Short Report: Genetic Analysis of Influenza A/H1N1 of Swine Origin Virus (SOIV) Circulating in Central and South America

Merly Sovero, Josefina Garcia,* V. Alberto Laguna-Torres, Jorge Gomez, Washington Aleman,
Wilson Chicaiza, Melvin Barrantes, Felix Sanchez, Mirna Jimenez, Guillermo Comach,
Ivette Lorenzana de Rivera, Alma Barboza, Nicolas Aguayo, and Tadeusz Kochel

*U.S. Naval Medical Research Center Detachment, Lima, Peru; Dirección General de Epidemiología, Ministerio de Salud, Lima, Perú;
Hospital Vernaza, Ecuador; Hospital Vozandes, Quito, Ecuador; Hospital Solano, Buenos Aires, Argentina; Hospital Infantil
Manuel de Jesus Rivera, Managua, Nicaragua; Hospital Santa Ana Metapan, El Salvador; LARDIDEV-Biomed-UC, Maracay,
Venezuela; Universidad Nacional Autónoma de Honduras, Tegucigalpa, Honduras; ONG Rayos de Sol, Asuncion, Paraguay*

Abstract. Since the first detection of swine origin virus (SOIV) on March 28, 2009, the virus has spread worldwide and oseltamivir-resistant strains have already been identified in the past months. Here, we show the phylogenetic analysis of 63 SOIV isolates from eight countries in Central and South America, and their sensitivity to oseltamivir.

INTRODUCTION

The swine origin influenza A/H1N1 virus (SOIV) emerged both in Mexico and the United States in March 2009, and has rapidly spread worldwide.^{1,2} This virus is not related to previous or current human seasonal influenza viruses and its genome is a product of a combination of four influenza virus gene segments from North American swine and avian influenza, human influenza, and Eurasian swine influenza.^{3,4}

Since its emergence, some genotypic mutations have been reported, for example, resistance to Oseltamivir (PROMED) was detected in Denmark, Japan, and Israel.^{5–7} In view of the situation, the World Health Organization (WHO) suggests the monitoring of changes in the nucleotide sequence of the virus as part of individual case reports.⁸ This measure is important for case management and vaccine development, and for detecting critical changes in this virus, which may give origin to a more pathogenic strain or to a resistant strain that may be highly transmissible.

Here, we show the phylogenetic analysis of the hemagglutinin gene of 63 SOIV samples and their sensitivity to oseltamivir.

MATERIAL AND METHODS

Specimen collection, isolation, and identification of influenza viruses. Patient recruitment, sampling, and viral isolation were performed as previously described⁹ by the NMRCD Collaborative Surveillance Network. Five thousand four hundred thirteen nasopharyngeal and throat swab specimens were collected from April to July 2009 from Argentina ($N = 215$), Ecuador ($N = 318$), El Salvador ($N = 142$), Honduras ($N = 141$), Nicaragua ($N = 464$), Paraguay ($N = 332$), Peru ($N = 3,702$), and Venezuela ($N = 99$).

RNA extraction and reverse transcription-polymerase chain reaction (RT-PCR). Nucleic acid was extracted with the use of a viral RNA kit (QIAamp, Qiagen, Valencia, CA), and real-time RT-PCR performed for SOIV detection according to WHO recommendations.¹⁰

Sequencing and phylogenetic analysis. A total of 1,581 samples resulted positive to real-time RT-PCR, an approximate 10% of these specimens were randomly selected from each

contributing country and the samples were then amplified by RT-PCR, sequenced, and analyzed. Amplification was carried out using the SUPERScript III Platinum One-Step RT-PCR (Invitrogen, San Diego, CA) with the specific primers and reaction conditions for the hemagglutinin gene for phylogenetic analysis,¹¹ and for the neuraminidase gene to monitor specific mutations for detecting resistance to Oseltamivir.¹²

Direct sequencing of RT-PCR products was carried out with the use of the Big Dye terminator cycle sequencing kit (version 3.1, Applied Biosystems) on a Genetic Analyzer System (version 3130xL, Applied Biosystems).

Gene sequences were assembled, aligned, and edited using Sequencher (version 4.8, Gene Codes Corp.) and Clustal X softwares, and phylogenetic analyses were performed using the neighbor-joining and maximum likelihood algorithms software (molecular evolutionary genetics analysis [MEGA] version 4). For the neighbor-joining analyses, distance was used and bootstrap values were calculated on the basis of 1,000 replicates to place confidence values on grouping within trees. Phylogenetic analyses and the evolutionary distances were performed using the Kimura two-parameter model as a model of nucleotide substitution and using the neighbor-joining method to reconstruct phylogenetic trees (MEGA version 4). Nucleotide Sequence Database accession nos. on GenBank: CY045101 to CY045142.

RESULTS

The sequences of 63 SOIV A/H1N1 isolates were compared with those originally reported from Mexico and the United States in April, at the beginning of the pandemic (Table 1). Our results show that all isolates were genetically similar to the original strains. The phylogenetic analysis reveals great similarity at the hemagglutinin (Figure 1) and neuraminidase. The strains were homogeneous and showed sequence similarity higher than 99%. The number of samples with identical sequences shown in parenthesis in Figure 1 on the right of one representative strain. No significant differences at the amino acid level were found.

The genetic analysis of the neuraminidase gene shows that all SOIVs analyzed lack the H274Y mutation, which is well known to confer resistance to Oseltamivir. No other substitutions described in the literature as conferring resistance to Oseltamivir were found in these isolates.¹³

*Address correspondence to Josefina Garcia, Av. Venezuela, S/N Bellavista Lima, Callao 2, Peru. E-mail: josefina.garcia@med.navy.mil

TABLE 1
Number of isolates sequenced by country

Country	Total samples	A H1N1 positive	Sequenced
Argentina	215	63	5
Ecuador	318	54	5
El Salvador	142	28	2
Honduras	141	9	2
Nicaragua	464	75	7
Paraguay	332	137	9
Peru	3,702	1,211	32
Venezuela	99	4	1
Total	5,413	1,581	63

CONCLUSIONS

Pandemic influenza A viruses were first in Mexico³ during this pandemic and thereafter it spread throughout the country and the entire world in < 4 months. The genetic analysis of SOIVs from Central and South America were similar to the original SOIV strains. Nevertheless, replacement of isoleucine by valine in position 477 was detected, and although this may not generate a critical change in the biological property of this virus, it may be a signal of how rapid this strain can mutate.

Considering the emergence of resistant strains to Oseltamivir in several countries worldwide^{5,6} and, although those resistant strains have not been detected in our surveillance network, it is highly important to continue the molecular surveillance of SOIV strains to detect critical genetic changes, which may generate resistant strains to first line therapy.

Received December 14, 2009. Accepted for publication May 17, 2010.

Acknowledgments: We are grateful to the Peruvian Ministry of Health and to the General Direction of Epidemiology (DGE) of Peru for their support and collaboration.

Financial support: This study was funded by the U.S. Department of Defense Global Emerging Infections Surveillance and Response System (DoD-GEIS), a division of the Armed Forces Health Surveillance Center, WORK UNIT NUMBER: 847705.82000.25GB. B0016.

Disclaimer: The views expressed in this article are those of the authors and do not necessarily reflect the official policy or position of the Department of the Navy, Department of Defense, or the U.S. Government. None of the authors have a financial or personal conflict of interest related to this study. The corresponding author had full access to all data in the study and final responsibility for the decision to submit this publication.

Disclosure: The study protocol was approved by the Naval Medical Research Center Institutional Review Board (Protocol NMRCD.2002.0019) in compliance with all applicable Federal regulations governing the protection of human subjects.

Copyright statement: Authors Tadeusz J. Kochel is a U.S. military service member; Merly Sovero and V. Alberto Laguna-Torres are employees of the U.S. Government. This work was prepared as part of their official duties. Title 17 U.S.C. § 105 provides that "Copyright protection under this title is not available for any work of the United States Government." Title 17 U.S.C. § 101 defines U.S. Government work as a work prepared by a military service member or employees of the U.S. Government as part of those person's official duties.

Authors' addresses: Merly Sovero, Josefina Garcia, V. Alberto Laguna-Torres, and Tadeusz Kochel, U.S. Naval Medical Research Center Detachment, Lima, Peru, E-mail: josefina.garcia@med.navy.mil. Jorge Gomez, Dirección General de Epidemiología, Ministerio de Salud, Lima, Perú. Washington Aleman, Hospital Vernaza, Ecuador. Wilson Chicaiza, Hospital Vozandes, Quito, Ecuador. Melvin Barrantes, Hospital Solano, Buenos Aires, Argentina.

FIGURE 1. Phylogenetic tree constructed using the neighbor-joining method and bootstrap analysis to determine the best-fitting tree for the gene. This figure is showing the phylogenetic relationship of the Influenza A H1N1 viruses found during an outbreak (based in the hemagglutinin region). The number strains with identical sequences are shown in parenthesis on the right of one representative strain. All the strains were part of only one cluster; hence, they are closely related to the worldwide circulating strains such as A/Texas, A/Mexico. For the comparison we have included strains reported from Mexico, Canada, and California in 2009.

Felix Sanchez, Hospital Infantil Manuel de Jesus Rivera, Managua, Nicaragua. Mirna Jimenez, Hospital Santa Ana Metapan, El Salvador. Guillermo Comach, LARDIDEV-Biomed-UC, Maracay, Venezuela. Ivette Lorenzana de Rivera, Universidad Nacional Autónoma de Honduras, Tegucigalpa, Honduras. Alma Barboza and Nicolas Aguayo, ONG Rayos de Sol, Asuncion, Paraguay.

REFERENCES

1. 2009. Swine influenza A (H1N1) infection in two children—southern California, March–April 2009. *MMWR Morb Mortal Wkly Rep* 58: 400–402.
2. World Health Organization, 2009. Influenza-like illness in the United States and Mexico. Available at: http://www.who.int/csr/don/2009_04_24/en/index.html.
3. Fraser C, Donnelly CA, Cauchemez S, Hanage WP, Van Kerkhove MD, Hollingsworth TD, Griffin J, Baggaley RF, Jenkins HE, Lyons EJ, Jombart T, Hinsley WR, Grassly NC, Balloux F, Ghani AC, Ferguson NM, Rambaut A, Pybus OG, Lopez-Gatell H, Alpuche-Aranda CM, Chapela IB, Zavala EP, Guevara DM, Checchi F, Garcia E, Hugonnet S, Roth C, 2009. Pandemic potential of a strain of influenza A (H1N1): early findings. *Science* 324: 1557–1561.
4. Garten RJ, Davis CT, Russell CA, Shu B, Lindstrom S, Balish A, Sessions WM, Xu X, Skepner E, Deyde V, Okomo-Adhiambo M, Gubareva L, Barnes J, Smith CB, Emery SL, Hillman MJ, Rivaller P, Smagala J, de Graaf M, Burke DF, Fouchier RA, Pappas C, Alpuche-Aranda CM, Lopez-Gatell H, Olivera H, Lopez I, Myers CA, Faix D, Blair PJ, Yu C, Keene KM, Dotson PD Jr, Boxrud D, Sambol AR, Abid SH, St George K, Bannerman T, Moore AL, Stringer DJ, Blevins P, Demmler-Harrison GJ, Ginsberg M, Kriner P, Waterman S, Smole S, Guevara HF, Belongia EA, Clark PA, Beatrice ST, Donis R, Katz J, Finelli L, Bridges CB, Shaw M, Jernigan DB, Uyeki TM, Smith DJ, Klimov AI, Cox NJ, 2009. Antigenic and genetic characteristics of swine-origin 2009 A (H1N1) influenza viruses circulating in humans. *Science* 325: 197–201.
5. PROMED-mail, 2009. Influenza A (H1N1)—worldwide (78): Tamiflu resistance, Denmark. Available at: http://www.promedmail.org/pls/otn/f?p=2400:1202:3285434049563252:NO:F2400_P1202_CHECK_DISPLAY,F2400_P1202_PUB_MAIL_ID:X,78150.
6. PROMED-mail, 2009. Influenza A (H1N1)—worldwide (85): Tamiflu resistance, Japan. Available at: http://www.promedmail.org/pls/otn/f?p=2400:1202:3285434049563252:NO:F2400_P1202_CHECK_DISPLAY,F2400_P1202_PUB_MAIL_ID:X,78237.
7. Mandelboim M, Hindyieh M, Segman-Meningher T, Mendelson E, 2010. Possible transmission of pandemic (H1N1) 2009 virus with Oseltamivir resistance. *Emerg Infect Dis* 16: 873–874.
8. World Health Organization, 2009. Human infection with pandemic (H1N1) 2009 virus: updated interim WHO guidance on global surveillance. Available at: http://www.who.int/csr/disease/swineflu/guidance/surveillance/WHO_case_definition_swine_flu_2009_04_29.pdf.
9. Laguna-Torres VA, Gomez J, Ocana V, Aguilar P, Saldarriaga T, Chavez E, Perez J, Zamalloa H, Forshey B, Paz I, Gomez E, Ore R, Chauca G, Ortiz E, Villaran M, Vilcarromero S, Rocha C, Chinchá O, Jimenez G, Villanueva M, Pozo E, Aspajo J, Kochel T, 2009. Influenza-like illness sentinel surveillance in Peru. *PLoS ONE* 4: e6118.
10. World Health Organization, 2009. CDC Realtime RTPCR (rRT-PCR) Protocol for Detection and Characterization of Swine Influenza (version 2009). Available at: www.who.int/csr/resources/publications/swineflu/CDCRealtimeRTPCR_SwineH1Assay-2009_20090430.pdf.
11. Hoffmann E, Stech J, Guan Y, Webster RG, Perez DR, 2001. Universal primer set for the full-length amplification of all influenza A viruses. *Arch Virol* 146: 2275–2289.
12. Garcia J, Sovero M, Torres AL, Gomez J, Douce R, Barrantes M, Sanchez F, Jimenez M, Comach G, de Rivera I, Agudo R, Kochel T, 2009. Antiviral resistance in influenza viruses circulating in Central and South America based on the detection of established genetic markers. *Influenza Other Respir Viruses* 3: 69–74.
13. McKimm-Breschkin J, Trivedi T, Hampson A, Hay A, Klimov A, Tashiro M, Hayden F, Zambon M, 2003. Neuraminidase sequence analysis and susceptibilities of influenza virus clinical isolates to zanamivir and oseltamivir. *Antimicrob Agents Chemother* 47: 2264–2272.