ESFLU Annual Meeting 2025
Copenhagen

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Influenza Network

Prevalence and genetic diversity of swine influenza virus in Spanish pig farms

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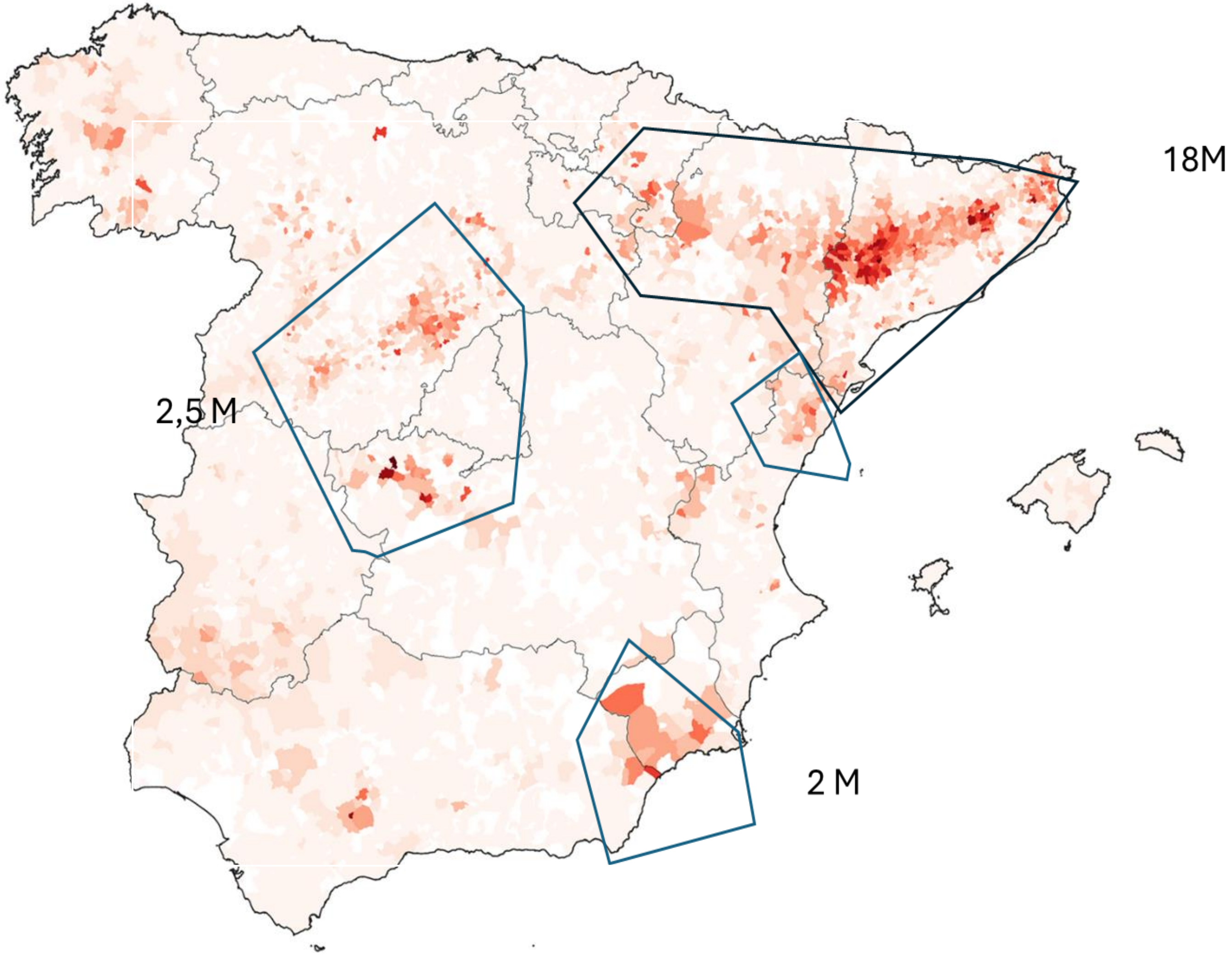
INTRODUCTION



- At a global level, most of the pig farms are endemically infected with influenza, driven by high replacement rates and the continuous introduction of susceptible animals

- Within Europe, Spain is one of the leading countries for pig production, with a total census of 34 million pigs in 2024

- In Spain, pig production is spread across the country with some areas with high density of farms

- Farms in these areas are often in close proximity to each other, which facilitates the spread and genetic exchange of virus strains.

Objectives of the study

1. Analyze the phylodynamics and phylogeography of Influenza A in pig production systems.

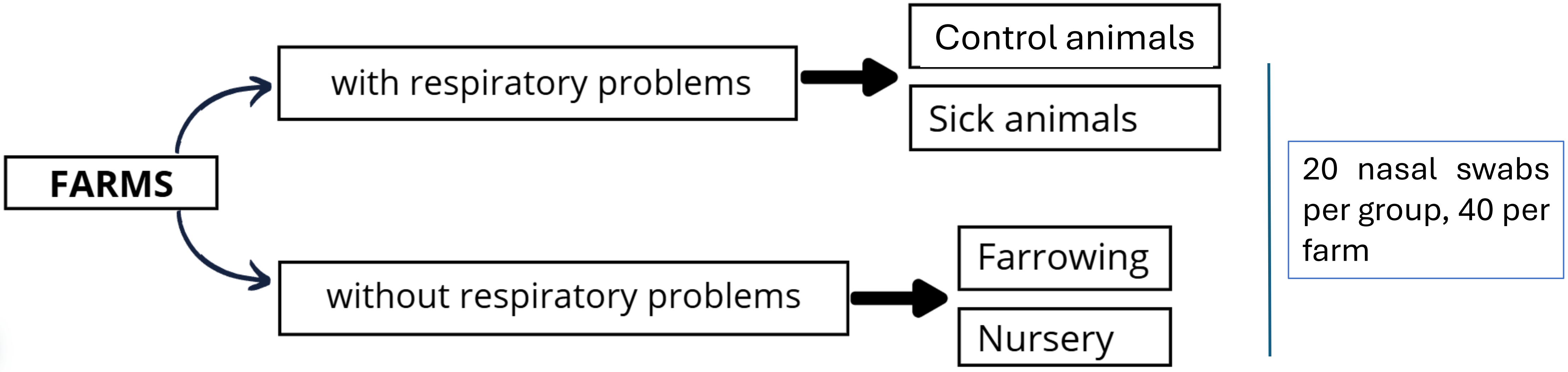
2. Identify potential routes of introduction and spread of swine influenza viruses between farms.

MATERIALS AND METHODS

Sample collection

Evaluation of about 80 farms of 5 large companies located across Spain

Samplings are scheduled every 6 months during 2 years (starting on March 2024)



20 nasal swabs per group, 40 per farm

+ Biosecurity survey of each farm

Sample processing

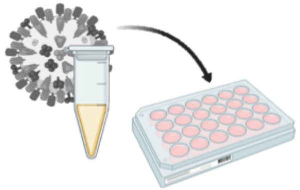
Viral RNA extraction

Viral RNA is extracted using a commercial kit - MagMax CORE Nucleic Acid Purification Kit

RT-qPCR

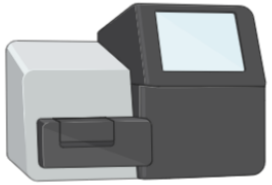
Primers and probe targeted to the IAV matrix gene (Busquets et al. 2010)

Virus isolation



Madin–Darby canine kidney (MDCK) cells are used for the isolation of IAV from RT-qPCR positive samples

Sequencing



MDCK-isolated viruses using Illumina® MiSeq platform

Phylogenetic analyses

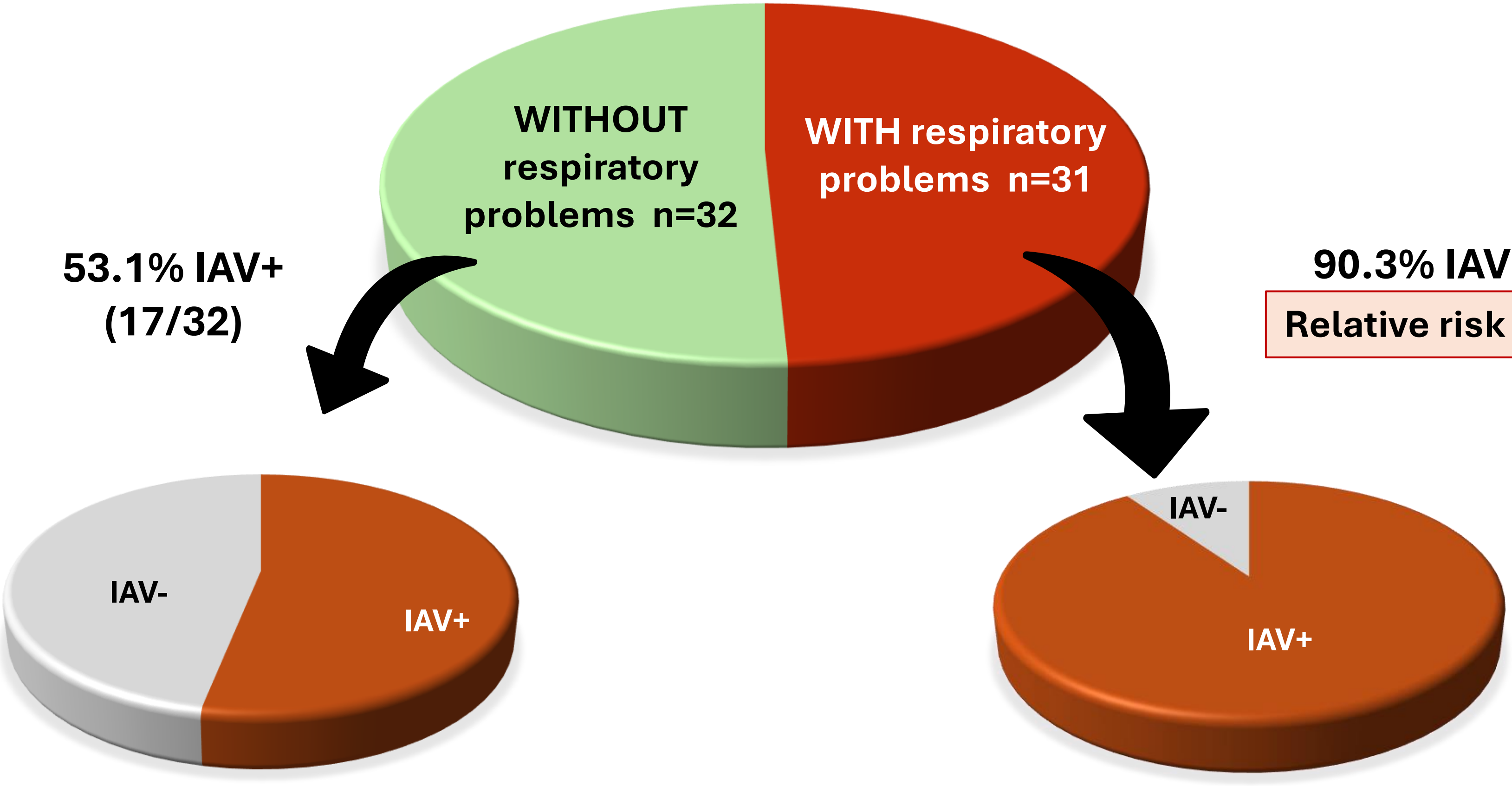
High-quality reads are filtered using IAV sequence references

If a viral quasi-species is obtained, genome consesus are generated

RESULTS

FIRST ROUND SAMPLING

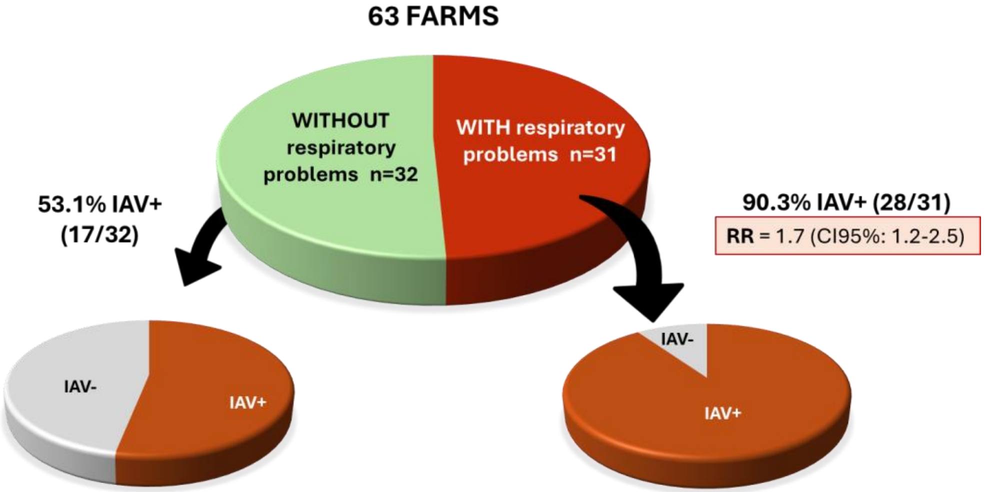
63 FARMS



53.1% IAV+ (17/32)

90.3% IAV+ (28/31)

Relative risk = 1.7 (CI95%: 1.2-2.5)



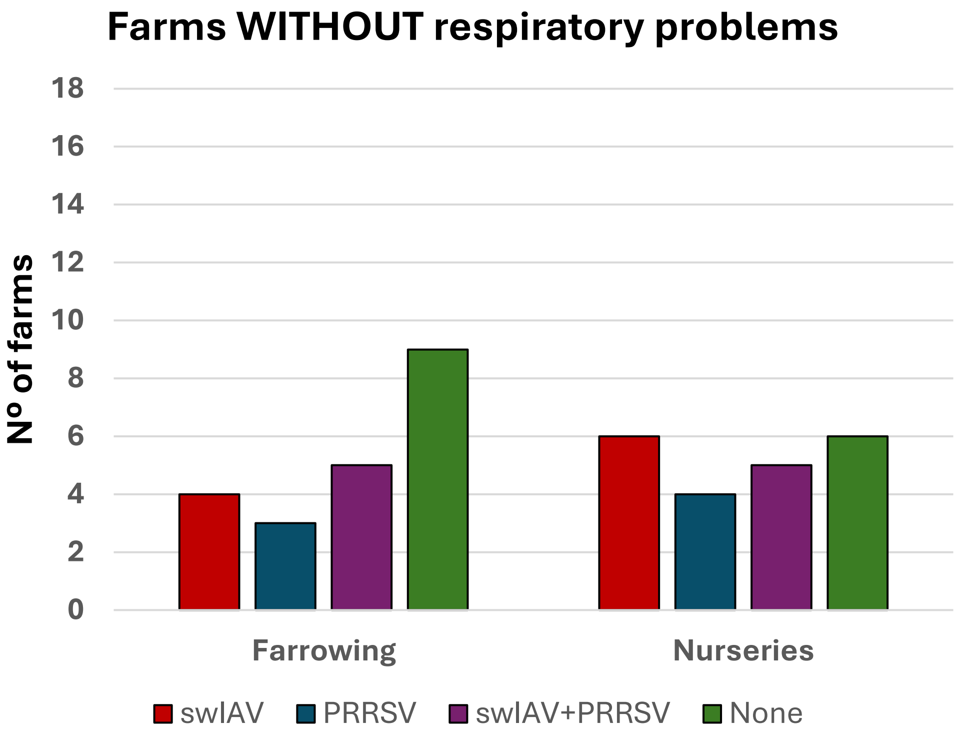
	IAV+
Farrowing only (n=11)	45.4% (5/11)
Farrowing + Nursery (n=10)	40% (4/10)
Nursery only (n=11)	72.7% (8/11)

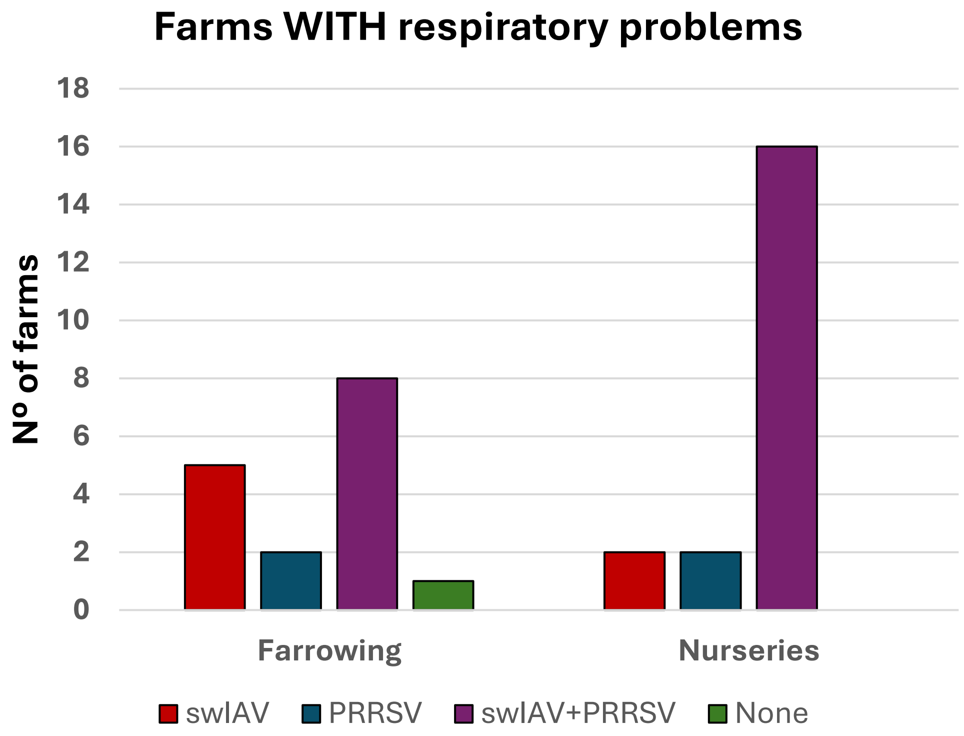
P=0.067

P=0.240

P=0.690

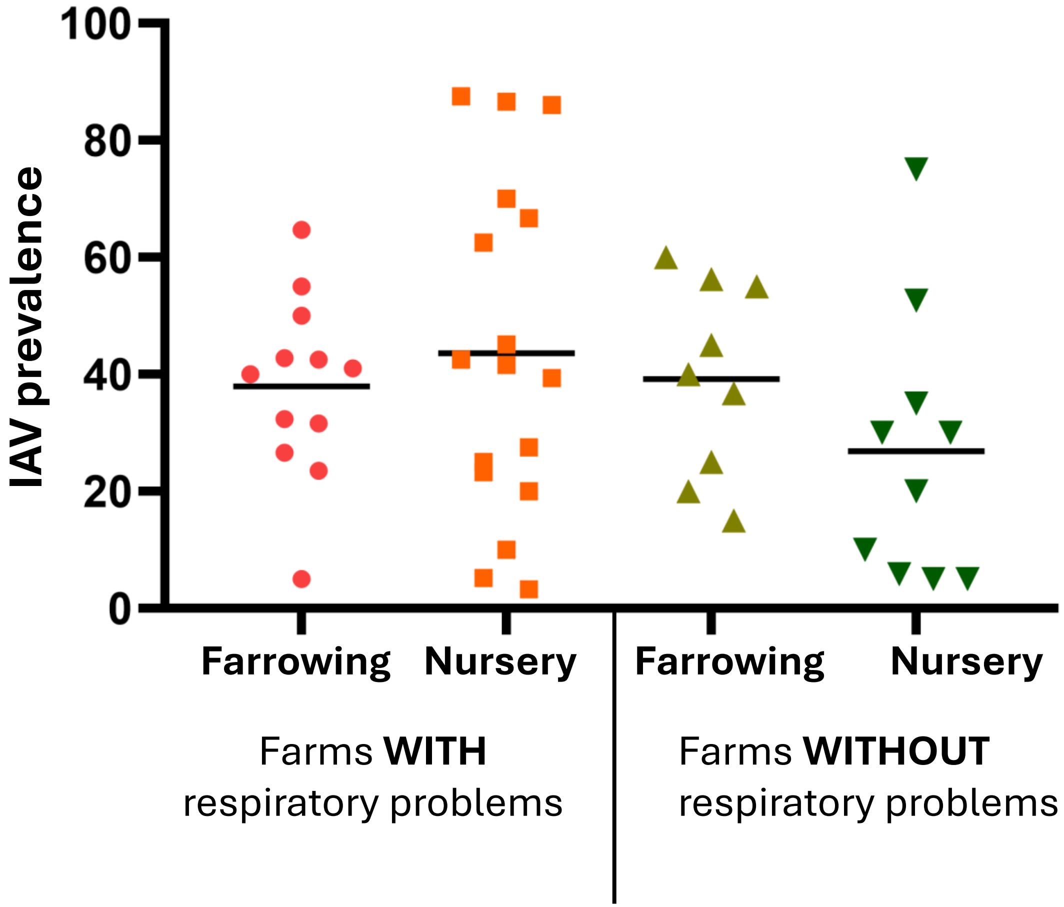
	IAV+
Farrowing only (n=11)	90.9% (10/11)
Farrowing + Nursery (n=5)	100% (5/5)
Nursery only (n=15)	86.6% (13/15)


Farms WITHOUT respiratory problems

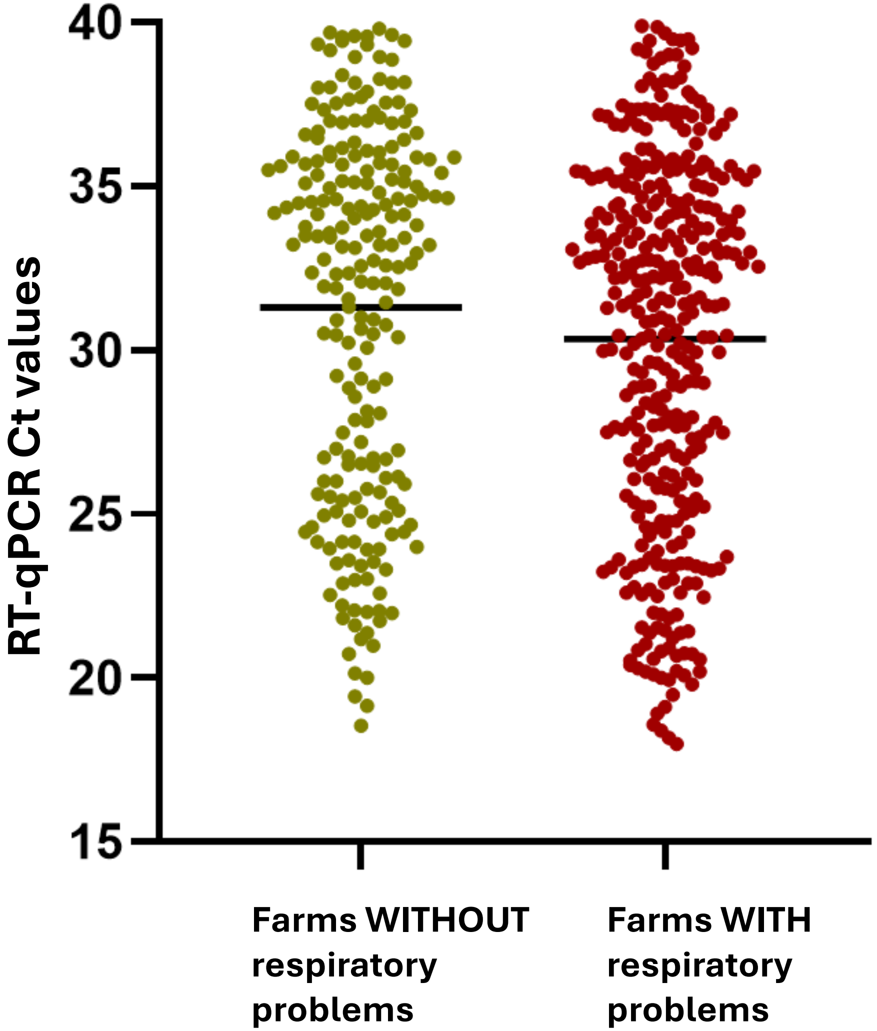

Farms WITH respiratory problems

Co-infections with PRRSV were predominant in clinically affected nurseries or farrowing units

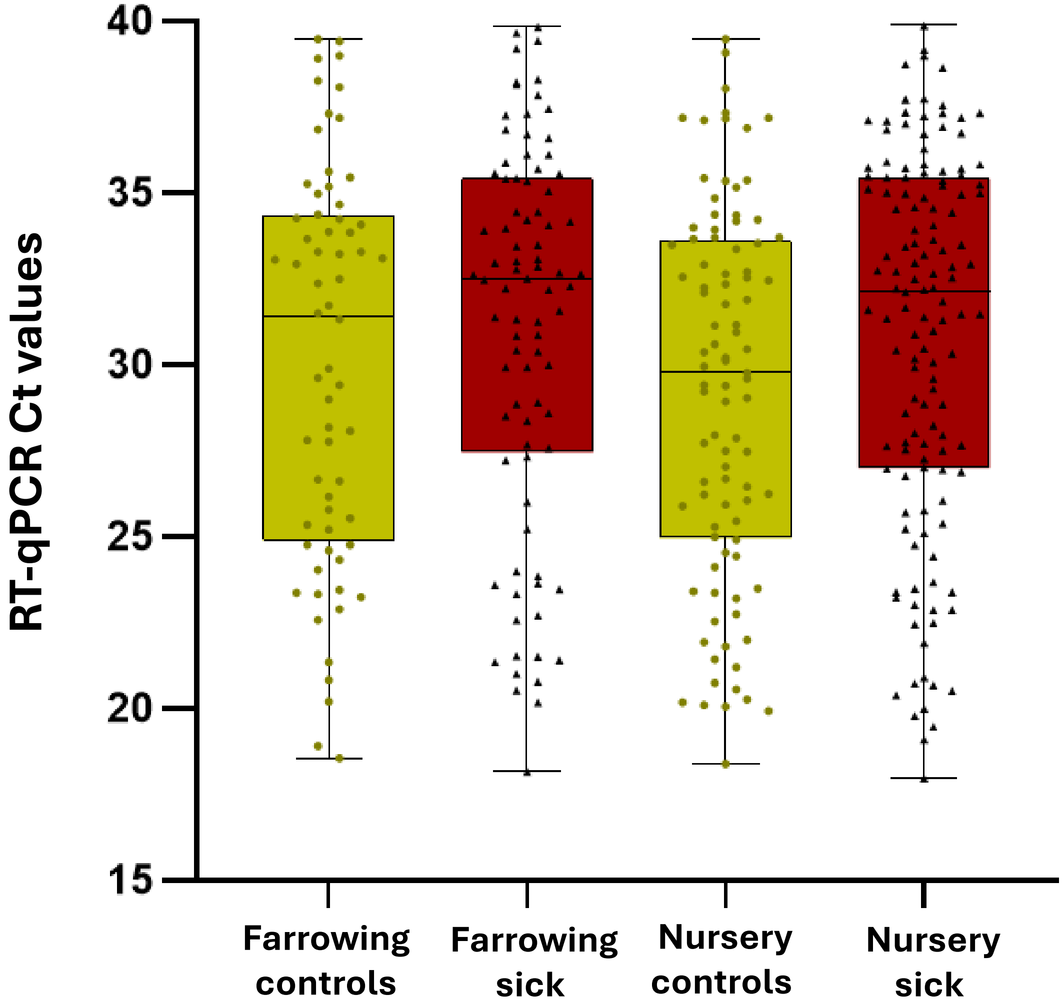
Prevalence of IAV in farms WITH and WITHOUT problems by age groups is non-significantly different



The distribution of Ct values for IAV in farms WITH and WITHOUT respiratory problems is non-significantly different



Distribution of swIAV RT-qPCR Ct values by group and age in farms WITH respiratory problems

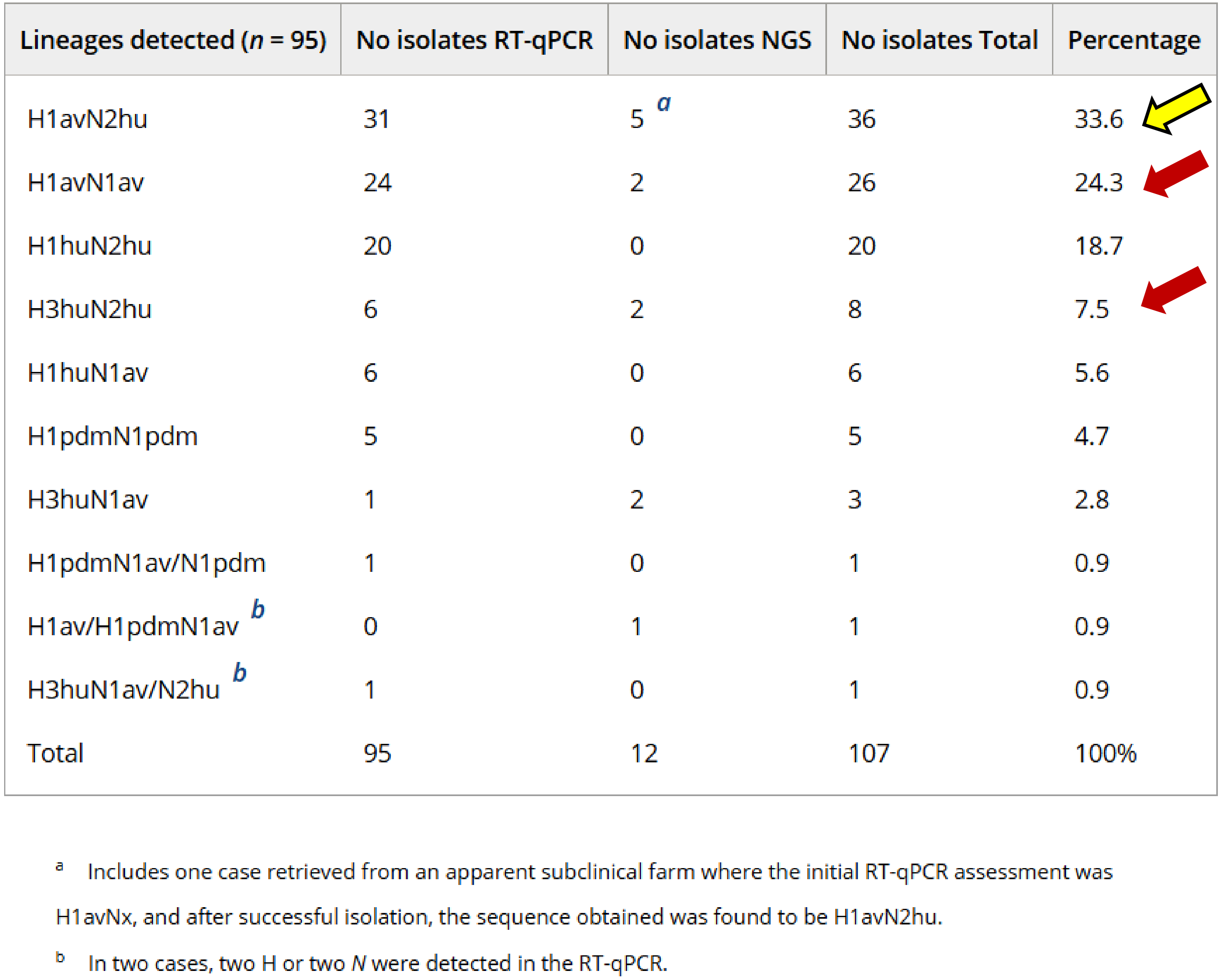


Detected lineages

Lineage detected	Isolates NGS	Percentage
H1avN1av	8	34.8
H3huN2hu	6	26.1
H1avN2hu	3	13
H1huN2hu	2	8.7
H3huN1av	1	4.4
H1huN1av/N2hu	1	4.4
H1pdmN1pdm	1	4.4
H3huN1av/N2hu	1	4.4
Total	23	100

In 5 farms co-circulation of 2 different lineages at the same moment

In 4 of these 5 farms the lineage present changed over consecutive samplings



Lineages detected (*n* = 95)	No isolates RT-qPCR	No isolates NGS	No isolates Total	Percentage
H1avN2hu	31	5 [a]	36	33.6
H1avN1av	24	2	26	24.3
H1huN2hu	20	0	20	18.7
H3huN2hu	6	2	8	7.5
H1huN1av	6	0	6	5.6
H1pdmN1pdm	5	0	5	4.7
H3huN1av	1	2	3	2.8
H1pdmN1av/N1pdm	1	0	1	0.9
H1av/H1pdmN1av [b]	0	1	1	0.9
H3huN1av/N2hu [b]	1	0	1	0.9
Total	95	12	107	100%

[a] Includes one case retrieved from an apparent subclinical farm where the initial RT-qPCR assessment was H1avNx, and after successful isolation, the sequence obtained was found to be H1avN2hu.

[b] In two cases, two H or two *N* were detected in the RT-qPCR.

Distribution of detected lineages between 2017 – 2019 (Sosa-Portugal et al. 2020)

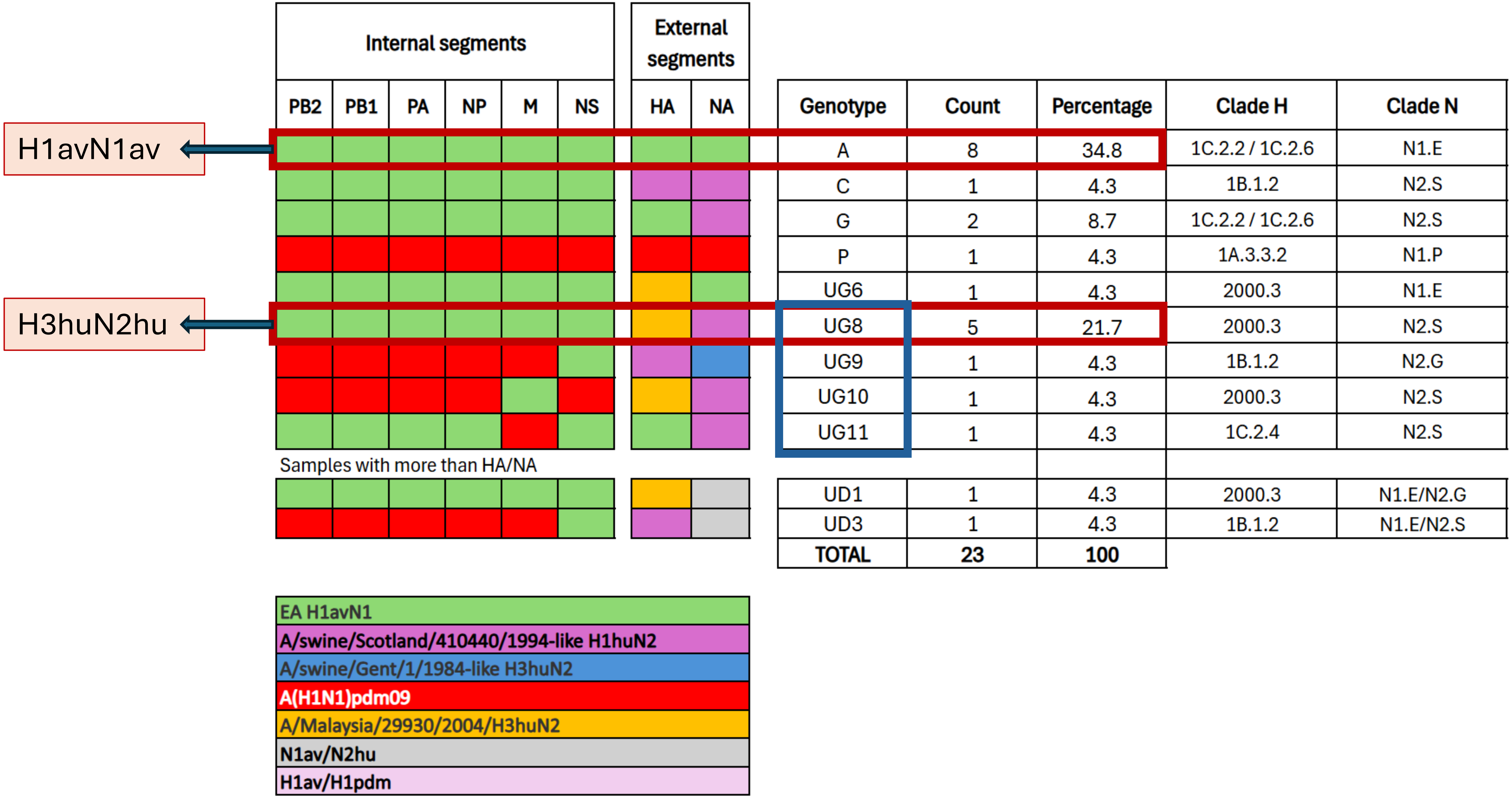
Lineages detected in farms WITH respiratory problems

Lineages detected	Isolates NGS	Percentage
H3huN2hu	3	**42.8**
H1avN1av	1	14.3
H1pdmN1pdm	1	14.3
H1huN2hu	1	14.3
H3huN1av	1	14.3
Total	7	100

Lineages detected in farms WITHOUT respiratory problems

Lineages detected	Isolates NGS	Percentage
H1avN1av	7	**43.7**
H1avN2hu	3	18.7
H3huN2hu	3	18.7
H1huN2hu	1	6.3
H1huN1av/N2hu	1	6.3
H3huN1av/N2hu	1	6.3
Total	16	100

Genotypes



H1avN1av ←

H3huN2hu ←

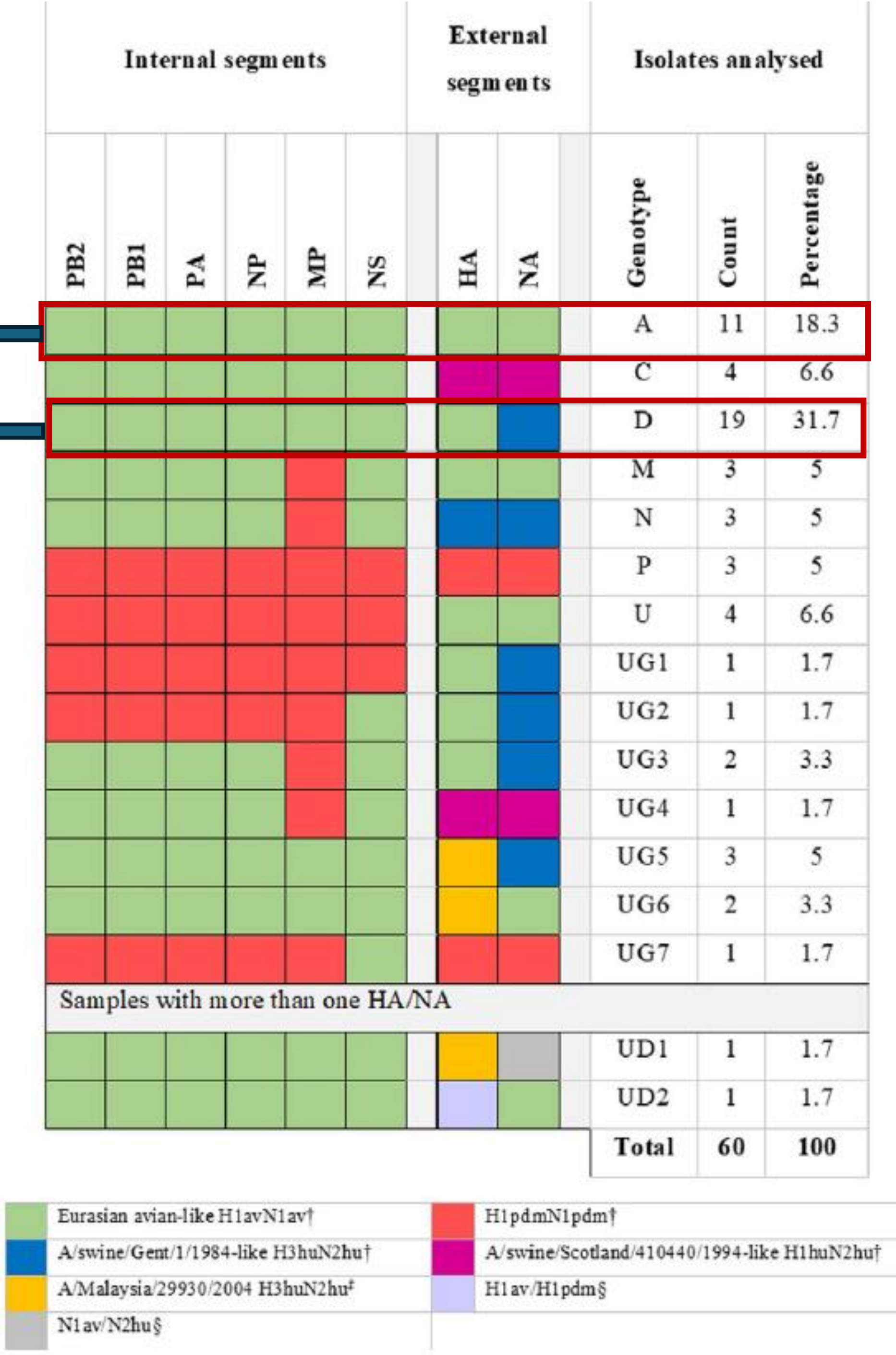
Internal segments						External segments						
PB2	PB1	PA	NP	M	NS	HA	NA	Genotype	Count	Percentage	Clade H	Clade N
🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	A	8	34.8	1C.2.2 / 1C.2.6	N1.E
🟩	🟩	🟩	🟩	🟩	🟩	🟪	🟪	C	1	4.3	1B.1.2	N2.S
🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟪	G	2	8.7	1C.2.2 / 1C.2.6	N2.S
🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	P	1	4.3	1A.3.3.2	N1.P
🟩	🟩	🟩	🟩	🟩	🟩	🟧	🟩	UG6	1	4.3	2000.3	N1.E
🟩	🟩	🟩	🟩	🟩	🟩	🟧	🟪	UG8	5	21.7	2000.3	N2.S
🟥	🟥	🟥	🟥	🟥	🟩	🟪	🟦	UG9	1	4.3	1B.1.2	N2.G
🟥	🟥	🟥	🟥	🟩	🟥	🟧	🟪	UG10	1	4.3	2000.3	N2.S
🟩	🟩	🟩	🟩	🟥	🟩	🟩	🟪	UG11	1	4.3	1C.2.4	N2.S
Samples with more than HA/NA												
🟩	🟩	🟩	🟩	🟩	🟩	🟧	⬜	UD1	1	4.3	2000.3	N1.E/N2.G
🟥	🟥	🟥	🟥	🟥	🟩	🟪	⬜	UD3	1	4.3	1B.1.2	N1.E/N2.S
								TOTAL	**23**	**100**		

- 🟩 EA H1avN1
- 🟪 A/swine/Scotland/410440/1994-like H1huN2
- 🟦 A/swine/Gent/1/1984-like H3huN2
- 🟥 A(H1N1)pdm09
- 🟧 A/Malaysia/29930/2004/H3huN2
- ⬜ N1av/N2hu
- ▨ H1av/H1pdm

H1avN1av

H1avN2hu

Genotypes 2017 – 2019 Spain (Sosa-Portugal et al. 2020)

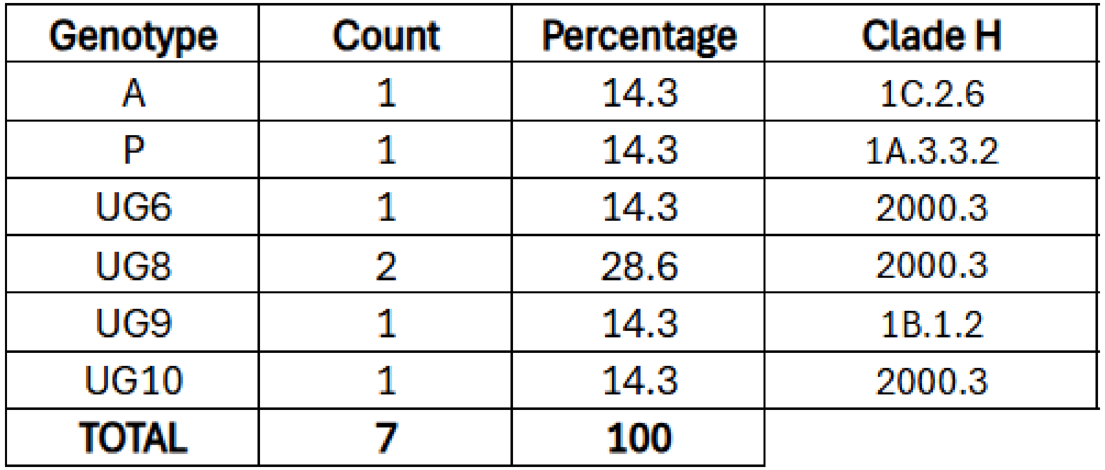


Internal segments						External segments		Isolates analysed		
PB2	PB1	PA	NP	MP	NS	HA	NA	Genotype	Count	Percentage
🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	A	11	18.3
🟩	🟩	🟩	🟩	🟩	🟩	🟪	🟪	C	4	6.6
🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟦	D	19	31.7
🟩	🟩	🟩	🟩	🟥	🟩	🟩	🟩	M	3	5
🟩	🟩	🟩	🟩	🟥	🟩	🟦	🟦	N	3	5
🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	P	3	5
🟥	🟥	🟥	🟥	🟥	🟥	🟩	🟩	U	4	6.6
🟥	🟥	🟥	🟥	🟥	🟥	🟩	🟦	UG1	1	1.7
🟥	🟥	🟥	🟥	🟥	🟩	🟩	🟦	UG2	1	1.7
🟩	🟩	🟩	🟩	🟥	🟩	🟩	🟦	UG3	2	3.3
🟩	🟩	🟩	🟩	🟥	🟩	🟪	🟪	UG4	1	1.7
🟩	🟩	🟩	🟩	🟩	🟩	🟨	🟦	UG5	3	5
🟩	🟩	🟩	🟩	🟩	🟩	🟨	🟩	UG6	2	3.3
🟥	🟥	🟥	🟥	🟥	🟩	🟥	🟥	UG7	1	1.7
Samples with more than one HA/NA										
🟩	🟩	🟩	🟩	🟩	🟩	🟨	▩	UD1	1	1.7
🟩	🟩	🟩	🟩	🟩	🟩	▨	🟩	UD2	1	1.7
								Total	**60**	**100**

🟩	Eurasian avian-like H1avN1av†	🟥	H1pdmN1pdm†
🟦	A/swine/Gent/1/1984-like H3huN2hu†	🟪	A/swine/Scotland/410440/1994-like H1huN2hu†
🟨	A/Malaysia/29930/2004 H3huN2hu‡	▨	H1av/H1pdm§
▩	N1av/N2hu§		

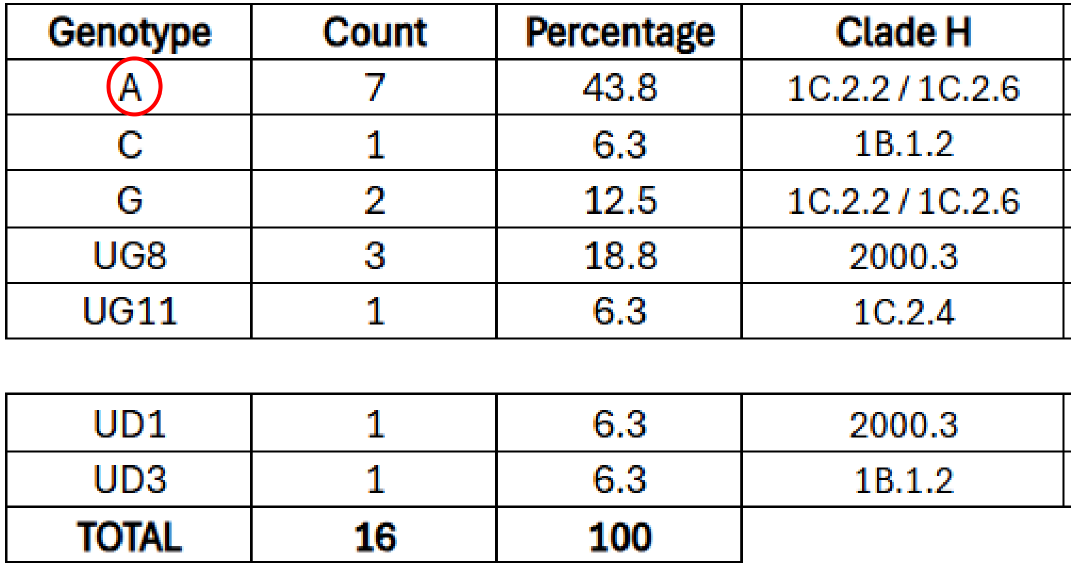
Genotype D, the third more common in that study has not been detected yet in the 2024-25 sampling

Genotypes detected in farms WITH respiratory problems



Genotype	Count	Percentage	Clade H
A	1	14.3	1C.2.6
P	1	14.3	1A.3.3.2
UG6	1	14.3	2000.3
UG8	2	28.6	2000.3
UG9	1	14.3	1B.1.2
UG10	1	14.3	2000.3
TOTAL	**7**	**100**	

Genotypes detected in farms WITHOUT respiratory problems



Genotype	Count	Percentage	Clade H
A	7	43.8	1C.2.2 / 1C.2.6
C	1	6.3	1B.1.2
G	2	12.5	1C.2.2 / 1C.2.6
UG8	3	18.8	2000.3
UG11	1	6.3	1C.2.4
UD1	1	6.3	2000.3
UD3	1	6.3	1B.1.2
TOTAL	**16**	**100**	

Conclusions (preliminary)

- swIAV is more likely to be found in farms with respiratory problems.
- A different pattern of co-infection with PRRSV is obseved depending on the status of the farm
- Apparently, in farms with respiratory problems the diversity of lineages seems to be higher
- A possible trend towards a decrease in H1avN2hu and increase in H3huN2hu compared to previous studies is observed in Spain

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Thank you for your attention!

Acknowledgements

Cefusa
GANADERIA PORCINA

BATALLÉ

AGAUR
Agència de Gestió d'Ajuts Universitaris i de Recerca

AGENCIA ESTATAL DE INVESTIGACIÓN

AGROCESA

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TRANSMISSIBLE DISEASES GROUP (UAB)

- Laia Aguirre
- Mònica Alberch
- Alberto Allepuz
- Vania Ciaravino
- Joaquim Castellà
- Martí Cortey
- Laila Darwich
- Marga Martín
- Enric Mateu
- Paula Rebollo
- Soledad Serena
- Montse Tello
- Ge Ye

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