Avanços em Sanidade, Produção e Reprodução de Suínos VII

SINSUI 2023

15° Simpósio Internacional de Suinocultura

Produção, Reprodução e Sanidade Suína

09 a 11 de maio de 2023 | Centro de Eventos da PUCRS

Editores

Fernando P. Bortolozzo - Ivo Wentz - Ana Paula G. Mellagi - Rafael da Rosa Ulguim - Gabriela P. Zanin - Dalila Mabel Schmidt Tomm - David E. S. N. Barcellos.

www.sinsui.com.br

Characterization of Influenza A subtypes in nursery and finishing pigs in integration in the South of Brazil.

Goslar MS[*1,2], Lara AC[1], Aguiar FC[1], Fornari BF[1] & Zanella JCR[3]

*[1] Animal Health laboratory, JBS Foods, Seara, Brazil [2]Pro-Rector of Research, Graduate Studies and Innovation, Federal Institute of Santa Catarina (IFC) Campus Concórdia, Brazil [3] Brazilian Agricultural Research Corporation (EMBRAPA), Concórdia, Brazil *Corresponding author: mariana.goslar@seara.com.br*

Keywords: diagnosis; respiratory diseases; RT-qPCR; subtyping; viruses.

Introduction

Influenza A virus (IAV) is an RNA virus that causes respiratory diseases and is zoonotic in nature. It is considered endemic in commercial pig farms. Faced with the various antigenic variations and genetically distinct lineages in the country, H1N1, H1N2, H3N2 pandemic (Pan) and human-like (Hu) subtypes. Rapid and accurate diagnostic tools are needed to know the subtypes present in each herd, in order to monitor and control the disease more assertively. This study aims to detect the presence of the virus in integrated swine herds of an agroindustry in South Brazil and mapping them through the characterization of circulating subtypes.

Material and methods

In total, 1988 samples from nursery and finishing pigs were analyzed in a laboratory of an agrobusiness in the west of Santa Catarina. Nasal swabs and lungs from clinical cases were collected in four different integration units of this agribusiness in the States of Santa Catarina and Rio Grande do Sul, between April and December 2021. RT-qPCR for IAV detection was used for screening. So RNA amplification was performed with primers described by the WOAH (2015)[1] and according to the Animal Health laboratory's internal methodology, using the commercial kit of AgPath-ID™ One-Step RT-PCR reagents (Thermo Fisher Scientific Inc, CA, USA), both for detection of the Influenza virus and for the characterization of its subtypes. Positive samples were selected for multiplex RT-qPCR of IAV subtypes as the criteria determined by Haach et al., (2020)[2]. Only samples classified as Cycle Threshold (CT) ≤30 were evaluated by multiplex RT-PCR subtype analysis. The primers of neuraminidase (NA) and hemagglutinin (HA) glycoprotein and the method to subtype identification used in this study were described by Haach et al., (2020)[2]. Frequency descriptive analysis and statistical analysis of virus identification were calculated by Excel 2013 (Microsoft Corporation, USA) and SPSS 18.8 software (IBM Company, USA), respectively. Using Pearson's non-parametric chi-square test we assess the significant difference in the proportions of events ($p \leq 0.01$).

Results and discussion

The IAV was detected in 16.65% (331) of cases. Zone A was the region most positive (27.68%) for IAV, followed by Zone C (23.90%), B (15.82%) and D (7.08%), figure 1. All regions were significantly different ($p \leq 0.01$) from each other for the presence of IAV. The most positive sample type was lung (84.21%), compared to nasal swab (16%). Our detection rates in nasal swabs were higher than those found by Dibarbora (2016)[4]. This successful outcome, might be due to the greater number of samples and the circumstance that the samples were collected, simply from animals with respiratory signs. A total of 110 IAV positive samples were analyzed by multiplex RT-qPCR subtypes. We were able to subtype 57.27% for at least one surface glycoprotein identified (HA or NA). Therefore two subtypes were detected: H1N1pan (3.51%) and one H1huN1pan subtype sample, lower than Chiaponi *et al*., (2012)[3] that determined viral subtype of clinical samples, without viral isolation, in 56% of the cases. Hemagglutinin H3hu was the most commonly found glycoprotein, 27 samples, followed by N1pan (22), H1hu (14), H1pan (2) and N2hu (1). In Figure 2, it is demonstrated the subtypes detected per region. Hemagglutinin H3hu was almost exclusively found in Rio Grande do Sul, with only one sample from Santa Catarina

(Zone D). Zone A was the most diverse and was also the only one where the N2hu subtype was detected.

Conclusion

Our work was able to detect IAV in most of the studied farms and in all regions. Occurrence rate was significantly different, since Zone A presented the highest positivity. The H3hu subtype gene presented the highest predominance, found mostly in Rio Grande do Sul. However, the N1pan glycoprotein gene, was well distributed in both States. Therefore, our results provide important information on the IAV circulation in swine herds helping to better interpret its epidemiology and applicable to the One Health concept, considering the potential for zoonotic and interspecies transmission of the Influenza virus.

References

(1) WOAH (2015). Manual of diagnostic tests and vaccines for terrestrial animals. In OIE (World Organisation for Animal Health), Influenza A virus of Swine Chapter 3.8.7. **(2) Haach, V.; Gava, d.; Cantao, M. E.; et al.** Evaluation of two multiplex RT-PCR assays for detection and subtype differentiation of Brazilian swine influenza viruses. Brazilian Journal of Microbiology, 51, n. 3, p. 1447-1451, Sep 2020. Article. **(3) Chiapponi, C.; Moreno, A.; Barbieri, I.; Merenda, M. Et Al.** Multiplex RT-PCR assay for differentiating European swine influenza virus subtypes H1N1, H1N2 and H3N2. Journal of Virological Methods, 184, n. 1-2, p. 117-120, Sep 2012. Article. **(4) DIBÁRBORA, M.** Caracterización del virus de influenza en cerdos en Argentina. Orientador: PEREDA, A., PERFUMO, C. 2016. 109 f. Universidad de Buenos Aires, Facultad de Ciencias Veterinarias**. (5) Schaefer, R. Gava, D.; Reis, J.; Zanella, C.** Como identificar e controlar a influenza em suínos. Embrapa Suínos e Aves, Concórdia. 36 p.; 21 cm. 2019. Documentos / Embrapa Suínos e Aves, ISSn 01016245; 207.

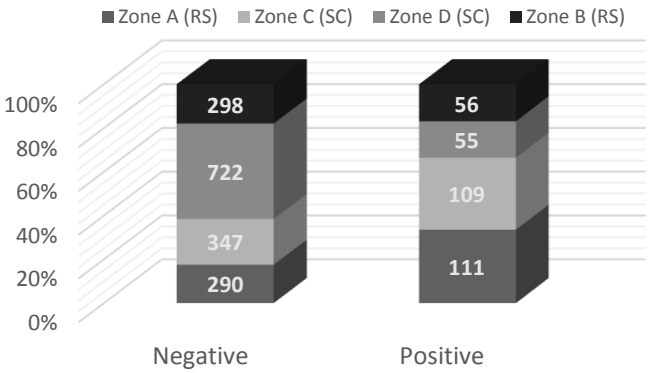

Figure 1. Positive and negative samples (n=1988) for the Influenza A virus in four different integration units in the States of Santa Catarina (SC) and Rio Grande do Sul (RS).

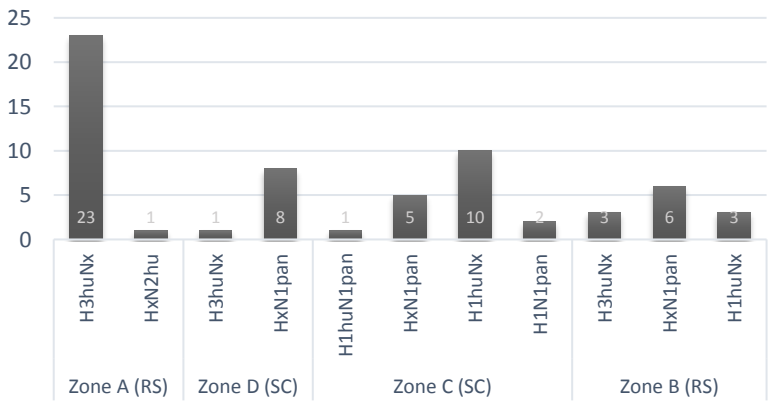

Figure 2. Influenza A virus subtypes identified by integration units of Santa Catarina (SC) and Rio Grande do Sul (RS) States. When the hemagglutinin (HA) or neuraminidase (NA) could not be identified, its identity was replaced by x (H_x or N_x). Pandemic (pan) and human-like (hu).