Occurrence of influenza A virus subtypes in Brazil

Ana Paula Mori[1], Rui Carlos Peretti[1], Rogério Ferraz[1], Gustavo Mazer Ferraz[1], Danielle Gava[2], Vanessa Haach[3], Rejane Schaefer[2]

[1]INATA – Laudo Laboratório Avícola LTDA, Uberlândia, MG, Brazil; [2] Embrapa Suínos e Aves, Concórdia, SC, Brazil; [3] Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil.
E-mail: ruiperetti@laudolab.com.br

Introduction

Even after one decade of the first report of pandemic H1N1 (H1N1pdm) influenza virus (IAV) in swine, the disease is highly prevalent in pig farms. Currently, IAV is considered one of the most important primary pathogens in swine respiratory infections in the country (4). IAV is a fast evolving RNA segmented virus which is prone to reassort and accumulate point mutations. Genetically and antigenically diverse H1N1, H1N2 and H3N2 IAVs circulate in pig's farms worldwide with limited cross-protection among distinct virus subtypes and lineages (1). Human seasonal-origin H1N1, H1N2 and H3N2 IAVs, along with H1N1pdm, are widespread in pig farms located in several Brazilian states (3).

The aim of this study was to evaluate the occurrence of IAV in swine from 2017 to 2020 and to update information about IAV in Brazil.

Materials and Methods

From January of 2017 to February of 2020, 1349 nasal swabs and/or lung tissue samples were collected from pigs showing respiratory clinical signs and submitted to Laudo veterinary laboratory for IAV testing. Pig samples were from nursery and finishing farms located in four Brazilian states (Rio Grande do Sul, RS; Santa Catarina, SC; Paraná, PR and Minas Gerais, MG). Viral RNA was extracted using PreAmp (Simbios) kit and tested for IAV/M gene by RT-qPCR (5). IAV positive samples were submitted to viral isolation in SPF-embryonated chicken eggs and to amplification of hemagglutinin (HA) and neuraminidase (NA) genes by RT-qPCR (2).

Results

From 1349 samples tested by RT-qPCR, 473 (35.1%) were positive for IAV. Sixty-four (64) IAVs were isolated in eggs and subtyped by RT-qPCR (Table 1). H1N1 was detected in 20 (31.3%) samples, followed by H1N2 in 17 (26.6%) and H3N2 in 12 (18.8%) samples. Partial subtyping was determined for 15 (23.4%) samples (HxN2: 10 samples; HxN1pdm: 3 samples; huH1Nx: 1 sample; H1pdmNx: 1 sample). The RT-qPCR employed for IAV subtyping is also able to differentiate the IAV lineages. Therefore, 19 out of 20 H1N1 were of H1N1pdm virus lineage. One H1N1 virus was detected with primers and probe designed for an H1N1 virus derived from human

seasonal IAV that circulated in late 2000s (2). Novel reassortant H1N2 virus with H1 gene of H1N1pdm was detected in four isolates from SC state. Moreover, co-infection with two virus subtypes was detected in six out of 64 (9.4%).

Table 1. Subtyping results for IAV positive samples collected from 2017 to 2019.

Subtypes determined by RT-PCR	States				
	RS	SC	PR	MG	Total
H1N1pdm	4	8	4	3	19
huH1N1	1	0	0	0	1
huH1N2	2	4	2	5	13
H1pdmN2	0	4	0	0	4
huH3N2	4	2	2	4	12
Partial subtyping	2	7	1	5	15

Conclusions and Discussion

The present study shows the wide circulation of H1N1, H1N2 and H3N2 virus subtypes in pig farms located in four Brazilian states, representing 79.9% of Brazilian pork production. After one decade of its emergence in pigs, H1N1pdm is still causing outbreaks in swine. Previous studies showed the predominance of H1N1pdm in swine since 2009 (3,4), confirming its high fitness in swine. Partial subtyping of 23.4% samples possibly reflects the need to update primers to follow IAV changes observed recently. Following the emergence of H1N1pdm, novel reassortant H3N2 and H1N2 viruses arose in swine (3) and, since 2015 H1pdmN2 virus has been detected in swine in Brazil.

Considering these results, the IAV dynamics and the mutation ability of the virus, monitoring of influenza in swine is important to understand and control the disease more effectively.

References

1. Anderson TK et al. 2016. mSphere 1(6):e00275-16.
2. Haach V et al. 2019. J Virol Methods 269: 43-48.
3. Nelson MI et al. 2015. Nat. Commun. 6, 1–11.
4. Rech RR et al. 2018. Zoonoses Public Hlth, 65(1):e155-e161.
5. Zhang J & Harmon KM. 2014. Methods Mol Biol 1161: 277-293.