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Article

# Molecular epidemiological investigation and genetic variation analysis of porcine reproductive and respiratory syndrome virus

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**Abstract:** Porcine reproductive and respiratory syndrome (PRRSV) is a reproductive disorder and respiratory infectious disease of pigs caused by virus (PRRSV). Its clinical characteristics are fever and anorexia in sows, abortion in late pregnancy, and easy to give birth to stillbirth; Piglets with respiratory symptoms and increased mortality before weaning, commonly known as blue-ear disease, can also cause immunosuppression, infection and other diseases immune failure. The constant variation of PRRSV has caused huge economic losses to China's pig industry chain.

**Keywords:** pig breeding; respiratory syndrome; virus immunosuppression; infectious diseases

## Introduction

At present, it is found that the mutation rate of NSP2 and ORF5 genes of PRRSV is high. A total of 23 PRRSV strains with partial deletion of NSP2 gene and complete ORF5 gene have been amplified. Therefore, the genetic evolution and variation rule of PRRSV can be understood by monitoring the NSP2 and ORF5 genes. Based on the PRRSV NSP2 and ORF5 genes, the PRRSV molecular epidemiology monitoring was carried out on the pig lung, spleen, liver and lymph nodes collected from the diseased pig farm, and the genetic evolution tree was drawn and its genetic variation trend was analyzed with reference to the published PRRSV gene sequence at home and abroad. Attention should be paid to clinical diagnosis and vaccine prevention and control in the future. The PRRSV live and attenuated vaccines (PRRSV MLV) on the market are anti-human PRRSV and anti-human PRRSV, respectively. Although PRRSV-MLV can cause certain protective effects on the body, it does not have broad-spectrum cross-immunity to various virus strains in humans and animals, resulting in insufficient immunity to exogenous viruses [1]. In addition, five commercial vaccines were evaluated, and the results showed that the current vaccines did not have sufficient protective effect on NADC30 sample strains. We obtained 132 PRRSV positive clinical samples in 2021, and completed 88 cases, of which NADC30-like was the main one, accounting for 98.86%. The homology of GP5 and Nsp2 with the reference strain NADC30 was 86.6%–95.2%, 51.3%–90.9%, respectively, indicating that PRRSV has undergone some variation, and new PRRSV may occur in the future, so it is necessary to carry out molecular epidemiology research on PRRSV.1.

# 1. Materials and methods

## 1.1. Clinical sample dissection

In 2021, a total of 1035 clinical samples from 20 provinces across the country were collected, all of which were oral swabs, blood and dissected organs of dead pigs suspected of PRRSV infection (**Figure 1**).

**Figure 1.** Dead pigs suspected of PRRSV infection were oral swabs, blood and dissected organs

## 1.2. Main reagents

RPMIMedium1640 medium and double antibody were purchased from gibco; Fetal bovine serum was purchased from AusGeneX.

## 1.3. Design and synthesis of sequencing primers

In this study, ORF5 and NSP2 gene fragments were detected at the same time. According to the reference sequence registered on GenBank, the primers were designed according to the reference sequence registered by the following primers, with the amplification length of 696 and 999.

## 1.4. RT-PCR identification

According to the nucleic acid extraction instructions, the nucleic acid was extracted from clinical samples, and then the target gene was amplified by ORF5 and NSP2 amplification primers. The RT-PCR reaction condition was: reverse transcription at 45℃ for 30 minutes; CDNA pre-denatured at 95℃ for 5min; Denaturation at 95℃ for 30s; Annealed at 59℃, extended at 72℃ for 30s; 72℃5 min; 4℃ 5min. The above three temperatures have a total of 35 cycles. Finally, the amplified target gene fragments were sent to Qingke Biotechnology Co., Ltd. for sequencing.

### 2.1. Clinical sample dissection and RT-PCR identification

According to the disease found by anatomical examination, there is no characteristic injury, and according to the concurrent and secondary infection, the injury is also different; Because it targets alveolar macrophages, pathological sections show diffuse lung parenchyma, alveolar inflammation and interstitial fibrosis with interstitial pneumonia. 132 positive samples were detected clinically by RT-PCR.

### 2.2 Virus isolation

Through 2–3 generations of blind transmission, 33 strains were obtained which could be stably passaged, and the pathological changes of PAM were observed as shrinking, splitting and falling off. By sequencing the cell fluid of the two strains, GP5, NSP2 and other target sequences can be amplified. If no target sequence is found in the cell fluid of other strains, it is considered as PRRSV negative.

### 2.3. Epidemiological statistical analysis

After a series of epidemiological surveys, it was found that the positive rate of Jiangxi was the highest in the country, followed by Shanxi, Inner Mongolia and Jiangsu. The experiment shows that piglets of different ages have certain sensitivity to this disease, but the most of them are 25 days and 80 days. These cases are mainly common cases, while highly pathogenic cases are rare [2]. In terms of sample types, blood samples and section samples are the main samples, and blood samples are the main ones.

### 2.4. Genetic variability analysis of Nsp2 gene

The target gene amplified from clinical samples was sent to the sequencing company for sequencing, and 70 Nsp2 sequences were obtained.

#### 2.4.1. System evolution tree display

We conducted a systematic genetic analysis of 70 Nsp2 genes of PRRSV in the Americas and divided them into two subgroups: 1, 2 (Subgroup 1, 2). Subgroup I is a common PRRSV, represented by NADC30 and MN184C. Subgroup II is a typical virus, mainly VR2332. The 65 gene loci of type I subgroup are close to NADC30 and HENAN-HEB/XINX gene loci, but close to CH-1a and CH-1R gene loci, and close to other representative gene loci. Among them, 6 genes belong to subgroup II and are close to JXA1.

#### 2.4.2. Analysis of Nsp2 homology and genetic evolution

Through homology comparison, 65 of them in subgroup I have 71.3%–90.4% similarity with NADC30 gene. The homology between the six gene sequences of subgroup II and JXA1 gene is between 86.3% and 91.8%. The gene sequences of the two viruses are different from HP-PRRSV and classical PRRSV. Through the sequence comparison of Nsp2 gene, it was found that the Nsp2 gene had a large variation, and there were intermittent amino acid deletions in all sequences. Our previous study showed that 65 sites in subgroup I were different from NADC30, and 6 sites in subgroup II were different from JXA1, but no new sites were found. 2.5

Genetic variability analysis of GP5 gene. In this study, a total of 53 GP5 gene sequences were measured, and the phylogenetic tree results were similar to Nsp2, which was divided into subgroup I represented by NADC30 and subgroup II represented by VR2332 (subgroup 1, 2).

### 2.5. System evolution tree display

NADC30 virus strains include HENAN-HEB, XINX and XINX, among which MN184C is NADC30-like type, but 46 genes are significantly different from other typical strains. The five gene sequences in subgroup II are closely related to JAX1.

#### 2.5.1. Analysis of GP5 homology and genetic evolution

The results showed that the 53 fragments of GP5 had high homology, reaching 79.4%~100%. Subgroup I has the strongest homology with the 48 sequences of NADC30 virus, accounting for 86.6%–95.2%, accounting for 86.6%–95.2% of all sequences; Four of the second sub-group are highly similar to JXA1 and VR-2332, accounting for 99.1–99.4% and 99.2%; Through comparison, it was found that these two subgroups were quite different from HPPRRSV in genome. Comparing the different segments of IGP5 gene of subgroup, it was found that the sequence of IGP5 gene in different segments was roughly the same, but there were also some mutations or losses. The amino acid residues of subgroup I changed in 33,151,192,221 segments, while subgroup II only changed in 33 segments. Within 72 hours, all identified strains did not have other mutations that were consistent with the characteristics of the reference strains, that is, P and L type mutations. The sequence of SD210724A-2 of subgroup II is consistent with that of JAX1, and the sequence of others is close to that of NADC30. In NADC30, 546,878 and other amino acid sequences also have similar amino acid sites. The GP5 gene as a whole belongs to a relatively conservative population, but this study found that there are six amino acid sites missing at the 73 and 74 sites of the GP5 gene, including HB1226B-1/- 3, SD210724A-4/- 5, and SD210601.

## 3. Conclusion

To sum up, it shows that PRRSV still presents a national epidemic trend, and pigs in different periods are easy to be infected, and there is no obvious seasonal epidemic. At present, the immune protection period of PRRSV live vaccine is usually only 4–5 months [3], so the occurrence of disease can be reduced by vaccination or other reasonable methods to prevent disease and control secondary infection at the appropriate time [4]. However, at present, there are still a large number of live attenuated vaccines being abused, and at the same time, there will be a variety of new strains of virus, which leads to the occurrence of pig blue-ear disease in China is very complex and difficult to control. Therefore, we should strictly control the production and use of pig blue-ear disease vaccine. As for the test area this time, blood samples are mainly used, which can not only protect the tested pigs to the maximum extent, but also make it more convenient. However, the higher the number of serum samples, the lower the detection rate, which may be related to the time of onset, collection stage and other factors. More attention should be paid to future detection.

To sum up, at present, NADC30-likePRRSV has replaced HP-PRRSV as the main epidemic strain in China [5,6]. As for the situation that the existing vaccine cannot provide complete protection to the NADC30-like strain, we should strengthen the investigation of its epidemic law and genetic variation for this new strain. This survey carried out a detailed analysis of the genome sequence of a large number of virus strains, providing important data support for further research and prevention and control of such viruses.

**Conflict of interest:** The authors declare no conflict of interest.

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