OFFLU Swine Influenza Virus technical meeting
27 – 28 February 2019
OIE Headquarters, Paris, France

Janice Reis Ciacci Zanella

Embrapa Swine and Poultry
Concordia, SC

BRAZIL

Influenza A Virus in Swine - Brazil

- Since 2009: Frequent outbreaks of acute respiratory disease in pigs caused by H1N1pdm
- Currently, influenza seroprevalence in pig heard is estimated in 78.1%
- H1N1pdm, human seasonal origin H1N2 and H3N2 influenza viruses are widespread in pig herds in Brazil, where they continue to evolve
- FLUAVs of human seasonal virus origin have been circulating in swine for more than a decade.

Influenza A Virus in Swine - Brazil

- A novel introduction of a human N1 in swine was detected; the most closely related human influenza virus circulated in humans between 1977 and 2009. The human-to-swine transmission probably has occurred in 2006.
- All H1N2 and H3N2 viruses sequenced so far have the internal gene segments derived from H1N1pdm
- These particular H3N2 and H1N2 swIAV clades appear to be specific to Brazil.

offlu
OIE FAO
network of expertise on animal influenza

FLUAV in Brazil

- Partial and complete gene sequences were generated for 79 FLUAVs.
- Sequence analysis of gene segments H1s, H1pdm, H3, N1 and N2.

Genotypes of IAV found in pigs in Brazil

Subtype	Year of isolation	PB2	PB1	PA	HA	NP	NA	M	NS	sw clade classif (IRD)	Total
H1N1	2009, 2010, 2011, 2012, 2013, 2014, 2015, 2016	pdm	pdm	pdm	pdm	pdm	pdm	pdm	pdm	1A3.3.2 (npdm)	45
H1N2	2011, 2013, 2014, 2015, 2016	pdm	pdm	pdm	huH1 2002	pdm	huN2 1998	pdm	pdm	1B2.2 (delta like)	18
H1N1	2011, 2014, 2015	pdm	pdm	pdm	huH1 2006	pdm	huN1 2006	pdm	pdm	Other-Human-1B.2 (delta 2)	3
H3N2	2011, 2014, 2015, 2016	pdm	pdm	pdm	huH3 1998	pdm	huN2 1998	pdm	pdm		13

H1pdm (H1N1): Multiple separate human-to-swine transmissions of H1N1pdm have occurred since 2009.

H1s (H1N2): Transmission of human seasonal H1N2 viruses to pigs occurred in the early 2000s (2002-2003).

H1s (H1N1): Transmission of human seasonal H1N1 into swine approx in 2006.

H3 (H3N2): Introduction of human H3N2 virus into swine in Brazil during late 1990s.

N2 (H1N2 and H3N2): Two different introductions of N2 segment from huH3N2 viruses into swine (late 1990s).

*NELSON M.I.; SCHAEFER R. et al. Emerging Infectious Diseases. v.21, n.8, 2015. SCHMIDT C. et al. Genome Announc. 2(6):e01319-14. 2014. RAJÃO D.S. et al. Influenza and Other Respiratory Viruses. v.7, n.5, 2013.

Detection and characterization of FLUAV in swine in a production system in Santa Catarina, Brazil

Maicom Vinícios Ferreira, DVM, MSc.

Três Sítios:

Weaner

- 22,000 sows
- 47 swine producers

Nursery

- 60,000 weaners/month
- 25 swine producers

Growing-Finishing

- 230,000 m^2
- 300 swine producers

Sampling and methods

- Prevalence: 40%
- Confidence level: 95%
- Accuracy level: 5%
- Finite population formula: 60,000 piglets
- Sampling: 367

FÓRMULA

$$n = \frac{Z^2 \times P \times Q \times N}{e^2 \times (N-1) + Z^2 \times P \times Q}$$

1) Onde:	Valor
2) Z = Nível de Confiança	95%
3) P = Quantidade de Acerto esperado (%)	40%
4) Q = Quantidade de Erro esperado (%)	60%
5) N = População Total	60.000
6) e = Nível de Precisão (%)	5%
Tamanho da amostra (n) =	367

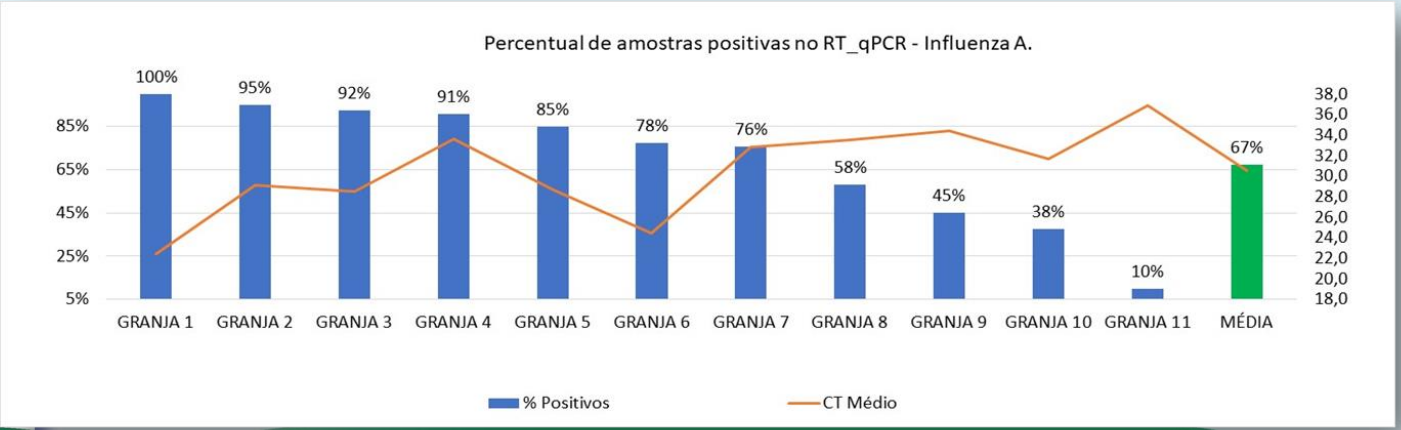
Farm	City	Latitude	Longitude	Nº Piglets	Sampling
1	CONCORDIA	27°18'02"	52°03'11"	2000	40
2	CONCORDIA	27°17'08"	52°02'58"	2020	40
3	SEARA	27°15'23"	51°53'20"	1280	26
4	JABORÁ	27°09'21"	51°48'20"	1585	32
5	CONCORDIA	27°01'15"	51°54'50"	2015	40
6	JABORÁ	27°06'44"	52°14'47"	2020	40
7	CONCORDIA	27°11'14"	51°45'43"	1640	33
8	CONCORDIA	27°07'03"	53°07'40"	3010	50
9	LINDOIA DO SUL	27°16'36"	51°03'12"	2008	40
10	ARABUTÃ	27°09'46"	51°50'23"	1600	32
11	CONCORDIA	27°05'47"	52°22'52"	2590	50
11					**423**

- Sampling of 423 pigs
- Elisa, HI
- Nasal swab: RT_qPCR
- Subtyping: multiplex PCR

Results

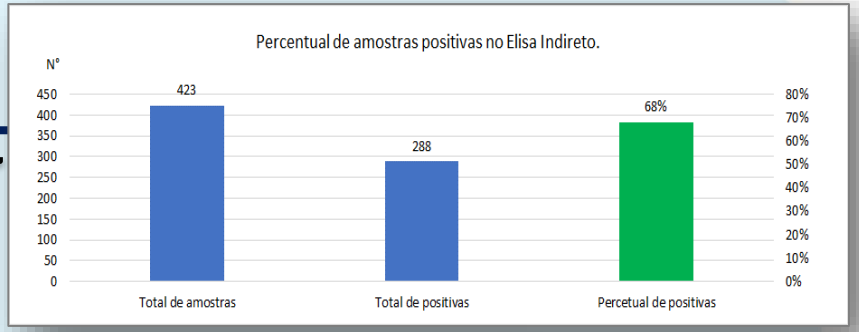
- RT_QqPCR for FLUAV

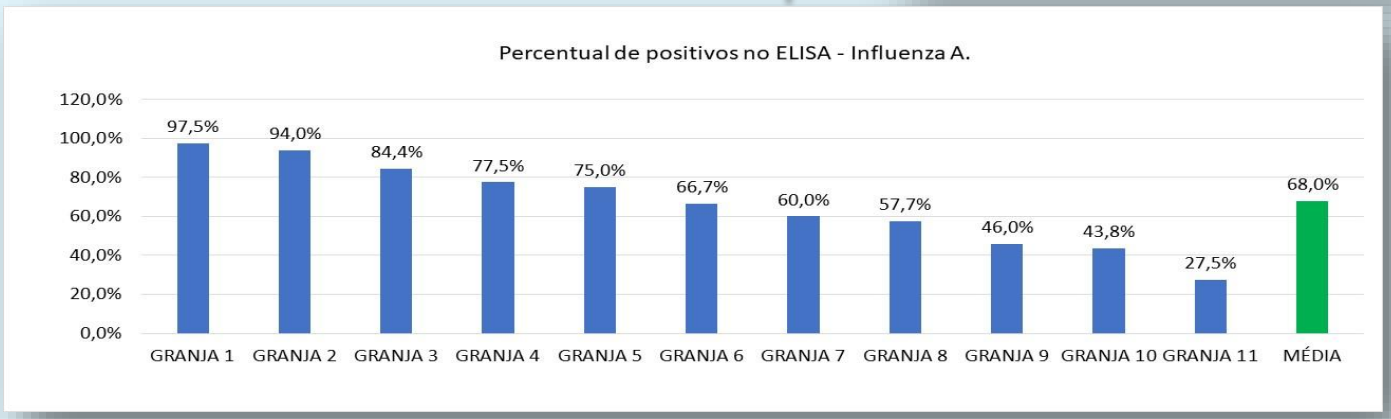
Farm	Nº Piglets	Sampling	Date	Age	Nº Origens	Nº Positive	% Positive
1	2000	40	16/08/2018	7	5	40	100,0%
2	2020	40	03/07/2018	12	4	38	95,0%
3	1280	26	24/07/2018	23	2	24	92,3%
4	1585	32	10/07/2018	19	5	29	90,6%
5	2015	40	10/07/2018	12	4	34	85,0%
6	2020	40	23/08/2018	11	5	31	77,5%
7	1640	33	24/07/2018	12	5	25	75,8%
8	3010	50	08/08/2018	17	8	29	58,0%
9	2008	40	28/06/2018	14	4	18	45,0%
10	1600	32	24/09/2018	25	4	12	37,5%
11	2590	50	03/07/2018	26	4	5	10,0%
		423		**16,2**	**4,55**	**285**	**67,4%**



Results

- Elisa: 68% positive



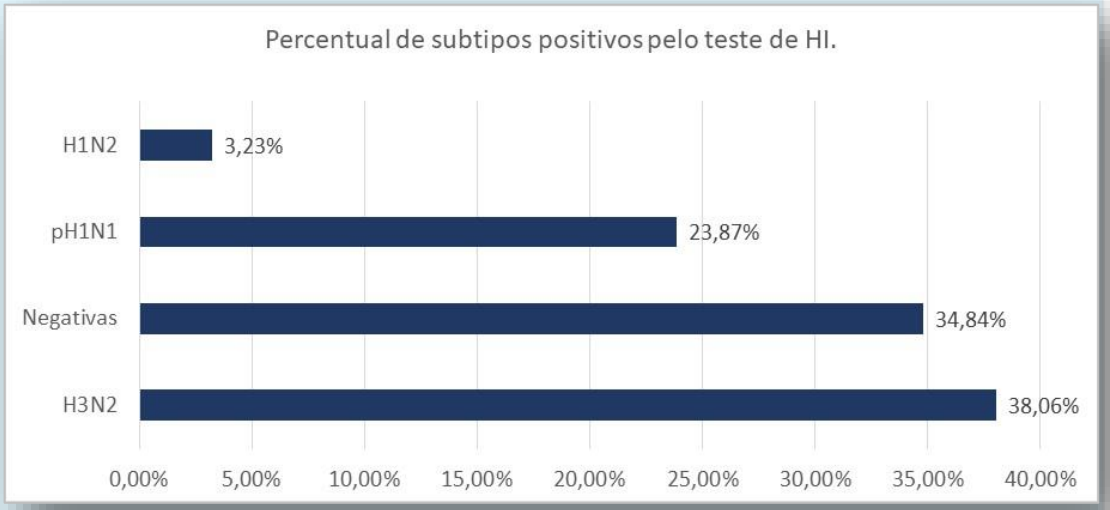


- HI: H3N2, pN1N1, H1N2

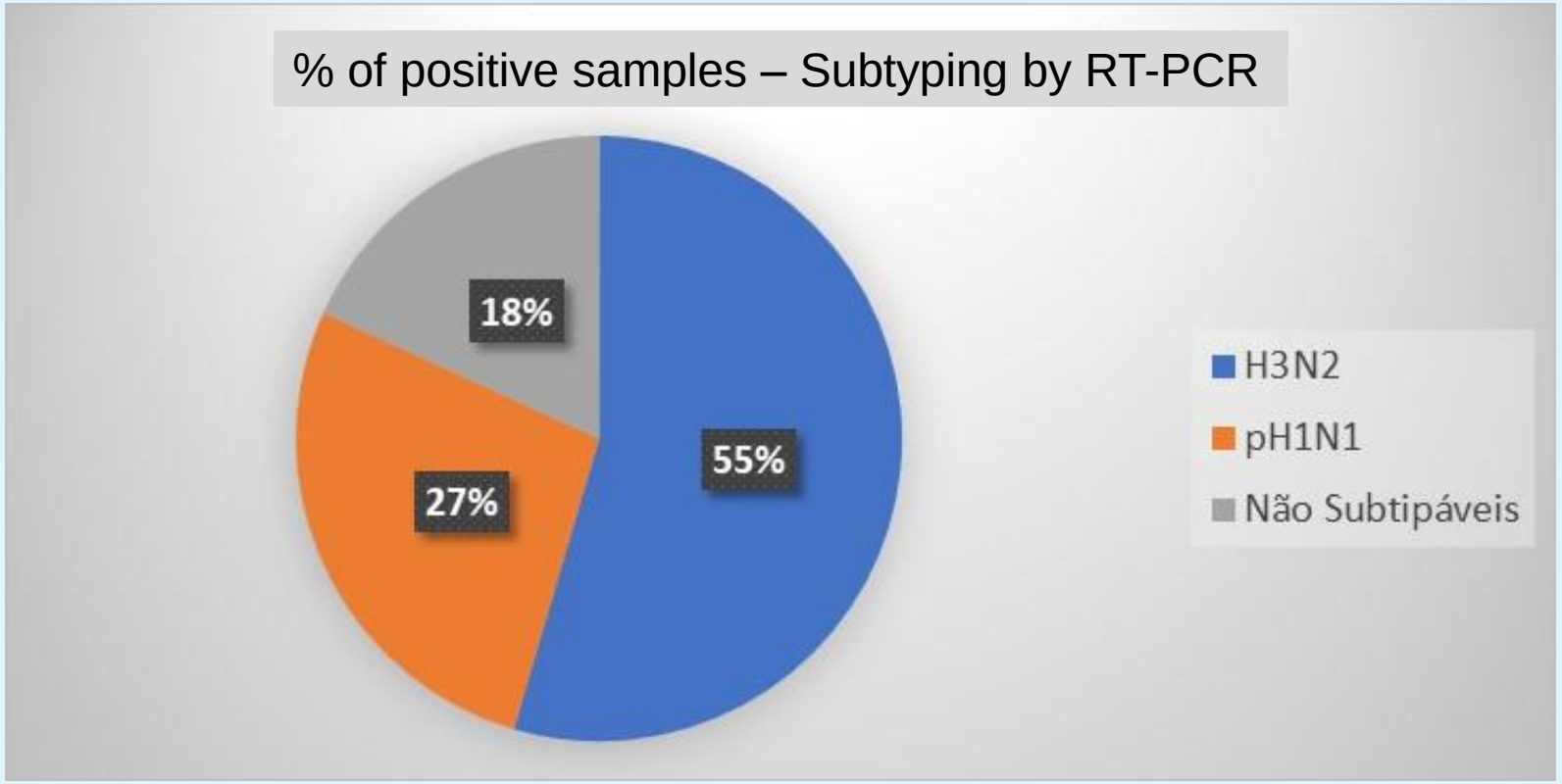
Results - HI

- H1N2- 1B2.2
- H1N1- 1A3.3.2
- H3N2 unclassified, isolated in 2015

- pH1N1 + H1N2 + H3N2 : 4
- pH1N1 + H1N2 : 4
- pH1N1 + H1N2 + H3N2 : 4
- H1N2 + H3N2 : 7
- pH1N1 + H3N2 : 27
- pH1N1: 59
- H1N2: 7
- H3N2 : 82
- Unclassified: 41



Results - Subtyping



Antigenic Cartography

1) Production of hyperimmune serum in piglets

Sera were produced against 13 samples of influenza virus:

- 2 H1N1 (1A.3.3.2/npdm*)
- 2 H1N1 (Other-Human-1B.2/ delta2)
- 2 H1N2 (1B2.2/delta-like)
- 1 H1N2 (1A.3.3.2/npdm)
- 6 H3N2.

*According to Global Swine H1Classification (tool available in IRD).

- In 2019, more hyperimmune serum is expected to be produced against new influenza samples of the H3N2 subtype.

2) Propagation of control viruses donated by the NADC / USDA

- Eleven IAVs (5 H1N1, 1 H1N2 and 5 H3N2)
- All viruses were propagated in SPF eggs or MDCK cells, and are stored at -70 ° C.

A/Swine/Iowa/1973 (H1N1)
A/Swine/Kentucky/02086/2008 (H1N1)
A/Swine/Minnesota/02011/2008 (H1N2)
A/Swine/Illinois/00685/2005 (H1N1)
A/Swine/Ohio/511445/2007 (H1N1)
A/California/04/2009 (H1N1)

A/Swine/Texas/4199-1/1998 (H3N2)
A/Swine/Colorado/23619/1999 (H3N2)
A/Swine/Minnesota/01146/2006 (H3N2)
A/Swine/New York/A01104005/2011 (H3N2)
A/Swine/Iowa/A01480656/2014 (H3N2)

3) Panel of porcine sera produced against major IAV cluster and sub-clusters (provided by the NADC / USDA).

4) Training in antigenic cartography, carried out at Embrapa Swine and Poultry on March 2018.

Instructor: Sara Lopes (University of Cambridge, UK).

Ongoing and Future Projects

- Genomic sequences deposited at Genbank: lack analysis and publication results (in 2019).
- New sequencing will be carried out in 2019 (viruses isolated between 2016 and 2019).
- Continue to monitor influenza in pigs.
- Increase sequencing capabilities and the phylogenetic analysis of Brazilian swine influenza virus sequences.
- Test vaccine protocols.

Acknowledgements

Swine Health group:
Ana Paula Bastos
Danielle Gava
Maurício E. Cantão
Marisete F. Schiochet
Marcos Morés
Neide L. Simon
Rejane Schaefer

Collaborators:
Amy L. Vincent (ARS/USDA)
Carine K. Souza (ARS/USDA)
Martha I. Nelson (FIC/NIH)
Maicom V. Ferreira (IFC)
Nicola Lewis (UC/UK)
Sara Lopes (UC/UK)
Rachel R. Rech (Texas A & M)
Lana T. Fernandes (Cedisa)
Vanessa Haach (UFRGS)
OFFLU

Funding:
Embrapa
CNPq
MISMS/FIC
ARS/USDA

Thank you

janice.zanella@embrapa.br

Embrapa
MINISTRY OF AGRICULTURE, LIVESTOCK AND FOOD SUPPLY
PÁTRIA AMADA BRASIL GOVERNO FEDERAL