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

Ariel Pereda, Agustina Rimondi, Javier Cappuccio, Ramon Sanguinetti, Matthew Angel, Jianqiang Ye, Troy Sutton, Marina Díabobra, Valeria Olivera, Maria I. Craig, Maria Quiroga, Mariana Machuca, Andrea Ferrero, Carlos Perfumo, Daniel R. Perez

aLaboratorio Aves y Porcinos, Instituto de Virología CICVyA – Instituto Nacional de Tecnología Agropecuaria (INTA), Buenos Aires, Argentina. 
bCatedra de Patología Especial, Facultad de Cs. Veterinarias, Universidad Nacional de La Plata, Buenos Aires, Argentina. cServicio Nacional de Sanidad y Calidad Agroalimentaria, Buenos Aires, Argentina. dVirginia-Maryland Regional College of Veterinary Medicine and Department of Veterinary Medicine, University of Maryland, College Park, MD, USA.

Correspondence: Ariel Pereda, Laboratorio Aves y Porcinos, Instituto de Virología CICVyA – Instituto Nacional de Tecnología Agropecuaria (INTA), CC25 (1712) Castelar, Buenos Aires, Argentina. E-mail: apereda@cnia.inta.gov.ar

Accepted 6 March 2011. Published Online 7 April 2011.

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.

Please cite this paper as: Pereda et al. (2011) Evidence of reassortment of pandemic H1N1 influenza virus in swine in Argentina: are we facing the expansion of potential epicenters of influenza emergence? Influenza and Other Respiratory Viruses 5(6), 409–412.

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. In addition, 21 countries reported infections with H1N1pdm in swine populations. 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.

Recently, Vijaykrishna et al. 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).
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, H1N1, H3N1, H1N2, H2N3, and human-like H1 were detected in swine in North America. Coincidentally, one of these TRIG cassette viruses led to the emergence of the pandemic (H1N1) 2009 virus. 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. During the 2009 pandemic, the southern hemisphere was hit with the H1N1pdm virus. In Argentina, 1,390,566 human cases and 617 human deaths were reported from May to December 2009. Coincidentally, the H1N1pdm virus was isolated from swine in Argentina after pig farmers showed clinical signs of H1N1pdm infection. 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 (16–25%). 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%), and from farm B, eight pigs (38%), 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). 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...
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); 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).

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.

**Acknowledgements**

The authors thank Andrea Puebla and her group for their technical sequencing support. This work was partially supported by the USDA and by the NIAID, Center for Research on Influenza Pathogenesis (CRIP) through University of Maryland College Park contract No. HHSN266200700010C, by Proyecto Específico INTA Exoticas y Emergentes (AESA201731), by the European Community (Proyecto Integrado Cadena Carne Aviar – BiotecSur), by the Ministerio de Ciencia, Tecnologia e Innovacion Productiva from Argentina, and by Secretaria de Ciencia y Tecnica, Universidad Nacional de La Plata.

**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.

**References**

1 World Health Organization. Pandemic (H1N1) 2009: update 89. Available at http://www.who.int/csr/dor/2010_02_26/en/index.html (Accessed 26 February 2010).
2 Dawood FS, Jain S, Finelli L et al. Emergence of a novel swine-origin influenza A (H1N1) virus in humans. N Engl J Med 2009; 360:2605–2615.
3 Pereda A, Cappuccio J, Quiroga MA et al. Pandemic (H1N1) 2009 outbreak on pig farm, Argentina. Emerg Infect Dis 2010; 16:304–307.
4 Pasma T, Joseph T. Pandemic (H1N1) 2009 infection in swine herds, Manitoba, Canada. Emerg Infect Dis 2010; 16:706–708.
5 Smith GJ, Vijaykrishna D, Bahl J et al. Origins and evolutionary genomics of the 2009 swine-origin H1N1 influenza A epidemic. Nature 2009; 459:1122–1125.
6 Vijaykrishna D, Poon LL, Zhu HC et al. Reassortment of pandemic H1N1/2009 influenza A virus in swine. Science 2010; 328:1529.
7 Yoon KJ, Janke BH. Swine influenza: etiology, epidemiology, and diagnosis; in Morilla A, Yoon KJ, Zimmerman JJ (eds): Trends in Emerging Viral Infections of Swine. Ames: Blackwell Publishing, 2002; 23–28.
8 Webby RJ, Swenson SL, Krauss SL et al. Evolution of swine H3N2 influenza viruses in the United States. J Virol 2000; 74:8243–8251.
9 Richt JA, Lager KM, Janke BH et al. Pathogenic and antigenic properties of phylogenetically distinct reassortant H3N2 swine influenza viruses cocirculating in the United States. J Clin Microbiol 2003; 41:3198–3205.
10 Webby RJ, Rossow K, Erickson G, Sims Y, Webster R. Multiple lineages of antigenically and genetically diverse influenza A virus co-circulate in the United States swine population. Virus Res 2004; 103:67–73.
11 Olsen CW, Karasin AI, Carman S et al. Triple reassortant H3N2 influenza A viruses, Canada, 2005. Emerg Infect Dis 2006; 12:1132–1135.
12 Ma W, Gramer M, Rossow K, Yoon KJ. Isolation and genetic characterization of new reassortant H3N1 swine influenza virus from pigs in the midwestern United States. J Virol 2006; 80:5092–5096.
13 Lekcharoensuk P, Lager KM, Vemulapalli R, Woodruff M, Vincent AL, Richt JA. Novel swine influenza virus subtype H3N1, United States. Emerg Infect Dis 2006; 12:787–794.
14 Karasin AI, Landgraf J, Swenson S et al. Genetic characterization of H1N2 influenza A viruses isolated from pigs throughout the United States. J Clin Microbiol 2002; 40:1073–1079.
15 Choi YK, Goyal SM, Farnham MW, Joo HS. Phylogenetic analysis of H1N2 isolates of influenza A virus from pigs in the United States. Virus Res 2002; 87:173–179.
16 Ma W, Vincent AL, Gramer MR et al. Identification of H2N3 influenza A viruses from swine in the United States. Proc Natl Acad Sci USA 2007; 104:20949–20954.
17 Vincent AL, Ma W, Lager KM et al. Characterization of a newly emerged genetic cluster of H1N1 and H1N2 swine influenza virus in the United States. Virus Genes 2009; 39:176–185.
18 Peiris JS, Poon LL, Guan Y. Emergence of a novel swine-origin influenza A virus (S-OIV) H1N1 virus in humans. J Clin Virol 2009; 45:169–173.
19 Cappuccio J, Pereda A, Insarralde L et al. An outbreak of influenza virus H3N2 subtype in a farm in Argentina; in D’Allaire S, Friendship R (eds): Proceedings of the 21st IPVS Congress. Vancouver, Canada: International Pig Veterinary Society, 2010; 563.
20 Ministerio de Salud. Alerta epidemiologico. Available at http://www.msal.gov.ar/htm/site/alerta-epidemiologico.asp (Accessed 30 December 2009).
21 Centers for Disease Control and Prevention (CDC). 2009. Available at http://www.who.int/csr/resources/publications/swineflu/realtimeptpcr/en/index.html (Accessed 30 April 2009) (revision 1).

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 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, 0.1 substitutions per site.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.