Current Challenges in PRRSV Strain Identification:
The Importance of Differentiating Major Strains

Over the past 30 years, Porcine reproductive and respiratory syndrome virus (PRRSV) has remained one of the most significant and persistent threats to the global swine industry. Since its emergence in the early 1990s, the virus has spread extensively and is now considered endemic in most pig-producing regions worldwide. The virus remains difficult to control and continues to spread at a fast rate, not only due to its ability to cause severe disease, but also by its remarkable genetic variability and ability to rapidly evolve.

PRRSV is an enveloped, positive-sense, single-stranded RNA virus belonging to the Arteriviridae family.[1] It infects pigs across all stages of production, from piglets to breeding-age sows, leading to a wide variety of clinical manifestations. These range from mild or asymptomatic infections to severe disease with high morbidity and mortality, depending on the strain, host factors and co-infections.

Porcine reproductive and respiratory syndrome (PRRS) is characterised by two primary disease presentations: reproductive failure in breeding animals and respiratory disease in growing pigs.[2] In breeding herds, infection can have devastating consequences. Pregnant sows infected with PRRSV may experience late-term abortions, stillbirths and the birth of weak or mummified foetuses (Figure 1). Additional symptoms include fever, anorexia, reduced conception rates and in severe cases, cyanosis of the ears, abdomen and vulva.

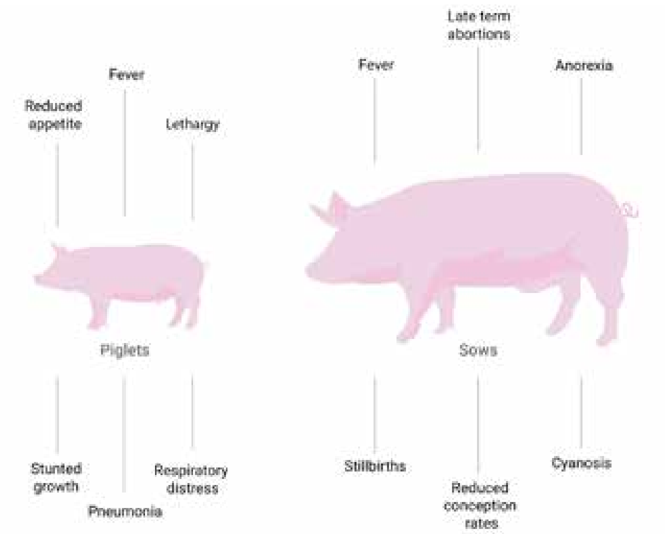

Figure 1. Clinical signs associated with PRRSV infection in piglets and sows. Common clinical manifestations observed in piglets include reduced appetite, fever, lethargy, stunted growth, pneumonia and respiratory distress. In sows, clinical signs may include fever, anorexia, late-term abortions, stillbirths, reduced conception rates and cyanosis.

A key challenge in managing PRRSV is that symptoms in adult pigs are often mild or inconsistent. Many infected sows do not display obvious or strong symptoms, allowing them to remain within the herd and act as reservoirs for ongoing viral transmission. This silent spread significantly complicates disease control efforts and highlights the need for robust surveillance strategies.

In piglets and growing pigs, the respiratory form of the disease is more prominent with more distinguishable symptoms. Infected animals may display high fever, lethargy, reduced appetite and respiratory distress, in some cases progressing to pneumonia. One of the most economically significant impact is reduced growth performance. Infected piglets frequently exhibit stunted growth, decreased weight gain and poor feed efficiency, all of which negatively affect production outcomes.

In addition to its direct symptoms, PRRSV is also known for its immunosuppressive properties. Infection with PRRSV can impair immune responses, meaning pigs are more susceptible to secondary infections. Co-infections with pathogens such as respiratory viruses and opportunistic bacteria are common and can exacerbate disease severity, leading to increased mortality rates. This compounding effect often leads to increased antibiotic use on farms, contributing to rising production costs and the broader global issue of antimicrobial resistance.[1]

Genetic Diversity and the Challenge of Strain Differentiation

One of the most notable features of PRRSV is its extraordinary genetic diversity, and this is an elemental reason that the virus remains difficult to control. PRRSV has adapted to diverse environments and immune pressures through variation and recombination events.[3] The virus is broadly classified into two major genotypes: Type 1 (European or EU) and Type 2 (North American or US). These genotypes were first identified in the early 1990s and have since evolved independently.[4]

Despite causing similar clinical symptoms, the two genotypes share only approximately 60% nucleotide similarity across their genomes.[5] This significant genetic divergence highlights the importance of accurate diagnostic methods capable of distinguishing between them, particularly for surveillance and biosecurity purposes.

The genetic variability of PRRSV is driven by a combination of high mutation rates, recombination events and selective pressures from host immune responses and vaccination. As a result, new variants continue to emerge, often with increased virulence, transmissibility, or antigenic properties.

There are two genomic regions that are particularly important in understanding PRRSV diversity, encoding the non-structural protein 2 (NSP2) and glycoprotein 5 (GP5). NSP2 is the largest non-structural protein and its encoding region is frequently used as a genetic marker due to its high variability. However, high mutation rates within this region can also complicate its use in strain classification. GP5 is a highly variable glycoprotein that plays a key role in virus-host interactions and immune recognition.

GP5 is highly immunogenic but also highly variable, limiting cross-protective immunity between strains. The variability of GP5 is particularly problematic for vaccine design and natural immunity. Pigs infected with one strain often do not develop effective immunity against other strains, making reinfection an ongoing risk complicating long-term control strategies.

Emergence and Impact of Highly Pathogenic PRRSV

The genetic variability of PRRSV has led to the emergence of highly pathogenic variants, most notably highly pathogenic PRRSV (HP-PRRSV). HP-PRRSV was first identified in China in 2006 as a derivative of the North American genotype and since has rapidly spread across the region and become the dominant epidemic strain.[6]

A defining feature of HP-PRRSV is a discontinuous 30-amino acid deletion in NSP2, although additional mutations across the genome also contribute to its phenotype. These genetic changes are believed to have conferred selective advantages, including increased virulence and transmissibility.

HP-PRRSV infections are associated with severe disease, including high fever, extensive lung pathology, and significant mortality rates in both piglets and adult pigs. The economic impact of these outbreaks has been substantial, particularly in regions with high pig production density.

The emergence of HP-PRRSV illustrates the virus's capacity for rapid evolution and highlights the importance of continuous monitoring and accurate strain identification.

Transmission and Global Spread

PRRSV is transmitted through a variety of direct and indirect routes, contributing to its widespread distribution. Direct transmission occurs through contact with infected animals, while indirect transmission can occur via contaminated biological materials such as semen, nasal secretions and urine.

Indirect transmission via equipment, clothing and farm infrastructure also plays a significant role in disease spread, particularly in systems with inadequate biosecurity measures. The virus can also be spread vertically from pregnant sows to offspring, producing persistently infected piglets.

The global spread of PRRSV has been driven by international trade, animal movement and varying levels of biosecurity across regions. In Europe, PRRSV-1 strains predominate, while in Asia, both PRRSV-2 and HP-PRRSV are widely circulating. These regional differences further emphasise the need for diagnostic tools capable of precise strain differentiation.

Previous Advancements

Despite decades of research, there is currently no fully effective treatment for PRRSV.[7] Vaccination remains the primary tool for disease control, with several vaccine types available, including modified live vaccines (MLVs), inactivated vaccines, subunit vaccines, DNA vaccines and viral vector vaccines.

While inactivated vaccines are generally safer, MLVs are the most widely used due to their ability to induce relatively strong immune responses. However, their use is associated with several challenges. MLVs provide limited protection against genetically diverse or heterologous strains, may revert to virulence, and impose a risk of recombination with circulating field strains.

There is growing evidence that MLV strains can recombine with field strains of PRRSV, particularly vaccines made against genotype II variants. This recombination can lead to the emergence of novel strains with enhanced fitness or virulence, potentially contributing to new outbreaks. Specifically, the JXA1 vaccine shares a high sequence similarity to a HP-PRRSV variant, and it is thought that recombination between the vaccine strain and wild type gave rise to this new strain, having gained selective advantages from both parental strains.[4]

Additionally, current vaccines primarily reduce disease severity rather than prevent infection or transmission. Economic constraints also limit widespread vaccination, particularly in growing pigs, meaning that vaccination strategies are often focused on breeding herds.

Implications for Farmers, Veterinarians and Diagnostic Laboratories

The ongoing challenges associated with PRRSV have significant implications for all stakeholders in the swine industry. For farmers, the economic impact includes reduced productivity, increased mortality, higher veterinary costs and greater reliance on antibiotics. It is estimated that PRRSV caused $1.2B in losses in the US pork industry from 2016–2020.[8] While vaccination and routine testing are widely recommended for farmers, their implementation is often hindered by high costs and limited labour availability. Key focus areas for PRRSV research include managing disease symptoms, understanding transmission pathways and working towards elimination at the herd and regional level.

Eradication of PRRSV from an infected herd is a complex and time-consuming process. Estimates suggest that it can take several years to eliminate the virus, even under controlled conditions without reintroduction.[9] This highlights the importance of early detection, continuous monitoring and strict biosecurity measures.

In the UK, PRRSV is a reportable disease and surveillance programmes have been implemented to monitor its prevalence and spread. Strict import controls are in place to prevent the introduction of new strains, particularly PRRSV-2, which has not yet been detected in the UK. These regulations require testing of animals and semen prior to importation, emphasising the critical role of accurate detection.

For veterinarians and diagnostic laboratories, the ability to rapidly and accurately detect and differentiate PRRSV strains is essential for informed decision-making and effective disease management.

The Role of qPCR in PRRSV Detection

The overarching goal within the swine industry is to improve prevention and elimination efforts, and progress toward effective PRRSV control. Achieving this relies heavily on effective and reliable surveillance and diagnostics.

Differentiation of PRRSV from other swine pathogens can be a significant challenge due to overlapping clinical presentations. The virus shares similarities with other swine viruses which can complicate accurate diagnosis without laboratory confirmation. This is further complicated by the increasing movement of animals between herds, which facilitates transmission and makes containment more difficult.

Precise detection methods are critical for both surveillance and diagnosis of PRRSV, as undetected outbreaks can rapidly devastate herds. Fast and accurate detection enables early intervention, including isolation of infected animals, limiting transmission, protecting productivity and reducing the risk of significant economic losses.

Many different methods are available for PRRSV detection. These include molecular approaches such as quantitative PCR (qPCR), CRISPR-based assays, loop-mediated isothermal amplification (LAMP) and next-generation sequencing (NGS), as well as serological methods like enzyme-linked immunosorbent assay (ELISA) and indirect fluorescent antibody (IFA) assays.[7] However, each method has limitations.

Serological assays are useful for monitoring herd exposure and immune response, but detection can be delayed as positive results only show after antibodies have formed. In addition, they cannot distinguish between prior exposure, vaccination and active infection, limiting their use in real-time decision making.

NGS provides detailed genetic insight and is valuable for strain characterisation and epidemiological investigations. However, it is cost intensive, has longer turnaround times and can be less sensitive in samples with low viral loads. It also requires specialised data analysis, making it less suitable for rapid, routine testing.

qPCR has become a cornerstone of PRRSV diagnostics due to its high sensitivity, specificity and speed. Most surveillance strategies recommend routine sampling and testing for PRRSV using qPCR across activities such as animal movement, herd monitoring and elimination efforts. By directly detecting viral RNA, qPCR enables the identification of active infections, often before clinical signs become apparent.

This is particularly important given that PRRSV can be detected as early as one day post-infection. Rapid detection allows for timely intervention, reducing the risk of widespread transmission within a herd. qPCR can also detect very low levels of the virus and results are available within hours of testing the sample. At a relatively low cost per sample, qPCR serves as the gold standard for high-throughput testing.

Establishing the true PRRSV status of a population remains challenging, as sensitivity can vary depending on sample type, such as serum, oral fluids and processing fluids. However, when paired with an effective extraction method, qPCR offers the flexibility to reliably test across a wide range of sample types, supporting more comprehensive and accurate surveillance programmes (Figure 2).

Advancements in Multiplex qPCR Testing

Advances in molecular diagnostics have led to the development of multiplex qPCR assays, which allow simultaneous detection and differentiation of multiple PRRSV strains within a single test.

Multiplex assays capable of simultaneous detection and differentiation of PRRSV-1, - 2, and highly pathogenic variants in a single test provide a complete, real-time PCR solution for veterinary diagnostic laboratories and swine producers (Figure 3). Rapid turnaround times and compatibility with open qPCR platforms also help to facilitate scalable and flexible deployment. Assays such as these offer significant advantages in terms of efficiency, cost-effectiveness, and accuracy, providing increased confidence in diagnostic outcomes.

These tools are particularly valuable in regions where multiple strains are circulating, providing clear strain-level insight to inform rapid decision-making, while maintaining gold-standard sensitivity. By supporting accurate strain identification, multiplex qPCR enhances herd health monitoring, strengthens biosecurity measures, and enables earlier intervention. This shift from reactive outbreak response to proactive disease management represents a critical step forward for the industry.

Next-Generation Swine Health Strategies

PRRSV continues to pose a major challenge to the global swine industry due to its genetic diversity, evolving strains and complex transmission dynamics. The emergence of highly pathogenic variants and the limitations of existing vaccines further complicate control efforts.

Accurate strain identification and differentiation is essential for effective surveillance, diagnosis and disease monitoring,

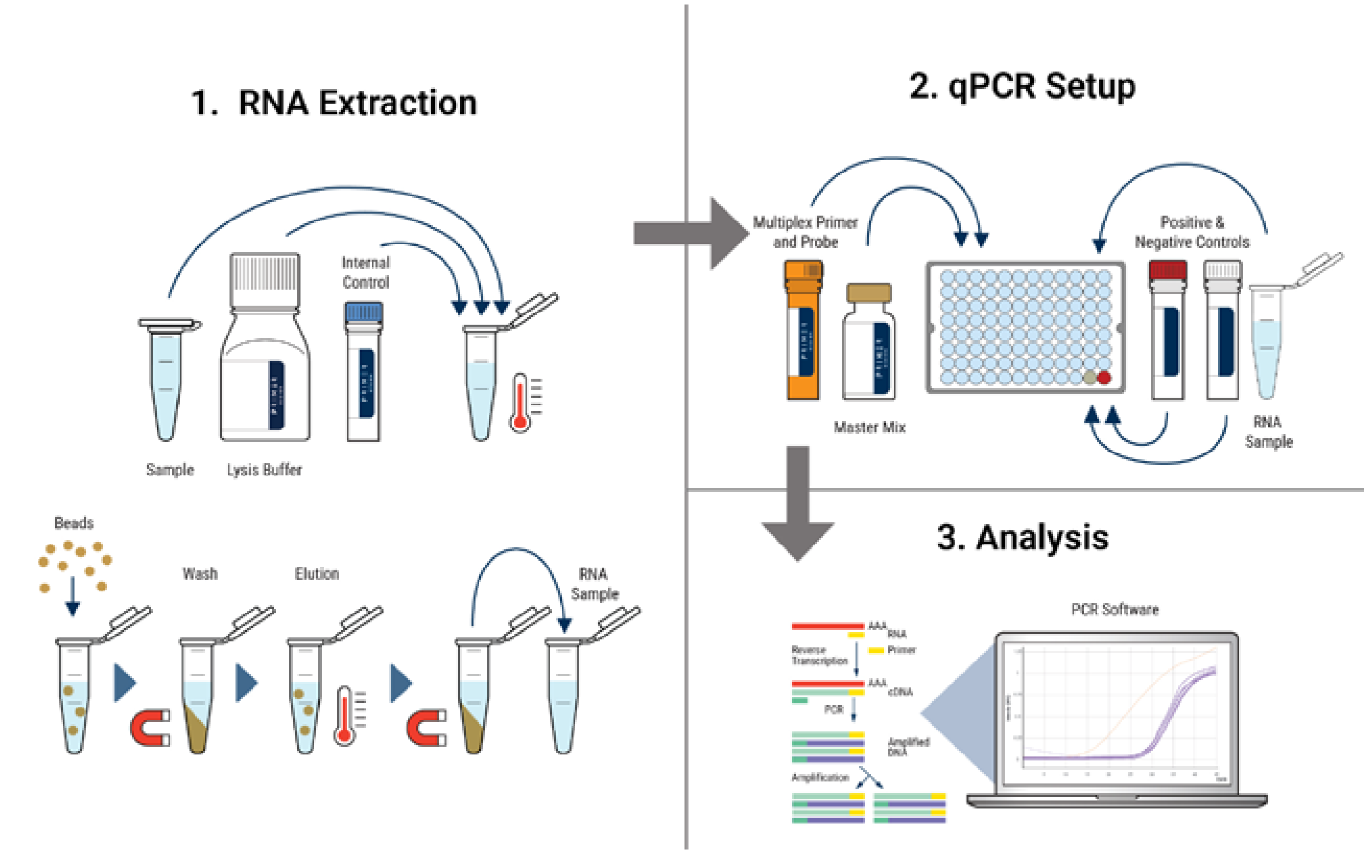

Figure 2. Overview of the qPCR workflow for PRRSV detection. Schematic representation of the qPCR testing workflow, including (1) RNA extraction from samples using lysis buffer and magnetic bead purification, (2) qPCR reaction setup with master mix, multiplex primers and probes, controls and extracted RNA and (3) amplification analysis using PCR software for detection and interpretation of target nucleic acids.

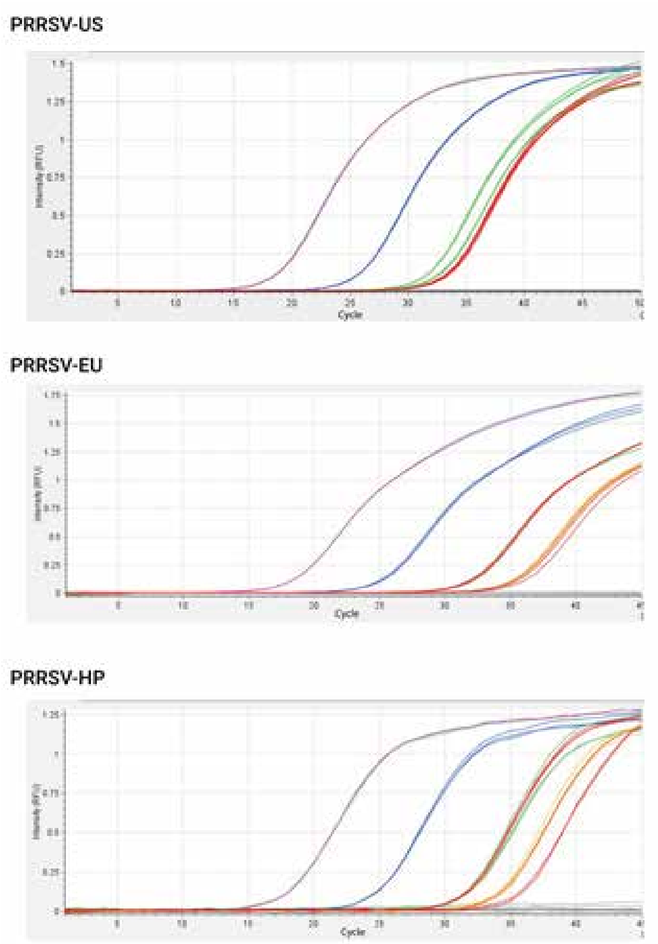

Figure 3. Amplification curves for PRRSV assays using synthetic template controls. Amplification curves demonstrating successful detection of synthetic PRRSV template controls across multiple target concentrations for PRRSV-US, PRRSV-EU, and PRRSV-HP assays. Representative curves show detection of 10^8 copies (blue), 10^4 copies (orange), 100 copies (red), and 10 copies (purple), with amplification efficiencies within the acceptable range of 90–110%.

yet remains difficult with traditional diagnostic approaches. Advances in molecular diagnostics, particularly qPCR and multiplex testing, are transforming the ability to detect and characterise PRRSV infections with speed and precision.

For swine breeders, farmers, veterinarians and diagnostic laboratories, adopting these advanced diagnostic tools offers a clear pathway to improved disease control, enhanced biosecurity and reduced economic impact. Continued investment in diagnostic innovation, combined with coordinated global surveillance efforts, will be essential in addressing the ongoing threat posed by PRRSV.

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