

IMPROVED DIAGNOSTICS AND SURVEILLANCE IDENTIFY NOVEL REASSORTANT SWINE INFLUENZA A VIRUSES IN CHILE

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Influenza A Virus (IAV) circulates endemically in nature, representing a constant concern to public health and animal production systems worldwide. The emergence of the 2009 H1N1 influenza A pandemic (A(H1N1)pdm09) highlighted the need of conducting systematic surveillance studies, and evidenced the significant gap of knowledge of the swine IAVs (SwIAV) circulating in pigs worldwide at the time of the outbreak. This also highlighted the role of swine as important reservoirs for the generation of endemic strains with zoonotic and pandemic potential. Despite this, comprehensive information of SwIAV circulating in Latin America is still lacking. While Chile has strong ecological barriers and high stringency controls regarding livestock trading, IAV is endemic and has been consistently detected in pigs. This has caused great concerns for the swine industry, because of the on-going clinical and production losses. Nevertheless, few surveillance efforts have been conducted in Chile and there is limited information regarding the genetic diversity and origin of swIAVs. Determining the viral subtypes and genotypes circulating in swine is key for the development of appropriate vaccine approaches and control measures for the swine industry, and it is crucial for identifying novel strains with pandemic potential. We developed improved diagnostic tools to detect endemic viruses and characterized the diversity and origin of SwIAV and its prevalence in Chilean swine production farms. From December 2013 to June 2015, we sampled 27 farms representative of intensive swine production systems located between the Valparaiso and Araucania administrative regions. Animals were mainly found to be susceptible to IAV infection at 50 to 110 days of age. Serological analysis of 718 serum samples obtained from 3 different farms showed 91-59% prevalence to SwIAV, with 18-67% positivity to A(H1N1)pdm09-like strains and 29-58% positivity to a novel SwH1N2 virus. We also obtained a total of 1016 nasal swabs (NS, 85%) and 176 oral fluids (OF, 15%) that were tested by Real-time RT-PCR (qPCR) and virus isolation. Of these, 295 samples (25%) were positive to swIAV (23% of NS and 38% of OF). A large number of farms (21 out of 27, 78%) were positive in at least one visit, and most farms showed temporal co-circulation of at least 2 viruses or mixed infections. We subtyped selected positive samples and sequenced 51 complete viral genomes using the Illumina platform. Nineteen additional hemagglutinins (HA) genes were sequenced by Sanger. We performed Bayesian Evolutionary Analyses to reconstruct the phylogenies of the viral segments. This identified the circulation of 2 predominant swIAV genotypes in Chile, the A(H1N1)pdm09-like (35%) strain and a novel SwH1N2 virus (45%). This H1N2 virus is unique to Chile since it is genetically distinct from the H1 virus clusters seen in North America and is not related to any previously reported IAV. The genome of this swH1N1 virus contains genes from 3 different human contemporary viruses. Its H1 and N2 genes are derived from human H1N1 and H3N2 viruses from the mid 90's, respectively, suggesting that both of these human viruses were likely introduced into the Chilean swine population during that time. All the internal genes are from the A(H1N1)pdm09 strain, indicating that multiple and recent reassortment events gave rise to this novel reassortant virus. Of interest, we identified additional reassortant viruses that also contain the internal genes derived from the A(H1N1)pdm09 strain. These included an H1N1 containing the novel SwH1 and NA gene derived from the A(H1N1)pdm09-like strain, an H1N2 virus containing an H1 derived from the A(H1N1)pdm09 strain and the novel SwN2, a SwH3N2 virus and a human-like H3N2 virus, among others. Additionally, our analyses also revealed at least 3 independent human-to-swine introductions of the A(H1N1)pdm09 strain within the last 4 years in Chile. Overall this indicates that close human-swine interactions greatly contribute to the genetic diversity and emergence of IAVs in Chilean swine. This is the first comprehensive molecular epidemiological study of swIAV in Chile demonstrating the co-circulation of multiple viral strains in intensive swine production systems. Our data emphasizes the value of conducting long-term SwIAV surveillance in Latin America, a poorly studied region of the world.

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