Emergence of a novel PRRSV-1 strain in mainland China: a recombinant strain derived from the two commercial modified live viruses Amervac and DV

Qi Sun[1], Hu Xu[1], Chao Li[1], Bangjun Gong[1], Zhen Li[2], Zhijun Tian[1], and HongLiang Zhang[1]

[1]Chinese Academy of Agricultural Sciences Harbin Veterinary Research Institute
[2]Pingdingshan Center For Animal Disease Control And
Prevention Pingdingshan 467000 China

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Abstract

Porcine reproductive and respiratory syndrome virus 1 (PRRSV-1) is one of the main pathogens causing porcine reproductive and respiratory syndrome (PRRS). In recent years, the detection number of PRRSV-1 in China has gradually increased, and the PRRSV-1 strains reported in China belong to subtype I (Global; Clade A-L). In the present study, a novel PRRSV-1 strain, TZJ2134, was found during epidemiological surveillance of PRRSV-1 in Shandong Province in China. We obtained two fragments of TZJ2134: TZJ2134-L12 (located at nt 1672-nt 2112 in the partial Nsp2 gene) and TZJ2134-(A+B) (located at nt 7463-nt 11272 in the partial Nsp9, complete Nsp10 and partial Nsp11 genes). Phylogenetic and recombination analyses based on the two sequences showed that TZJ2134 is a recombinant strain derived from two commercial PRRSV-1 modified live vaccine (MLV) strains (the Amervac vaccine and DV vaccine strains), forming a new recombinant subgroup of DV+Amervac-like isolates with other strains. However, PRRSV-1 MLV is not currently allowed for use in China. We speculated that TZJ2134 might have been introduced from Europe. This study is the first to provide information on two recombinant PRRSV-1 MLV strains in China and provides new data for the epidemiological study of PRRSV-1 in China.

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Qi Sun[a1], Hu Xu [a1], Chao Li[a], Bangjun Gong[a], Zhen Li[b], Zhi-Jun Tian[a], Hongliang Zhang[a*]

[a] State Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Harbin 150001, China

[b] Pingdingshan Center For Animal Disease Control And Prevention, Pingdingshan 467000, China

[1] These authors contributed equally to this work.

* Corresponding author contact information: Harbin Veterinary Research Institute, CAAS, No. 678 Haping Road, Xiangfang District, Harbin 150001, China.

Tel.: +86-13624503578

E-mail address: zhanghongliang01@caas.cn

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KEYWORDS: PRRSV-1, Vaccine, Recombination, Novel strains.

1 INTRODUCTION

Porcine reproductive and respiratory syndrome (PRRS) is a highly contagious disease causing substantial economic losses in the swine industry worldwide. It is mainly characterized by widespread reproductive failure in pregnant sows and respiratory symptoms in pigs of all ages (Butler et al., 2014). PRRSV is divided into two species, namely, Betaarterivirus suid 1 (PRRSV-1) and Betaarterivirus suid 2 (PRRSV-2) (Walker et al., 2020), which share only 50–60% nucleotide sequence identity (Murtaugh, Faaberg, Laber, Elam, & Kapur, 1998) and have attracted increasing attention due to the high incidence of PRRSV mutation and recombination. Based on the phylogenetic analysis of ORF5 nucleotide sequences and the global PRRSV classification system, PRRSV-1 is divided into 4 subtypes: subtype I (Global; Clade A-L), subtype I (Russian), subtype II, and subtype III. PRRSV-2 can be divided into 9 subtypes: lineages 1˜9 (Shi et al., 2010).

PRRSV-1 and PRRSV-2 have significant differences in geographical distribution. PRRSV-2 was isolated in 1992 in America (ATCC-VR2332, the North American prototypic strain) (Wensvoort et al., 1992) and is mainly prevalent in North America and Asia(Brar, Shi, Murtaugh, & Leung, 2015). PRRSV-1 was isolated in 1991 in the Netherlands (Lelystad virus, the European prototypic strain) (Collins et al., 1992) and is mainly prevalent in Europe. However, only subtype I (Global; Clade A-L) has spread to continents other than Europe. The remaining subtypes have been reported only in Eastern European countries and Russia (Stadejek, Stankevicius, Murtaugh, & Oleksiewicz, 2013). In 2011, Chen et al. isolated PRRSV-1 strains (BJEU06-1, NMEU09-1) in China, which was the first report of wild PRRSV-1 isolates in mainland China (Chen et al., 2011). To date, PRRSV-1 has been prevalent in at least 20 provinces in China (Chen et al., 2020; Gao et al., 2017; Liu et al., 2017; X. Wang et al., 2016; Q. Zhang et al., 2020; Zhou et al., 2015). In recent years, the detection number of PRRSV-1 in China has gradually increased. The PRRSV-1 strains reported in China all belong to subtype I (Global) and can be divided into four subgroups (NMEU09-1-like, Amervac-like, HKEU16-like, and BJEU06-1-like isolates) (Chen et al., 2017). However, compared with the large number of PRRSV-2 infections in China, the number of PRRSV-1 infections detected was relatively low (Lin et al., 2020; Zhai et al., 2018). We speculated that the reason might be that PRRSV-1 is not given much attention because it is not the main epidemic strain in China, and there is evidence that most PRRSV-1-infected pigs in China exhibit mild clinical symptoms (Ming et al., 2017; X. Wang et al., 2016). Even so, the effect of PRRSV-1 on pigs should not be ignored.

Vaccination is a key strategy for PRRSV-1 prevention and control. In the late 1990s, PRRSV-1 MLV was used in Europe(Chae, 2021). Nevertheless, the particularly high variability of PRRSV and the possibility of vaccine revertant PRRSV emerging in pigs vaccinated with PRRSV MLV could result in recombination between different MLV strains (Eclercy et al., 2019; Kvisgaard et al., 2020) or recombination between MLV strains and wild-type PRRSV strains (Chen et al., 2017; Marton et al., 2019; Wang et al., 2019). This indicates the importance of the rational use of vaccines. In this study, a novel PRRSV-1 strain was identified in the epidemiological investigation of PRRSV in China; this strain was derived from recombination of two commercial PRRSV-1 MLV strains (Amervac vaccine strain and DV vaccine strain).

2 MATERIALS AND METHODS

In 2021, a clinical (lung) sample, TZJ2134, was collected in Shandong Province of China from a pig with suspected PRRSV infection. Tissue sample processing, RNA extraction, cDNA preparation, RT–PCR and genome sequencing were performed as described previously (Leng et al., 2014; Zhang. et al., 2015). TZJ2134 was identified as PRRSV-1 by RT–PCR with the primer L12. Based on the complete genomic sequence of PRRSV-1, eight primer pairs were designed for RT–PCR amplification and sequencing (Table S2), and two overlapping fragments of TZJ2134 were amplified by RT–PCR. Each PCR product was purified with the E.Z.N.A.® Gel Extraction Kit - Omega Bio-Tek, cloned into pMD18-T according to the manufacturer's instructions, and then submitted to Comate Bioscience Co., Ltd. (Changchun, China) for sequencing.

Sequence analysis was performed with DNASTAR (version 7.1) software. The resulting sequences were assembled using SeqMan. Phylogenetic trees and molecular evolutionary analyses were constructed by using the neighbor-joining method in MEGA 7.0. with 1000 bootstrap replicates for each node (Kumar, Stecher, & Tamura, 2016). The generated phylogenetic tree was annotated using iTOL online software (https://itol.embl.de/) (Letunic & Bork, 2021). Sixty-five strains of PRRSV-1 available in GenBank were used for comparative sequence analyses in this study.

To test for recombination, the obtained sequence was screened using recombination detection program 4 (RDP4) (Martin et al., 2015). Seven different algorithms (RDP, GeneConv, BootScan, MaxChi, Chimera, SiScan, and 3Seq) embedded in the RDP4 software package with Bonferroni correction were utilized to detect recombination events and breakpoints. Detection using four or more of the seven methods implemented in RDP4 was taken as significant evidence for recombination. Recombination events were further confirmed by SimPlot 3.5.1 (Lole et al., 1999), and boot scanning analysis was performed with a 200-bp window, sliding along the genome alignment with a step size of 20 bp. The other three strains (PRRS-FR-2014-56-11-1, DK-2011-05-23-9 and OLot/91 strain) with high homology to TZJ2134-(A+B) were analyzed by SimPlot 3.5.1 (Lole et al., 1999) using the same method.

3 RESULTS AND DISCUSSION

In 2021, TZJ2134 was collected in Shandong Province in China and showed positivity for PRRSV-1 by detection using primer L12 (Table S1). Subsequently, a sequence of TZJ2134 (TZJ2134-L12) was obtained by amplification with the detection primer L12, located in the partial Nsp2 gene (nt 1672-nt 2112 of DV) (Figure 1C). Phylogenetic analysis showed that all Chinese PRRSV-1 isolates belonged to subtype 1 and could be divided into four subgroups (Amervac-like, BJEU06-1-like, HKEU16-like, and NMEU09-1-like isolates) (Figure 1). TZJ2134-L12 belonged to DV-like isolates and shared the highest sequence identity (99.54%) with the DV vaccine strain (Table 1).

To obtain the complete genome of TZJ2134, we designed eight pairs of primers for amplification. Unfortunately, probably due to the low viral load, the whole-genome sequence could not be obtained after repeated attempts, and only two overlapping fragments of TZJ2134 were amplified by using the primers Ly-E and Ly-F (Table S2). Subsequently, the resulting sequences of two overlapping fragments were assembled into a contig (named TZJ2134-(A+B)). Further genetic evolution and homology analyses showed that TZJ2134-(A+B) located at nt 7463-nt 11272 (3810 nt in length) in partial Nsp9, complete Nsp10 and partial Nsp11 (Figure 1C) shared 97.1% nucleotide identity with the DV vaccine strain and 97.4% nucleotide identity with the Amervac vaccine strain (Table 1). To establish a genetic relationship between TZJ2134-(A+B) and other PRRSV-1 isolates, we constructed a phylogenetic tree based on 65 PRRSV-1 strains (Table S1). Phylogenetic analysis showed that TZJ2134-(A+B) was intermediate between Amervac-like isolates and DV-like isolates and formed a separate subgroup (DV+Amervac-like isolates) with PRRS-FR-2014-56-11-1, DK-2011-05-23-9 and OLot/91 strains (Figure 1B).

RDP4 and SimPlot (version 3.5.1) were used to test for recombination of TZJ2134-(A+B). The RDP4 analysis results showed that TZJ2134-(A+B) was a recombinant strain from Amervac and DV vaccine strains with a potential crossover event spanning Nsp10. Additionally, the recombination event was further confirmed by SimPlot 3.5.1, which showed that the recombination breakpoint was approximately located in Nsp10 (nt

9423) (Figure 2A). Based on the putative recombination breakpoint (nt 9243), we divided TZJ2134-(A+B) into two fragments, TZJ2134-A (nt 7463-nt 9423) and TZJ2134-B (nt 9423-nt 11272), for phylogenetic and homology analyses. The results revealed that the homology between the two fragments and the corresponding parent viruses showed high similarity (Table 1). TZJ2134-A shared the highest nucleotide identity (99.17%) with the DV vaccine strain (Table 1) and belonged to DV-like isolates (Figure 2B). TZJ2134-B shared the highest nucleotide identity (99.73%) with the Amervac vaccine strain (Table 1) and belonged to Amervac-like isolates (Figure 2C). Both the DV and Amervac vaccine strains were PRRSV-1 MLV strains. To the best of our knowledge, only two reports, from France and Denmark, have described recombination events between two PRRSV-1 MLV strains (Kvisgaard et al., 2020; Renson et al., 2017). One of them, PRRS-FR-2014-56-11-1, was the first recombinant strain derived from the Amervac vaccine strain and the DV vaccine strain described previously, with recombination events occurring at nt 500 to nt 1370, nt 3646 to nt 4272 and nt 4972 to nt 8430 in ORF1, as determined using RDP4 (Renson et al., 2017). Homology analysis showed that TZJ2134-(A+B) has the highest nucleotide identity (97.6%) with PRRS-FR-2014-56-11-1. PRRS-FR-2014-56-11-1 and TZJ2134-(A+B) are intermediates between Amervac-like isolates and DV-like isolates with DK-2011-05-23-9 and OLot/91 strains in the phylogenetic tree (Figure 1B). The recombinant and phylogenetic analysis results showed that all three viruses were recombinant strains derived from the Amervac vaccine strain and DV vaccine strain but with different recombinant patterns (Figure S1) and formed a novel subgroup (DV+Amervac-like isolates) in the phylogenetic tree (Figure 1B).

In the late 1990s, PRRSV-1 MLVs were usually used to control PRRSV-1 infection in Europe (Chae, 2021). PRRSV-1 MLVs used worldwide include Porcilis PRRS (MSD), Amervac PRRS (Laboratories Hipra S.A.), ReproCyc PRRS EU (Boehringer Ingelheim), Ingelvac PRRSFLEX EU (Boehringer Ingelheim), Pyrsvac-183 (SYVA Laboratories), and Ingelvac PRRSFLEX® EU (Boehringer Ingelheim) (Nan et al., 2017). The Amervac vaccine strain is usually produced from the Amervac PRRS vaccine introduced by Hipra, and the DV vaccine strain is usually produced from the Porcilis® PRRS vaccine introduced by MSD. Both vaccines are often used in western Europe. The present findings confirm that animals infected with the recombinant strain showed a viremia level 10- to 100-fold higher in than that in animals infected with the Amervac or DV vaccine strain, in both inoculated and contact pigs (Eclercy et al., 2019). However, TZJ2134 may have had a low viral load, so the complete genome sequence could not be obtained. This study provides the first genetic evidence of the recombination of the Amervac vaccine strain with the DV vaccine strain in China. As the largest pork importer in the world, in China, the pig industry is vulnerable to the influence of the foreign pig industry (Brockmeier et al., 2012; van Geelen et al., 2018; H. L. Zhang et al., 2018). TZJ2134 may be highly likely to be introduced from Europe via pig trade after recombination abroad, as PRRSV-1 MLV is not currently allowed for use in mainland China. Although recombination of MLV strains is rarely reported, the existence of TZJ2134 is a reminder that surveillance against PRRSV-1 should be strengthened in China.

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ETHICAL APPROVAL

Sampling procedures were performed in accordance with the guidelines of the Animal Ethics Committee of the School of Harbin Veterinary Research Institute of the Chinese Academy of Agricultural Sciences. The Animal Ethics Committee Approval Number was SYXK(Hei) 2011022.

CONFLICT OF INTEREST

The authors declare no potential conflicts of interest with respect to the research, authorship and publication of this article.

DATA AVAILABILITY STATEMENT

All data applicable to this article are available in this study.

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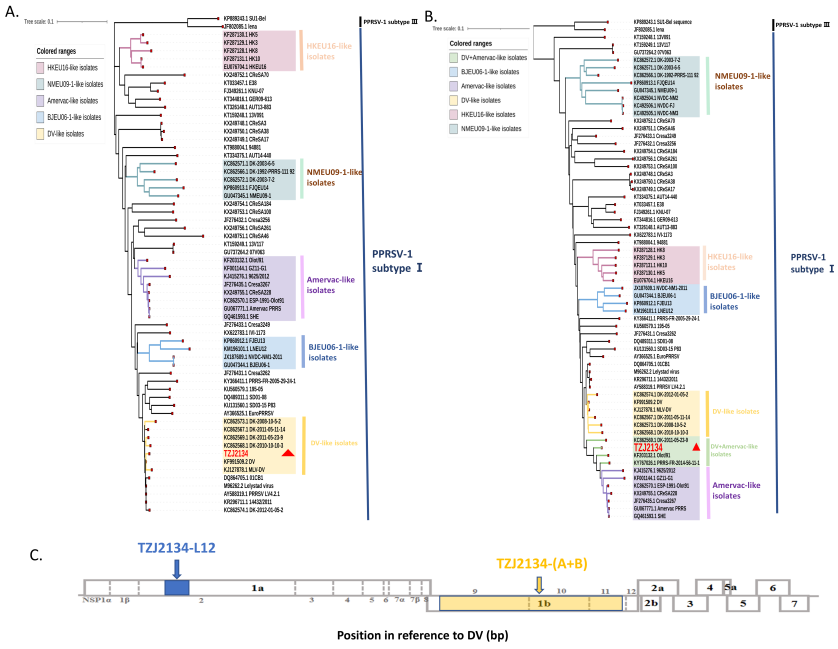

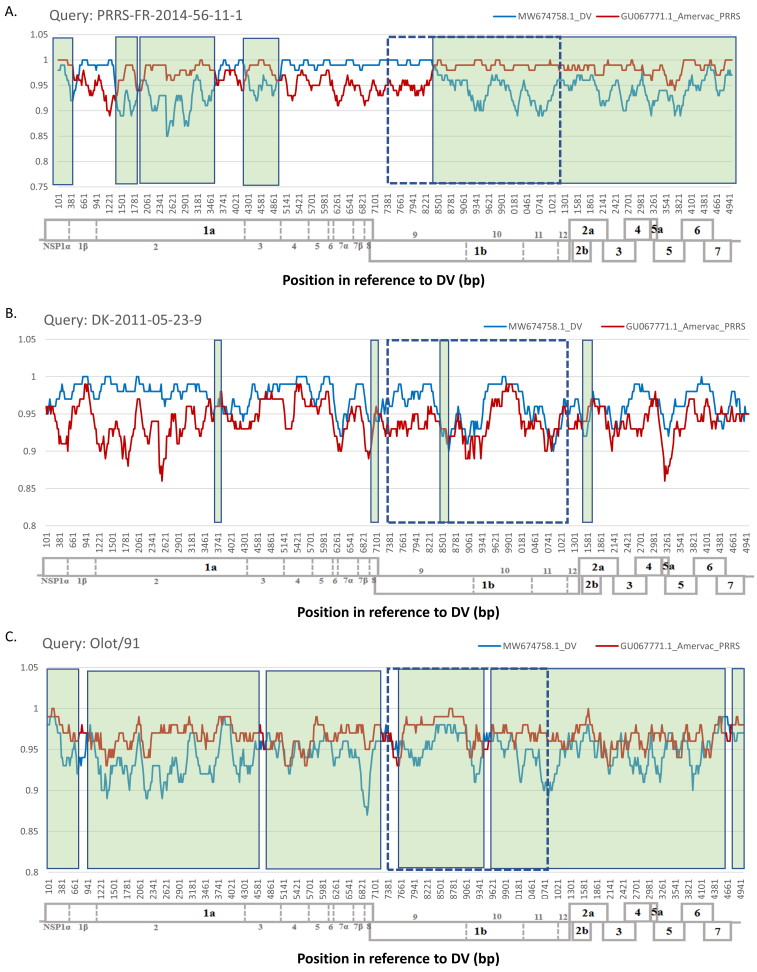

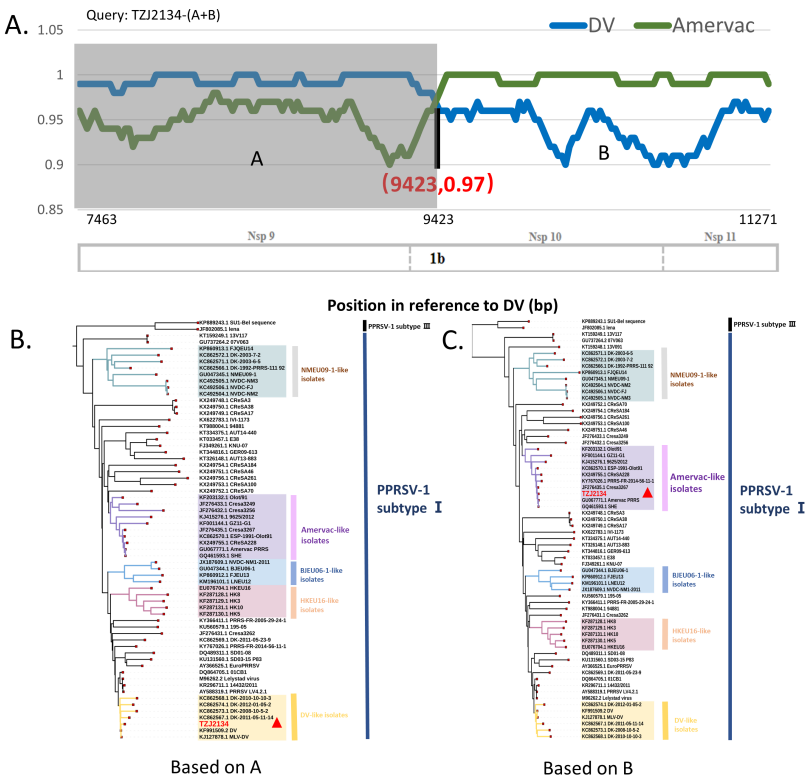

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TABLE S2.docx available at https://authorea.com/users/484541/articles/570291-emergence-of-a-novel-prrsv-1-strain-in-mainland-china-a-recombinant-strain-derived-from-the-two-commercial-modified-live-viruses-amervac-and-dv