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Short Communication

Serological and molecular prevalence of swine influenza virus on farms in northwestern Mexico

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ABSTRACT

The aim of this study was to provide an overview of the epidemiological status of swine influenza viruses in pigs from northwestern Mexico in 2008–2009. A serological and molecular survey was conducted in 150 pigs from 15 commercial farms in Sonora, Mexico (northwestern region of Mexico). The serological data showed that 55% of the sera were positive for the H1N1 subtype, 59% for the H3N2 subtype, and 38% for both subtypes. Overall, 16.6% (25/150) of the samples were positive for type A influenza by qRT-PCR. The phylogenetic analysis of the H1 viruses circulating in northwestern Mexico were grouped into cluster α, from five other clusters previously described. The influenza virus H1 circulating in northwestern Mexico showed 97–100% identity at the nucleotide level among them, 89% identity with other North American strains, 88% with strains from central Mexico, and 85% with the pandemic A/H1N1p2009 virus. Meanwhile, a closer relationship with some influenza viruses from North America (97% nucleotide identity) was found for H3 subtype. In conclusion, our results demonstrated a high circulation of strains similar to those observed in the North American linage among commercial farms in northwestern Mexico, involving of a different lineage virus different to the influenza pandemic of 2009.

1. Introduction

Swine influenza virus (SIV) belongs to the *Orthomyxoviridae* family and contains 8 segments of single-stranded, negative-sense RNA, encoding 11 proteins. Hemagglutinin (HA) and neuraminidase (NA) are surface viral proteins that provide a capability for virus selection, recognition, propagation, and antigenicity and are targets for diagnostic tests (Thacker and Janke, 2008). Swine influenza is a seasonal respiratory disease that is easily spread among pigs, is distributed worldwide and is economically important. SIV is a primary agent that may allow entry of other pathogens into the host (Easterday and Van Reeth, 2006). It also represents a zoonotic risk for people who are in close contact with pigs (Gray et al., 2007; Lopez-Robles et al., 2012).

In early 2009, a swine-origin virus of the H1N1 subtype caused an influenza pandemic that reached level 6 and spread rapidly among humans (WHO, 2009). This novel influenza virus was detected by the CDC in samples from the USA, Mexico and Canada at the same time (Dawood et al., 2009). Unfortunately, no genetic data on SIV in Mexico had been reported prior to the outbreak. After the pandemic, an influenza virus isolated from swine in Mexico was analyzed and was found to be phylogenetically

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related to the pandemic strain. It has been suggested that the virus could be transmitted from humans to pigs (Escalera-Zamudio et al., 2012). Although another study performed in Mexico using porcine serum samples collected from 2000 to 2009 conducted by hemagglutination inhibition (HI) assay, demonstrated cross-reactivity with a pandemic A/H1N1p2009 virus (Saavedra-Montanez et al., 2013). It remains unknown whether this virus was circulating among the porcine population in Mexico prior to the pandemic. The aim of the present study was to provide a serologic and molecular overview of SIV in pigs from northwestern Mexico.

2. Materials and methods

2.1. Sample size and collection

A cross-sectional study was conducted in 15 commercial farms selected from three cities with the major pork production in Sonora state in the northwestern region of Mexico (Hermosillo, Cd. Obregón and Navojoa), during October 2008 to March 2009. All farms reported positivity to respiratory disease complex, of those 14 farms had an influenza immunization protocol for sows and none reported vaccination in growing pigs. The sample size ($n = 150$) was estimated considering a 30% prevalence of swine influenza infections in farms and a confidence level of 95% (Cannon and Roe, 1982). The samples were from unvaccinated 1–19-week-old weaning or fattening pigs. The study includes 21, 89, 18 and 22 samples from pigs from 1 to 4, 5 to 8, 9 to 12 and $\geq$13 weeks old, respectively. Blood samples ($n = 150$) were collected for serum antibody testing. Nasal swabs ($n = 150$) were also collected in cryovials containing viral transport medium (Hank' solution and 10% glycerol, supplemented with 10,000 U/mL penicillin G, 2 mg/mL streptomycin, 1 mg/mL gentamicin and 0.02 mg/mL amphotericin B).

2.2. Serological tests

A commercial enzyme-linked immunosorbent assay (ELISA) kit (IDEXX Laboratories, Inc., Westbrook, MASS.) was used for the detection of SIV specific antibodies following the recommendations of the manufacturer. Samples with an S/P ratio (net optical density of test sample/net optical density of positive control) equal or greater to 0.4 were considered positive for antibody against SwH1N1 and 0.3 for SwH3N2.

2.3. RNA extraction

Viral RNA was extracted from 140 μL of each nasal swab sample using a QIAamp viral RNA minikit (QIAGEN Inc., Valencia, CA, USA), following the protocols recommended by the manufacturer. The RNA was stored at −80 °C until used.

2.4. Real time RT-PCR (RT-qPCR)

An RT-qPCR assay targeting the M gene (influenza type A) was performed with 5 μL of RNA as described previously (Spackman et al., 2002), using the QIAGEN One-Step RT-PCR kit according to the manufacturer's protocol. Negative and positive controls were included with each run.

2.5. RT-PCR and sequencing

The influenza A-positive samples were further subtyped via conventional RT-PCR using previously described primers (Choi et al., 2002b). Further sequencing was conducted in the Genetic Analysis Technology Core of the University of Arizona, and phylogenetic analyses were performed to determine evolutionary relationships via the neighbor-joining method with 1000 bootstrap replicates using MEGA v.4 (Tamura et al., 2007). The northwestern Mexico strains were compared with previously reported H1 sequences and analyzed with the same criteria proposed by Lorusso et al. (2011).

2.6. Data analysis

To estimate statistically significant differences, Fisher's exact test was carried out to compare the determined SIV antibody titers based on age, geographic location and production system. To assess differences in the percentages of viral detection, a chi squared (X^2) test was applied. Both analyses were performed with a confidence level of 95% in NCSS version 2007 (NCSS Statistical Software, Kaysville, UT, USA).

3. Results

3.1. Seroprevalence

Sampling location included 15 swine farms from North, Central and South of Sonora State, Mexico, with at least 3 km of distance between them. The sampled farms were among 300–2500 sows. In all farms at least one positive sample for any of the two subtypes was observed. Serological analysis revealed that 55% of the sera samples were positive for the H1N1 subtype, 59% for the H3N2 subtype, and 38% for both subtypes. The antibody titers were higher for the H3N2 (S/P values of 0.676 ± 0.046) than for the H1N1 subtype (S/P values of 0.516 ± 0.037; $p < 0.05$; Fig. 1a). Anti-H1N1 subtype antibody levels showed an age-dependent decrement ($p < 0.05$; Fig. 1b). In contrast, anti-H3N2 subtype antibody levels tended to decrease from 1 to 12 weeks and rise through the 13th week (Fig. 1b).

3.2. Molecular survey

The molecular analyses revealed that 16.6% (25/150) of the samples were positive for type A influenza (matrix gene detection), of which 22 out of 25 (88%) pigs had clinical signs of respiratory disease such as cough, fever, and growth retardation. Among all of the positive samples, only 6 could be subtyped by RT-PCR, 4 of which showed the H1 subtype and 2 if which showed the H3 subtype. Due to the low viral loads present in the samples, only four of these samples were sequenced, 3 subtype H1 samples: CIAD14C of 812 bp (A/SW/SON/CIAD14C/08), GenBank accession no. KF997857; CIAD18C of 705 bp (A/SW/SON/CIAD18C/08),