

# Refining genetic classification of global PRRSV-1 ORF5 sequences and characterization of their geographic distributions



Wannarat Yim-im<sup>1</sup>, Tavis Anderson<sup>2</sup>, Jan Böhmer<sup>3</sup>, Jordi Baliellás<sup>4</sup>, Tomasz Stadejek<sup>5</sup>, Phillip Gauger<sup>1</sup>, Karen Krueger<sup>1</sup>, Cornelis Vermeulen<sup>6</sup>, Rianne Buter<sup>6</sup>, Aliaksandr Kazlouski<sup>7</sup>, Tongqing An<sup>8</sup>, Jianqiang Zhang<sup>1</sup>

<sup>1</sup>College of Veterinary Medicine, Iowa State University, USA; <sup>2</sup>National Animal Disease Center, USDA-ARS, USA; <sup>3</sup>IVD Gesellschaft für Innovative Veterinaerdiagnostik mbH, Seelzer-Letter, Germany; <sup>4</sup>Grup de Sanejament Porci (GSP), Lleida, Spain; <sup>5</sup>Institute of Veterinary Medicine, Warsaw University of Life Science, Poland; <sup>6</sup>Royal GD (GD Animal Health), The Netherlands; <sup>7</sup>Vitebsk State Academy of Veterinary Medicine, Viciebsk, Belarus; <sup>8</sup>Harbin Veterinary Research Institute, Chinese Academy of Agricultural Science, China

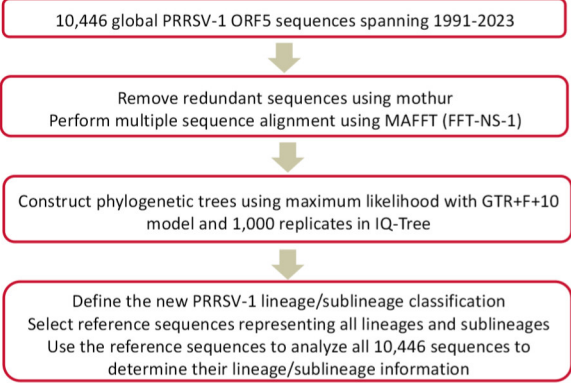
## INTRODUCTION

Based on ORF5 sequences, previous studies classified PRRSV-1 into four subtypes. Subtype 1 was further classified into 12 clades (A-L) or into three lineages with lineage 1 including clades 1A-1G and lineage 3 including clades 3A-3G. Researchers also classified PRRSV-1 detected in South Korea into subtypes 1A, 1B, and 1C. None of these classification systems have been widely accepted for use. The different classification systems have caused confusions when characterizing PRRSV-1 in molecular epidemiology studies. In this study, we proposed a statistically supported PRRSV-1 genetic classification system based on 10,446 global PRRSV-1 ORF5 sequences spanning 1991-2023.

## OBJECTIVES

To refine PRRSV-1 ORF5-based genetic classification system using the available global PRRSV-1 ORF5 sequences and characterize global distributions of PRRSV-1 lineages/sublineages.

## MATERIALS AND METHODS



## RESULTS AND DISCUSSION

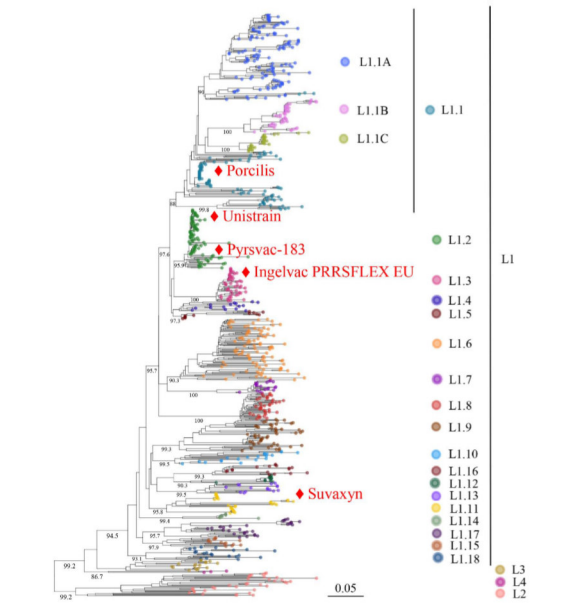
- Global PRRSV-1 ORF5 sequences were classified into four genetic lineages (L1-L4) with lineages 1, 2, 3 and 4 corresponding to the original subtypes 1, 2, 3 and 4. Lineage 1 sequences were further classified into 18 sublineages (L1.1-L1.18) and some unclassified L1 clades (Fig 1).
- The average pairwise nucleotide distance at the inter-lineage levels was in the range of 17.46% (between L3 and L4) to 21.46% (between L2 and L4). The average pairwise nucleotide distance at the intra-lineage level was in the range of 11.12% within lineage L3 to 17.55% within lineage L2.
- Among 18 L1 sublineages, the average intra-sublineage genetic distance was in the range of 3.51-11.93% and the average between-sublineage genetic distance was in the range of 8.39-18.32%.

## RESULTS AND DISCUSSION (cont.)

- With the flexibility of the proposed PRRSV-1 genetic classification, L1.1 could be further divided into three groups (L1.1A-L1.1C) and unclassified groups (Fig 1).
- Among the 10,446 PRRSV-1 ORF5 sequences analyzed in this study, most of them were L1 (10,384; 99.41%) and only a few sequences belonged to L2 (39; 0.37%), L3 (20; 0.19%) or L4 (3; 0.03%). L1 has a global distribution whereas L2, L3, and L4 were exclusively found in Eastern Europe (Table 1).
- In Europe, 17 out of 18 L1 sublineages (excluding L1.13) were identified. L1.3-L1.5, L1.7-L1.9, L1.12, L1.14-L1.16, and L1.18 sequences were exclusively distributed in Europe. In North America, only L1.1 was detected. In Asia, L1.1, L1.2, L1.6, L1.10, L1.11, L1.13 (confined to China), and L1.17 were identified (Table 1).
- A set of PRRSV-1 ORF5 reference sequences representing the refined classifications are available for future diagnostic and epidemiological applications.
- This study provides a benchmark describing the current genetic diversity and geographic distributions of PRRSV-1 at the lineage/sublineage levels. The refined ORF5-based classification system can be used to characterize PRRSV-1 genetic evolution in future studies.

**Table 1.** Geographic distributions of PRRSV-1 ORF5-based lineages and sublineages

Classification	Number in this study	Sample of seq	Collection year	Country or region distribution (number of sequences)	Continental level
Lineage 1	10,384	1991-2023; N/D (135)			
L1.1	4,256	1991-2023; N/D (42)		AUT (18), BEL (15), CHN (1), CZE (10), DEU (16), DEU-NLD-DNK (617), DNK (53), ESP (57), FRA (8), GBR (164), HRV (6), HUN (187), ITA (4), KOR (7), LTU (1), LVA (1), NLD (282), POL (24), SVK (1), SVN (50), THA (156), USA (2,556), Undefined (22)	Europe, Asia, North America, Undefined
L1.2	1,033	1991-2023; N/D (24)		AUT (7), BEL (1), BLR (1), CHN (16), CZE (30), DEU (2), DEU-NLD-DNK (541), DNK (7), ESP (189), FRA (3), HUN (73), KOR (53), NLD (66), POL (12), PRT (1), ROU (6), SVN (3), THA (14), TWN (3), VNM (3), Undefined (2)	Europe, Asia, Undefined
L1.3	680	2004-2023; N/D (15)		DEU (3), DEU-NLD-DNK (468), ESP (5), HUN (7), NLD (181), Undefined (16)	Europe, Undefined
L1.4	15	1994-2011		POL (15)	Europe
L1.5	44	1991-2021; N/D (8)		DEU (4), DEU-NLD-DNK (7), ESP (17), HUN (6), POL (1), Undefined (9)	Europe, Undefined
L1.6	1,384	1992-2022; N/D (25)		AUT (1), CHE (1), DEU (29), DEU-NLD-DNK (425), DNK (2), ESP (94), HRV (1), HUN (27), KOR (784), NLD (3), POL (3), SVK (2), SVN (7), Undefined (5)	Europe, Asia, Undefined
L1.7	161	2013-2021		AUT (3), DEU-NLD-DNK (129), ESP (1), HUN (14), NLD (12), SVN (2)	Europe
L1.8	822	2013-2023		DEU-NLD-DNK (396), ESP (7), HUN (4), NLD (414), SVN (1)	Europe
L1.9	957	2003-2023; N/D (1)		BEL (3), BGR (3), DEU (7), DEU-NLD-DNK (591), ESP (6), HUN (25), NLD (294), POL (25), SVN (2), Undefined (1)	Europe, Undefined
L1.10	59	1992-2017		CHN (20), CZE (3), DEU (2), DEU-NLD-DNK (1), DNK (28), HUN (5)	Europe, Asia
L1.11	330	1994-2023; N/D (7)		BEL (8), CHN (3), DEU-NLD-DNK (99), ESP (9), KOR (59), NLD (55), THA (95), Undefined (2)	Europe, Asia, Undefined
L1.12	12	2010-2011		ROU (12)	Europe
L1.13	48	2006-2023		CHN (48)	Asia
L1.14	8	2003-2016		DEU (7), DEU-NLD-DNK (1)	Europe
L1.15	14	2009-2015		HUN (2), SRB (12)	Europe
L1.16	26	1995-2017		CZE (19), ESP (2), HUN (3), SVK (2)	Europe
L1.17	116	1991-2023		CHN (5), ESP (66), ITA (43), NLD (2)	Europe, Asia
L1.18	66	1996-2017		ITA (56), SVN (10)	Europe
Unidentified sublineage in L1	353	1992-2023		BEL (1), BLR (1), DEU-NLD-DNK (39), DNK (2), ESP (178), HKG (5), HUN (1), ITA (82), NLD (24), POL (9), SVK (3), USA (1), Undefined (7)	Europe, North America, Undefined
Lineage 2	39	1997-2022		BLR (7), LTU (7), RUS (25)	Europe
Lineage 3	20	2004-2022		BLR (20)	Europe
Lineage 4	3	2004		BLR (3)	Europe
<b>Grand total</b>	<b>10,446</b>				



**Fig 1.** Phylogenetic tree showing the newly proposed PRRSV-1 lineages and sublineages based on global ORF5 sequences. Tip points are presented in different colors. Bootstrap values are shown for the major clades. Five commercial PRRSV-1 modified live virus vaccines Porcilis PRRS, Unistrain PRRS, Pysvac-183, Ingelvac PRRSFLEX EU, and Suvaxyn PRRS MLV are classified in the sublineages L1.1, L1.2, L1.3, and L1.11, respectively.