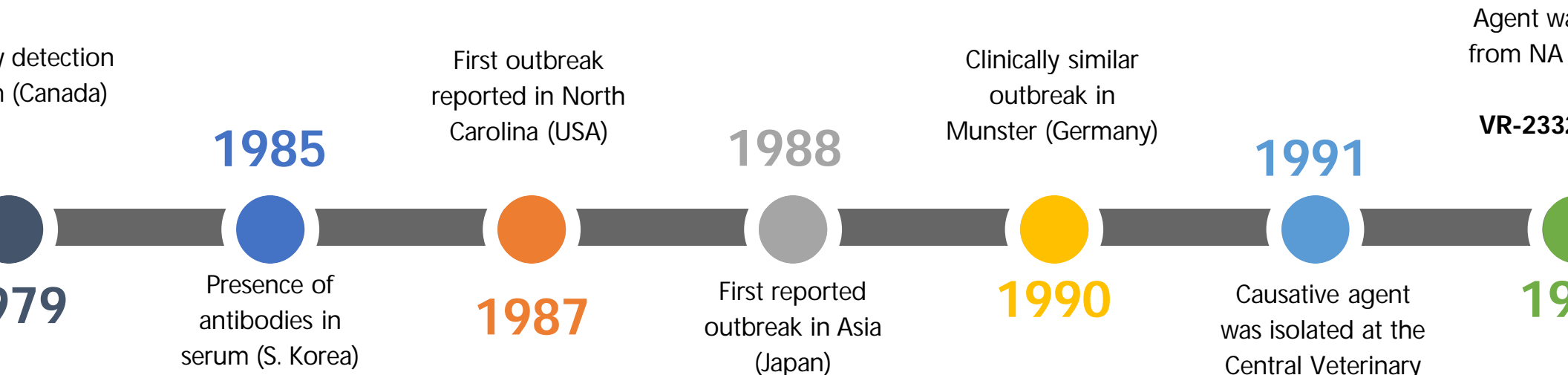


DEEPER UNDERSTANDING OF PRRS GENETIC DIVERSITY STRAINS & LINEAGES

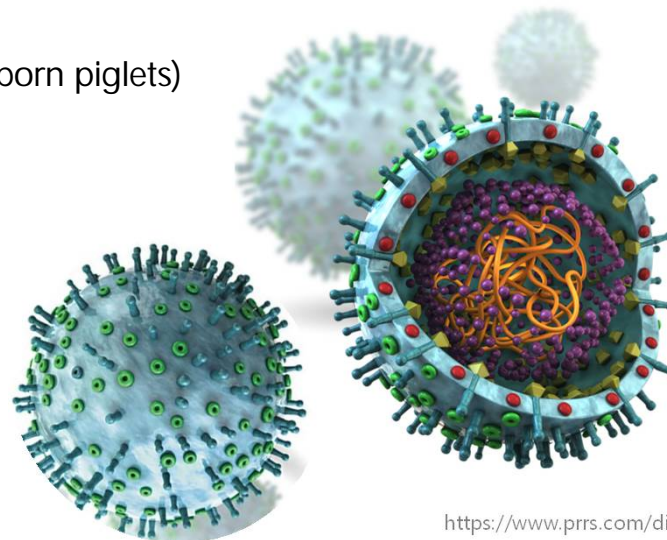
DISEASE ORIGIN

Porcine Reproductive and Respiratory Syndrome



Pathologies:
Failure in sows (abortions, mummified fetuses and weak born piglets)
Disease in young pigs

Swine Fever (SMD)
Fertility and respiratory syndrome (SIRS)
Reproductive and respiratory syndrome (PRRS) 1992



Lelystad virus (porcine alveolar macrophage)

<https://www.prrs.com/disease-control/virus>

THE VIRUS

Porcine Reproductive and Respiratory Syndrome

Classification

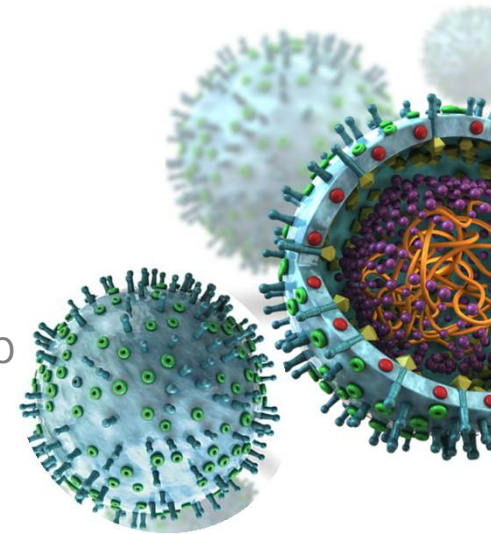
Arteriviridae

Arterivirus

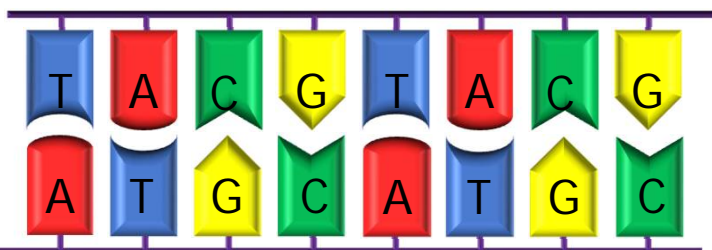
Nidovirales

veloped virus (45-70 nm diameter)

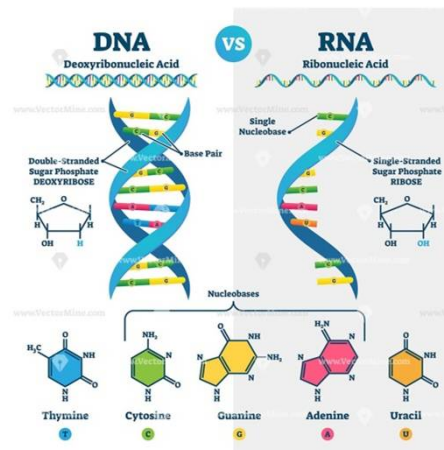
stranded, non-segmented, positive-sense RNA (12.7 to 15.7 kb) ASF 170-190 kb



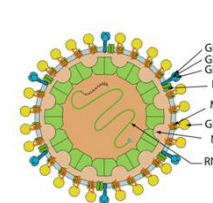
<https://www.prrs.com/disease-control>



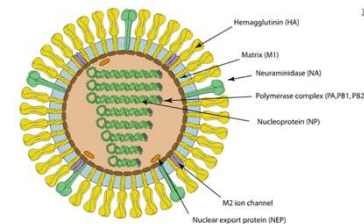
Double-stranded



Single-stranded



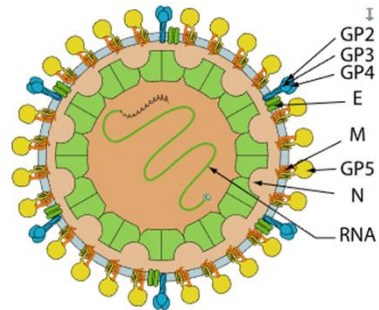
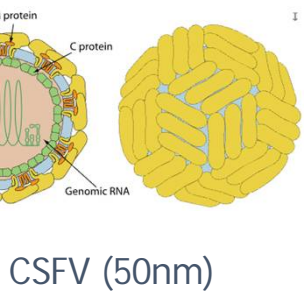
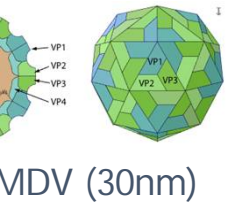
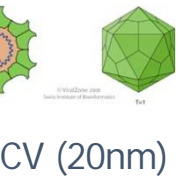
Non-segmented



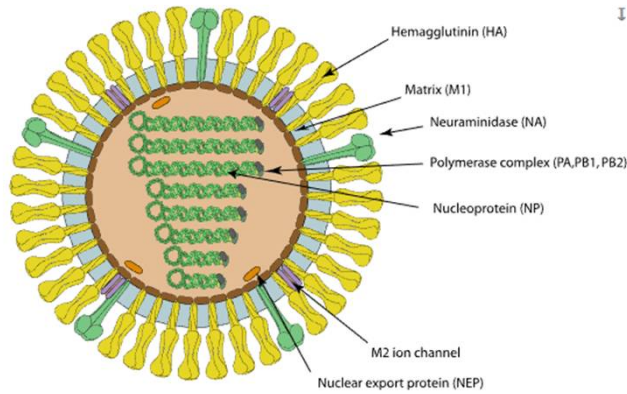
Segmented

THE VIRUS

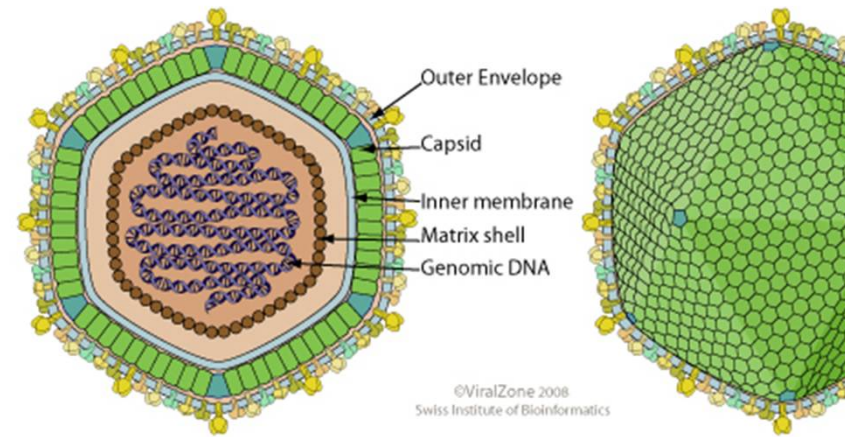
Porcine Reproductive and Respiratory Syndrome



PRRSV (45-60nm)



Influenza (80-120nm)



ASFV (175-215nm)

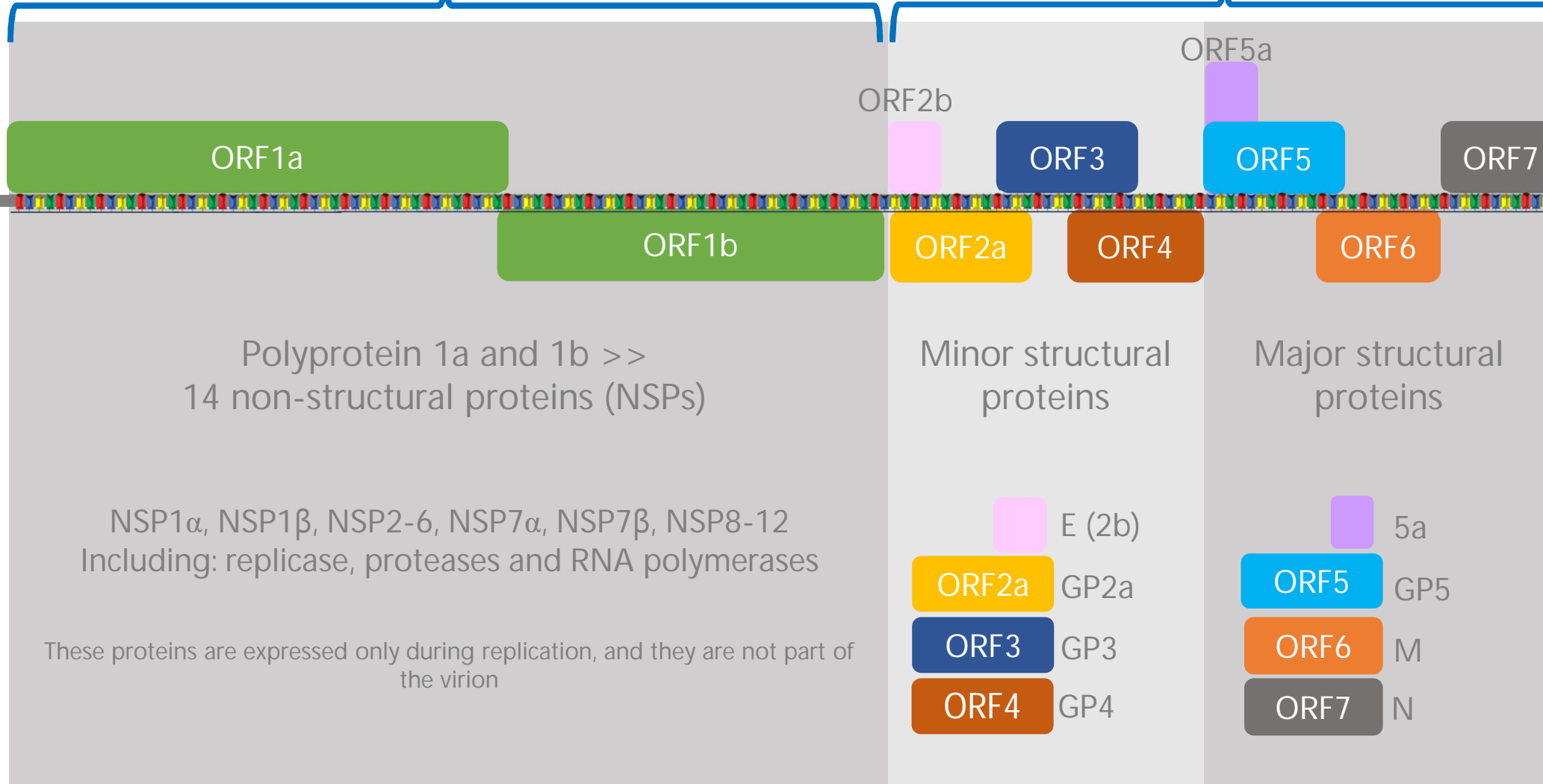
THE VIRUS

Porcine Reproductive and Respiratory Syndrome

Viral Genome

75-80%

20-25%



Polyprotein 1a and 1b >>
14 non-structural proteins (NSPs)

NSP1 α , NSP1 β , NSP2-6, NSP7 α , NSP7 β , NSP8-12
Including: replicase, proteases and RNA polymerases

These proteins are expressed only during replication, and they are not part of the virion

Minor structural proteins

Major structural proteins

E (2b)

ORF2a GP2a

ORF3 GP3

ORF4 GP4

5a

ORF5 GP5

ORF6 M

ORF7 N

THE VIRUS

Porcine Reproductive and Respiratory Syndrome

genome

structural protein
(NSP)

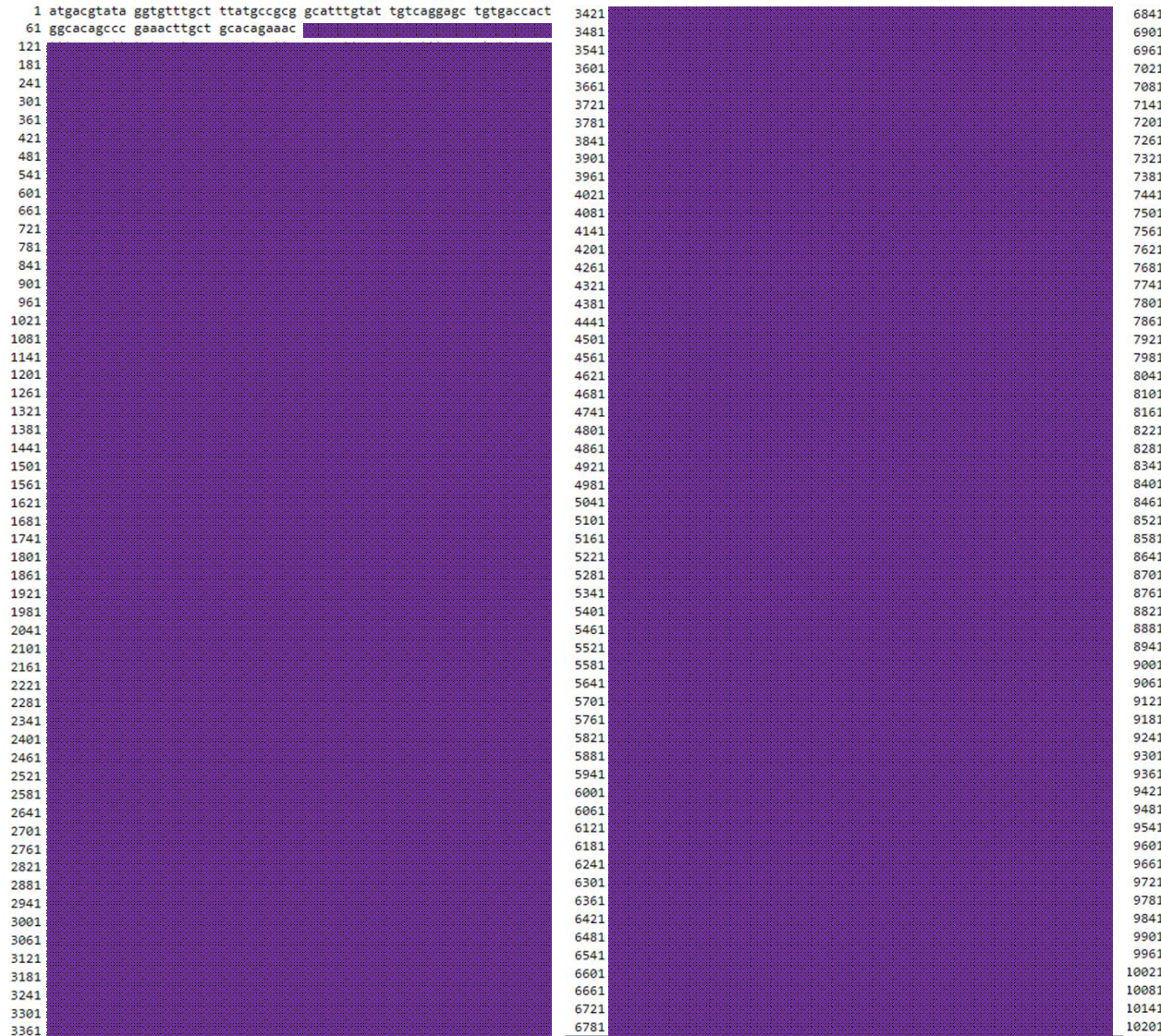
ORF1a

ORF1b

ate SD11-21_P100

porary L1 vaccine

[m.nih.gov/nuccore/KU131568](https://www.nih.gov/nuccore/KU131568)



THE VIRUS

Porcine Reproductive and Respiratory Syndrome

genome

ORF1b

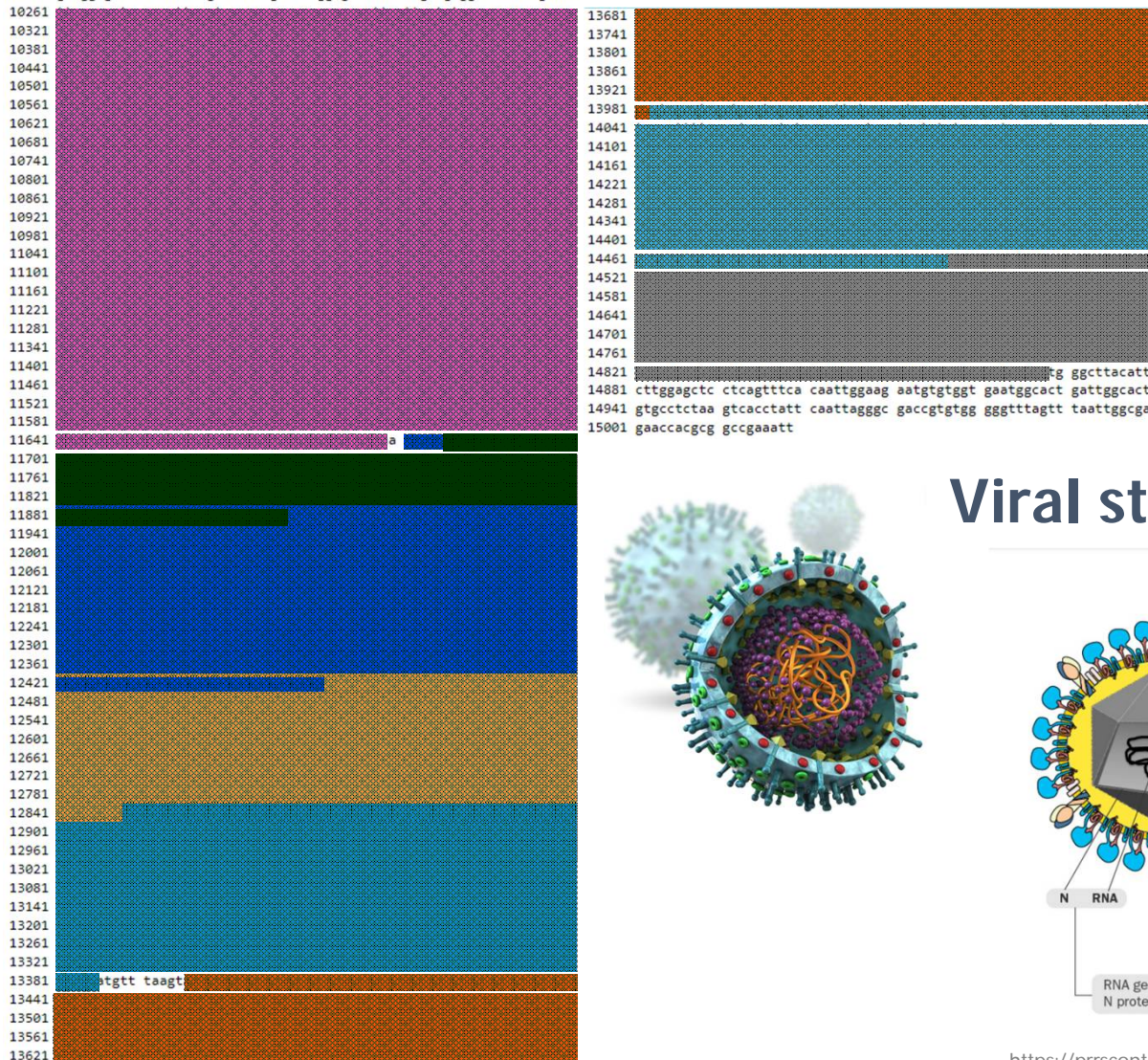
structural proteins

ORF2a

ORF2b

ORF3

ORF4



Major structural proteins

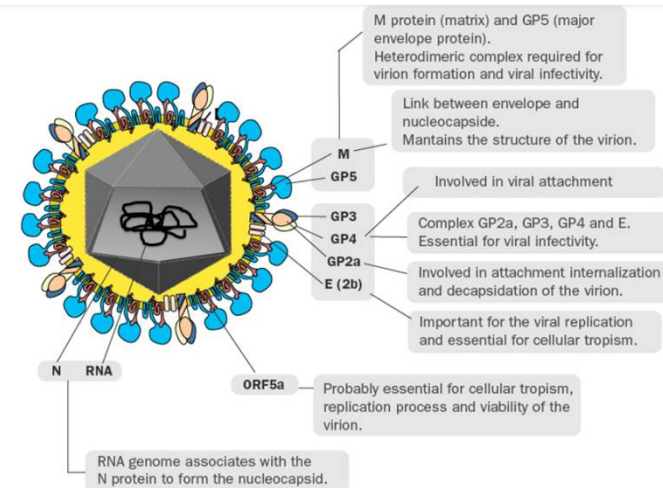
ORF5 - viral attachment protein (G)

ORF6

ORF7



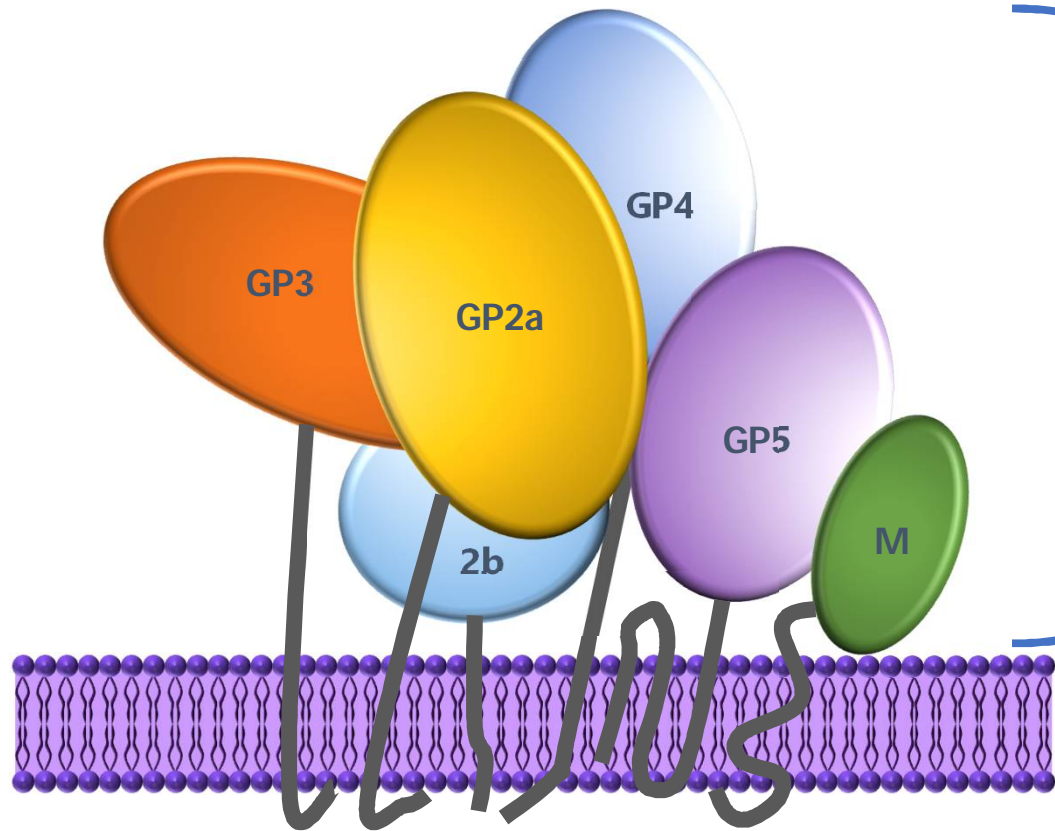
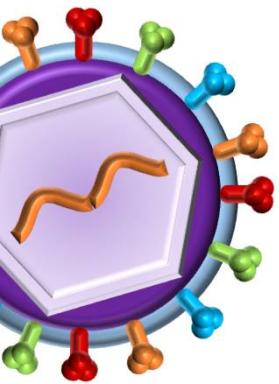
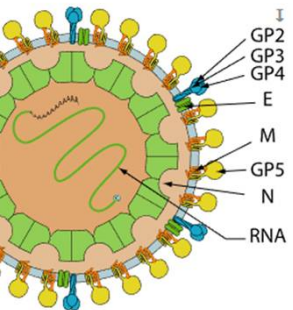
Viral structure



THE VIRUS

Porcine Reproductive and Respiratory Syndrome

Viral structure



Spike protein complex

GP5 – identified as the neutralizing epitope of PRRSV responsible for the neutralizing antibody activity (Lopez et al. 2004)

PRRSV envelope

Lunney et.al. 2016

GENETIC DIVERSITY

Porcine Reproductive and Respiratory Syndrome

5'- The-quick-brown-fox-jumps-over-the-head-of-the-lazy-dog -3'

Variant, Lineage, Strain

Variant – a single point of change on the genome compared to the original virus

5'- **quick**-The-brown-fox-jumps-over-the-head-of-the-lazy-dog -3'

5'- The-quick-**BROWN**-fox-jumps-over-the-head-of-the-lazy-dog -3'

5'- The-quick-brown-fox-**jumps**-over-the-head-of-the-lazy-dog -3'

Lineage – a collection of variants that define a specific line of the virus

5'- **quick**-**The**-**BROWN**-fox-jumps-over-the-head-of-the-lazy-dog -3'

5'- The-quick-**BROWN**-fox-jumps-over-the-head-of-the-lazy-dog -3'

5'- The-quick-**BROWN**-fox-**jumps**-over-the-head-of-the-lazy-dog -3'

Strain – a lineage that has acquired new properties (transmissibility, infectivity, pathogenicity, immunogenicity)

5'- The-quick-**BROWN**-fox-jumps-over-the-head-of-the-lazy-dog -3'

5'- The-quick-**BLACK**-fox-**runs**-over-the-head-of-the-lazy-dog -3'

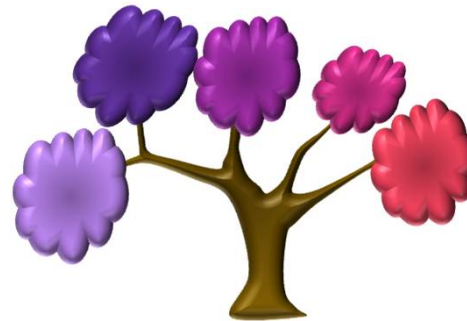
5'- The-quick-**BROWN**-fox-jumps-over-the-**tail**-of-the-lazy-dog -3'

GENETIC DIVERSITY

Porcine Reproductive and Respiratory Syndrome

1: European or type 1 (Lelystad virus)
4 different subtypes (I, II, III, IV)

2: North American or type 2 (VR-2332)
Lineage 1-9 (11)



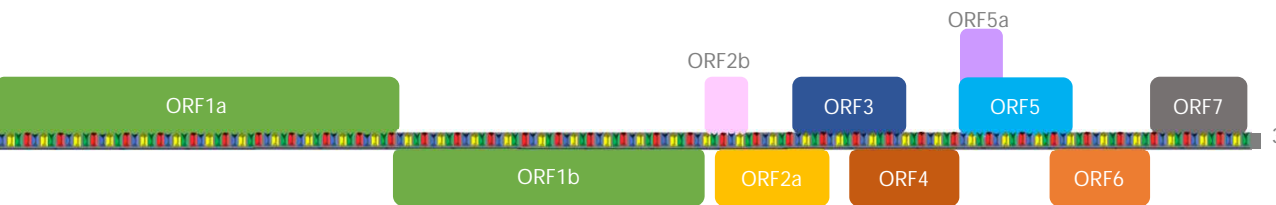
ORF5

- L1A
- L1C
- L1Dalpha
- L1Dbeta
- L1E
- L1F
- L1G
- L1H
- L5
- L6
- L8
- PRRSV1
- undetermined

Variant – a single point of change on the genome compared to the original virus

Lineage – a collection of variants

Strain – a lineage that has acquired new properties



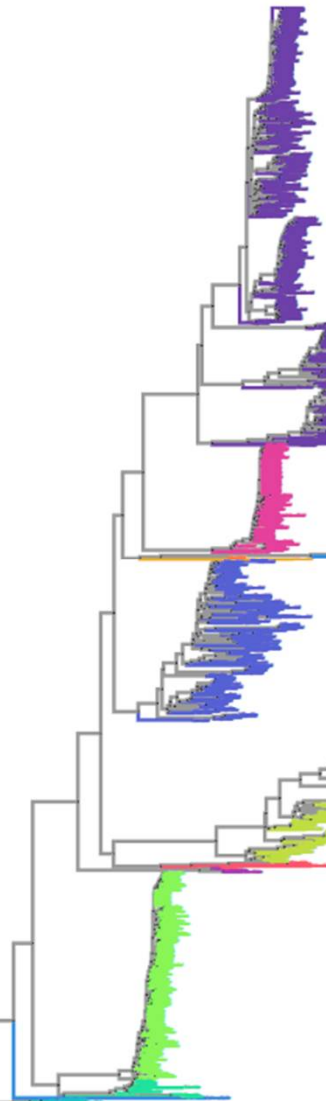
Phylogenetic analyses based on ORF5

Extremely high genetic diversity

PRRSView sequences between 17FEB2023 and 21 MAR 2023

<https://prsv.vdl.iastate.edu/>

Paploski 2019



1: European or type 1 (Lelystad virus)
4 different subtypes (I, II, III, IV)

2: North American or type 2 (VR-2332)
Lineage 1-9 (11)

ISU PRRSView

PRRSV Lineages

This Porcine Reproductive and Respiratory Syndrome Virus project (ISU PRRSView) uses lineages designated by Paploski et al. Please refer to the 'How to cite' section in **General Information** for reference.

Lineage ^(3,4,5)	Description
L1	Family of PRRSV viruses that originated in eastern Canada and first appeared in the USA in the late 1990s and early 2000s. In China, it is also known as NADC30-like lineage. This lineage includes sublineages L1A-H. Prevacent vaccine belongs to lineage 1
L1A	Most prevalent lineage from 2015 to present. This lineage re-emerged in 2015 coinciding with the spread of the 1-7-4 RFLP-type. Common RFLPs: 1-7-4, 1-8-4, 1-4-4, 1-7-2, 1-7-3, 1-10-4, 1-6-4, 1-3-4, 1-4-3
L1B	Lineage 1B increased substantially in detection in 2013, then subsequently declined in 2016 and 2017. Common RFLPs: 1-18-2,
L1C	Most prevalent PRRSV lineage from 2011 to 2014. Common RFLPs: 1-4-4, 1-3-4, 1-3-2, 1-2-4, 1-4-2
L1D	Earliest emergent L1 group. Currently a less frequently detected lineage containing two distinct sublineages L1Dalpha and L1Dbeta.

GENETIC DIVERSITY

Porcine Reproductive and Respiratory Syndrome

L1Dbeta	Poorly resolved polyphyletic sublineage of L1D sequences. Vaccine: Prevacent (Elanco) Common RFLPs: 1-8-4,
L1E	This lineage is less frequently detected. Common RFLPs: 1-3-2,
L1F	Most prevalent between 2002-2008, but has been rarely detected since 2014. Common RFLPs: 1-8-4,
L1G	Lineage appeared to have emerged from the 1B lineage. Common RFLPs: 1-26-2, 1-37-2,
L1H	Regularly detected lineage with increasing genetic diversity. Common RFLPs: 1-8-4, 1-4-4
L2	Lineage originated in eastern Canada. Not routinely detected.
L3	This lineage is primarily found in Asia. Emerged in Asia around the late 1980s. Introduced from North America. Not routinely detected.
L4	This lineage is primarily detected in Japan. Emerged in Asia around the late 1980s. Introduced from North America. Not routinely detected.
L5	Detected in North America in the early 1990s. Also, referred to as BJ-4-like. Ingelvac PRRSV uses a lineage 5 isolate. VR-2332 belongs to lineage 5. Vaccine: Ingelvac MLV (Boehringer Ingelheim) Common RFLPs: 2-5-2
L6	Originated in the USA, though is rarely detected. Common RFLPs: 1-1-2,
L7	Originated in the USA. Vaccine strain PrimePac and its parental strain, Neb-1, belong to lineage 7. Vaccine: PRIME PAC (Merck Animal Health) Common RFLPs: 1-4-4,
L8	Originated in the USA. Emerged in 1996 and have been responsible for several outbreaks in the field. Also known as CH-1a-like. Fostera vaccine belongs to lineage 8. Vaccine: Ingelvac ATP (Boehringer Ingelheim), Fostera (Zoetis) Common RFLPs: 1-3-2, 1-4-2,
L9	Originated in the USA. Emerged in 1996 and have been responsible for several outbreaks in the field. Most prevalent lineage from 2009 to 2010, but its occurrence fell to 0.5% of all lineages identified per year after 2014. Common RFLPs: 1-4-2,
PRRSV1	Formerly known as EU PRRSV. PRRSV1 genetic evolution has continued to diverge from other lineages. Small outbreaks are observed in the US.

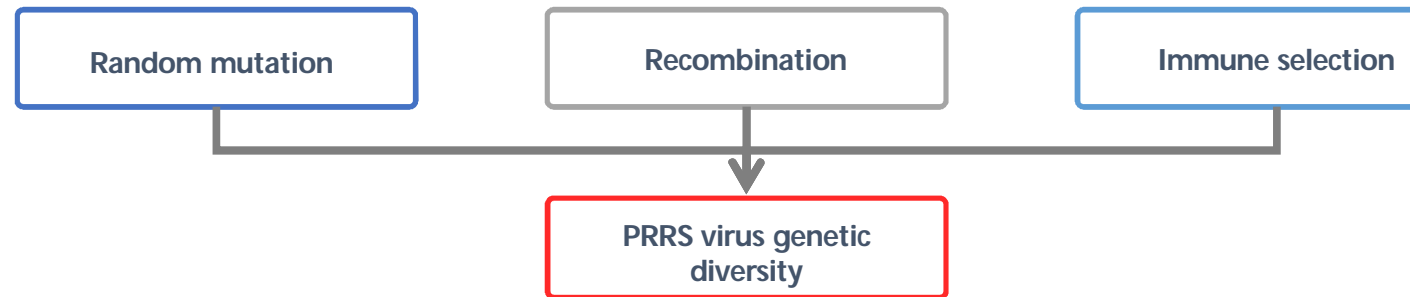
GENETIC DIVERSITY

Porcine Reproductive and Respiratory Syndrome

Random Mutation

Highest mutation rate compared to among well-known viruses

Its RNA polymerase does not have the ability to correct the inherent common errors that occur during the transcription of RNA



polymerase

The-quick-brown-fox-jumps-over-the-head-of-the-lazy-dog

The-quick-brown-fox-jumps-over-the-head-fo-the-lazy-dog

GENETIC DIVERSITY

Porcine Reproductive and Respiratory Syndrome

QUASISPECIES

A virus replicating with a high mutation rate generating a diverse mutant repertoire over the course of few generations

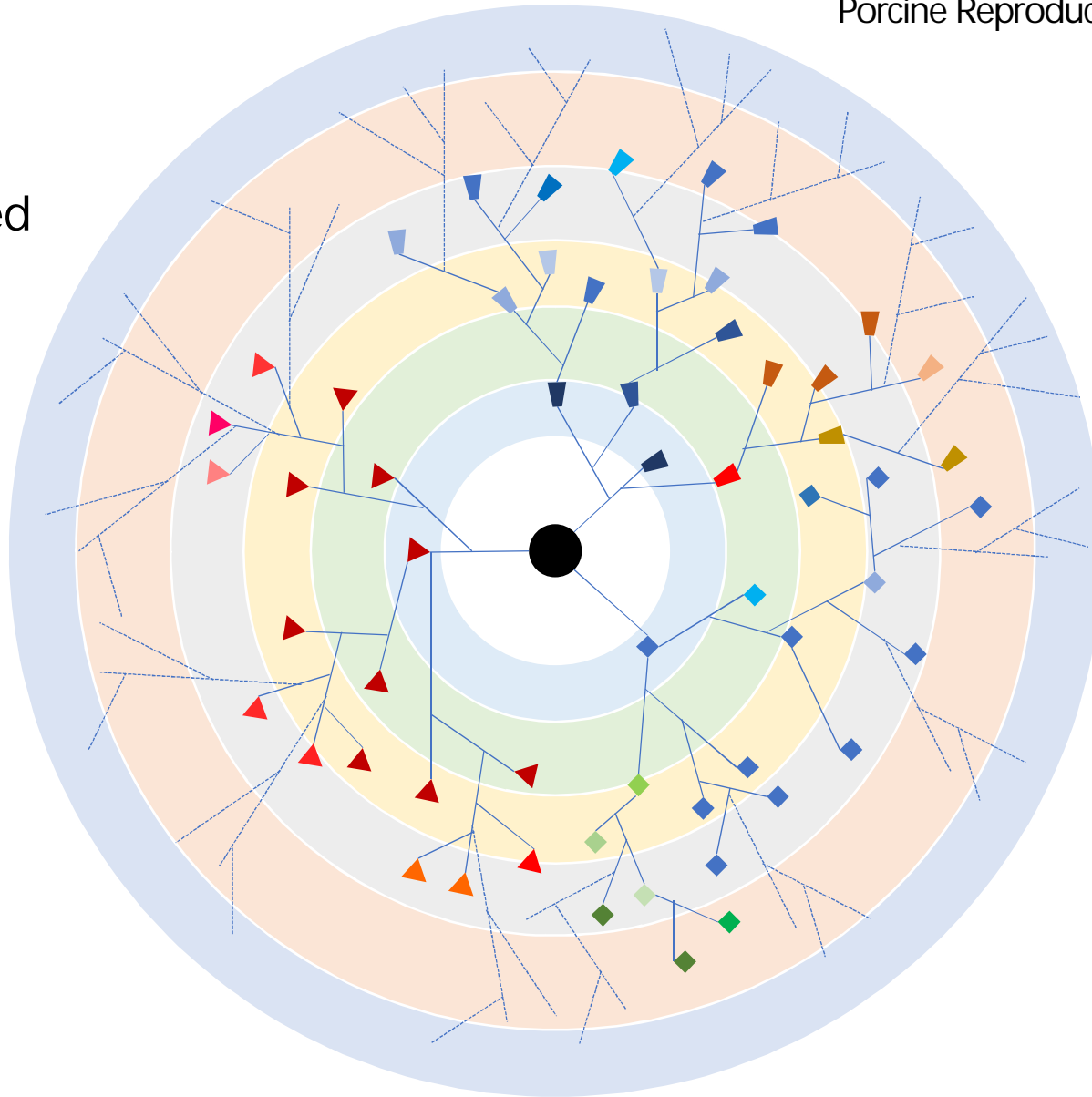


GENETIC DIVERSITY

Porcine Reproductive and Respiratory Syndrome

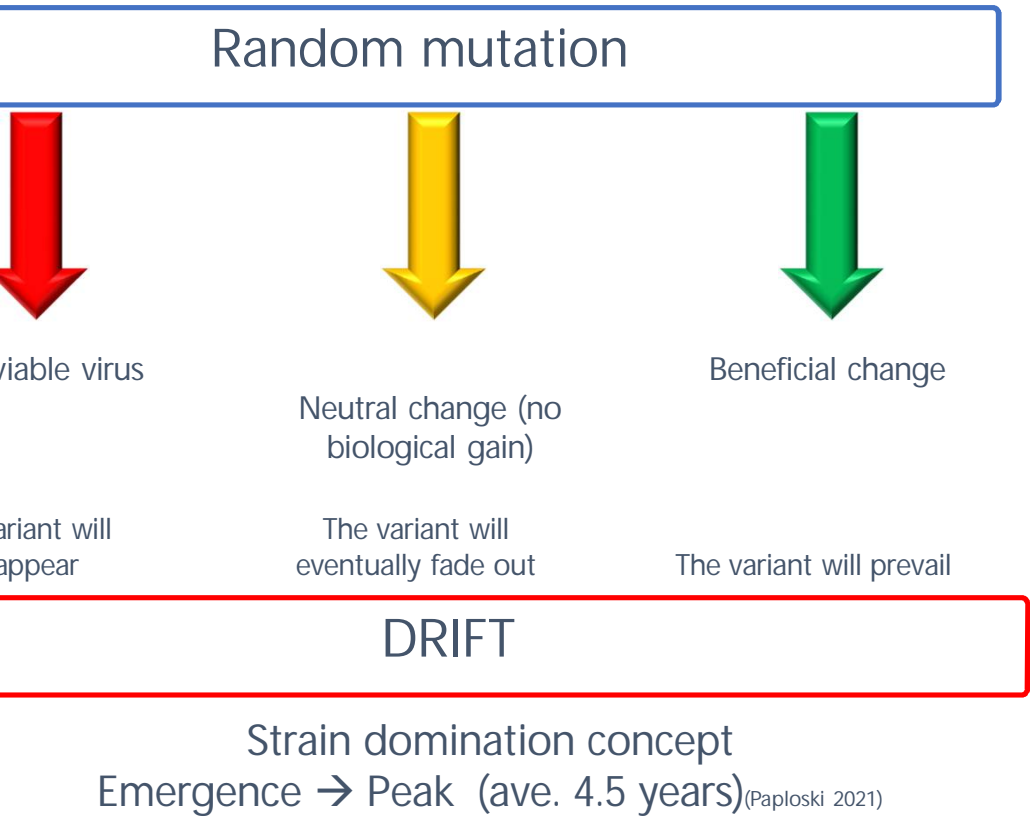
ASISPECIES

Group of closely related
different variant



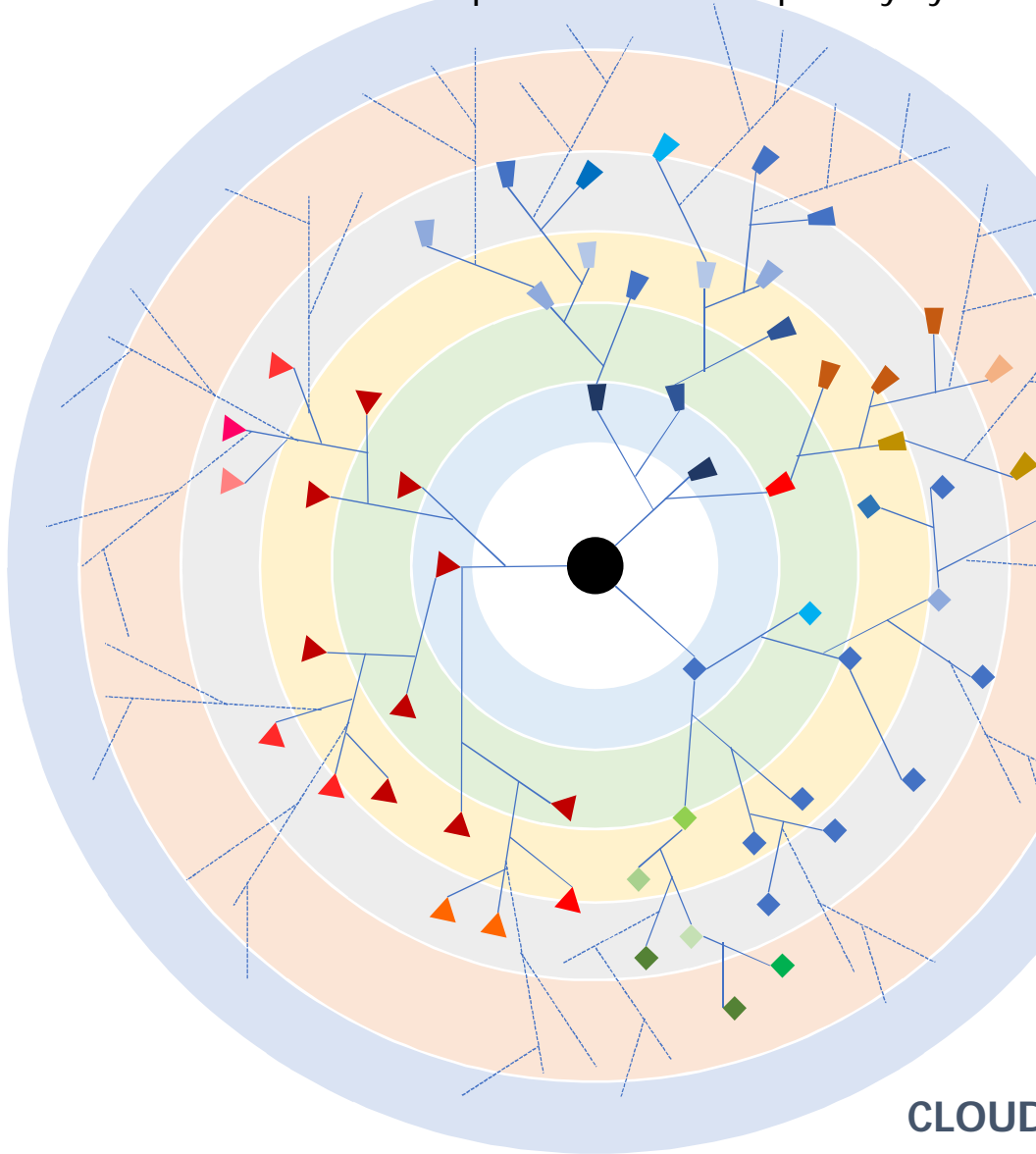
CLOUD

QUASISPECIES



GENETIC DIVERSITY

Porcine Reproductive and Respiratory Syndrome



Recombination

Exchange of genetic material between two or more strains
A major force of the evolution of RNA

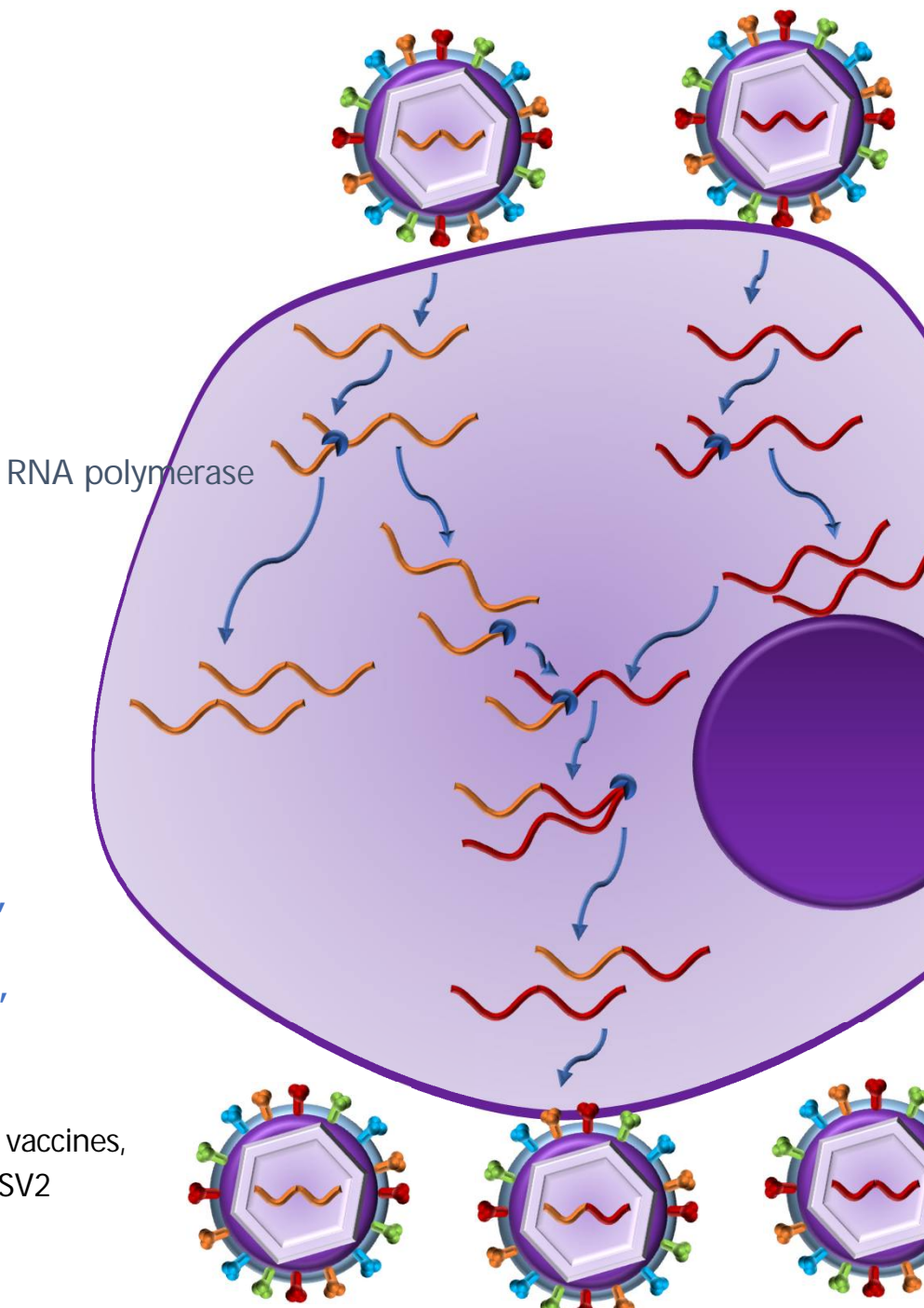
The quick-BROWN-fox-jumps-over-the-head-of-the-lazy-dog -3'

The quick-BLACK-fox-runs-over-the-head-of-the-lazy-dog -3'

The quick-BROWN-fox-runs-over-the-head-of-the-lazy-dog -3'

The quick-BLACK-fox-jumps-over-the-head-of-the-lazy-dog -3'

Recombination has been demonstrated between field isolates, between vaccines,
and between field isolates and vaccines, for both PRRSV1 and PRRSV2



GENETIC DIVERSITY

Porcine Reproductive and Respiratory Syndrome

Immune Selection

Selection pressure cause by the favoring parental strain that can evade the host immune response



5'- The-quick-brown-fox-jumps-over-the-head-of-the-lazy-dog -3'

5'- The-quick-BROWN-fox-jumps-over-the-head-of-the-lazy-dog -3'
5'- The-quick-BROWN-fox-jumps-over-the-head-of-the-lazy-dog -3'
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5'- The-quick-BROWN-fox-jumps-over-the-head-of-the-lazy-dog -3'

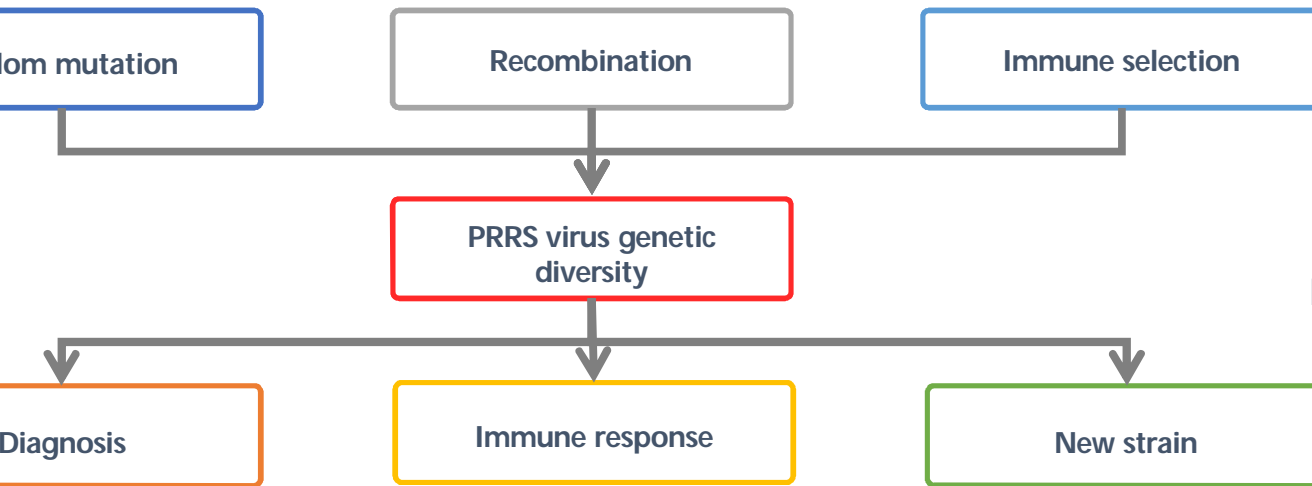


GENETIC DIVERSITY

Porcine Reproductive and Respiratory Syndrome

Consequence of genetic diversity

Impact in diagnosis, immune response, emergence of new strains, virulence, cell tropism, epidemiology etc



PRRSV1 Subtype III Lena strain (Belarus, 2007)

PRRSV1 Subtype I (Italy, 2003)

PRRSV1 Subtype I AUT13-883 and AUT14-440(Australia)

PRRSV1 Subtype I Rosalia (Spain, 2020)

PRRSV2 JA 142 and SDSU73 (1996)

PRRSV2 MN 184 (2001 -2006)

PRRSV2 MN 414 (2014)

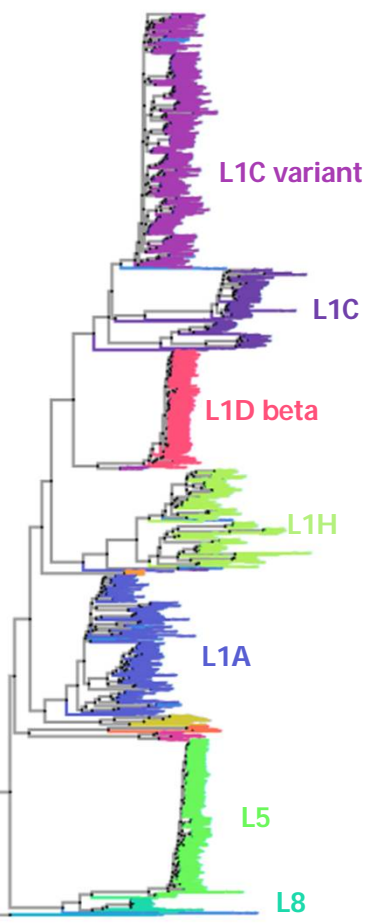
PRRSV2 HP-PRRSV (2006)

PRRSV2 HP-PRRSV JL580 and HLJ 58 (2013-2014)

PRRSV2 L1C 144 and 124 (2020)

view

- L1A
- L1C
- L1Cvariant
- L1Dalpha
- L1Dbeta
- L1E
- L1F
- L1G
- L1H
- L5
- L7
- L8
- PRRSV1
- undetermined



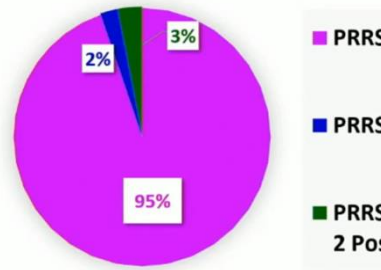
Description of Lineages

PRRSView sequences between 14/07/2023 and 10/08/2023

ate.edu/

GENETIC DIVERSITY -

PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME

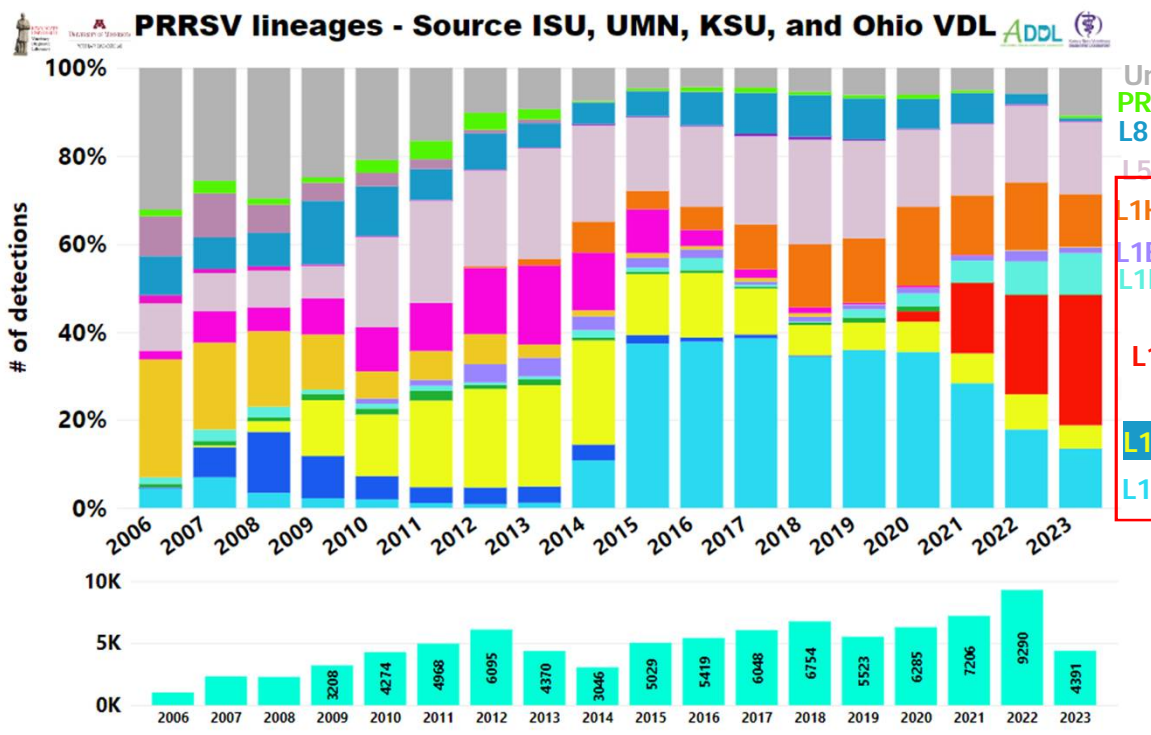


Trevisan et al. PLo

☐ Mainly PRRSV type 2 (US)

☐ Lineage 1 – predominant lineage

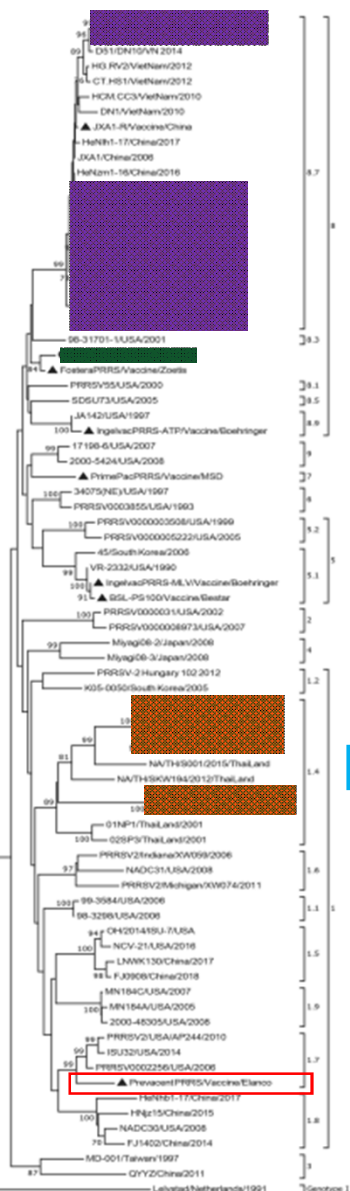
☐ L1C variant – first detection in 2020 and increasing ever since



PRRSV lineages - Source ISU, UMN, KSU, and Ohio VDL

ADDL

<https://fieldepi.org/domestic-swine-disease-monitoring-program/>



L8

L1

B

GENETIC DIVERSITY -VIETNAM

PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME

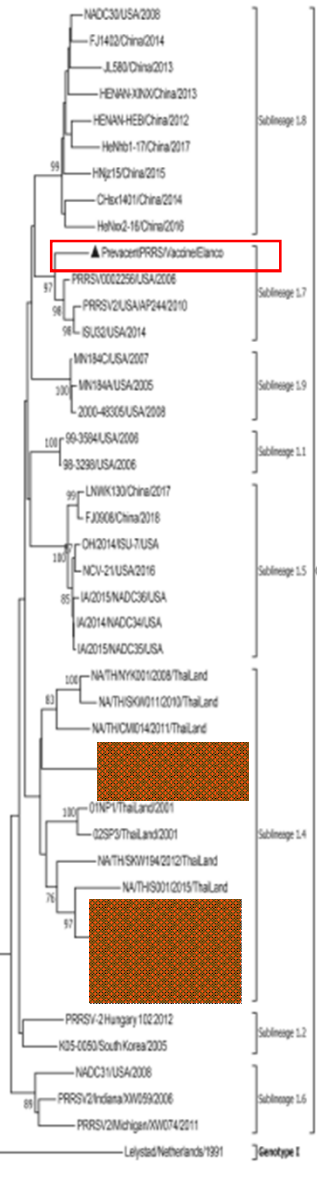
PRRSV type 1 (EU) and PRRSV type 2 (US) co-infection (Chae et al. 2021)

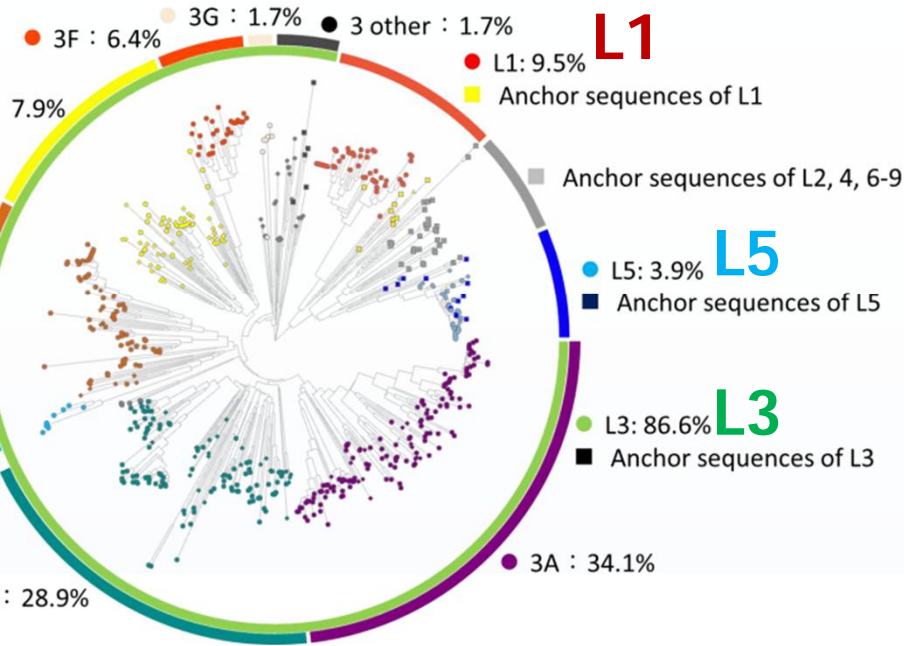
Lineage 8 – sublineage 8.7, (HP-PRRSV)

Lineage 1 – sublineage 1.4, NADC-like group, first time reported in Vietnam

Vaccine-like strain – lineage 8

L1.4





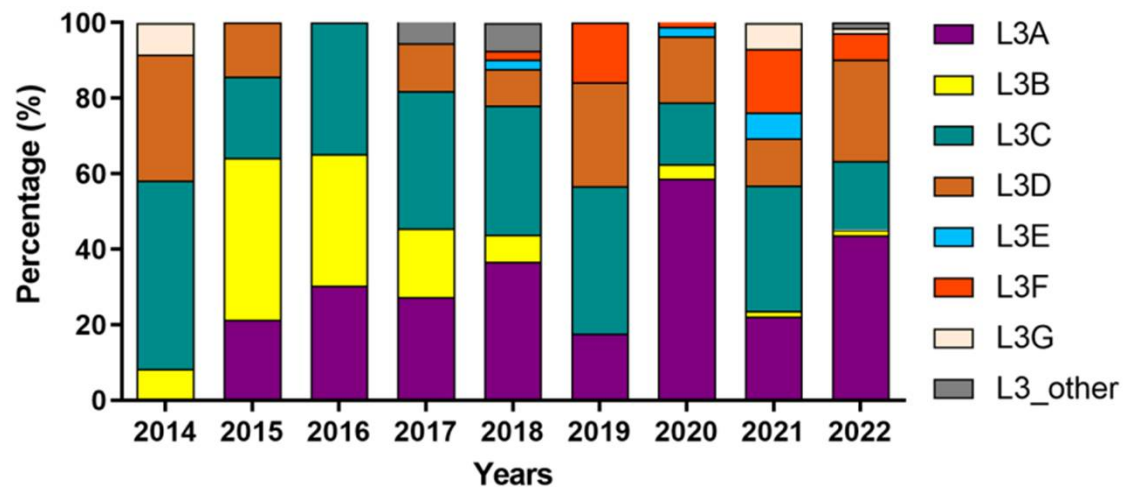
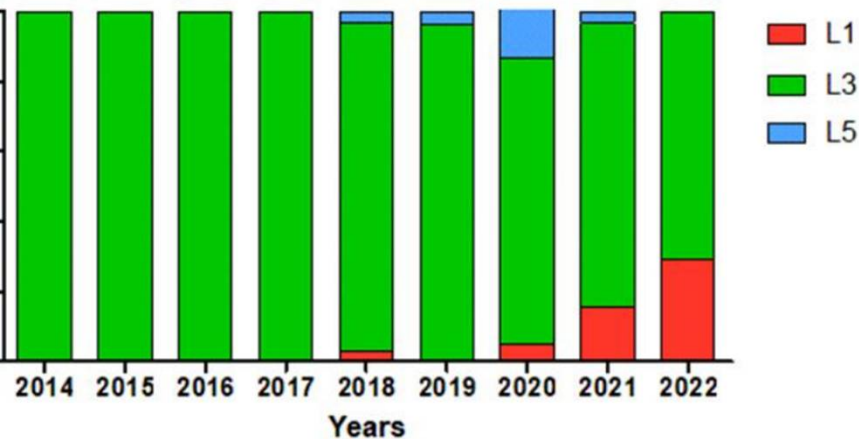
GENETIC DIVERSITY - TAIWAN

PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME

Lineage 3 (86.6%) – dominant lineage from 2014-2022
 predominant sublineages are L3A (34.1%) and L3C (28.9%)

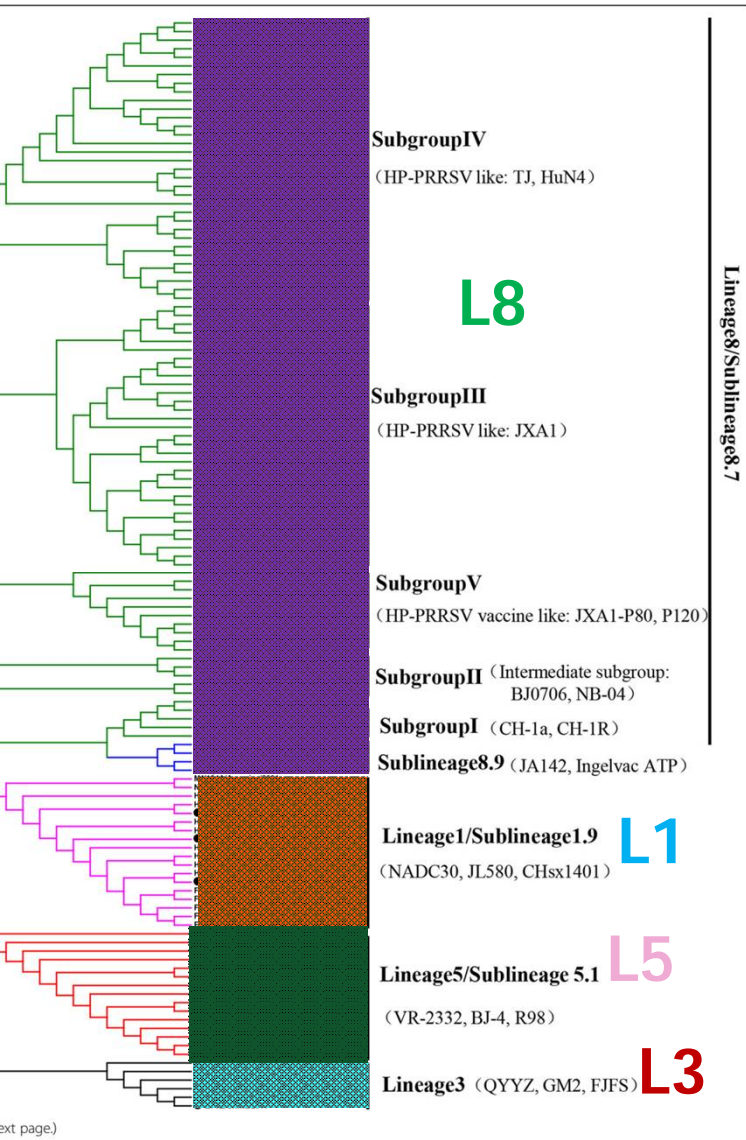
Lineage 1 (9.5%) – gradually increasing since 2020

Lineage 5 (3.9%) – vaccine lineage



GENETIC DIVERSITY – CH

PORCINE REPRODUCTIVE AND RESPIRATORY SYNDR



❑ PRRSV2 is clustered into four lineages

❑ **Lineage 1** – spreads rapidly since 2013

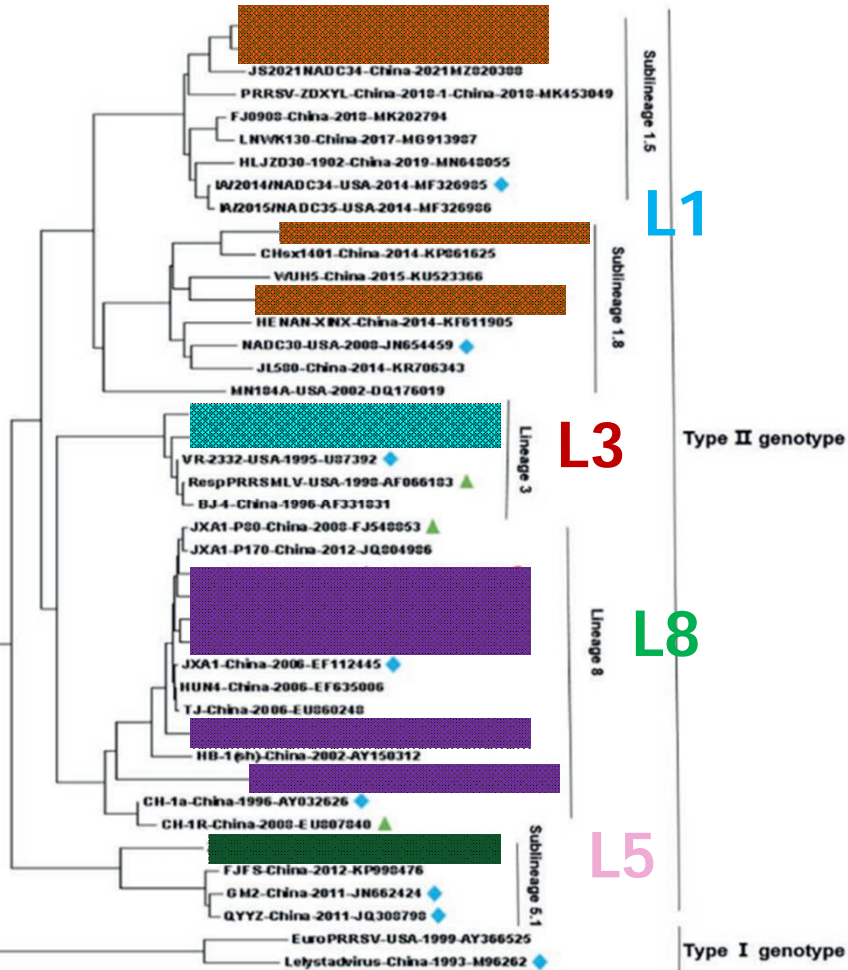
❑ **Lineage 3**

❑ **Lineage 5**

❑ **Lineage 8** – predominant in 1996-2016

GENETIC DIVERSITY – EASTERN CHINA

PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME



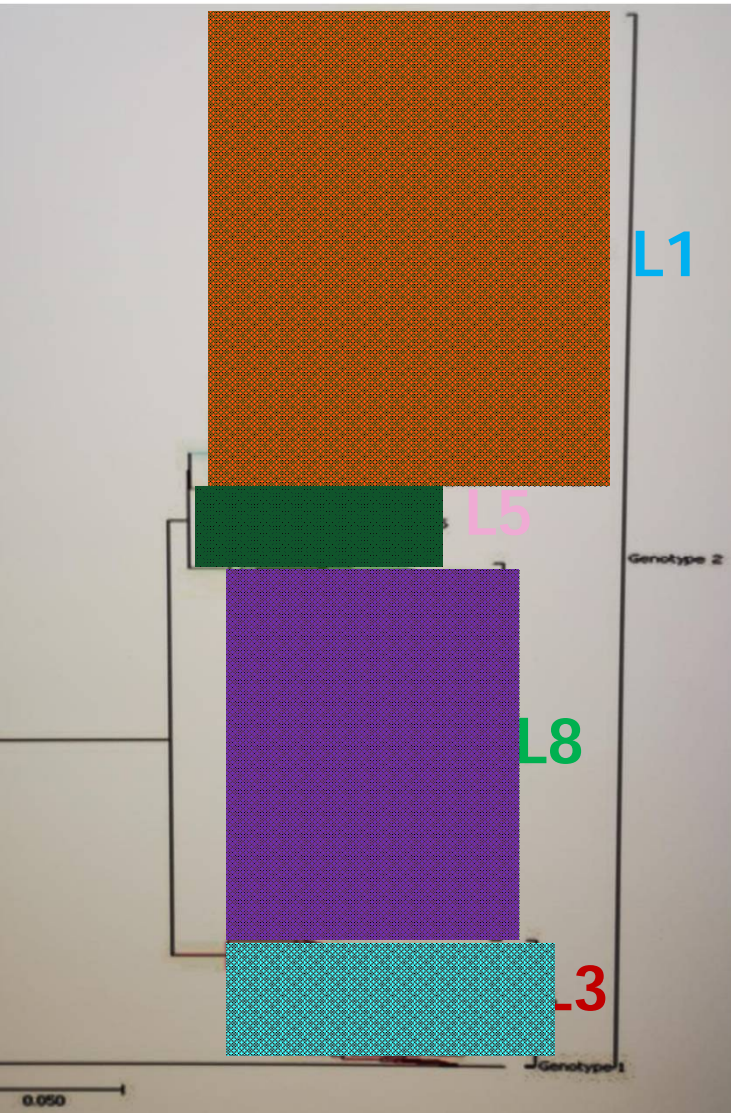
Lineage 8 – HP-PRRSV remained the predominant circulating strain in the field

Lineage 1 – sublineage 1.5 and 1.8, NADC 30-like and NADC 30-like detection increased in the recent years

Lineage 3

Lineage 5

Currently, [redacted] and [redacted] are used for the prevention and control of PRRS in China. In previous studies and this study indicated that [redacted] [redacted] [redacted]. Thus, additional prevention strategies must be developed.



GENETIC DIVERSITY – CHINA

PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME

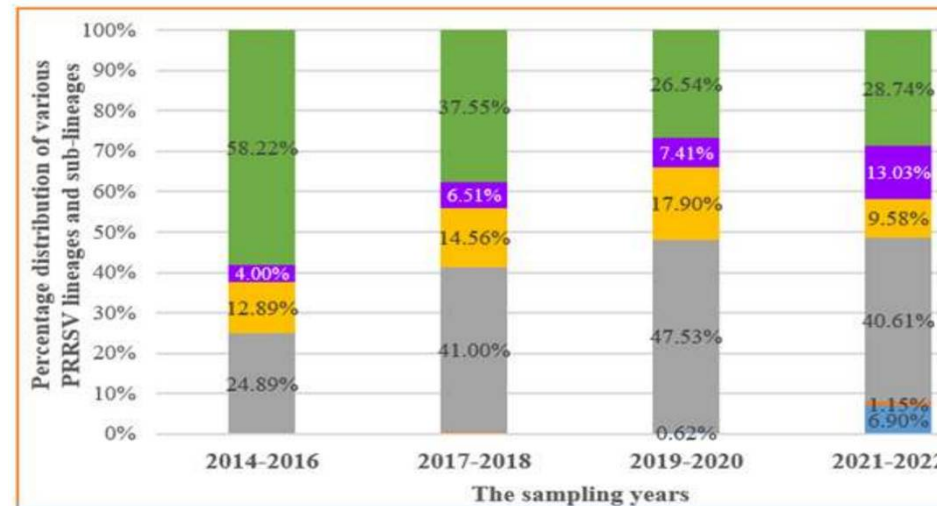
□ 909 ORF5 sequences

□ **Lineage 1 (369)** – sub-lineage L1A, L1B, and L1C; showed a clear tendency to spread widely

□ **Lineage 3 (121)**

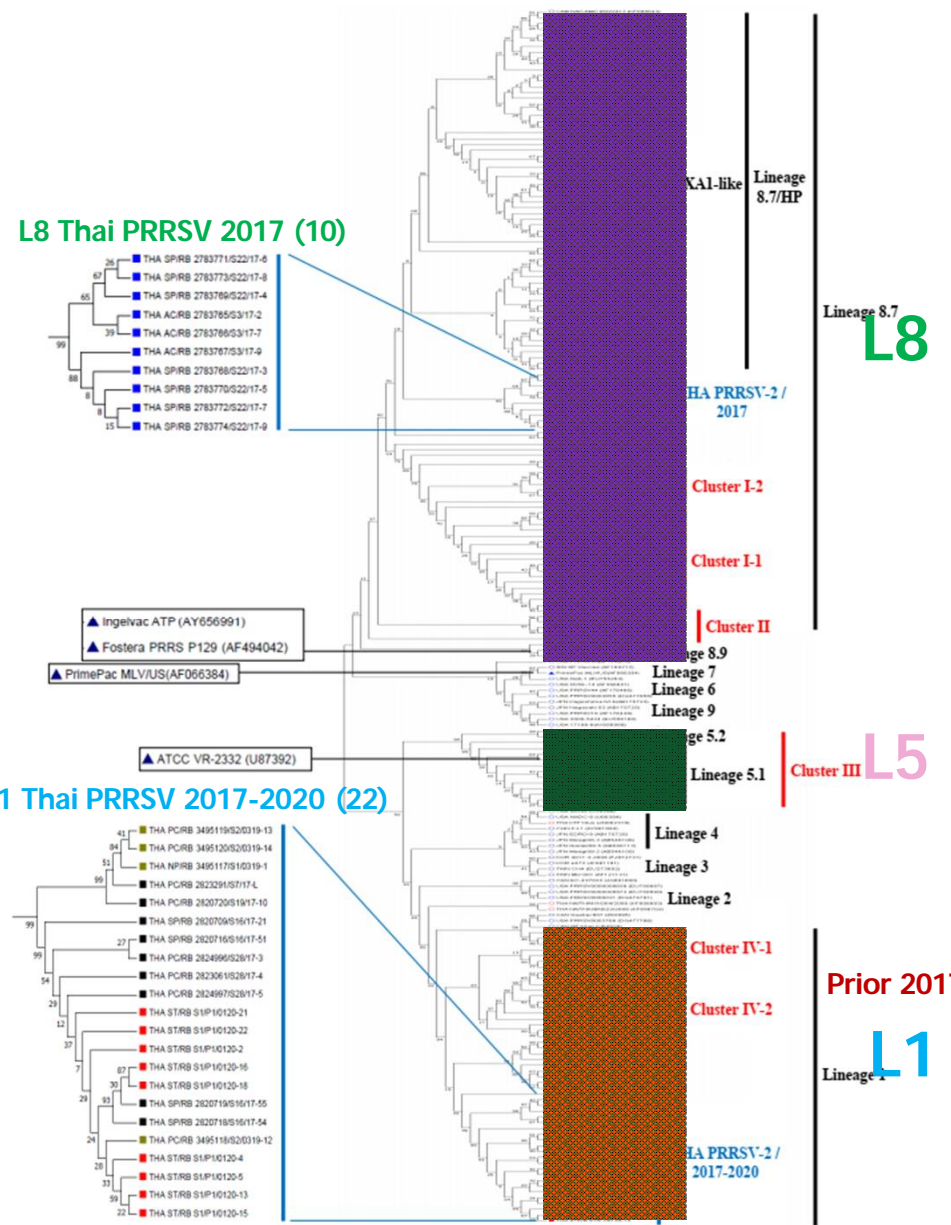
□ **Lineage 5 (72)**

□ **Lineage 8 (347)** – HP-PRRSV remained the predominant circulating PRRSV in the field



GENETIC DIVERSITY -THAILAND

PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME



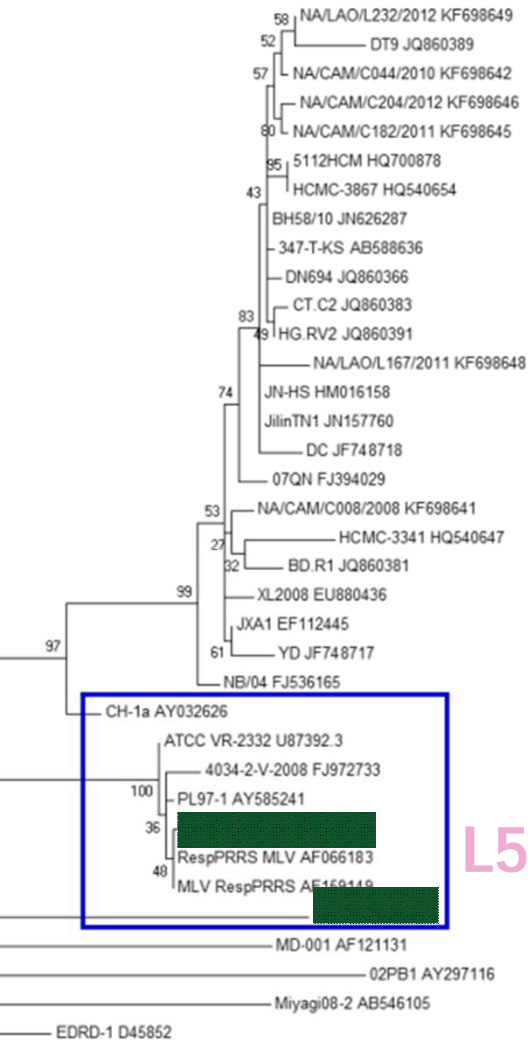
- ❑ PRRSV type 1 (EU) and PRRSV type 2 (US) co-infection (Chae et al. 2021)
- ❑ **Lineage 1** – Cluster IV-1 and IV-2; 22 isolates in 2017-2020
- ❑ **Lineage 5** – Cluster III
- ❑ **Lineage 8** – Cluster I-1, I-2 and Cluster II; Sublineage 8.7 (HP) 10 isolates in 2017

GENETIC DIVERSITY -MALAY

PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME

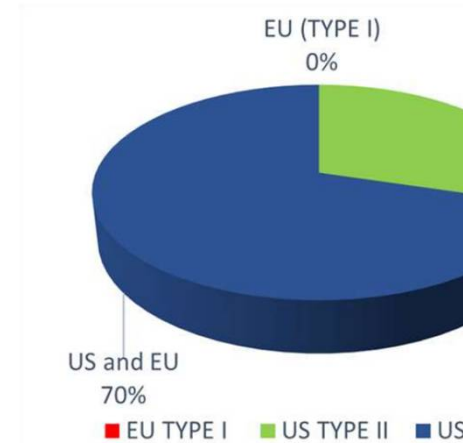
❑ PRRSV type 1 (EU) and PRRSV type 2 (US) co-infection (Yong et al. 2022)

❑ Lineage 5 – Sar01/2013 (KU512850) and Sel01/2014 (KU512851)



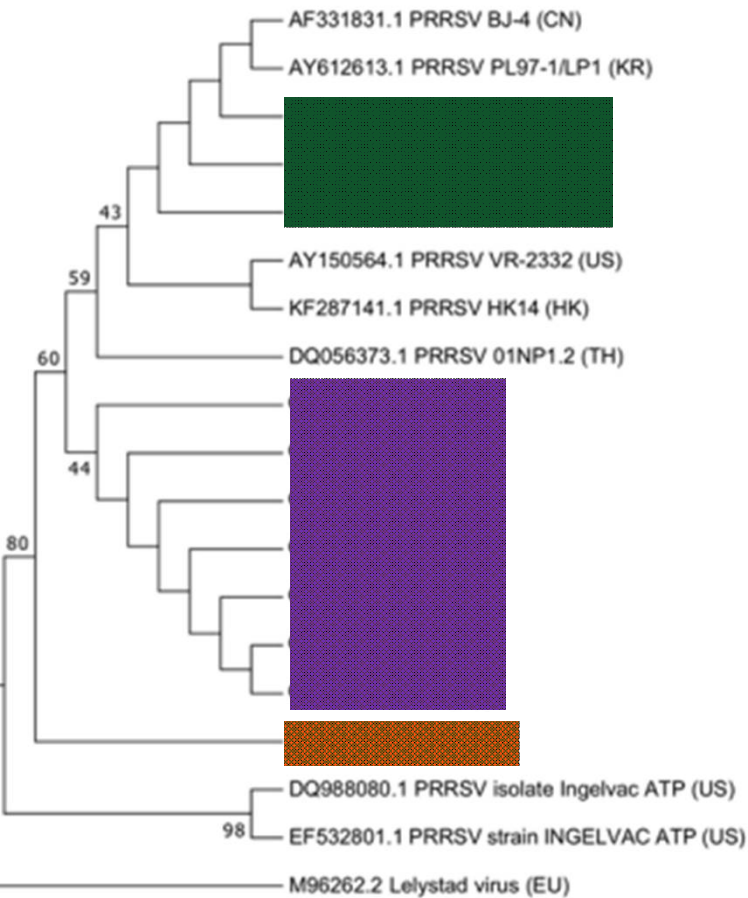
King et. al. 2017

as it has been documented that PRRSV strains differ in virulence [22, 52–55] and vary genetically [56–59] suggesting that the ORF5 gene derived from Case 2 (Sel01/2014), Central Malaysia (KU512851) may be a derivative of the Ingelvac PRRS MLV vaccine, a possible recombinant of the vaccine virus and a wild-type virus, or a truly wild-type virus that is partially homologous to the original parent vaccine strain, VR2332, which may be still circulating in the field [55].



GENETIC DIVERSITY -PHILIPPINE

PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME



ORF 6

❑ PRRSV type 1 (EU) and PRRSV type 2 (US) co-infection

❑ **ORF 6** analysis with two clusters (Parayao et al. 2021)

❑ **Cluster 1** – related to commercial MLV vaccine (L5?)

❑ **Cluster 2** – ???

Table 2. PRRSV positive samples detected using two RT-PCR methods.

Origin	Pool No.	Farm type	Real-time RT-PCR	Endpoint RT-PCR	Genotype
Biñan	P01	Commercial	-	+	NA strain
	P02	Commercial	-	+	NA strain
	P03	Commercial	-	+	NA strain
	P08	Commercial	-	+	NA strain
Nagcarlan	P15	Small-hold	+ (C: 28.36)	+	NA strain
	P23	Small-hold	+ (C: 29.66)	+	NA strain
San Pedro	P25	Commercial	-	+	NA strain
	P33	Commercial	-	+	NA strain
Siniloan	P34	Commercial	-	+	NA strain
	P37	Commercial	-	+	NA strain
	P39	Commercial	-	+	NA strain
	P55	Commercial	-	+	NA strain
Sta Cruz	P58	Small-hold	-	+	NA strain
	P59	Small-hold	+ (C: 26.27)	+	NA strain
	P60	Commercial	-	+	NA strain
	P61	Commercial	-	+	NA strain
	P65	Commercial	-	+	NA strain
	P67	Commercial	-	+	NA strain
	P72	Small-hold	-	+	NA strain
PRRSV positives detected			3/77 (3.9%)	19/77 (24.7%)	

GENETIC DIVERSITY -PHILIPPINE

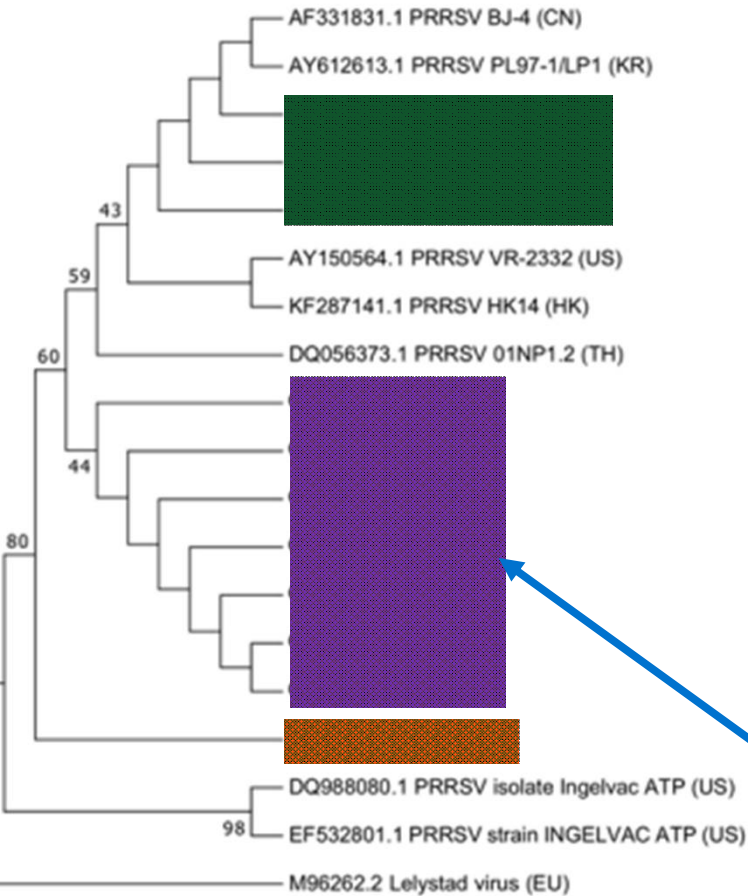
PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME

❑ PRRSV type 1 (EU) and PRRSV type 2 (US) co-infection

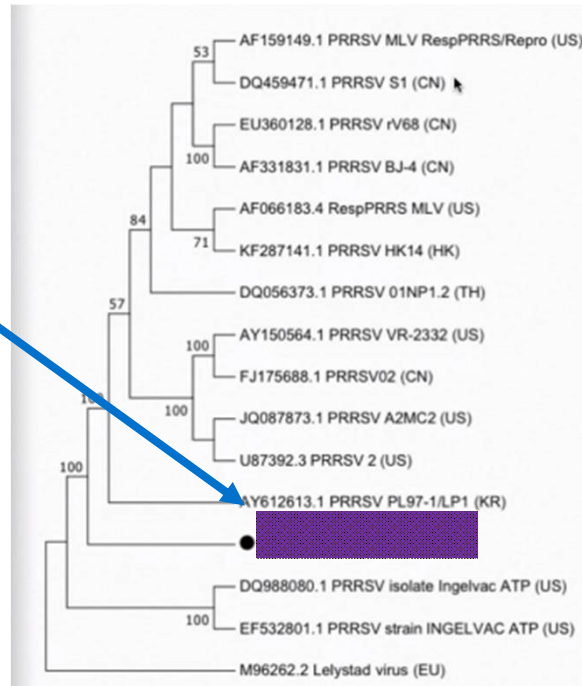
❑ **ORF 6** analysis with two clusters (Parayao et al. 2021)

❑ **Cluster 1** – related to commercial MLV vaccine (L5?)

❑ **Cluster 2** – ???



ORF 6



Whole genome sequences

Phylogenetic relationship of PRRSV Laguna strain with selected Asian and modified live PRRSV strains using whole genome sequences

GENETIC DIVERSITY -PHILIPPINES

PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME

❑ PRRSV type 1 (EU) and PRRSV type 2 (US) co-infection

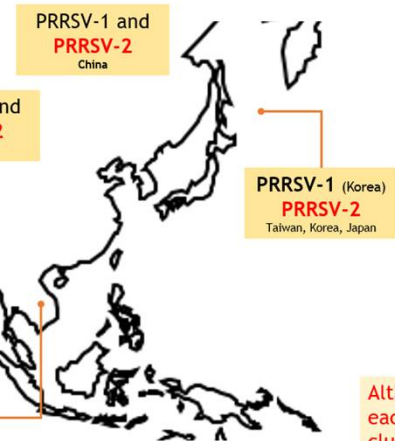
❑ ORF 6 analysis with two clusters (Parayao et al. 2021)

❑ **Cluster 1** – related to commercial MLV vaccine (L5?)

❑ **Cluster 2** – ???

❑ L1 and L5 sublineage 5.1 (Nilubol. 2023)

Asia and Southeast Asia



PRRSV-2

China; 4 lineages (1, 3, 5 and **8.7HP**)

- Lineage **5** (BJ-4-like) shares 99.8% identity with RespPRRS MLV.
- Lineage 1 (NADC30-like).

Vietnam

- Lineages 1, sub-lineages **8.7** and **5.1**.

Philippines

- Lineages 1 and sub-lineage **5.1**.

- **What are dominant lineages in this region?**

- sub-lineages **1**, **8.7** and **5.1**.
- **major factors accelerating genetic diversity in this region?**

Although clustered in the same lineage, PRRSV in each country has evolved separately into a separated cluster genetically distinct from other countries

SUMMARY

Porcine Reproductive and Respiratory Syndrome

High genetic diversity and will continue to mutate

Lineage classification of PRRSV2 is based on ORF5

- Glycoprotein 5 (GP5) -> Neutralizing antibodies

Lineage 1 to 9 (11)

- Increase detection of L1 isolates

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