OFFLU Technical Meeting

Coordinating world-wide surveillance for influenza in swine

FAO headquarters, Rome, Italy
April 16-17, 2013

Brazil update

Janice Reis Ciacci Zanella

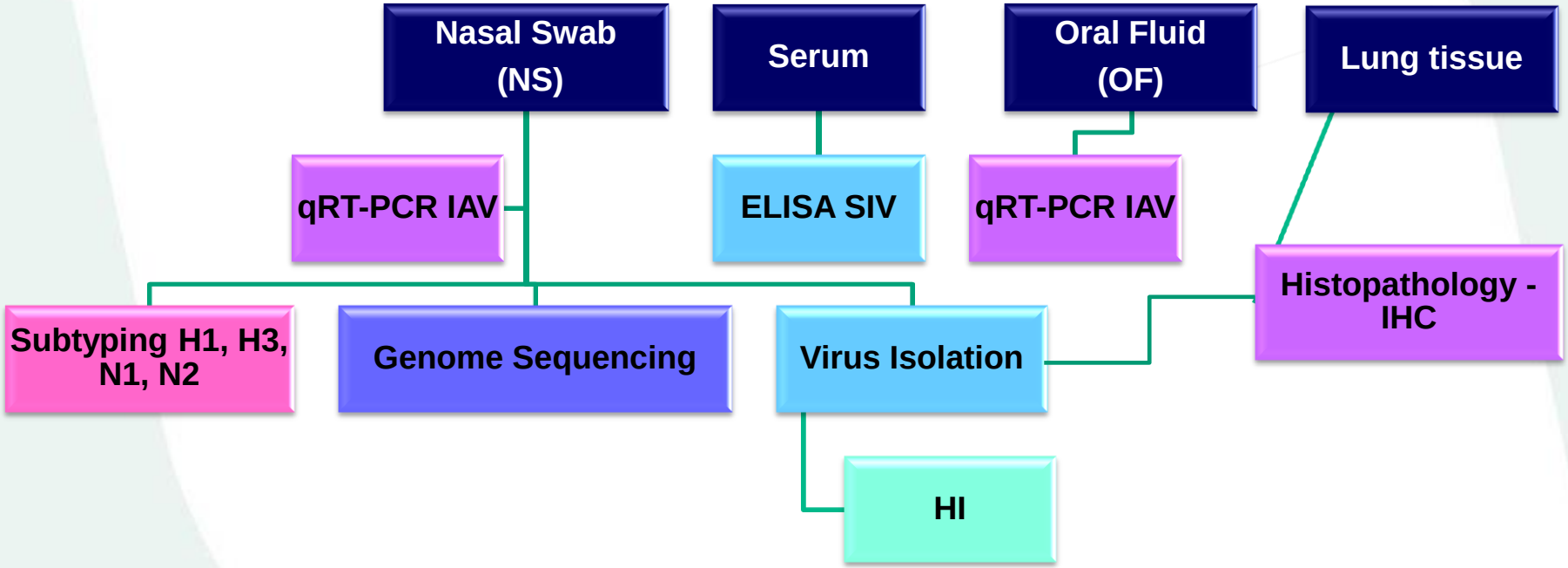
Brazilian Agricultural Research Corporation – EMBRAPA

Embrapa Swine and Poultry Research Center

Project funded by CNPq/MAPA: **"Diagnostic, molecular characterization and pathogenesis studies of infectious agents economically important for the Brazilian Swine Production".** 02/2009 – 02/2012

Embrapa Swine & Poultry

Comercial farms 2009	CNPq Project 2009 to 2012	Comercial farms 2011-2012*	Quarantine Boars	Captive wild pig	Monteiro Feral Pigs	Diagnostic Lab - CEDISA
•Plasma, OF •1 protocol •1000 plasma samples •257 OF samples •51 farms	•Serum, NS, OF •16 farms •87 pools •435 samples	•Serum, SN, FO •49 farms •288 pools •1440 samples	•Serum, OF •1 protocol •52 OF samples •471 serum samples	•Lungs, NS •2 protocols •60 lung samples	•Serum, NS, GS •2 protocols •152 samples	•Lungs •86 protocols

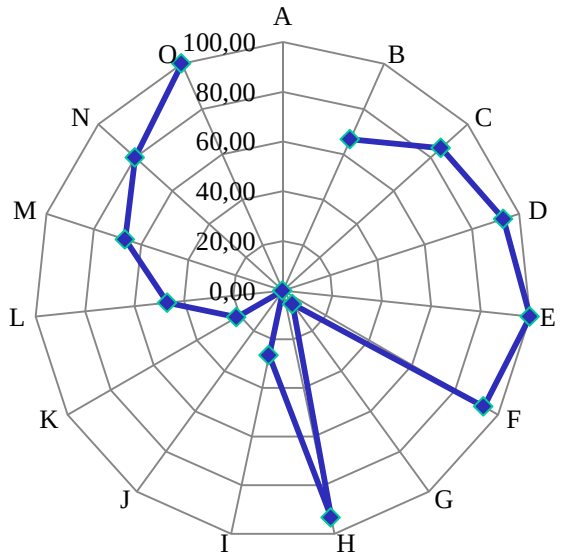


*Comercial farms 2011-2012: 8 (PR), 7 (RS), 3 (SP), 11 (SC), 10 (MG), 5 (MS) e 5 (MT)= 49
Total: 62 farms

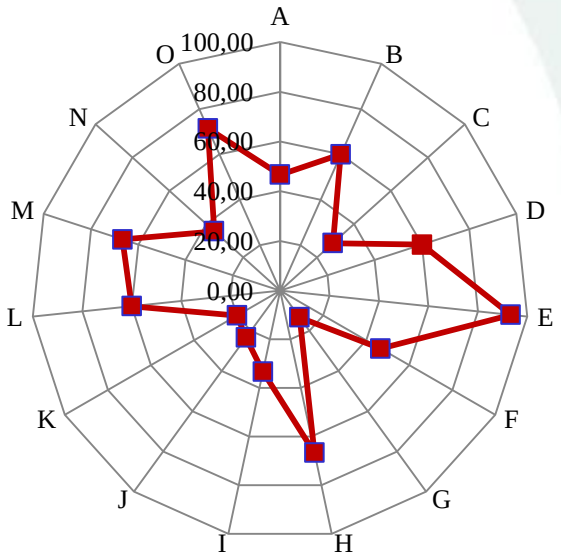
Serology – CNPq – 15 farms

Embrapa Swine & Poultry

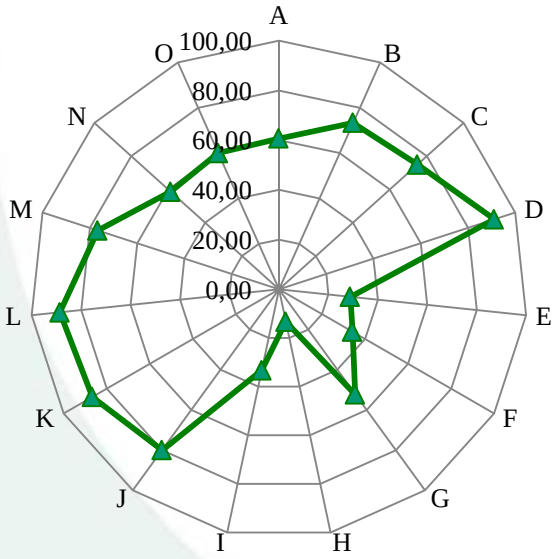
suckling



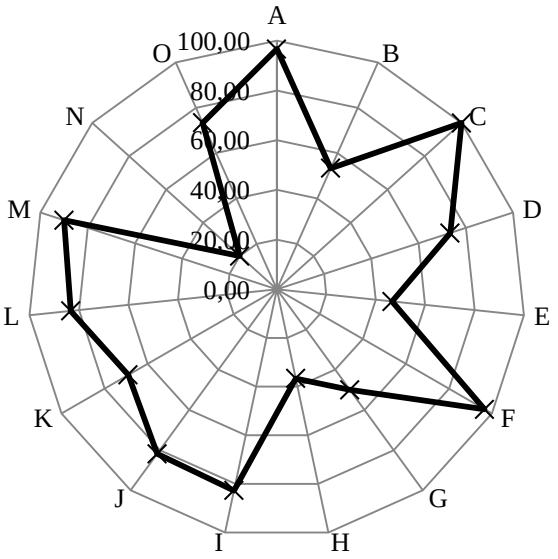
nursing



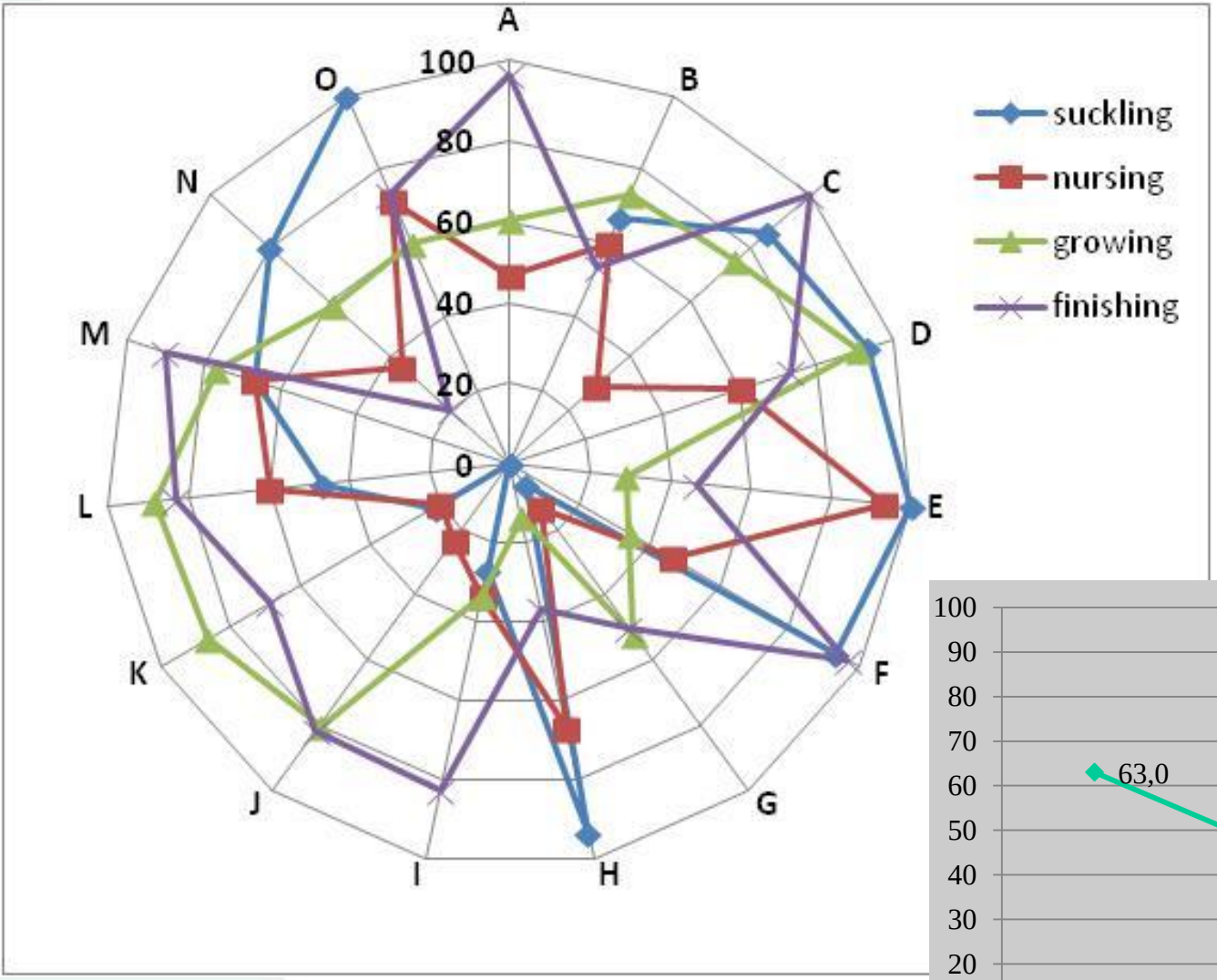
growing

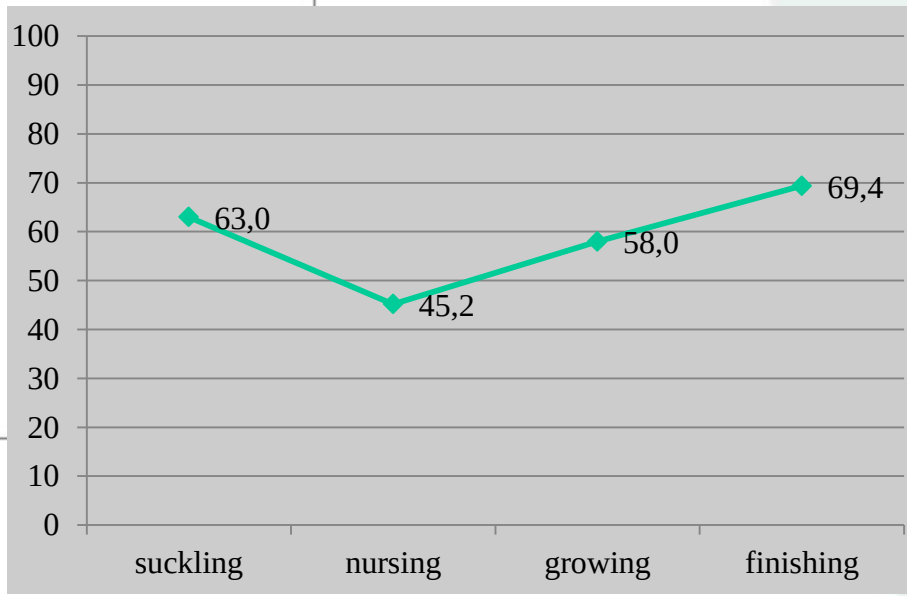


finishing



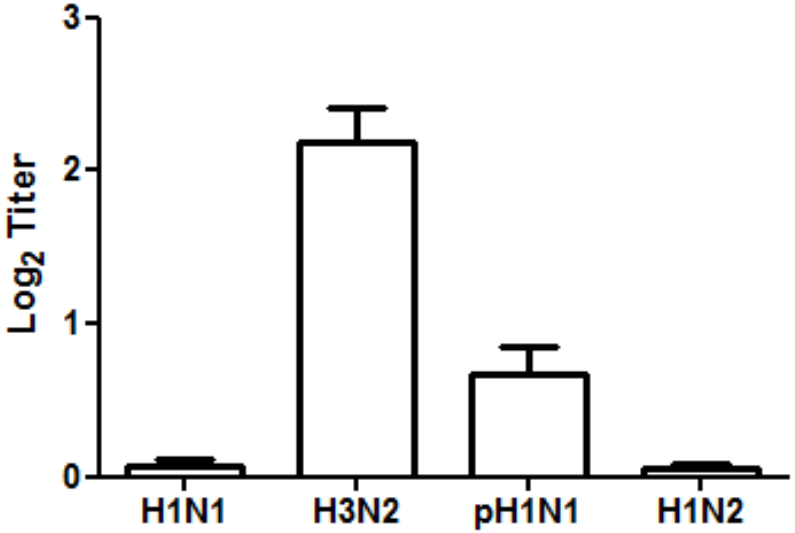
Serology – CNPq – 15 farms





HI results CNPq FF swine farms

Figure 1: HI analyses FF farms



HI results	Number of positive farms
H3N2 , pH1N1	5 /14
H3N2	5 /14
H1N1 , H3N2 , pH1N1	2 /14
H3N2 , H1N2	1 /14
H1N1 , H3N2 , H1N2	1 /14

IHC results CNPq FF swine farms

FARM	Protocol	IHC IAV	IHC Pasteurella multocida	IHC PCV2	TOTAL	HI
1	72/2010	0/15	4/15	0/15	15	ND
12	17/2011	0/4	1/4	0/4	4	H1N1, H3N2, pH1N1
11	14/2011	0/7	1/7	0/7	7	H3N2, pH1N1
13	43/2011	0/13	3/13	0/13	13	H3N2, pH1N1
14	278/2011	0/12	9/12	0/12	12	H1N1, H3N2, H1N2
16	88/2012	1/10	4/10	0/10	10	ND
TOTAL		1/61	22/61	0/61	61	

Mores et al., unpublished

INFLUENZA A VIRUSES INFECTION IN SWINE HERDS IN BRAZIL IN 2011 – 2012

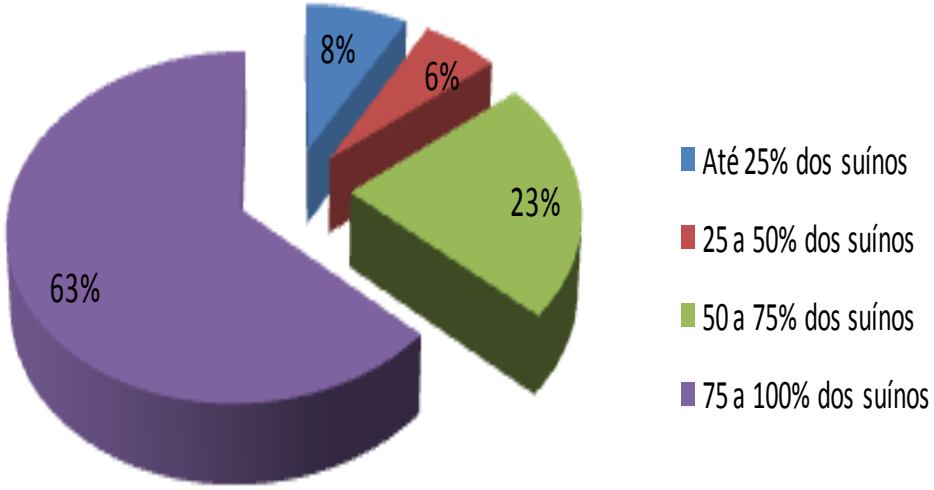
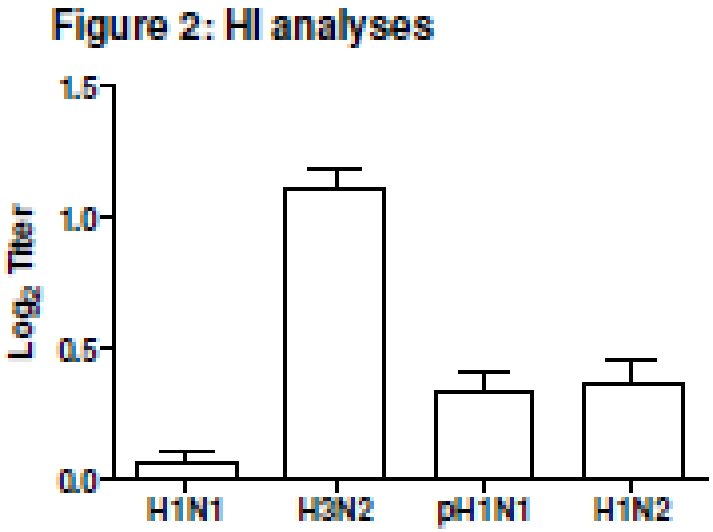


Figure 1 – Percentage of Elisa positive growing swine (8 – 12 week old pigs) in 49 Brazilian swine farms



Comercial farms 2011-2012: 8 (PR), 7 (RS), 3 (SP), 11 (SC), 10 (MG), 5 (MS) e 5 (MT)= 49 - 75% of swine production

INFLUENZA A VIRUS DETECTION IN NASAL SWABS AND ORAL FLUIDS FROM PIGS BY QUANTITATIVE REAL-TIME RT-PCR

Embrapa Swine & Poultry

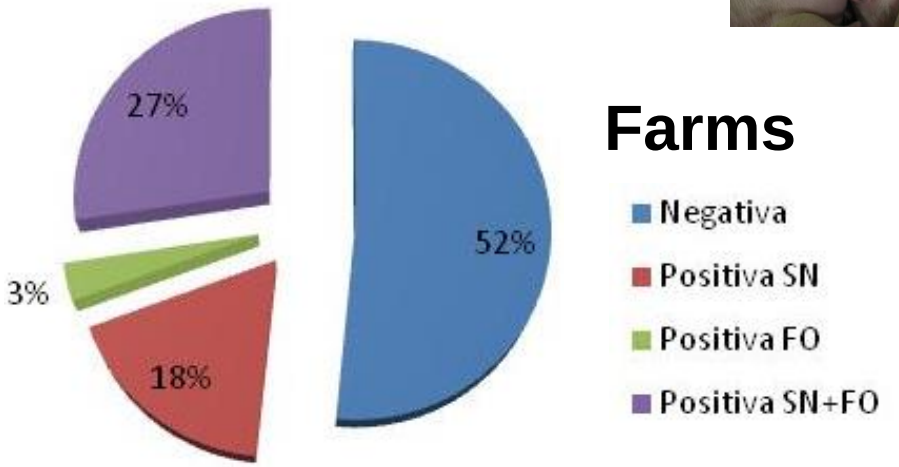
Samples

- 30 pigs/farm (8-12 week-old)
- NS pools of 5 pigs

	Nasal Swab	Oral Fluid
Samples	1875	158
Pools	375	62
Farms	62	62

Influenza A virus

- 66/375 NS pools
- 19/62 OF pools



INFLUENZA A VIRUS DETECTION IN NASAL SWABS AND ORAL FLUIDS FROM PIGS BY QUANTITATIVE REAL-TIME RT-PCR

Embrapa Swine & Poultry

Nasal Swab	Oral Fluid	IAV	pH1N1
Negative	Negative	35	36
Negative	Positive	3	0
Positive	Negative	8	11
Positive	Positive	16	15
	n	**62**	**62**
	Positive Oral Fluid (%)	30,65	24,19
	Positive Nasal Swab (%)	38,71	41,94
	Global concordance	**82,26**	**82,26**
	sensitivity	66,67	57,69
	specificity	92,11	100,00
	predictive value +	84,21	100,00
	predictive value -	81,40	76,60
	Kappa	0,613	0,613

The Global Concordance of nasal swab pools and oral fluid was 82.26% for the two tests and the Kappa index was of 0.613.
The sensitivity of real-time PCR for IAV and pH1N1 were respectively 66.67% and 57.69% for the oral fluid relative to nasal swab, whereas specificity was 92.11 and 100%, respectively .
So the oral fluid test is very specific and less sensitive.

Viral isolation samples

- **From lungs or nasal swabs: total of 50 IAV**

- Nasal swabs: 107/10, 83/10, 72/11 (7 samples SN).

  Total: 03 samples

- Lungs: 104/09, 12a/10, 83/10, 89/10, 107/10, 131/10 (2 samples), 136/10, 170b/10 (2 samples), 170c/10, 170d/10, 170e/10 (3 samples), 170f/10, 170h/10, 31/11 (2 samples), 66/11, 70/11, 71/11, 85/11, 95/11, 138/11, 146/11, 146b/11, 152/11, 173/11, 18/12, 37/12 (6 samples), 42/12, 263/12.
  Total: 47 samples

** genome sequencing data*

Subtyping – HA - NA

Protocol	Nasal swabs	Band quality	H1 - H3	N1 - N2
184/11	1	Good	**Untyped**	**Untyped**
184/11	5	Very good	**Untyped**	**Untyped**
185/11	6	Very good	**H1**	**N2**
185/11	7	Good	**Untyped**	**N2**
185/11	21	Good	Untyped	Untyped
185/11	22	Very good	Untyped	N2
231/11	1	Good	Untyped	N2
231/11	3	Good	H1	Untyped
231/11	4	Good	H1	Untyped
231/11	14	Good	Untyped	N2
232/11	13	Very good	**H1**	**N2**
232/11	14	Very good	**H1**	**N2**
232/11	26	Good	H1	N2
232/11	27	Good	H1	N2
232/11	29	Very good	H1	N2
232/11	30	Good	H1	Untyped
319/11	28	Good	Untyped	Untyped
355/11	6	Good	Untyped	Untyped
355/11	7	Good	Untyped	Untyped
365/11	6	Very good	**H1**	**N2**
365/11	7	Very good	**H3**	**N2**
365/11	9	Very good	**H1**	**N2**
366/11	28	Good	Untyped	N2
Total: 23 samples			**H1 (11) - H3 (01)**	**N2 (14)**

Embrapa Swine & Poultry

Diagnostic laboratory- CEDISA – 2009-2012

86 lungs : suckling (6%), nursing (65%), and fattening (29%) pigs with respiratory signs from pig farms in southern Brazil were submitted to a diagnostic laboratory for necropsy and/or histopathological examination and screening of respiratory agents involved in the porcine respiratory disease complex (PRDC).

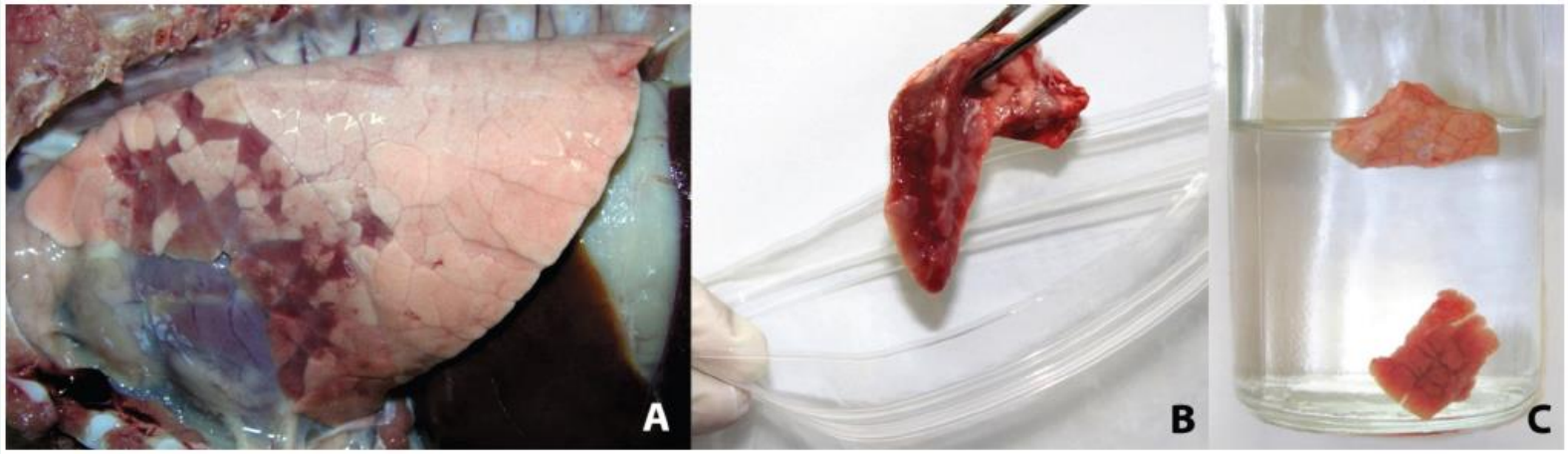
Virology:

influenza A - RT-PCR (M gene): 56/86 lungs (65%) – 44/86 typical histopathological lesions – 25/86 + IHC

A(H1N1)pdm09 - qRT-PCR: 53/56 lungs (95%)

PCV2 – PCR: 14% positive

PRRSV - qRT-PCR: all negative



Schaefer et al., 2013, Silva et. al, unpublished

Diagnostic laboratory- CEDISA – 2009-2012

Bacteriology: *Mycoplasma hyopneumoniae – IHC:* 27/86 (31%) - coinfection of influenza A and *Mhyo* was seen in 17 (20%) cases

Bacterial isolation:

Pasteurella multocida type A (8/18), followed by *Haemophilus parasuis* (4/18), *Actinobacillus pleuropneumoniae* (3/18), and *Salmonella choleraesuis* (3/18).

Histopathological lesions associated with influenza virus: 43/86 (50%) had characterized by necrotizing bronchiolitis/bronchitis and/or bronchointerstitial pneumonia with bronchiolar/bronchial hyperplasia. In those cases, 58% (25/43) were positive in the IHC for influenza A antigen.

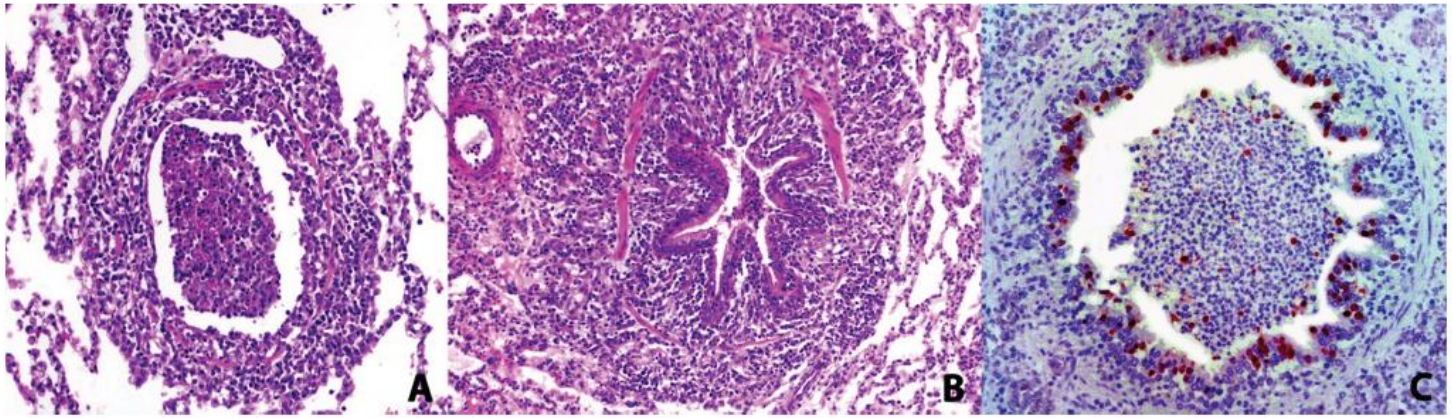


Fig.6. Pulmão suíno com lesão microscópica de influenza A. (**A**) Bronquiolite necrosante. HE, obj.10x.(**B**) Pneumonia broncointersticial. HE, obj.10x. (**C**) Marcação do antígeno viral (nucleoproteína) no núcleo das células epiteliais bronquiolares íntegras e descamadas dentro do lúmen. O lúmen do bronquíolo está parcialmente obliterado por neutrófilos degenerados. Imuno-histoquímica, obj.20x.

Schaefer et al., 2013, Silva et. al, unpublished

Genome Sequencing

Embrapa Swine & Poultry

- Sequencing data for HA, M and NA (some genomes with complete genome, other partial sequencing).

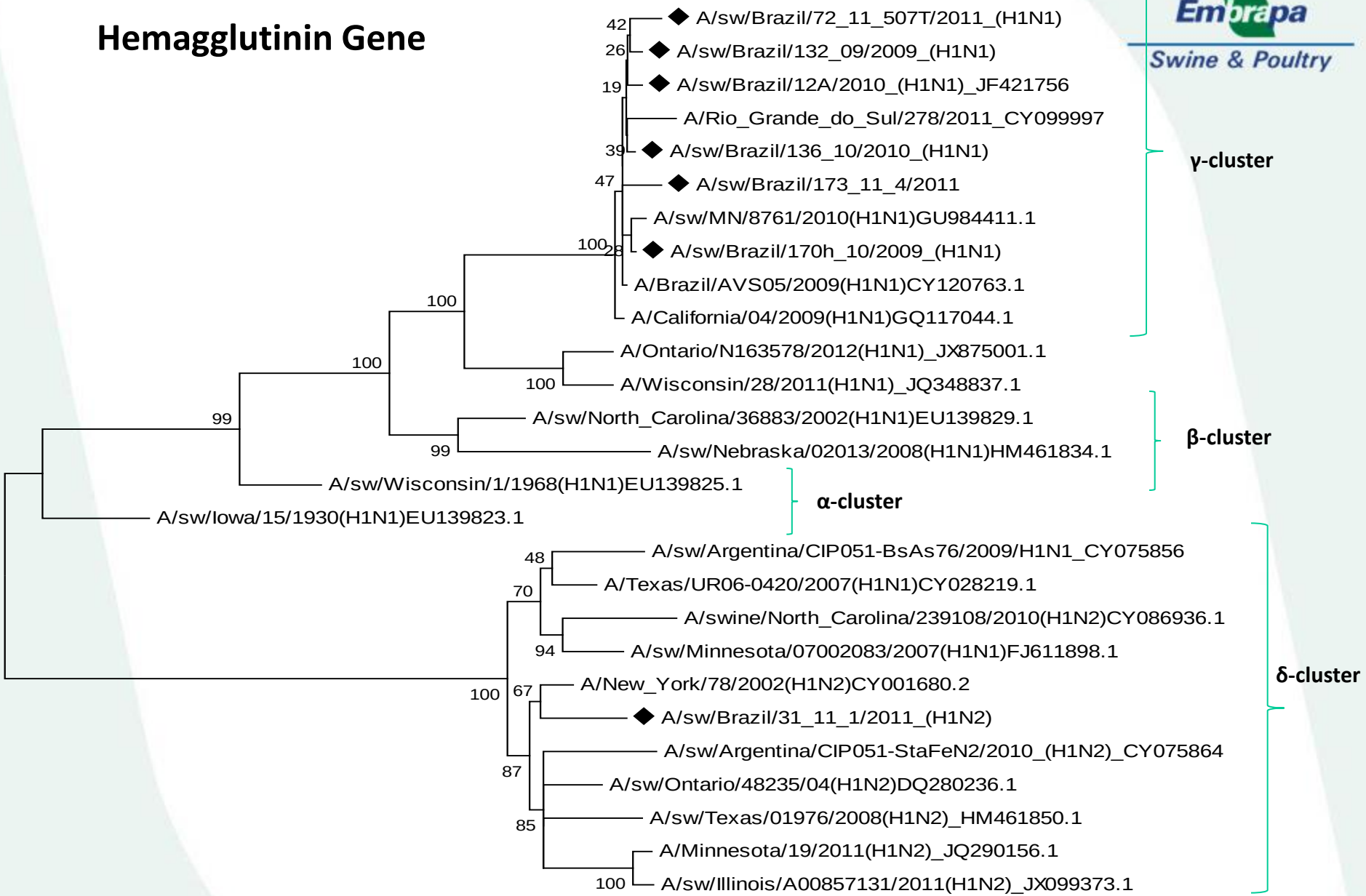
  HA, NA and M : samples 12a-10, 72-11-507T, 95-11, 104-09-S1, 104-09-S7, 104-09-S8, 107-10, 132-09, 136-10, 170e-10, 170h-10 e 173-11-4. **Total: 12 samples**

  NA and M: 18-12, 66-11, 70-11-2, 71-11, 83-10, 85-11, 98-10, 106-09, 131-10-1, 131-10-2, 152-11-1, 173-11-11 e 72-11-440. **Total: 13 samples**

  All 8 segments: sample 31-11-1.

- **Total of 26 samples sequenced**

Hemagglutinin Gene



Schaefer et al., unpublished

software MEGA 5.05 , Maximum Likelihood, no. of bootstrap replications 1000

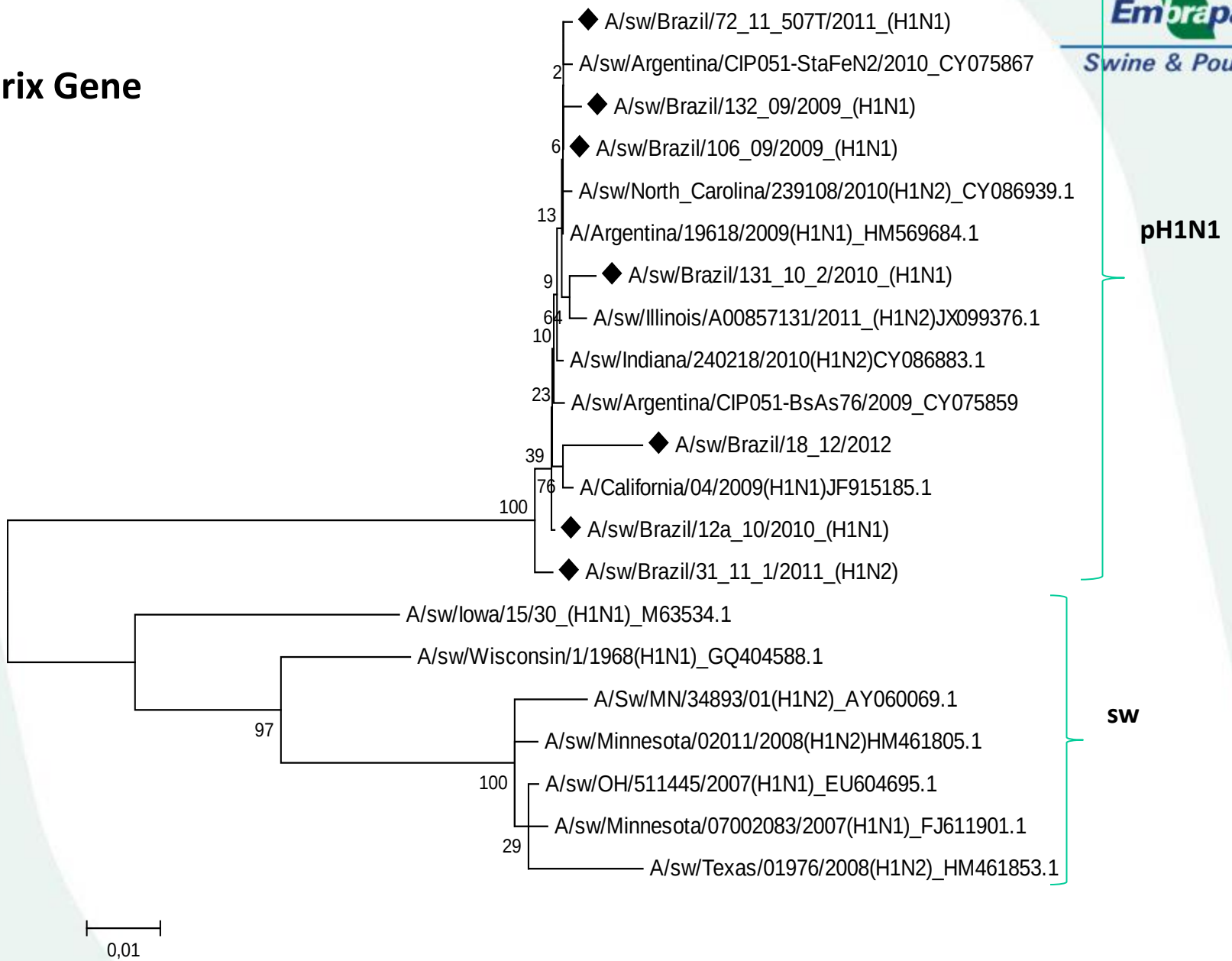
Sequence Identity Matrix- Hemagglutinin Gene

Seq->	72_11_507T/2011	107_10/2010	132_09/2009	136_10/2010	170h_10/2009	170e_10/2009	173_11_4/2011	31_11_1/2011
72_11_507T/2011	ID	0,986	0,989	0,99	0,99	0,99	0,982	0,757
107_10/2010	0,986	ID	0,993	0,995	0,995	0,994	0,987	0,758
132_09/2009	0,989	0,993	ID	0,997	0,997	0,996	0,987	0,759
136_10/2010	0,99	0,995	0,997	ID	0,998	0,998	0,989	0,759
170h_10/2009	0,99	0,995	0,997	0,998	ID	0,998	0,99	0,759
170e_10/2009	0,99	0,994	0,996	0,998	0,998	ID	0,989	0,76
173_11_4/2011	0,982	0,987	0,987	0,989	0,99	0,989	ID	0,759
31_11_1/2011	0,757	0,758	0,759	0,759	0,759	0,76	0,759	ID

8 IAVs- 75 to 99% of nucleotide identity (BioEdit 7.0)

Schaefer et al., unpublished

Matrix Gene



Schaefer et al., unpublished

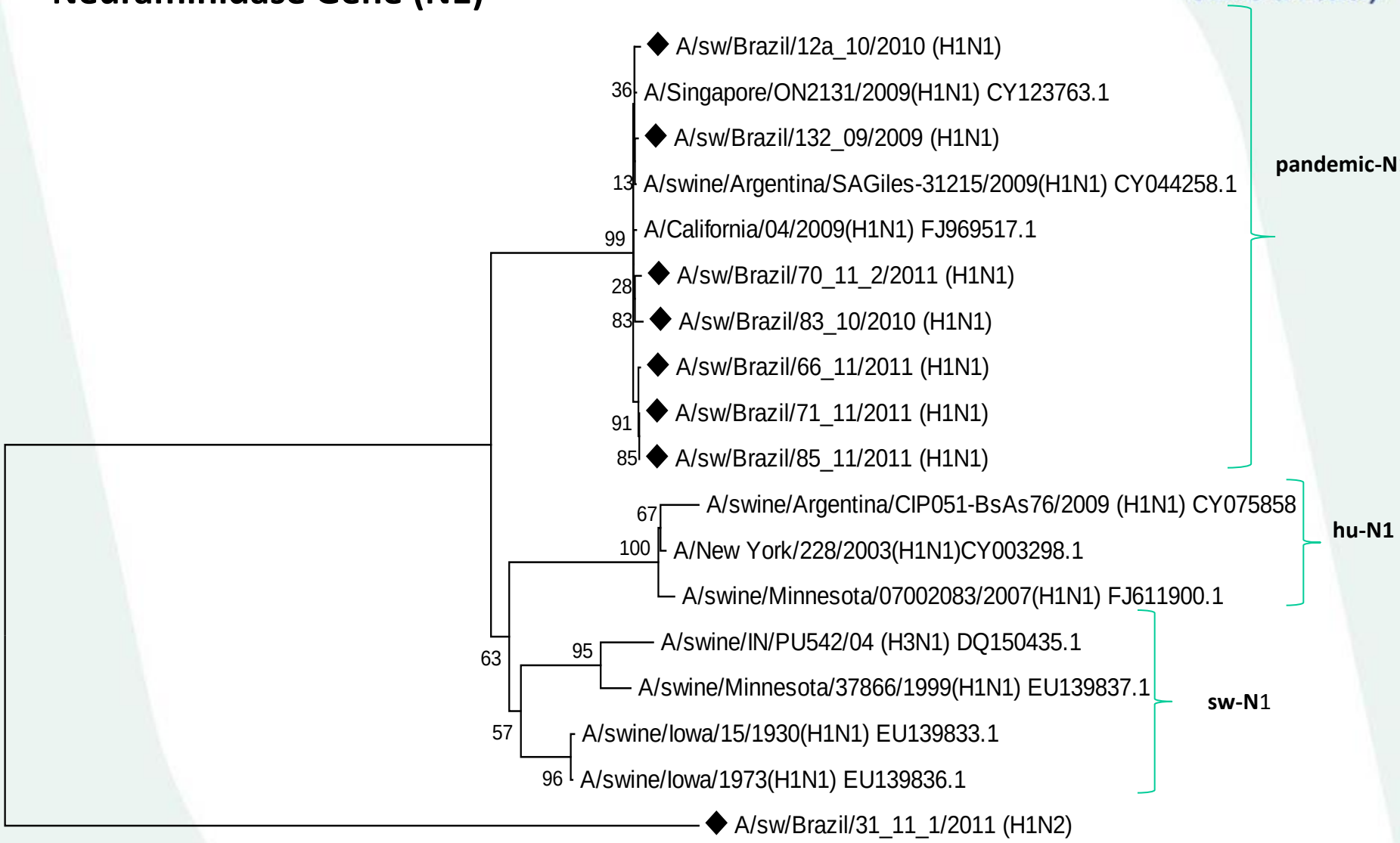
Sequence Identity Matrix- Matrix Gene

Seq->	12a_10	72_11_507T	95_11	107_10	132_09	136_10	170h_10	170e_10	173_11_4	104_09_S1	104_09_S7	104_09_S8	18_12
12a_10/2010	ID	0,997	0,995	1	0,996	0,997	0,998	0,998	0,996	0,998	0,997	0,998	0,985
72_11_507T	0,997	ID	0,995	0,997	0,996	0,997	0,998	0,998	0,996	0,998	0,997	0,998	0,985
95_11/2011	0,995	0,995	ID	0,995	0,993	0,995	0,996	0,996	0,993	0,996	0,995	0,996	0,983
107_10	1	0,997	0,995	ID	0,996	0,997	0,998	0,998	0,996	0,998	0,997	0,998	0,985
132_09	0,996	0,996	0,993	0,996	ID	0,996	0,997	0,997	0,995	0,997	0,996	0,997	0,984
136_10	0,997	0,997	0,995	0,997	0,996	ID	0,998	0,998	0,996	0,998	0,997	0,998	0,985
170h_10/2009	0,998	0,998	0,996	0,998	0,997	0,998	ID	1	0,997	1	0,998	1	0,986
170e_10/2009	0,998	0,998	0,996	0,998	0,997	0,998	1	ID	0,997	1	0,998	1	0,986
173_11_4	0,996	0,996	0,993	0,996	0,995	0,996	0,997	0,997	ID	0,997	0,996	0,997	0,986
104_09_S1	0,998	0,998	0,996	0,998	0,997	0,998	1	1	0,997	ID	0,998	1	0,986
104_09_S7	0,997	0,997	0,995	0,997	0,996	0,997	0,998	0,998	0,996	0,998	ID	0,998	0,985
104_09_S8	0,998	0,998	0,996	0,998	0,997	0,998	1	1	0,997	1	0,998	ID	0,986
18_12	0,985	0,985	0,983	0,985	0,984	0,985	0,986	0,986	0,986	0,986	0,985	0,986	ID

22 IAVs- 98 to 100% of nucleotide identity (BioEdit 7.0)

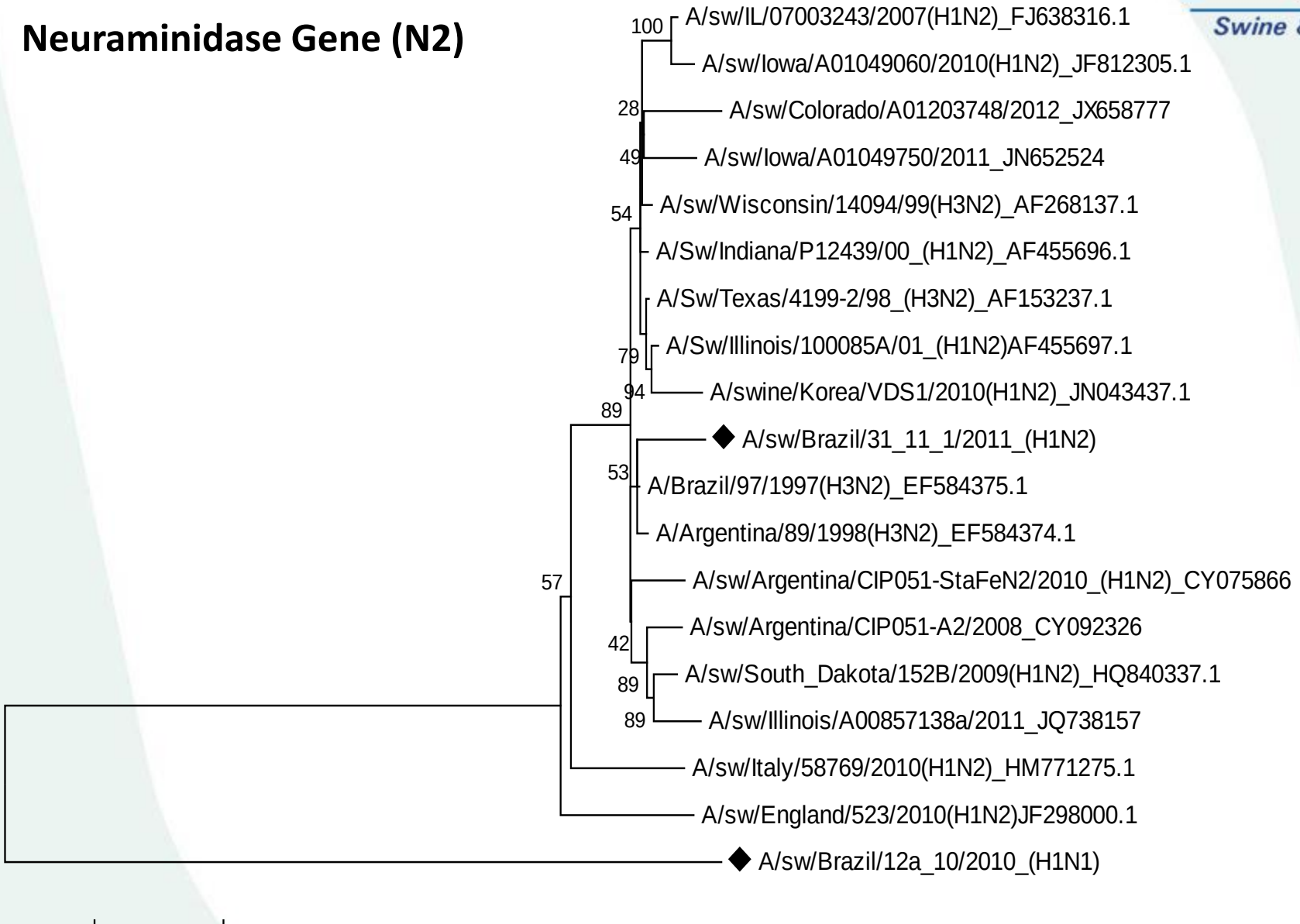
Schaefer et al., unpublished

Neuraminidase Gene (N1)



Schaefer et al., unpublished

Neuraminidase Gene (N2)



Schaefer et al., unpublished


10 KEEP CALM
20 CUTOFF += 0.01
30 GOTO 10