of information regarding the circulation of SIVs in South America, we cannot discard the possibility that ancestors of the H1N1pdm or other SIVs have been present in this part of the world. More importantly, these findings suggest an ever-expanding geographic range of potential epicenters of influenza emergence with public health risks.

Keywords H1N1 2009 pandemic virus, pigs, reassortment.

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In 2009, a new H1N1 influenza virus emerged in North America that led to the first pandemic of the 21st century – herein referred to as H1N1pdm.[1,2] In addition, 21 countries reported infections with H1N1pdm in swine populations.[3,4] The H1N1pdm genome is similar to other swine influenza viruses (SIVs) of the H1N1 subtype circulating in North America. These viruses are triple reassortants with genes derived from avian (PB2 and PA), human (PB1), and classical SIVs; however, the H1N1pdm is unique in the sense that the NA and M genes are derived from Eurasian SIVs.[5]

Recently, Vijaykrishna *et al.*[6] reported a novel reassortant SIV in swine in China derived from a triple reassortant H1N1 virus carrying an hemagglutinin (HA) gene derived from an Eurasian SIV and the NA gene from the H1N1pdm virus. This observation suggests that H1N1pdm viruses provide the opportunity for additional reassortment events in swine, which is not necessarily surprising considering the origin of the virus. Nevertheless, it highlights the potential of the H1N1pdm virus to reassort and generate additional strains with the potential to infect humans.

Swine influenza is a viral disease caused by type A influenza viruses of the family Orthomyxoviridae. Type A influenza viruses are highly infectious pathogens that affect a variety of bird and mammalian species. The genome of type A influenza viruses contain eight segments of negative-sense single-stranded RNA. The segmentation of the genome allows reassortment and production of novel viruses. There are two major surface glycoproteins, HA and neuraminidase (NA), which are distinguished in subtypes based on their antigenic and genetic characteristics – 16 HA and nine NA subtypes have been described so far.

Swine influenza virus infections range from asymptomatic to severe and the disease can be exacerbated by management practices (e.g. poor ventilation) and secondary infections (e.g. Porcine Reproductive and Respiratory Syndrome).[7]

Since the late 1990s, in North America, the landscape of SIVs has become increasingly heterogeneous with the introduction of the triple reassortant internal genes cassette (TRIG cassette) derived from the H3N2 triple reassortant viruses carrying the PB1 (and HA and NA) gene derived from human H3N2 influenza viruses, PB2 and PA genes from an avian influenza virus, and the rest of the genes from the classical SIV. Since then, TRIGs have shown great flexibility to generate novel reassortants that have become established in the continental swine population. Thus, TRIG cassette reassortants of influenza subtypes H3N2,[8–11] H1N1,[10] H3N1,[12,13] H1N2,[14,15] H2N3,[16] and human-like H1[17] were detected in swine in North America. Coincidentally, one of these TRIG cassette viruses led to the emergence of the pandemic (H1N1) 2009 virus.[18]

In South America, little is known about the incidence of SIV and whether TRIG cassette viruses have been introduced. Evidence of the introduction in swine of a wholly human H3N2 virus was detected in 2008 in Argentina, although there is no further evidence that this virus continues to circulate in swine.[19] During the 2009 pandemic, the southern hemisphere was hard hit with the H1N1pdm virus. In Argentina, 1 390 566 human cases and 617 human deaths were reported from May to December 2009.[20] Coincidentally, the H1N1pdm virus was isolated from swine in Argentina after pig farmers showed clinical signs of H1N1pdm infection.[3] In Argentina, vaccines against SIV are neither licensed nor used.

In this report, we describe the occurrence of two novel SIVs in pigs in Argentina derived by reassortment of the H1N1pdm virus with human-like H1 SIVs. These viruses are the result of two independent reassortment events between the H1N1pdm and SIVs. Two pig farms reported clinical respiratory disease in fattening pigs, in October 2009 (Buenos Aires province, farm A, 21 000 pigs) and May 2010 (Santa Fe province, farm B, 12 000 pigs), respectively. No increases in mortality were reported in these farms, although increased morbidity near 10% (farm A) and 20% (farm B) was observed in comparison with previous farm records (1·6–2·5%). Clinical signs included fever, cough, dyspnea, and lethargy. From these farms, 26 pigs from farm A and 21 pigs from farm B were necropsied and samples were collected for diagnostic studies (virology and histopathology). From farm A, nine pigs (34·6%), and from farm B, eight pigs (38·1%), had lung lesions compatible with SIV infection. The microscopic findings were characterized by bronchiolar necrosis and infiltration of neutrophils that obstruct the small airway. Edema into the alveoli and interlobular connective tissue was also observed. Nasal swabs and lung tissue samples collected from these 17 pigs were processed for real-time reverse transcription–PCR (rRT–PCR). Viral RNAs were extracted from swab suspensions (QIAamp Viral RNA Mini kit; QIAGEN, Valencia, CA, USA) and used for three independent rRT–PCR tests: influenza type A (InfA) directed to the matrix (M) gene, swine influenza (SwInfA) directed to NP gene, and pandemic (H1N1) 2009 virus directed to the HA gene (SwH1).[21] The nine samples from farm A were positive for the InfA and SwInfA tests, but negative for the SwH1 test. From farm B, eight samples showed positive results for the InfA and SwInfA tests, but only three samples were positive for the SwH1 test. Two samples, one from farm A, and one from farm B that were InfA/SwInfA positive but SwH1 negative were grown in tissue culture in Madin-Darby Canine Kidney cells, and two different SIVs were isolated. RT–PCR analysis of these samples confirmed the previous observations (InfA/SwInfA positive, SwH1 negative). The isolates were labeled A/Swine/Argentina/CIP051-BsAs76/2009 (H1N1) from farm A and A/Swine/Argentina/CIP051-SantaFe/2010 (H1N2) from farm B – herein referred to as BsAs/H1N1 and StaFe/H1N2, respectively. Full sequence segments were obtained by using appropriate set of primers and are available through GenBank, accession nos. CY075853 through CY075868. Molecular characterization of these two viruses indicated that the internal genes were similar to the TRIG cassette found in H1N1pdm viruses (Table 1). However, the surface genes

Table 1. Schematic table showing the lineages of reassortant swine viruses identified

Virus	1 (PB2)	2 (PB1)	3 (PA)	4 (HA)	5 (NP)	6 (NA)	7 (M)	8 (NS)
Swine human-like H1	NA-Av	Hu	NA-Av	Hu	cH1N1	Hu	cH1N1	cH1N1
H1N1 2009 pdm	NA-Av	Hu	NA-Av	cH1N1	cH1N1	EA-Sw	EA-Sw	cH1N1
BsAs/H1N1	NA-Av (93–95%)	Hu (84–85%)	NA-Av (90–92%)	Hu (92–97%)	cH1N1 (93–96%)	Hu (89–92%)	EA-Sw (91–95%)	cH1N1 (92–96%)
StaFe/H1N2	NA-Av (93–95%)	Hu (83–84%)	NA-Av (90–92%)	Hu (93–95%)	cH1N1 (94–96%)	Hu (91–93%)	EA-Sw (89–94%)	cH1N1 (92–96%)

Virus lineages are identified in each box.
In between parenthesis are the ranges of percentages of identity of the two reassortants to each lineage.
NA-Av, North America Avian; Hu, Human; EA-Sw, Eurasian Swine; cH1N1, classical swine H1N1; HA, hemagglutinin.

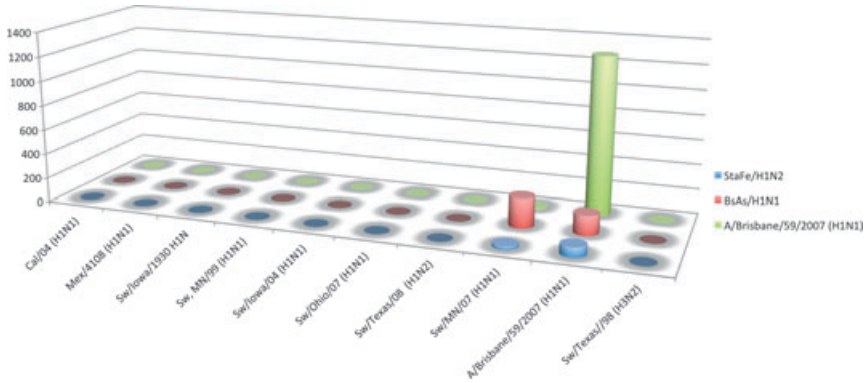

Figure 1. Hemagglutination inhibition (HI) assay with sera against antigenically distinct swine influenza viruses and human influenza viruses. *Y*-axis: HI titer; *X*-axis: Antibodies used for HI assay in this study include ferret sera against human seasonal strain A/Brisbane/59/2007 (H1N1); swine sera against H1N1pdm strain A/California/04/2009 (H1N1), A/Mexico/4108/09 (H1N1), A/swine/Iowa/15/1930 (H1N1) (classical swine H1), A/swine/Minnesota/27866/99 (H1N1) (α cluster swine H1), A/ swine/Iowa/00239/04 H1N1 (β cluster swine H1), A/swine/Ohio/511445/07 (H1N1) (γ cluster swine H1), A/swine/Texas/01976/08 (H1N2) (δ1 cluster swine H1, Human-Like lineage), A/Swine/Minnesota/07002083/07 (H1N1) (δ2 cluster swine H1 Human-Like lineage), and A/swine/Texas/4199-2/98 (H3N2).

indicated that these viruses were novel reassortant viruses and thus explained the rRT–PCR results. The HA genes of these viruses were similar to the human-like H1 SIVs, whereas the NA genes were similar to the human-like N1 (BsAs/H1N1) and human-like N2 (StaFe/H1N2) SIVs (Table 1). Phylogenetic analysis confirmed these initial observations (Figure S1). It is also important to note that the phylogenetic analysis of the M gene shows that these two viruses cluster together with Eurasian SIVs and with pandemic viruses (Figure S1). To further characterize these viruses, hemagglutination inhibition assays were carried out with a panel of convalescent SIV swine sera, convalescent ferret sera against seasonal and H1N1pdm viruses, and a panel of monoclonal antibodies developed against the H1N1pdm strain A/California/04/2009 (H1N1)(Figure 1). Hemagglutination inhibition analysis confirmed the antigenic relationship between the BsAs/H1N1 and StaFe/H1N2 and human-like SIVs of the delta 2 cluster.[17]

To our knowledge, this is the first report of reassortment of the H1N1pdm virus with human-like SIVs in South America. These observations are consistent with prior observations of the malleability of the pandemic TRIG cassette to reassort with other influenza viruses. In addition, we show indirect evidence of the presence of human-like SIVs in the Argentinean pig population – because of the presence of segments (HA and NA) derived from these viruses in the new reassortants SIVs described in this report. It must be noted that because of the lack of information regarding the circulation of SIVs in South America, we cannot discard the possibility that ancestors of the H1N1pdm or other SIVs have been circulating in this part of the world. Continued surveillance of the swine population in Argentina and elsewhere is warranted to better understand the ecology of influenza viruses in these hosts and to prevent the emergence of viruses with pandemic potential.

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Addendum

A. Pereda, C. Perfumo, and D. R. Perez contributed to the concept and design of the study; A. Rimondi, M. Angel, J. Ye, T. Sutton, M. Dibárbora, V. Olivera, and M. I. Craig analyzed and interpreted the virological and molecular data; J. Cappuccio, M. Quiroga, and M. Machuca performed necropsies, analyzed, and interpreted the histopathological data; A Ferrero provided technical support; A. Pereda, C. Perfumo, and D. R. Perez revised and approved the final version of the manuscript.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1: Phylogenetic tree of hemagglutinin (1a), NA (1b), and MP (1c) genes. Unrooted trees were generated by the neighbor-joining method in 11 the PAUP* program. Numbers above branches indicate neighbor-joining bootstrap values. Not all supports are shown because of space constraints. Analysis was based on full-length segments. Viruses characterized in this study are highlighted in bold, and swine influenza clusters are identified in colored boxes. H1N1pdm corresponds to H1N1 2009 pandemic virus, cH1N1a to classical swine H1N1, rH1N1b to triple reassortant swine H1N1, H1N2c to triple reassortant swine H1N2, Hu-H1d to human-like swine H1, Hu-N1 or Hu-N2 to human-like swine N1 and N2, respectively, and Sw-N1 to swine N1. Scale bar, 0Æ1 substitutions per site.

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