Communication

Severe Mutations on Open Reading Frame (ORF5) of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV)2 in Korean Pig Farm

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Received: 4 August 2025; Revised: 8 September 2025; Accepted: 29 September 2025; Published: 30 September 2025

Abstract: Porcine reproductive and respiratory syndrome virus (PRRSV)2 is a highly contagious Ribonucleic acid (RNA) virus of the Arteriviridae family that causes reproductive and respiratory disorders in pigs, posing a major economic threat to the swine industry in Republic of Korea and worldwide. Recently, a PRRSV2-related virus was isolated from a pig farm in Gyeonggi-do, Republic of Korea, and its open reading frame 5 (ORF5, also known as GP5; glycoprotein 5) was sequenced to characterize the strain. Sequence alignment showed only 57.7% identity to PRRSV1 references, consistent with PRRSV2 rather than PRRSV1; however, surprisingly, due to the severe mutation in ORF5, this sequence does not belong to any known PRRSV2 strain with an average identity of 87.4%. These findings raise concerns about the effectiveness of currently available PRRSV2 vaccines in Republic of Korea and suggest that vaccines derived from newly isolated strains may be required for more effective protection.

Keywords: porcine reproductive and respiratory syndrome virus (PRRSV)2; RNA virus; open reading frame 5 (ORF5); mutation; vaccine

1. Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV)2 is a highly contagious positive sense, single-stranded RNA virus belonging to the Arterivirus family [1,2]. PRRSV2 primarily affects the respiratory tract of young pigs and can cause reproductive failure in breeding animals. Two genotypes of PRRSV2 have been proposed, European PRRSV1 and North American PRRSV2, which cause largely similar pathogenicity but have very high genetic diversity. PRRSV1 and 2 share only 55–70% sequence identity [3,4]. Unlike coronaviruses, arteriviruses are known to evolve rapidly due to the lack of proofreading enzymes, such as the Exoribonuclease (ExoN), which coronaviruses possess and are essential for survival. Therefore, there is considerable genetic variation within each strain of PRRSV2. At least nine lineages have been proposed for North American PRRSV2 [5–7].

PRRSV2 infection is considered the disease that causes the greatest economic damage to the global pig industry. It is estimated that in the United States alone, damages amount to approximately $664 million annually [8–11]. PRRS first appeared in Europe in 1991 but became prevalent in Western Europe and North America in the late 1980s, causing respiratory disease in young pigs and reproductive failure in sows, leading to abortions, mummified fetuses, and weak piglets [12–15].

European PRRSV1 can be phylogenetically divided into at least three, if not four, subtypes. For example, there is a pan-European PRRSV1 type 1, Eastern European PRRSV1 types 2 and 3, and Belarusian and Latvian PRRSV1 type 4. Type 1 is subdivided into at least 12 clades. Interestingly, despite the increasing genetic diversity of PRRSV1, strains similar to those found in the early 1990s are still circulating [7,16,17]. Despite intensive research on viruses associated with Porcine high fever disease (PHFD), the genetic basis for this increased pathogenicity is not yet fully understood. Recent studies suggest that mutations in nonstructural proteins 10 may lead to increased viral virulence [18]. Several

reports have also described differences in virulence between PRRSV1 strains, finding that Eastern European subtype 3 strains are significantly more pathogenic than subtype 1 strains [19–23].

Vaccine development and vaccination are the basis for the management of most viral diseases, including PRRS. However, despite the development of various PRRSV2 vaccines, including inactivated or modified live virus (MLV) vaccines, none have yet proven sufficiently effective in controlling the spread of this disease. Among available vaccines, MLV vaccines are the most widely used. Representative commercial PRRSV-2 vaccines include Ingelvac PRRS® MLV, derived from lineage 5 (variant 5A.1), and Prevacent® PRRS MLV, derived from lineage 1 (variant 1D.2). These vaccines provide clinical protection but have limited cross-protective efficacy against genetically diverse field strains and carry potential risks of recombination or reversion to virulence [24]. One of the challenges in developing an improved vaccine is the lack of understanding of the animal immune response to PRRSV2 infection and the fact that the virus is an RNA virus with a high mutation rate. For PRRSV-1, vaccines such as those derived from the Lelystad virus are widely used [25]. However, unlike PRRSV2, cellular immune responses appear to be important in achieving protection, and antibody responses appear to be less useful overall for PRRSV1 [26,27].

The importance of virus-neutralizing antibody production remains largely unclear, partly because it is difficult to assess accurately with current in vitro assay methods. At the very least, a better understanding of these common features of the immune response across genotypes is essential to providing an intelligent approach to improved vaccine design. In this study, we present the cloning of the ORF5 gene of a Korean local farm. The amino acid sequence and DNA sequence were found to be significantly mutated, suggesting that the efficacy of current vaccines may be limited in preventing the spread of PRRSV2 epidemics in Korean farms.

2. Material and Methods

2.1. Isolation of Total RNA and Reverse Transcription Polymerase Chain Reaction (RT-PCR)

Whole blood cells from five piglets were collected and total RNA was isolated using RNAiso (Takara, Kusatsu, Japan). Total RNA (2 μg) from each sample was reverse transcribed using 1 μL of M-MuLV reverse transcriptase (Cosmo Genentech, Seoul, Republic of Korea) in a 20 μL reaction volume according to the attached protocol. We performed PCR of ORF5 of PRRSV2 (accession NO. PP921531.1) with sense primer: 5′-TCAGGTATGTT GGGGAAATGC-3′; reverse primer: 5′-GAGCTGTCA TAGCAGAAGTC G-3′. The PCR products were loaded onto a 1% agarose gel electrophoresis unit and examined under ultraviolet (UV) illumination.

2.2. Cloning of ORF5 and DNA Sequencing

TA cloning of ORF5 PRRSV2 was performed to ligate the PCR product of ORF5 from whole blood cells of piglets from pig farms in Republic of Korea. The ligated TA cloning plasmid vector was transformed into DH5α competent cells as previously described [28]. Positive clones were screened using the same forward and reverse primers used for RT-PCR. The positive clones were prepared for plasmid isolation (Cosmo Genentech), and the ORF5 insert was released from the TA cloning vector using Hind III restriction enzyme (Takara). One positive clone was sent for DNA sequencing analysis (Cosmo Genentech).

2.3. Align Amino Acid Sequence of ORF5 PRRSV2

Eleven different ORF5 PRRSV2 amino acid sequences (Table 1) were aligned using Clustal Omega a multiple sequence alignment program (https://www.ebi.ac.uk/jdispatcher/msa/clustalo?stype=protein (accessed on 7 July 2025) to determine their similarity to ORF5 PRRSV2 cloned from a Korean pig farm. In addition, two sequence alignments were performed using SIM-Protein Sequence Alignment Tool (https://web.expasy.org/sim/ (accessed on 17 July 2025)) to compare the newly cloned ORF5 PRRSV2 with ORF5 PRRSV1 (Figure 1).

Table 1. Eleven ORF5 genes, Information on the 11 ORF5 genes includes gene ID, protein ID, origin, reference number, and homology with the newly isolated ORF5 from a Korean local farm (Kulf). Identity (ID).

ORF5				
Gene ID	**Protein ID**	**Origin**	**Reference**	**Kulf % Identity**
PP658207.1	WZH58094.1	Minnesota 56187, USA	JOURNAL Submitted (11 April 2024) Diagnostics, Research and Development	88.50%
AY424271.1	AAR88269.1	Minnesota 55108, USA	J. Virol. 78 (7), 3684–3703 (2004)	87.40%

Table 1. *Cont.*

ORF5				
Gene ID	**Protein ID**	**Origin**	**Reference**	**Kulf % Identity**
PP409069.1	WYX89014.1	Gansu province of China	Submitted (27 February 2024) Description of Host Antiviral Infection	87.00%
OP866757.1	XAJ10782.1	Xin Jiang 830023, China	Journal Submitted (19 November 2022) Technique Center, Animal College	86.00%
MK287894.1	QDL52618.1	Mizoram 796014, India	Journal Submitted (12 December 2018) Veterinary Pathology	85.40%
MK453049.1	QGT31804.1	Jilin 130122, China	Journal Submitted (25 January 2019) Academy of Military Medical Sciences Institute of Military Veterinary	89.40%
MK820650	QGD14175.1	Zoetis Michigan 49007, USA	Journal Submitted (22 April 2019) Verterinary Medicine Research & Development	86.40%
AF159149.1	AAG02138.1	New York 11944, USA	Journal Arch. Virol. 145 (6), 1149–1161 (2000)	84.50%
PP740377.1	XAI71514.1	Batangas 4234, Philippines	Journal Submitted (17 April 2024) Research, BioAssets Corporation	86.50%
PP921531.1	XCA47559.1	Seoul 08823, South Korea	Journal Submitted (14 June 2024) Veterinary Medicine School, Seoul National University	88.50%
JQ656131	AFY24382.1	Anyang, Gyeonggi 13998, South Korea	Submitted (9 February 2012) Viral Disease Division, Animal, Plant and Fisheries Quarantine and Inspection Agency	92.00%

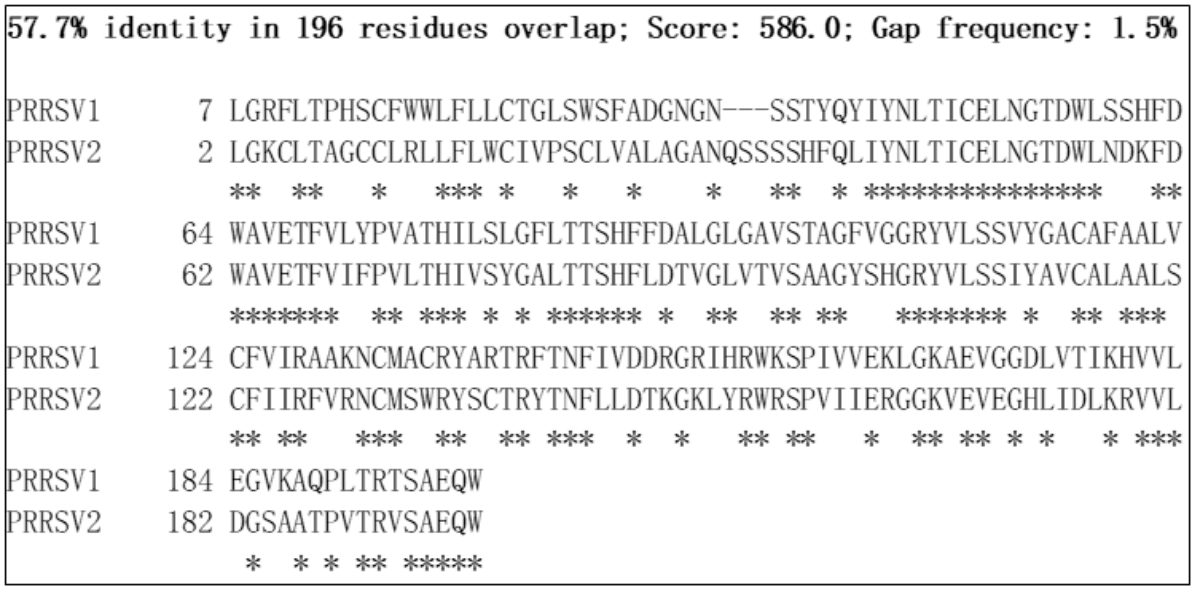

Figure 1. Comparison of PRRSV1 ORF5 to Kulf, Amino acid sequence alignment of newly isolated ORF5 (Kulf) compared to PRRSV1 ORF5**,** 'PRRSV2' refers to the amino acid sequence that was newly obtained from a Korean pig farm (Kulf). * indicates positions where the two sequences are identical.

3. Result and Discussion

Globally, PRRSV2, a positive single-stranded RNA virus, represents a formidable pathogen that imposes significant economic losses on the swine industry. A serious problem is the very fast mutation of PRRSV2 that escapes current vaccine strategies [24]. Because the virus evolves regionally and locally, continuous monitoring of country-specific isolates is crucial. In this context, we characterized a newly isolated strain from a Korean pig farm to evaluate its genetic divergence, and compared its ORF5 amino acid sequence with representative PRRSV1 sequences (Figure 1) as well as with 11 different PRRSV2 sequences (Table 1). The ORF5 amino acid sequence of the isolate shared only 57.7% identity with PRRSV1 references, but showed 84.5–92% identity with representative PRRSV2 strains (Figure 1, Table 1), indicating that it belongs to PRRSV2 but has accumulated substantial mutations. Notably, none of the 12 PRRSV2 ORF5 sequences analyzed were identical to each other,

reflecting the high variability of this gene (Figure 2). Table 1 presents eleven different ORF5 gene IDs, protein IDs, origins, references, and homology with the newly isolated ORF5.

While Kulf clearly belongs to PRRSV2 rather than PRRSV1, it does not cluster with any recognized PRRSV2 lineage and instead forms a distinct branch, suggesting the emergence of a putative new sub-lineage showing the relatively long branch length (Figure 3). Surprisingly, the closest strain (Protein ID AFY24382.1; Accession No. JQ656131), isolated by the Virus Division of the Animal, Plant and Fisheries Quarantine Agency in Anyang, Gyeonggi Province, in 2012, shared 92% identity (Table 1), whereas a more recent Korean strain (Protein ID XCA47559.1; Accession No. PP921531.1) from Veterinary Medicine School, Seoul National University, Seoul 08823, Republic of Korea a year ago in 2024 was shared only 88% identity (Table 1) with the newly isolated ORF5 PRRSV2 strain (also named Kulf). Phylogenetic analysis revealed that the strain (Protein ID AFY24382.1; Accession No JQ656131) was the closest phylogenetic partner of the newly isolated ORF5 PRRSV2 (Figure 3). In contrast, another Korean strain (Protein ID 134 XCA47559.1; Accession No. PP921531.1) was positioned on a separate basal branch of the tree, indicating that PRRSV2 strains circulating in Republic of Korea encompass distinct evolutionary lineages rather than forming a single cluster. These data suggest that, despite Republic of Korea being a very small country, ongoing rapid mutations are already isolating PRRSV2 strains into sub-strains.

Figure 2. An amino acid sequence alignment of 11 PRRSV2 ORF5 isolates, including a newly isolated ORF5 from a Korean farm (Kulf). * indicates positions where the two sequences are identical.

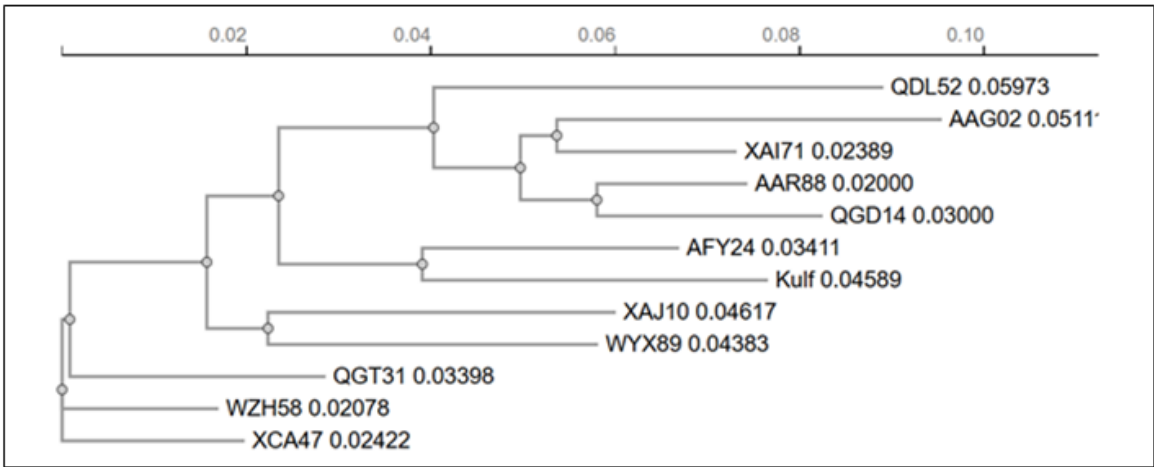

Figure 3. Phylogenetic analysis of 11 PRRSV2 with Kulf, phylogenetic analysis revealed that the strain (Protein ID AFY24382.1; Accession No JQ656131) is the closest PRRSV2 ORF5 of newly isolated ORF5 PRRSV2. Identity (ID), Number (No).

PRRSV, which causes significant economic losses to pig farms worldwide and domestically, has been managed using PRRSV vaccines as a preventive strategy for domestic pig farms. However, the results of this study raise questions about the effectiveness of vaccines currently used in Republic of Korea, as evidenced by the severe PRRSV2 ORF5 mutation. This paper underscores the need for more in-depth research and the development of novel vaccines to control PRRSV2 infection more effectively.

Author Contributions: H.K., W.H., M.Y., S.L., D.S., J.H., M.J.-C. and S.K.: designed and analyzed the data; H.K., W.H., J.H. and S.K.: performed the experiments; H.K. and S.K.: funding acquisition; H.K., W.H., M.Y., S.L., D.S., J.H., M.J.-C. and S.K.: examined the data; H.K. and S.K.: edited the manuscript; H.K. and S.K.: designed the study, supervised the project, and wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by YbdY biotech and Kulf Co., Ltd. internal grants.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

ExoN	Exoribonuclease
GP5	Glycoprotein 5
ORF 5	Open reading frame 5
ID	Identification
No	Number
PHFD	Porcine high fever disease
PRRSV	Porcine reproductive and respiratory syndrome virus
RNA	Ribonucleic acid

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