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NOTE

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First detection of NADC34-like porcine reproductive and respiratory syndrome virus strains in Japan

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ABSTRACT. Porcine reproductive and respiratory syndrome virus (PRRSV) is an important pathogen in the swine industry. We report for the first time the detection of NADC34-like PRRSV strains in Japan. Serum samples from 18 piglets in Okinawa Prefecture were analyzed for the open reading frame 5 (ORF5) gene encoding glycoprotein 5, which revealed that all samples belonged to sublineage 1.5 (L1A) of Lineage 1. Phylogenetic analysis, restriction fragment length polymorphism patterns, and glycosylation site characteristics confirmed the presence of NADC34-like strains that caused severe outbreaks in the U.S. and Asia. Despite its known pathogenicity, the farm showed only a transient increase in mortality. These findings highlight the need for further investigation into the pathogenicity of PRRSV strains and their potential for the emergence of new variants in Japan.

KEYWORDS: lineage, phylogenetic, porcine reproductive and respiratory syndrome virus, swine

Porcine reproductive and respiratory syndrome virus (PRRSV) is a major pathogen that causes significant economic losses for the global swine industry. It impairs the reproductive performance of sows and induces a range of pathological conditions, including respiratory symptoms, particularly during the weaning and fattening stages. PRRSVs are classified into two primary genotypes: PRRSV-1 (European type) and PRRSV-2 (North American type). PRRSV-2, in particular, is of concern because of its genetic diversity and high mutation rate [23]. Based on the genetic sequence of ORF5 genes, which encodes the major envelope glycoprotein GP5, PRRSV-2 was further divided into 11 distinct lineages (lineages 1–11). GP5 plays a pivotal role in viral infectivity, replication, and induction of neutralizing antibodies. Lineage 1 was further subdivided into 10 sublineages (L1A to L1J), each exhibiting unique pathogenic characteristics and geographical distribution [19].

Several highly pathogenic PRRSV-2 strains circulate in the U.S., many of which belong to these sublineages. Notably, sublineage 1.5 (L1A) includes the NADC34 strain, sublineage 1.8 (L1C) includes the NADC30 strain, and sublineage 1.9 (L1F) includes the MN184 strain [14]. Because of their heightened pathogenicity, these strains have caused significant outbreaks, posing a serious challenge to the swine industry. Recently, NADC30- and NADC34-like PRRSV strains have spread across Asia, including China, Korea, Thailand, and Taiwan, resulting in high mortality rates and severe economic losses [9, 13, 18]. PRRSV-2 was first identified in Japan in the early 1990s. Despite extensive vaccination efforts, the virus continues to persist in the swine population, raising concerns about vaccine efficacy and the potential emergence of novel strains [7].

In Japan, PRRSV-2 genetic classification was performed through cluster analysis, which revealed five major clusters [5, 20]. Compared with the widely adopted international lineage classification, Cluster 1 corresponds to Lineage 8, Cluster 2 to Lineage 5, Cluster 3 to Lineage 4, Cluster 4 to Lineage 1, and Cluster 5 to Lineage 4. From 1990 to 2000, Cluster 3 was the dominant strain in Japan; however, in recent years, strains from Cluster 4 have become increasingly prevalent. These newer strains are believed to be more pathogenic than their predecessors [5, 8]. The Cluster 4 strain in Japan (Jpn5-37) was first detected in 2008 and shares significant homology with the MN184 strain from Lineage 1 based on ORF5 gene similarity [4]. Despite further diversification of Lineage 1 into several sublineages, a detailed analysis of the sublineages within Lineage 1 has not been conducted in Japan. Moreover, the presence of NADC-like strains, as reported in the U.S. and Asia, remains underexplored. In this study, we report the first detection of NADC34-like PRRSV strains in Japan, identified through the lineage classification of strains collected from piglets in Okinawa Prefecture.

In 2022, serum samples were collected from 18 piglets (42–56 days old) on a pig farm in Okinawa Prefecture as part of a diagnostic

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investigation. According to a report from the farm, PRRSV began circulating among piglets in the early growth stage, with respiratory symptoms appearing at approximately 60 days of age; however, no significant clinical symptoms were observed in these 18 piglets. All samples were transported under refrigerated conditions and processed immediately upon arrival.

RNA isolation, RT-PCR, and Sequencing of PRRSV ORF5 was performed by SMC Co., Ltd. (Atsugi, Japan). The resulting sequences were aligned and subjected to phylogenetic analysis using ISU PRRSView, hosted by the Iowa State University [11, 12, 21]. Additionally, restriction fragment length polymorphism (RFLP) patterns were determined using the method described by Wesley *et al.*, employing three restriction enzymes (MluI, HincII, and SacII) and expressed as three-digit codes [16]. The representative strain codes belonging to Lineage 1 are as follows: NADC30 (1-4-4), NADC34 (1-7-4), and MN184 (1-8-4) [20]. N-linked glycosylation sites in ORF5 were predicted using NetNGlyc 1.0, hosted by the Technical University of Denmark [3].

Phylogenetic analysis of the ORF5 gene sequences from 18 samples (no. 31, 32, 33, 34, 35, 37, 61, 63, 67, 69, 70, 71, 72, 74, 75, 76, 77, and 80) was performed, with the corresponding GenBank accession numbers LC848471 to LC848488. It was revealed that all strains belonged to sublineage 1.5 (L1A) within Lineage 1 (Fig. 1A, 1B). The ORF5 gene sequence homology among the samples ranged from 91.5 to 99.8%, while homology with reference to Lineage 1 strains was 90.9–92.9% for NADC34, 86.0–88.6% for NADC30, 86.4–87.7% for MN184, and 86.1–87.6% for Jpn5-37. The highest homology was observed with the highly virulent NADC34 strain, first isolated in the U.S. in 2014, and is associated primarily with abortion outbreaks (Table 1) [1, 15].

In the RFLP analysis, 10 of the 18 samples exhibited the 1-7-4 pattern, whereas the remaining 8 samples showed the 1-4-4 pattern (Table 2). These findings suggested that two distinct RFLP patterns, both belonging to the same L1A lineage, were present on this farm. Previous studies have reported that within the sublineage L1A, 59.4% of ORF5 RFLP patterns were 1-7-4, followed by 11.4% with the 1-4-4 pattern, which is consistent with the findings of this study [19]. Conversely, the 1-4-4 pattern was most frequently observed in sublineage L1C (65%), whereas the 1-8-4 pattern was common in sublineage L1F (33.1%). However, it is important to note that RFLP patterns alone may not accurately reflect viral genotypes [19].

The GP5 protein of PRRSV-2 contains multiple N-linked glycosylation sites that are essential for viral assembly and host cell entry. GP5 is a transmembrane protein that contains a neutralizing epitope between amino acids 35 and 51. These regions are highly variable and often glycosylated, potentially affecting immunogenicity, depending on the glycosylation patterns [2]. Although the number of glycosylation sites varies across strains, two conserved regions (positions 44 and 51) are crucial for viral survival [10]. Despite the variability in glycosylation patterns across lineages, a glycosylation site at position 57 was uniquely retained in over 62% of L1A strains in the USA in 2015 [14]. In the present study, 12 of the 18 L1A samples exhibited glycosylation at position 57 (67%), reinforcing this characteristic feature of L1A (Table 2). On the other hand, glycosylation patterns exhibit annual variability even within the same lineage. In L1A, glycosylation at position 57 disappeared between 2013 and 2014 and only reappeared after 2015 [10, 14]. In this case, strains lacking glycan at position 57, as reported in previous studies, were present on the same farm [14]. The reemergence and coexistence of these strains are believed to be related to the circulation of different epidemic strains within the farm; however, this relationship remains unclear [10].

Although no single piece of information was conclusive, the combination of phylogenetic analysis (L1A), RFLP patterns (1-7-4 or 1-4-4), and glycosylation site characteristics allowed for the classification of the strains analyzed in this study as typical L1A (NADC34-like strains). This is the first detection of NADC34-like strains in Japan. NADC34-like strains have been detected in Peru (2015), China (2017), and Korea (2022) with widespread transmission across China since 2020, raising concerns regarding the emergence of new variant strains [6, 17, 22]. In the U.S., more than half of the PRRSV strains detected since 2006 belong to Lineage 1, with L1A accounting for 40.7–66.3%, underscoring the global spread of this lineage [19]. Although NADC34-like strains are known to cause severe clinical signs, particularly abortion, the pig farm in this study experienced only a transient increase in mortality and mild respiratory symptoms during the early fattening stage, with no other notable clinical signs [18]. Although the relationship between viral strain and pathogenicity remains poorly understood, further investigations combining lineage and glycosylation site analyses could provide valuable insights into PRRSV strain-pathogenicity correlations.

CONFLICT OF INTEREST. All authors have no conflicts of interest related to the content of this article.

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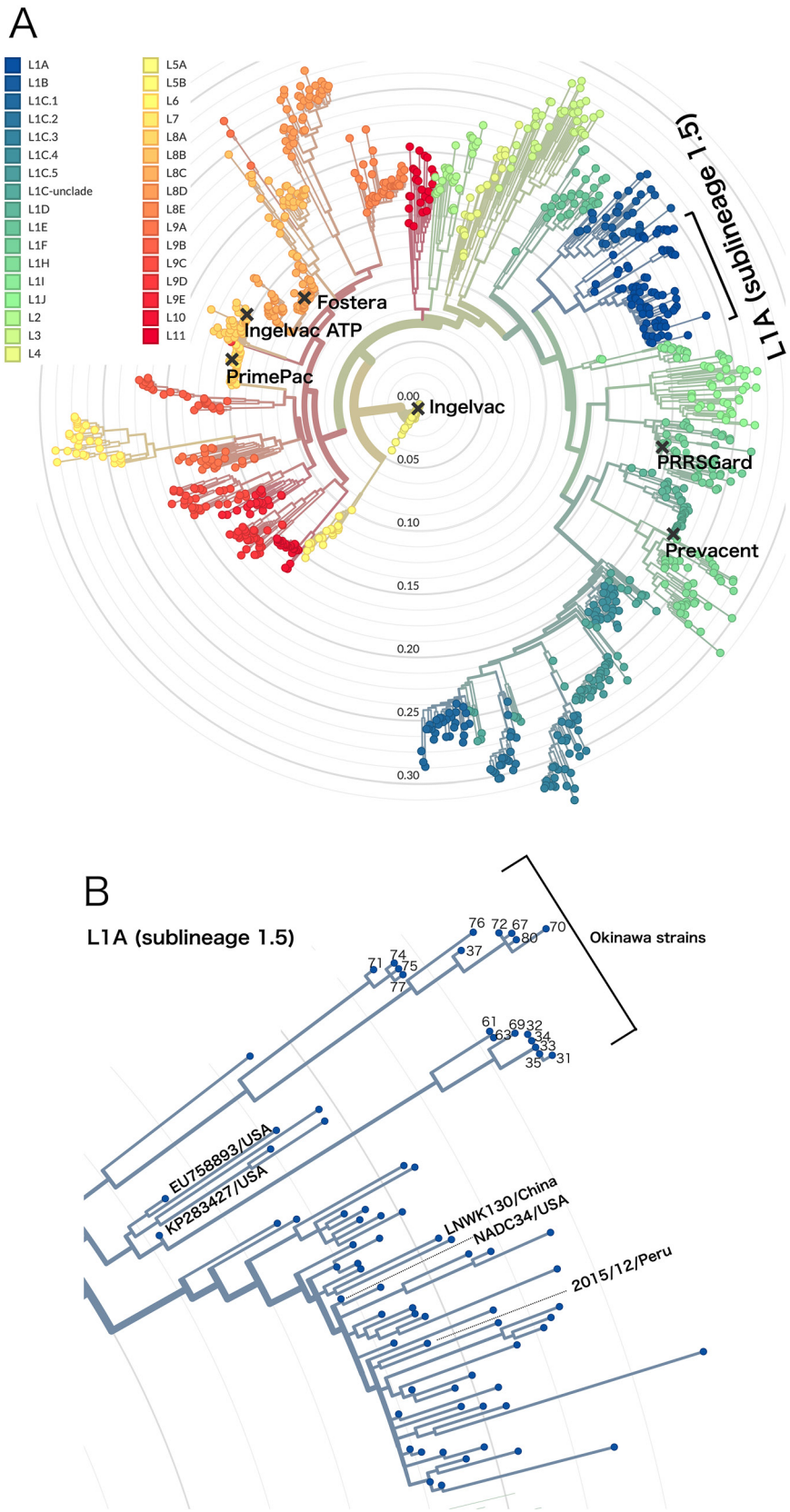

Fig. 1. Molecular phylogenetic tree based on the open reading frame 5 (ORF5) gene of porcine reproductive and respiratory syndrome virus. **A**: Phylogenetic tree showing all lineages from lineages 1 to 11. × symbols indicate representative vaccine strains of several lineages. The correspondence between cluster classification and lineage was as follows: cluster 1, L8; Cluster 2, L5; Cluster 3, L4; Cluster 4, L1; Cluster 5, L4. **B**: Enlarged view of L1A lineage. Numbers 31–35, 37, 61, 63, 67, 69, 70–72, 74–77, and 80 indicate the strains obtained in this study. KP283427, EU758893, and NADC34 refer to representative NADC34-like strains isolated in the United States. LNWK130 represents a strain isolated in China, and 2015/12 refers to a strain isolated in Peru.

Table 1. The open reading frame 5 (ORF5) gene homology between Okinawa-detected strains and representative Lineage 1 strains

Reference strain	Lineage	Homology (%)			
		Min	Max	Ave	SD
NADC34	L1A	90.9	92.9	91.9	0.6
NADC30	L1C	86.0	88.6	87.6	0.9
MN184	L1F	86.4	87.7	87.2	0.5
Jpn5-37	L1F	86.1	87.6	87.1	0.5

Min, minimum homology; Max, maximum homology; Ave, average homology.

Table 2. Restriction fragment length polymorphism (RFLP) pattern of the open reading frame 5 (ORF5) gene and N-glycosylation sites in the GP5 amino acid sequence

Strain	RFLP code	N-linked glycosylation sites*
31	1-7-4	30, 33, 44, 51, 57
32	1-7-4	30, 33, 44, 51, 57
33	1-7-4	30, 33, 44, 51, 57
34	1-7-4	30, 33, 44, 51, 57
35	1-7-4	30, 33, 44, 51, 57
37	1-7-4	32, 33, 44, 51
61	1-7-4	30, 33, 34, 44, 51, 57
63	1-4-4	30, 33, 34, 44, 51, 57
67	1-4-4	33, 34, 44, 51
69	1-7-4	30, 33, 34, 44, 51, 57
70	1-4-4	33, 34, 44, 51
71	1-7-4	30, 33, 44, 51, 57
72	1-4-4	33, 34, 44, 51
74	1-7-4	30, 33, 44, 51, 57
75	1-7-4	30, 33, 44, 51, 57
76	1-4-4	33, 34, 44, 51
77	1-7-4	30, 33, 44, 51, 57
80	1-4-4	33, 34, 44, 51
NADC34	1-7-4	32, 33, 44, 51, 57
NADC30	1-4-4	34, 44, 51
MN184	1-8-4	34, 51
Jpn5-37	1-8-4	44, 51

*Position number from the start codon.

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