The Origin of PRRSV and Advances in the Evolution and Pathogenicity of PRRSV 1

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Abstract Porcine reproductive and respiratory syndrome virus (PRRSV) has been mutating and evolving constantly since its emergence in the 1980s, which has brought inestimable economic losses to the global swine industry. The virus has two genotypes, of which genotype 1 PRRSV (PRRSV 1) first broke out in Germany and mainly prevailed in Europe, which can be clustered into four subtypes based on the ORF5 sequence. Although few cases of PRRSV 1 have been reported in China, the prevention and control of PRRSV should not be ignored. The origin of PRRSV, genetic evolution and pathogenicity of PRRSV 1 were retrospectively analyzed, in order to provide valuable evidences for molecular epidemiology and immune prevention and control of PRRSV 1.

Keywords Porcine reproductive and respiratory syndrome virus (PRRSV); PRRSV genotype 1; Origin; Variation; Evolution; Pathogenicity

Porcine reproductive and respiratory syndrome virus (PRRSV) is a spherical, capsulated, single strand plus RNA virus, with a particle size of 60–65 nm and a genome length of about 15 kb, which mainly causes reproductive disorders of sows and respiratory diseases of pigs of all ages clinically, commonly known as "blue ear disease" in China[1]. In 2016, International Committee on Taxonomy of Viruses (ICTV) officially released that PRRSV belongs to *Porartevirus*, Arteriviridae, Nidovirales, and PRRSV genotypes 1 and 2 were also reclassified into two species, named PRRSV 1 and PRRSV 2, respectively[2]. Since the outbreak of PRRSV, PRRSV 2 infection has been the main infection in China, while cases of PRRSV 1 infection have rarely been reported, so there is a lack of monitoring and evaluation of PRRSV 1. In recent years, due to the outbreak of African swine fever (ASF) in China, the number of eliminated breeding pigs in some regions has increased significantly, and the frequency of transregional and transnational purchase or transportation of breeding pigs has increased, which raises the pressure of prevention and control of PRRS in China and strengthens the risk of silent epidemic of PRRSV 1. PRRSV is featured by rapid mutation and evolution, and natural variation strains, vaccine evolution strains and recombinant strains emerge in an endless stream. However, there are few reviews on the molecular epidemiology and pathogenicity of PRRSV 1. In this paper, the origin of PRRSV, genetic evolution and pathogenicity of PRRSV 1 were reviewed, in order to provide the reference for immune control and prevention of PRRS.

1 Origin and Differentiation of PRRSV

There are few reports about the origin of PRRSV. In 1979, PRRSV antibody was detected positive in pig serum from Ontario, Canada, which was the earliest detection of PRRSV antibody to date. PRRSV antibodies were detected in swine herd in Iowa and Minnesota in 1985 and 1986, respectively. European researchers found that PRRSV infection in domestic swine herds in Europe probably first occurred in the mid-1980s. PRRS first broke out in North America and Europe in 1987 and 1990, respectively[2], indicating that PRRSV had been widely spread and prevalent in swine herbs before the outbreak. Through bioinformatics and big data analysis, it was found that PRRSV may appear several decades before the first outbreak, but the retrospective investigation of serum antibodies could only be detected in the 1980s. Most experts assumed that PRRSV existed in other hosts before it adapted to domestic pigs, that is, PRRSV was transmitted across species. Plagemann[3] speculated that PRRSV originated from the older lactate dehydrogenase elevating virus (LDV), which initially infected only rodents and then crossed the interspecies barrier to infect wild pigs in central Europe sometime in the 19th century, and then wild pigs transmitted PRRSV's ancestor virus to the Americas; after about 70 years of

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mutation and evolution, the virus was finally transmitted to domestic pigs. However, Reiner *et al.*[4] found that PRRSV sequences in wild boars were highly homologous when conducting molecular epidemiological investigation of PRRSV in 531 wild boars in Germany from 2004 to 2007, which was inconsistent with the hypothesis that PRRSV had evolved in wild boars for a long time. The research results got by Reiner *et al.*[4] are not representative for the following reasons. First, there are few studies on PRRSV in wild boar in modern times, let alone the real situation of PRRSV in wild boar in the 19th century. Second, Reiner *et al.*[4] studied the samples that were collected from 2004 to 2007; after years of selection pressure, the strain existed in wild boar is a dominant strain, which probably shares high homology; in addition, it is unclear whether PRRSV in wild boar comes from domestic pigs. Therefore, Plagemann[3] put forward that although PRRSV 1 and PRRSV 2 are very similar in terms of outbreak time, clinical symptoms and genome structure, their nucleotide sequence homology is only about 60%, indicating that the differentiation of PRRSV must have occurred before the outbreak. At present, there are two views on the differentiation of PRRSV: one holds that the differentiation of the two strains is only a nucleotide substitution with a very high mutation rate before the first outbreak, which lasted a short period; and the other suggests that the two strains had evolved over a longer period of time after early differentiation, and then erupted at the same time. The origin of PRRSV proposed by *G. Lagemann*[3] is in agreement with the second view. In order to obtain a more accurate origin and differentiation time of the virus, the most classical method is the molecular clock model, which calculates the time of the most common ancestor (tMRCA) based on the sequences of strains that can be obtained. According to the evolution rate of PRRSV, Hanada *et al.*[5] calculated that the differentiation time of the two PRRSV strains was from 1972 to 1988, and suggested that the differentiation of the two strains was caused by the sudden nucleotide substitution before the outbreak. Meanwhile, Forsberg[6] pointed out that the data and algorithm used by Hanada *et al.*[5] in their study were unreasonable, and the tMRCA obtained had a large deviation. Therefore, Forsberg[6] also recalculated according to the existing ORF3 sequence of PRRSV, and found that the tMRCA of PRRSV was around 1880; after further study and analysis, the tMRCA of PRRSV 1 was estimated to be 1946–1967. Shi *et al.*[7] calculated that the tMRCA of PRRSV 2 was from 1977 to 1981 by analyzing the genetic evolution of more than 8600 PRRSV sequences and combining the substitution rate of ORF5 nucleotide. Therefore, the view that the two PRRSV strains differentiated at an early stage had been accepted by the majority.

2 Genetic Variation and Evolution of PRRSV 1

2.1 Variation and evolution of natural epidemic PRRSV 1 strain PRRSV 1 broke out in Germany in 1990, and then spread throughout Europe within a few years. In 1991, Dutch scholars isolated Lelystad virus (LV) and completed its whole genome sequencing for the first time. LV strain was also identified as the prototype strain of PRRSV 1[8]. During the same period, PRRSV strains from Belgium, France, Germany, the United Kingdom and Spain were isolated successively, and it was found that the isolates all belonged to PRRSV 1 and were highly homologous to the prototype strain LV. Therefore, it was agreed that PRRSV 1 strains had high homology and little variation, which were also collectively referred to as “Lelystad-like” strain. Sequencing and further evolutionary analysis of PRRSV isolates from a wider area of Europe and at different times revealed that PRRSV 1 strains were much more diverse than thought. In 1996, when Suarez *et al.*[9] analyzed the sequence of 14 PRRSV 1 strains, they found that Italian strain "2156" had low homology with other strains and formed an independent branch in the evolutionary tree. In 2000, Oleksiewicz *et al.*[10] analyzed 26 isolates from Denmark and found that 10 of them had deletion and large variation in ORF3. In 2002, Forsberg *et al.*[11] showed that the variability and diversity of PRRSV 1 strains from Italy and Denmark were more complex than those from Western Europe, and they were divided into "Italian-like" and "Danish like" groups except the western European strains according to the homology of ORF5. Meantime, the analysis of mass data from Russia, the Czech Republic, Poland and Lithuania by different laboratories of different countries confirmed that the variation and diversity of PRRSV 1 strains was extremely complex and had a distinct regional character. In 2006, Stadejek *et al.*[12] systematically analyzed the genetic evolution of PRRSV 1 for the first time based on the existing ORF5 sequence and divided it into 4 subtypes, but subtype 4 only contained 3 sequences isolated from Belgium. In 2010, Shi *et al.*[13] conducted a systematic analysis again based on the ORF5 sequence included in GenBank, and divided PRRSV 1 into 3 subtypes; subtype 1 was distributed in many European countries, mainly in western Europe, and can be further divided into 12 evolutionary branches; subtypes 2 and 3 were mainly distributed in eastern Europe. Stadejek *et al.*[14] proved the existence of subtype 4, which was represented by Russian isolate VL, and the representative strains of subtype 1, 2 and 3 were prototype strains LV, BOR59 and highly pathogenic mutant strain Lena, respectively.

In addition to Europe, PRRSV 1 was also distributed and prevalent in other countries. In May 1999, Canada introduced 350 pigs from the Netherlands. Three months later, the pigs developed disease and were found to be positive for PRRSV antigen, and virus sequencing al-

so demonstrated that it was highly homologous with PRRSV 1 strain LV[15]. Subsequently, researcher in the United States also detected the presence of PRRSV 1 in clinical samples, and collectively referred to as the "Europe-like" strain. In 2004, when Ropp *et al.*[16] analyzed the Euro-PRRSV isolate, they found that it was highly homologous with LV strain, but there were 51 bases deletion in nsp2 coding region, indicating that PRRSV 1 had undergone mutation and evolution after being introduced to the United States. In 2005, the existence of PRRSV 1 was detected for the first time in South Korea, and the PRRSV 1 strain was also successively isolated in Thailand, Japan and China in 2004, 2009 and 2011[17-19]. Chinese isolates BJEU06-1 and NMEU09-1 had 5 and 2 amino acid deletions respectively in nsp2 coding region, as well as equal 8 amino acid deletions on ORF3[19].

2.2 Variation and evolution of PRRSV 1 vaccine strain and recombinant strain The vaccines most commonly used in the market are PRRSV 1 attenuated live vaccines (MLV), such as "Porcillis PRRS (Intervet)", "Amervac-PRRS (HIPRA)", "Pyrsvac-183 (SYVA)", "Suvaxyn (Zoetis)", *etc*. The nucleotide sequences of Chinese strain GZ11-G1 and Thai strain 02CB12 were highly homologous to PRRSV 1 MLV Amervac and Porcillis, respectively[20], suggesting that the two strains may be derived from the evolution of the vaccine strain or from the introduction of swine immunized with the vaccine. The nsp2 coding region of Danish isolates DK-2008 and DK-2012 had the same deletion of 74 amino acids as that of the vaccine strain Porcillis, and the genome had the highest homology, indicating that DK-2008 and DK-2012 were vaccine evolutionary strains of Porcillis[21]. In addition, several countries, including Germany, the United Kingdom, Poland and Hungary, had also reported the existence of vaccine evolutionary strains in swine herds[22-23]. In 2002, Forsberg *et al.*[11] found for the first time and reported the recombination of PRRSV 1 strain in the field, and further revealed that the recombination point was located on ORF6 via SimPlot software. In 2012, Frossard *et al.*[22] found the recombination of vaccine strains or vaccine evolutionary strains and field strains. In China, Chen *et al.*[24] also isolated HLJB1, a recombinant strain of Amervac vaccine strain and field strain BJEU06-1, from the serum of infected pigs. PRRS-FR-2014-56-11-1 isolated from France in 2014 was a recombination of attenuated live vaccines Porcillis and Amervac[25], which confirmed the recombination between vaccine strains. In 2019, Kvisgaard *et al.*[26] isolated a recombinant strain of two different vaccine strains from a boar station in Denmark, and the strain had spread to 38 surrounding swine herds through semen.

3 Genetic Variation and Evolution of PRRSV 1 in China

Since the outbreak of PRRS in China, PRRSV 2 has been the dominant strain that occurred and prevailed, while few cases of PRRSV 1 have been reported. Before 2011, there were only a few fragments of PRRSV 1 in GenBank, such as B13 and FJ603. In 2011, Chen *et al.*[19] first reported the complete genome sequences of 2 PRRSV 1 strains in China, BJEU06-1 and NMEU09-1. In 2015, Zhou *et al.*[27] reported 4 PRRSV 1 strains isolated in 2011, *i.e.*, NVDC-NM1-2011, NVDC-NM2, NVDC-NM3 and NVDC-FJ. Wang *et al.*[20] isolated a PRRSV 1 strain GZ11-G1 which was highly homologous to the nucleotide sequence of the vaccine strain Amervac from the samples collected in 2011, which may be related to the use of live attenuated vaccines introduced through an abnormal route in pig farms or the introduction of breeding pigs immunized by vaccines. In 2016, Zhang *et al.*[28] isolated 3 PRRSV 1 strains, *i.e.*, ZD1, WK14 and WG9; the ORF3 of ZD1 had 26 amino acids premature termination, which was also the first report of premature termination of structural proteins encoded by PRRSV; in addition, the isolated strain WK14 was a recombinant virus, and the parent strains were BJEU06-1 and NVDC-FJ, respectively. In 2017, Chen *et al.*[24] isolated HLJB1, a recombinant strain of Amervac vaccine and field strain BJEU06-1, from the serum of infected pigs. In 2020, an evolutionary strain of PRRSV 1 vaccine, PRRSV/NPFUST-2789-3W-2/TW/2018, was also isolated in Taiwan, China[29].

4 Pathogenicity of PRRSV 1

PRRSV 1 is clustered to four subtypes. The clinical symptoms of pigs infected by PRRSV 1 are similar to that infected by PRRSV 2, mainly including reproductive disorders of multiparous sows and replacement gilts as well as respiratory disorders, growth retardation and increased mortality of pigs of other ages. However, compared with PRRSV 2, much less efforts have been dedicated to the pathogenicity of various subtypes of PRRSV 1. Generally speaking, represented by the prototype strain LV (subtype 1) of PRRSV 1, the stains are low pathogenic strains with mild clinical symptoms and no lethality to piglets after infection[30-31]. However, PRRSV 1 has also witnessed the emergence of virulent/highly pathogenic strains during its genetic evolution. In 2007, Lena, a subtype 3 strain characterized by 29 amino acid deletion in nsp2 coding region, was isolated, which was clinically characterized by abortion and mummified fetus of sows and high mortality of suckling piglets, and the mortality rate of growing-finishing pigs could be as high as 70%; the results of the piglet pathogenicity test showed that Lena could cause typical clinical symptoms with a mortality rate of 40% (4/10), and its replication capacity and distribution volume in tissues were significantly higher than Belgium A (subtype 1)[32]. Stadejek *et al.*[33] compared the pathogenicity of subtype 2 strains BOR59 and ILI6 with subtype 1 strain 18794; the results showed

that BOR59 was the most pathogenic, being a highly pathogenic strain; ILI6 was of moderate virulence; and subtype 1 strain 18794 was a low pathogenic strain, suggesting that highly pathogenic strains also existed in subtype 2 and the pathogenicity of different strains within the same subtype was also different. Frydas *et al.*[30] showed that the pathogenicity enhanced strain of subtype 1 was isolated from Belgium and Italy.

There are many reports on the evolution of PRRSV 1 vaccine strain and its clinical pathogenesis, but there is only one literature available on pathogenicity test using isolated vaccine evolutionary strains. In 2016, Wang *et al.*[20] analyzed the pathogenicity of PRRSV 1 vaccine evolutionary strain GZ11-G1, and found that GZ11-G1 was not lethal to piglets but pathogenic, and could cause respiratory symptoms and transient temperature rise of piglets, whereas the proliferation capacity of GZ11-G1 was significantly higher than that of vaccine strain.

5 Prospects

It has been more than 30 years since the outbreak of PRRS in the 1980s. In practice, although biosafety, vaccine immunization, drug intervention, isolation and domestication, population closure, closed group and other measures have played an important role in the prevention and control of PRRS, PRRS has not been effectively controlled. The epidemic of PRRSV in China is dominated by PRRSV 2. In recent years, the exchange of personnel in pig related industries at home and abroad has increased, with frequent introduction, and PRRSV 1 strain has been detected and isolated in Fujian, Guangdong, Jiangxi and other regions of China, indicating an increased risk of PRRSV 1 dissemination and prevalence. Therefore, external biosafety prevention and control should be carried out resolutely, and introduction is forbidden from PPRSV 1 endemic areas; relevant detection and screening work should be done when introducing species from medium-low risk areas; live attenuated PRRSV 1 vaccine should not be used; and molecular epidemiological studies of PRRSV 1 should be carried out and detection/monitoring of PRRSV 1 strain should be strengthened to take preventive measures.

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