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Influenza A virus circulation in pig nurseries in the state of Santa Catarina, Brazil

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Abstract – The objective of this work was to evaluate the infection caused by influenza A virus (IAV) subtypes and its incidence in pig nurseries in the state of Santa Catarina, Brazil. A total of 423 nursery pigs were sampled in 11 farms, and IAV circulation, viral RNA, and antibodies were identified. Reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) was used to detect viral RNA in nasal swab samples (70.2%) and to subtype 33 viruses, of which 18 (54.5%) from six nurseries were the H3N2 virus, 6 (18.1%) from two nurseries were H1N1pdm, and 9 (27.2%) from three nurseries could not be subtyped. The enzyme-linked immunosorbent assay (ELISA) detected the presence of IAV antibodies (68%), which was confirmed by the hemagglutination inhibition test, revealing a higher prevalence of antibodies for the H3N2 virus (38.0%), followed by H1N1pdm (23.8%) and H1N2 (3.23%). The obtained data showed that 10.3% of the swine reacted to at least two viral antigens. There is a high prevalence of influenza A virus infection in all 11 piglets nurseries sampled by viral RNA and antibody detection. H3N2 and H1N1pdm, in this order, are the most detected viral subtypes in the 11 sampled nurseries.

Index terms: *Sus scrofa domesticus*, respiratory diseases, viral diseases, zoonosis.

Circulação de vírus influenza A em creches suínas no estado de Santa Catarina, Brasil

Resumo – O objetivo deste trabalho foi avaliar a infecção por subtipos do vírus influenza A (IAV) e a sua incidência em creches de suínos, no estado de Santa Catarina, Brasil. Foram amostrados 423 leitões de creche em 11 granjas, e foram identificados circulação de IAV, RNA viral e anticorpos. A transcriptase reversa seguida de quantificação por reação em cadeia da polimerase (RT-qPCR) foi utilizada para detectar o RNA viral em amostras de swab nasal (70,2%) e para subtipar 33 vírus, dos quais 18 (54,5%) de seis creches eram o vírus H3N2, 6 (18,1%) de duas creches eram o H1N1pdm e 9 (27,2%) de três creches não puderam ser subtipificados. O ensaio de imunoabsorção enzimática (ELISA) detectou a presença de anticorpos IAV (68%), a qual foi confirmada pelo teste de inibição da hemaglutinação, tendo revelado maior prevalência de anticorpos para o vírus H3N2 (38,0%), seguido por H1N1pdm (23,8%) e H1N2 (3,23%). Os dados obtidos mostraram que 10,3% dos suínos reagiram a pelo menos dois antígenos virais. Há alta prevalência de infecção pelo vírus influenza A em todas as 11 creches de leitões amostradas por RNA viral e detecção de anticorpos. H3N2 e H1N1pdm, nesta ordem, são os subtipos virais mais detectados nas 11 creches amostradas.

Termos para indexação: *Sus scrofa domesticus*, doenças respiratórias, doenças virais, zoonose.

Introduction

The influenza A virus (IAV) infects a variety of avian and mammalian species, including humans, pigs, and horses (Vincent et al., 2014). IAV is a segmented, negative-sense RNA virus, which enables it to be in constant evolution and to present a wide genetic diversity due to point mutations and genetic reassortment (Vincent et al., 2008).

In swine (*Sus scrofa domesticus*), the main clinical signs of the virus are coughing, sneezing, increased nasal discharge, and fever. Currently, three IAV hemagglutinin and neuraminidase subtypes – H1N1, H1N2, and H3N2 – circulate in the swine population worldwide, causing outbreaks of the respiratory disease (Vincent et al., 2020). Although the IAV subtypes in swine are restricted, distinct virus lineages are distributed globally (Anderson et al., 2021), which has led to the development of a swine H1 nomenclature system, based on the phylogenetic analysis of circulating virus sequences, to define the three major lineages, which are 1A, 1B, and 1C (Anderson et al., 2016). Furthermore, unique IAV clades have been associated with different geographical regions, reinforcing a high genetic evolution after IAV human-to-swine transmission (Vincent et al., 2014; Nelson et al., 2015a, 2015b; Baudon et al., 2017; Anderson et al., 2021).

In Brazil, since 2009, frequent outbreaks of an acute respiratory disease in pigs caused by H1N1pdm have been reported (Schaefer et al., 2011, 2015; Rajão et al., 2013; Ciacci-Zanella et al., 2015; Nelson et al., 2015b). H1N1pdm (2009 H1N1 pandemic or clade 1A.3.3.2), H1N2 (clade 1B.2.2), and H3N2 are widespread in swine herds in the country, where they continue to evolve (Ciacci-Zanella et al., 2015; Nelson et al., 2015b; Schaefer et al., 2015).

Among swine, nursery pigs are most affected by respiratory diseases, mainly caused by viruses (Ferreira et al., 2017). The porcine respiratory disease complex (PRDC) is an important disease for commercial swine production (Bochev, 2007; Rech et al., 2018). Globally, the most relevant pathogens in PRDC are IAV, porcine reproductive and respiratory syndrome virus (PRRSV), *Mycoplasma hyopneumoniae* (Mhyo), porcine circovirus type 2 (PCV2), and pyogenic bacteria (Bochev, 2007). Since PRRSV has not yet been reported in Brazil (Rech et al., 2018; Gava et al., 2022), IAV has been described as the main pathogen responsible for the majority of losses in the country's herd, including poor gain weight in swine and additional costs for antimicrobial treatments to control secondary bacterial infections (Rech et al., 2018). Therefore, the control of respiratory diseases, as well as the monitoring of influenza, considered a zoonosis, is fundamental, especially in the state of Santa Catarina, the largest producer and exporter of pork in Brazil.

The objective of this work was to evaluate the infection caused by IAV subtypes and its incidence in pig nurseries in the state of Santa Catarina, Brazil.

Materials and Methods

From June to September 2018, 11 nurseries, housing 21,268 piglets, in farms in the western region of the state of Santa Catarina, Brazil, were screened for the presence of IAV (Figure 1). Since these farms belong to an integrated-system company, all of them adopt the same structural biosecurity standards, including perimetral fence, change of clothes and shoes, and disinfection protocols. The piglets housed in each nursery were from different origins (weaned piglets from different sow farms and locations that were later grouped and redistributed), being clustered in a system with 35 days of lodging and with a downtime between batches of 7 days ("all-in all-out"). All piglets received the same feed and were subjected to the same sanitary and vaccination protocol against PCV2, Mhyo, *Glaesserella parasuis*, and *Pasteurella multocida*; pigs and dams were not vaccinated against IAV. The stocking density of the nurseries was three piglets per square meter. All procedures in swine were approved by the ethics committee on animal use of Institute Federal de Santa Catarina under protocol number 228-2017.

Herd sampling was conducted based on previous data, with a minimal prevalence of 40% into the herd, considering a 95% confidence level, 95% test sensitivity, 5% precision level, and 40% prevalence (Cannon, 2001); at least 30 pigs, aged from 33 to 52 days, were selected and sampled per farm. For sample size definition, the following equation for calculating a finite population was used: $n = Z2 \times P \times Q \times N / e2 \times (N - 1) + Z2 \times P \times Q$, where $Z2$ is the confidence level, P is the expected hit amount, Q is the expected amount of error, N is the total population, and e is the level of precision. In addition, sampling was stratified according to the size and geographic location of each

farm (Figure 1). Pigs with typical clinical signs of influenza infection, such as fever, respiratory distress, cough and prostration, were preferentially chosen for sampling, which was done by collecting blood and nasal swab samples from 423 piglets as described in Ciacci-Zanella et al. (2015).

Viral RNA was extracted from each nasal swab sample using the MagMAX kit (Ambion, Themo Fisher Scientific, Waltham, MA, USA) and then tested for the amplification of the IAV matrix gene by reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) according to Zhang & Harmon (2014). RT-qPCR positive samples were subjected to viral isolation in Madin-Darby Canine Kidney (MDCK) cells or in embryonated specific pathogen free (SPF) chicken eggs (ECE) as in Zhang & Gauger (2014). For IAV subtyping, three isolated viruses were selected from each nursery, totalizing 33 samples, which were also tested by RT-qPCR (Haach et al., 2020). The

serum – individual samples per assay – was separated from the blood samples and tested by the InfA Multi commercial kit for ELISA (BioCheck: Smart Veterinary Diagnostics, Scarborough, ME, USA), following the manufacturer's instructions.

For the hemagglutination inhibition (HI) test, 155 IAV antibody samples positive by ELISA were selected on the basis of a 95% confidence level, 5% accuracy level, and 40% prevalence (Ciacci-Zanella et al., 2015). The virus antigens used in the HI test are representative of the IAV subtypes that are circulating in Brazilian herds and that were deposited at Coleção de Microrganismos de Interesse para a Suinocultura e Avicultura, Embrapa's collection of microorganisms of interest for pig and poultry, and also previously sequenced by Nelson et al. (2015b) as: H1N1pdm (107/10; BRMSA0009; KF683614; clade 1A.3.3.2), H1N2 (31/11-1; BRMSA 1736; KF680296; clade 1B.2.2), and H3N2 (28/15-8; BRMSA1697; MH559963).
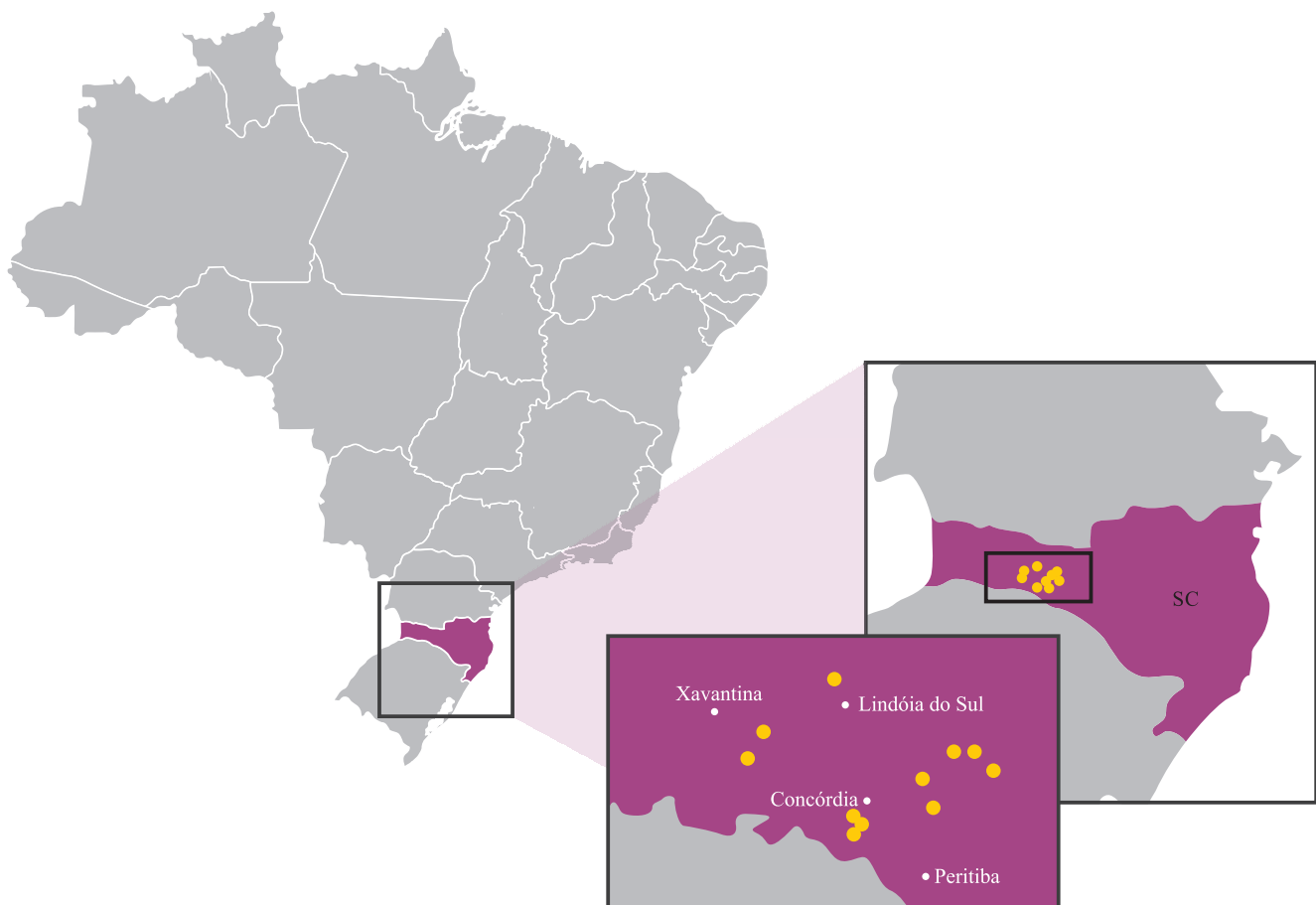

Figure 1. Map of Brazil, detailing the location of the 11 piglet nurseries in the western region of the state of Santa Catarina (SC).

Results and Discussion

Although H1N1pdm has been circulating in swine for 10 years, with established subtypes, IAV strains had not yet been characterized in pigs in the nursery phase. The analyzed herds/nurseries were separated into sites, with piglets remaining in the nurseries for about 40 days during weaning, starting at 21 or 28 days of age, until being transported to grower-finisher farms, which differ from the swine farms in other countries that are from wean to finish (Ramirez & Karriker, 2019). Furthermore, the farms used in the present study were mostly small and family owned, and the piglets in each nursery were from two to eight different origins (herds), from where they were sent to the nurseries, grouped, and then redistributed again into each nursery for housing (Table 1).

IAV infection was identified in all 11 nurseries since viral RNA was detected by RT-qPCR in 70.2% (297/423) of the nasal swab samples, with a cycle threshold ranging from 13.69 to 38.87 (Table 1). This higher detection rate of IAV is probably due to the selection and sampling of pigs already with respiratory clinical signs (coughing) and hyperthermia. A study conducted during an active surveillance of IAV in herds in the Midwest of the United States showed a low detection rate of influenza in piglets (≤5% of the samples) in the nursery and growing phases, with subclinical manifestations (Corzo et al., 2013). These differing results could be due to pig sampling during the acute phase of IAV infection in the present study, which leads to a higher viral detection and isolation (Allerson et al., 2013). The higher percentage of infected piglets (70.2%) here could also be explained by the absence of immunity to IAV. In their research, Pardo et al. (2018) concluded that maternally derived antibodies play an important role in the reduction of influenza infection and of the clinical presentation of the disease in weaned piglets.

A total of 23 IAVs, from eight farms, were isolated in MDCK cells or in ECE. Of the 33 IAV strains subjected to subtyping by RT-qPCR, 18 (54.5%) from six nurseries were positive for the H3N2 virus, 6 (18.1%) from two nurseries were positive for H1N1pdm, and nine (27.2%) from three nurseries could not be subtyped (Table 1). Of the 11 nurseries, 8 (72.7%) were positive for at least one viral subtype and 3 (27.2%) were negative for the three tested viral subtypes.

The most detected subtype in 6 of the 11 studied farms was H3N2. In Brazil, the H1N1pdm virus subtype was predominant in different pig categories – suckling, nursing, fattening pigs, and pregnant sows – from its

Table 1. Influenza A virus (IAV) detection by reverse transcriptase quantitative polymerase chain reaction (RT-qPCR), subtyping, and antibodies obtained by the enzyme-linked immunosorbent assay (ELISA) and the hemagglutination inhibition (HI) test in piglet (*Sus scrofa domesticus*) nurseries in different municipalities of the state of Santa Catarina, Brazil, from June to September 2018.

Nursery ID	Location (municipality)	Number of samples[1]	Piglet age (days)	Number of origins[2]	IAV by RT-qPCR[3]	Cq range[4]	Subtyping[5]	IAV by ELISA[6]	HI[7] H1N1[8]	HI[7] H1N2[9]	HI[7] H3N2[10]
1	Concórdia	40	42	4	18	25.63–38.87	H3N2	31	40	40–80	40–320
2	Concórdia	40	40	4	38	21.84–37.46	H3N2	24	<10	<10	40–320
3	Seara	50	54	4	5	35.01–37.96	ND[11]	23	40	<10	<10
4	Jaborá	32	47	5	29	26.48–37.40	ND[11]	27	40	<10	80–640
5	Concórdia	40	40	4	34	20.55–37.72	H3N2	39	40–80	<10	40–80
6	Jaborá	33	40	5	25	17.75–38.45	H3N2	22	80	<10	<10
7	Concórdia	26	51	2	26	17.92–36.60	H1N1pdm	15	40	<10	160
8	Concórdia	50	45	8	27	28.65–37.77	ND[11]	47	40–80	40	40–640
9	Lindóia do Sul	40	35	5	40	13.69–31.35	H3N2	29	40–80	<10	40–80
10	Arabuta	40	39	5	40	15.39–33.90	H3N2	9	40	40	40–160
11	Concórdia	32	53	4	15	20.19–37.99	H1N1pdm	22	80	<10	40

[1]Number of sampled piglets per nursery. [2]Number of sources of the weaned piglet before housing at the nursery. [3]RT-qPCR in nasal swab samples. [4]Cycle threshold or crossing point values. [5]IAV subtypes detected by RTq-PCR. [6]Number of positive samples per nursery using antibodies obtained by ELISA. [7]Hemagglutination inhibition test. [8]Not determined. [9–11]IAV subtypes. The results of the HI test were: negative, when lower than (<) 10 or 1:10; suspect, when 10–20 or 1:10 or 1:20; and positive, when 1:40 or higher.

introduction in the country in 2009 (Schaefer et al., 2011; Rajão et al., 2013) until 2015 (Ciacci-Zanella et al., 2015; Rech et al., 2018; Haach et al., 2020; Fraiha et al., 2021). However, since 2015, IAV outbreaks caused by the H3N2 virus have been reported more frequently among Brazilian pig herds (Haach et al., 2020; Fraiha et al., 2021).

In the ELISA, antibodies against IAV were detected in 68% (288/423) of the tested sera (Table 1). The percentage of piglets positive for IAV antibodies in the farms varied from 22.5 to 97.5%, and the HI test revealed a higher prevalence of antibodies against the H3N2 virus (59/155; 38.0%), followed by H1N1pdm (37/155; 23.8%) and H1N2 (05/155; 3.23%). In addition, of the 11 farms: 9 (81.8%) were found positive for H3N2, with titers ranging from 40–640; 10 (90.9%) were positive for H1N1pdm, with titers from 40–80; and 3 (27.2%) were positive for H1N2, with titers from 40–80. Three (27.2%) farms were positive for the three viral subtypes and 5 (45.4%) were positive for two virus subtypes. These results are indicative of the simultaneous circulation of distinct IAVs in the studied nurseries and are similar to the findings of previous works (Corzo et al., 2013; Rech et al., 2018; Silva et al., 2019), which highlighted the role of piglets, born from negative sows, as a source of IAV dissemination in pig herds.

In the present study, the rates of piglets with antibodies against H3N2 and H1N1pdm were of 38.0 and 23.8%, which are higher than the following values obtained in Brazil: 16.7% for H3N2 and 2.2% for H1N1 (clade 1A.1) in serological studies (Anderson et al., 2016); and 20% for H3N2 in analyzes in the state of Paraná (Caron et al., 2010; Resende et al., 2017). These differences could be due to the fact that, before the introduction of H1N1pdm, outbreaks of influenza in the country were not frequent (Ciacci-Zanella et al., 2015). According to a research carried out in 17 pig herds in the state of Minas Gerais (Rajão et al., 2013), 10.1% of the pigs showed antibodies for H3N2, 44.5% for H1N1 IAV of swine origin (H1N1 IAV-swine), and 38.3% for H1N1 of human origin. Of the 17 herds tested by these authors, 11 (64.7%) were considered positive for H1N1 IAV-swine, 4 (23.5%) for H3N2, and 10 (58.8%) for H1N1 human.

The data obtained here also revealed that 10.3% of the pigs reacted to at least two IAV antigens, and that 27 or 45% of the herds reacted to three or two of the tested subtypes, respectively. It should be noted that the antigens used in the present study are from the classic lineage of IAV-swine – H1N1 (A/swine/Iowa/15/1930/H1N1; clade 1A.1), H3N2 (A/swine/Iowa/8548-2/1998/H3N2), and H1N1 human reference strain (A/WSN/1933).

Since IAV may undergo reassortment, it poses a potential threat to humans, especially those who work with pigs, particularly in the nursery phase, for which, to date, there are no specific IAV control measures. In the present study, the variation in the detection of IAV RNA in the farms, which was from 10 to 100%, and of antibodies in the piglets, from 22.5 to 97.5%, may have been due to piglet origin, viral loads, virulence of IAV, and biosecurity measures (Silva et al., 2019). Some practices have shown positive or protective effects on the prevalence of the influenza virus in pigs, including the vaccination of pigs and farm staff (Oliveira & Iguti, 2010), the use of anti-bird netting, the delimitation of a specific facility for the acclimatization of gilts, and the internal replacement of breeders (Silva et al., 2019). Regarding vaccination, commercial or autogenous vaccines are a possibility, but the choice of one or another depends on the identification of the subtypes that are occurring in a given herd (Mancera Gracia et al., 2020; Vincent et al., 2020), which should be done by the genomic or antigenic analysis. Contrarily, the incidence of influenza could increase if certain practices – such as all-in all-out, limited downtime between batches, and controlled access of people to the farm – are not adopted (Silva et al., 2019).

Although South American countries, as Brazil, are important players in the production of animal protein, including pork, there are few studies of the subtypes circulating in their herds (Vincent et al., 2014; Nelson et al., 2015b; Cappuccio et al., 2017). In these countries, as in others, swine populations represent reservoirs for emerging IAV strains with zoonotic and possibly pre-pandemic potential (Henritzi et al., 2020). A recent study has highlighted the importance of monitoring influenza viruses with human pandemic potential that may undergo rearrangements in farms and infect pig farmers or slaughterhouses in Brazil (Resende et al., 2017). Through a representative population, the present work sought to identify and contribute to the characterization of IAV in pigs in the nursery phase, aiming to understand the infection and improve control measures, paving the way for genomic sequencing analyses and further studies for the antigenic characterization of IAVs.

Conclusions

1. There is a high prevalence of influenza A virus (IAV) infection in all 11 piglet (*Sus scrofa domesticus*) nurseries as shown by the detection of viral RNA and antibodies.

2. H3N2 and H1N1pdm, in this order, are the most detected viral subtypes in all 11 nurseries.

3. The diagnosis of influenza by reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) and the monitoring of the main virus subtypes circulating in the studied herd are important to evaluate the involvement of IAV in respiratory diseases in weaned pigs and, consequently, to contribute to the adoption of effective control measures.

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References

ALLERSON, M.; DEEN, J.; DETMER, S.E.; GRAMER, M.R.; JOO, H.S.; ROMAGOSA, A.; TORREMORELL, M. The impact of maternally derived immunity on influenza A virus transmission in neonatal pig populations. **Vaccine**, v.31, p.500-505, 2013. DOI: https://doi.org/10.1016/j.vaccine.2012.11.023.

ANDERSON, T.K.; CHANG, J.; ARENDSEE, Z.W.; VENKATESH, D.; SOUZA, C.K.; KIMBLE, J.B.; LEWIS, N.S.; DAVIS, C.T.; VINCENT, A.L. Swine influenza A viruses and the tangled relationship with humans. **Cold Spring Harbor Perspectives in Medicine**, v.11, a038737, 2021. DOI: https://doi.org/10.1101/cshperspect.a038737.

ANDERSON, T.K.; MACKEN, C.A.; LEWIS, N.S.; SCHEUERMANN, R.H.; VAN REETH, K.; BROWN, I.H.; SWENSON, S.L.; SIMON, G.; SAITO, T.; BERHANE, Y.; CIACCI-ZANELLA, J.; PEREDA, A.; DAVIS, C.T.; DONIS, R.O.; WEBBY, R.J.; VINCENT, A.L. A phylogeny-based global nomenclature system and automated annotation tool for H1 hemagglutinin genes from swine influenza A viruses. **MSphere**, v.1, e00275-16, 2016. DOI: https://doi.org/10.1128/mSphere.00275-16.

BAUDON, E.; PEYRE, M.; PEIRIS, M.; COWLING, B.J. Epidemiological features of influenza circulation in swine populations: a systematic review and meta-analysis. **PLoS ONE**, v.12, e0179044, 2017. DOI: https://doi.org/10.1371/journal.pone.0179044.

BOCHEV, I. Porcine respiratory disease complex (PRDC): a review. I. Etiology, epidemiology, clinical forms and pathoanatomical features. **Bulgarian Journal of Veterinary Medicine**, v.10, p.131-146, 2007.

CANNON, R.M. Sense and sensitivity: designing surveys based on an imperfect test. **Preventive Veterinary Medicine**, v.49, 141-163, 2001. DOI: https://doi.org/10.1016/S0167-5877(01)00184-2.

CAPPUCCIO, J.; DIBARBORA, M.; LOZADA, I.; QUIROGA, A.; OLIVERA, V.; DÁNGELO, M.; PÉREZ, E.; BARRALES, H.; PERFUMO, C.; PEREDA, A.; PÉREZ, D.R. Two years of surveillance of influenza A virus infection in a swine herd. Results of virological, serological and pathological studies. **Comparative Immunology, Microbiology and Infectious Diseases**, v.50, p.110-115, 2017. DOI: https://doi.org/10.1016/j.cimid.2016.12.005.

CARON, L.F.; JOINEAU, M.E.G.; SANTIN, E.; RICHARTZ, R.R.T. de B.; PATRICIO, M.A. de C.; SOCCOL, V.T. Seroprevalence of H3N2 influenza A virus in pigs from Paraná (South Brazil): interference of the animal management and climatic conditions. **Virus Reviews and Research**, v.15, p.1-11, 2010. DOI: https://doi.org/10.17525/vrr.v15i1.36.

CIACCI-ZANELLA, J.R.; SCHAEFER, R.; GAVA, D.; HAACH, V.; CANTÃO, M.E.; COLDEBELLA, A. Influenza A virus infection in Brazilian swine herds following the introduction of pandemic 2009 H1N1. **Veterinary Microbiology**, v.180, p.118-122, 2015. DOI: https://doi.org/10.1016/j.vetmic.2015.08.021.

CORZO, C.A.; CULHANE, M.; JULEEN, K.; STIGGER-ROSSER, E.; DUCATEZ, M.F.; WEBBY, R.J.; LOWE, J.F. Active surveillance for influenza A virus among swine, midwestern United States, 2009-2011. **Emerging Infectious Diseases**, v.19, p.954-960, 2013. DOI: https://doi.org/10.3201/eid1906.121637.

FERREIRA, J.B.; GRGIĆ, H.; FRIENDSHIP, R.; WIDEMAN, G.; NAGY, É.; POLJAK, Z. Longitudinal study of influenza A virus circulation in a nursery swine barn. **Veterinary Research**, v.48, art.63, 2017. DOI: https://doi.org/10.1186/s13567-017-0466-x.

FRAIHA, A.L.S.; MATOS, A.C.D.; CUNHA, J.L.R.; SANTOS, B.S.Á. da S.; PEIXOTO, M.V.C.; OLIVEIRA, A.G.G.; GALINARI, G.C.F.; NASCIMENTO, H.I. de J.; GUEDES, M.I.M.C.; MACHADO, A. de M.V.; COSTA, E.A.; LOBATO, Z.I.P. Swine influenza A virus subtypes circulating in Brazilian commercial pig herds from 2012 to 2019. **Brazilian Journal of Microbiology**, v.52, p.2421-2430, 2021. DOI: https://doi.org/10.1007/s42770-021-00550-y.

GAVA, D.; CARON, L.; SCHAEFER, R.; SILVA, V.S.; WEIBLEN, R.; FLORES, E.F.; LIMA, M. de; TAKEDA, G.Z.; CIACCI-ZANELLA, J.R. A retrospective study of porcine reproductive and respiratory syndrome virus infection in Brazilian pigs from 2008 to 2020. **Transboundary and Emerging Diseases**, v.69, p.903-907, 2022. DOI: https://doi.org/10.1111/tbed.14036.

HAACH, V.; GAVA, D.; CANTÃO, M.E.; SCHAEFER, R. Evaluation of two multiplex RT-PCR assays for detection and subtype differentiation of Brazilian swine influenza viruses. **Brazilian Journal of Microbiology**, v.51, p.1447-1451, 2020. DOI: https://doi.org/10.1007/s42770-020-00250-z.

HENRITZI, D.; PETRIC, P.P.; LEWIS, N.S.; GRAAF, A.; PESSIA, A.; STARICK, E.; BREITHAUPT, A.; STREBELOW,

G.; LUTTERMANN, C.; PARKER, L.M.K.; SCHRÖDER, C.; HAMMERSCHMIDT, B.; HERRLER, G.; BEILAGE, E.g.; STADLBAUER, D.; SIMON, V.; KRAMMER, F.; WACHECK, S.; PESCH, S.; SCHWEMMLE, M.; BEER, M.; HARDER, T.C. Surveillance of European domestic pig populations identifies an emerging reservoir of potentially zoonotic swine influenza A viruses. **Cell Host & Microbe**, v.28, p.614-627, 2020. DOI: https://doi.org/10.1016/j.chom.2020.07.006.

MANCERA GRACIA, J.C.; PEARCE, D.S.; MASIC, A.; BALASCH, M. Influenza A virus in swine: epidemiology, challenges and vaccination strategies. **Frontiers in Veterinary Science**, v.7, art.647, 2020. DOI: https://doi.org/10.3389/fvets.2020.00647.

NELSON, M.; CULHANE, M.R.; ROVIRA, A.; TORREMORELL, M.; GUERRERO, P.; NORAMBUENA, J. Novel human-like influenza A viruses circulate in swine in Mexico and Chile. **PLoS Currents**, v.7, 2015a.

NELSON, M.I.; SCHAEFER, R.; GAVA, D.; CANTÃO, M.E.; CIACCI-ZANELLA, J.R. Influenza A viruses of human origin in swine, Brazil. **Emerging Infectious Diseases**, v.21, p.1339-1347, 2015b. DOI: https://doi.org/10.3201/eid2108.141891.

OLIVEIRA, N.A.S.; IGUTI, A.M. O vírus Influenza H1N1 e os trabalhadores da suinocultura: uma revisão. **Revista Brasileira de Saúde Ocupacional**, v.35, p.353-361, 2010. DOI: https://doi.org/10.1590/S0303-76572010000200017.

PARDO, F.O.C.; SCHELKOPF, A.; ALLERSON, M.; MORRISON, R.; CULHANE, M.; PEREZ, A.; TORREMORELL, M. Breed-to-wean farm factors associated with influenza A virus infection in piglets at weaning. **Preventive Veterinary Medicine**, v.161, p.33-40, 2018. DOI: https://doi.org/10.1016/j.prevetmed.2018.10.008.

RAJÃO, D.S.; ALVES, F.; DEL PUERTO, H.L.; BRAZ, G.F.; OLIVEIRA, F.G.; CIACCI-ZANELLA, J.R.; SCHAEFER, R.; REIS, J.K.P. dos; GUEDES, R.M.C.; LOBATO, Z.I.P.; LEITE, R.C. Serological evidence of swine influenza in Brazil. **Influenza and Other Respiratory Viruses**, v.7, p.109-112, 2013. DOI: https://doi.org/10.1111/j.1750-2659.2012.00366.x.

RAMIREZ, A.; KARRIKER, L.A. Herd evaluation. In: ZIMMERMAN, J.J.; KARRIKER, L.A.; RAMIREZ, A.; SCHWARTZ, K.J.; STEVENSON, G.W.; ZHANG, J. (Ed.). **Diseases of swine**. 11th ed. Hoboken: J. Wiley & Sons, 2019. p.1-16. DOI: https://doi.org/10.1002/9781119350927.ch1.

RECH, R.R.; GAVA, D.; SILVA, M.C.; FERNANDES, L.T.; HAACH, V.; CIACCI-ZANELLA, J.R.; SCHAEFER, R. Porcine respiratory disease complex after the introduction of H1N1/2009 influenza virus in Brazil. **Zoonoses and Public Health**, v.65, e155-e161, 2018. DOI: https://doi.org/10.1111/zph.12424.

RESENDE, P.C.; BORN, P.S.; MATOS, A.R.; MOTTA, F.C.; CAETANO, B.C.; DEBUR, M. do C.; RIEDIGER, I.N.; BROWN, D.; SIQUEIRA, M.M. Whole-genome characterization of a novel human influenza A (H1N2) virus variant, Brazil.

Emerging Infectious Diseases, v.23, p.152-154, 2017. DOI: https://doi.org/10.3201/eid2301.161122.

SCHAEFER, R.; RECH, R.R.; GAVA, D.; CANTÃO, M.E.; SILVA, M.C. da; SILVEIRA, S.; ZANELLA, J.R.C. A human-like H1N2 influenza virus detected during an outbreak of acute respiratory disease in swine in Brazil. **Archives of Virology**, v.160, p.29-38, 2015. DOI: https://doi.org/10.1007/s00705-014-2223-z.

SCHAEFER, R.; ZANELLA, J.R.C.; BRENTANO, L.; VINCENT, A.L.; RITTERBUSCH, G.A.; SILVEIRA, S.; CARON, L.; MORES, N. Isolation and characterization of a pandemic H1N1 influenza virus in pigs in Brazil. **Pesquisa Veterinária Brasileira**, v.31, p.761-767, 2011. DOI: https://doi.org/10.1590/S0100-736X2011000900007.

SILVA, A.P.S.P.; COSTA, E. de F.; SILVA, G.S. e; SOUZA, C.K.; SCHAEFER, R.; VAZ JR., I. da S.; CORBELLINI, L.G. Biosecurity practices associated with influenza A virus seroprevalence in sows from southern Brazilian breeding herds. **Preventive Veterinary Medicine**, v.166, p.1-7, 2019. DOI: https://doi.org/10.1016/j.prevetmed.2019.02.013.

VINCENT, A.; AWADA, L.; BROWN, I.; CHEN, H.; CLAES, F.; DAUPHIN, G.; DONIS, R.; CULHANE, M.; HAMILTON, K.; LEWIS, N.; MUMFORD, E.; NGUYEN, T.; PARCHARIYANON, S.; PASICK, J.; PAVADE, G.; PEREDA, A.; PEIRIS, M.; SAITO, T.; SWENSON, S.; VAN REETH, K.; WEBBY, R.; WONG, F.; CIACCI-ZANELLA, J. Review of influenza A virus in swine worldwide: a call for increased surveillance and research. **Zoonoses and Public Health**, v.61, p.4-17, 2014. DOI: https://doi.org/10.1111/zph.12049.

VINCENT, A.L.; ANDERSON, T.K.; LAGER, K.M. A brief introduction to influenza A virus in swine. **Animal influenza virus**: methods and protocols. 3th ed. New York: Springer, 2020. p.249-271. (Methods in Molecular Biology, 2123). DOI: https://doi.org/10.1007/978-1-0716-0346-8_18.

VINCENT, A.L.; MA, W.; LAGER, K.M.; JANKE, B.H.; RICHT, J.A. Swine influenza viruses: a North American perspective. **Advances in Virus Research**, v.72, p.127-154, 2008. DOI: https://doi.org/10.1016/S0065-3527(08)00403-X.

ZHANG, J.; GAUGER, P.C. Isolation of swine influenza virus in cell cultures and embryonated chicken eggs. In: SPACKMAN, E. (Ed.). **Animal influenza virus**. 2nd ed. New York: Springer, 2014. p.265-276. (Methods in Molecular Biology, 1161). DOI: https://doi.org/10.1007/978-1-4939-0758-8_22.

ZHANG, J.; HARMON, K.M. RNA extraction from swine samples and detection of influenza A virus in swine by real-time RT-PCR. In: SPACKMAN, E. (Ed.). **Animal influenza virus**. 2nd ed. New York: Springer, 2014. p.277-293. (Methods in Molecular Biology, 1161). DOI: https://doi.org/10.1007/978-1-4939-0758-8_23.