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The Global Research Trend of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV): A Mini Review

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Abstract

The Porcine reproductive and respiratory syndrome (PRRS) is a significantly harmful illness in swine causing respiratory problems and reproductive failure in sows, gilts, and pneumonia in young pigs. PRRS is often diagnosed with available test methods to identify disease agent or to detect the immune response of virus. In spite of many extensive researches, there are still no specific treatments and many unknown relationships between this virus and other diseases in swine. Virus isolation and molecular techniques are possible to identify PRRS virus or its acid nucleic but the high genetic diversity of virus results to unwanted false-negative results. Although PRRS has spreads throughout the world, many researches still focus on updating the methods to quickly diagnose PRRSV and to generate an effective fast treatment. This review highlights the current global PRRS research trend, its detection and diagnosis.

Keywords: PRRS, PRRS virus, North America VR-2332 virus, European Lelystad virus, Blue-ear disease in swine

Introduction

The Porcine Reproductive and Respiratory Syndrome Virus (PRRSV)

Porcine reproductive and respiratory syndrome (PRRS) is a momentous diseases in swine which is distinguished by reproductive failure in sows and respiratory problems affecting all age pigs. The PRRS or mystery swine disease; blue-ear disease; swine reproductive and fertility syndrome was first discovered in USA industrial pigs with an unknown etiological agent in 1987. Until 1991 in The Netherlands, the etiological agent of PRRS was PRRS virus (PRRSV) - a RNA virus which was identified and isolated as a member of the order *Nidovirales*; family *Arteriviridae*; genus Arterivirus[1,2]. PRRSV genome shown in Figure 1 contains a single-stranded, positive-sense RNA with approximate size of 15 kbs which has the untranslated region in both side (5′-UTR and 3′-UTR following polyA tail), non-structural protein (Nsp) coding sequences[3-8]; structural protein coding sequence ORFs[9,10] and sequences of its virion consist of three major proteins: GP5 (glycoprotein), M (matrix protein), N (nucleocapsid protein) and three minor proteins as GP2, GP3 and GP4[11-14]. The ORF 1a and 1b located almost 75% of genome at the 5′ end after an untranslated region, which are responsible for protein in replication and translation. The expression of ORF5, ORF6 and ORF7, three major structural proteins: an envelope glycoprotein GP5 (25 kDa); an unglycosylated membrane protein M (18-19 kDa) and nucleoacapsid protein N with M_w of 15 kDa, respectively. In virion, both the GP5 and M protein were represented in heterodimeric complexes by disulfide bonds. The ORF2; ORF3 and ORF4 coded for three minor components: membrane-associated glycoproteins GP2 (29 kDa) and GP4 (31 kDa), highly glycosylated protein GP3 (42 kDa). In Figure 1, the GP3 referred to the North American IAF-Klop strain as a

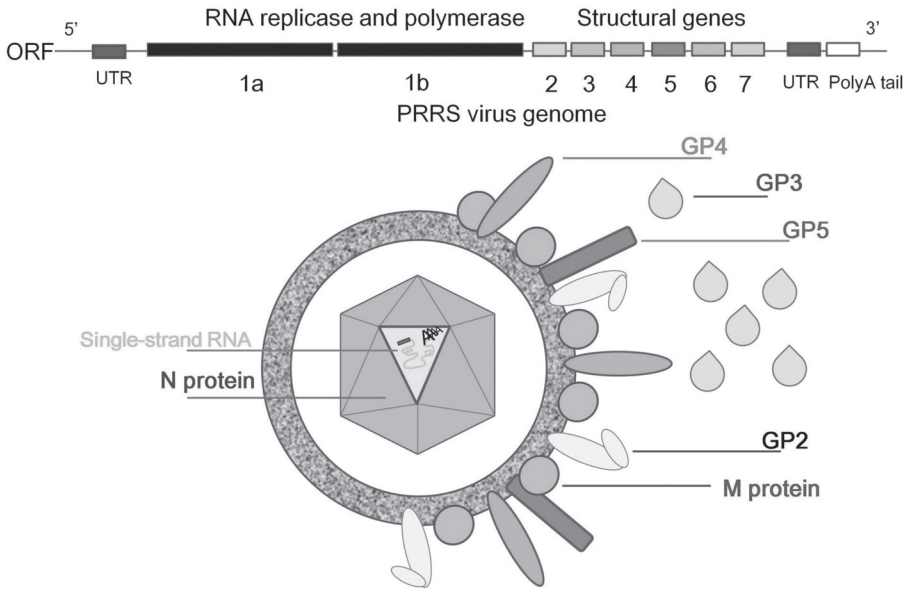

Figure 1. PRRSV genome contains a positive-sense RNA single strand.

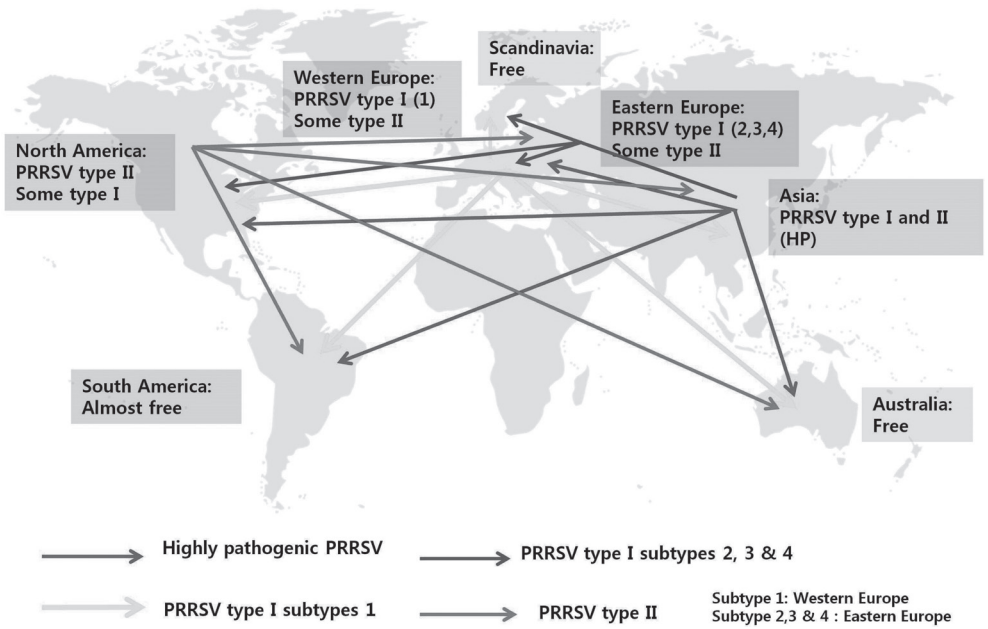

Figure 2. PRRS virus global distribution and hypothetical intercontinental transmission.

soluble and weakly membrane-associated protein[15].

After discovery, the virus soon spread rapidly all over the world from Canada (1987); Germany (late 1990); United Kingdom (1991) to Asia Korea (1985) and Japan (1988). The virus was isolated in two genotypes with different gene and antigen: genotype 1 (European, EU), with the prototype Lelystad virus representing the viruses mostly found in Europe and genotype 2 (North American, NA), represented by VR 2332, the prototype of strains originally discovered in North America and Asia[16-19]. During 2009-2010, the PRRSV isolated from southwestern China with genome analysis belonged to North American genotype 2. The study showed that China virus sequences with 30aa and extra

7aa deletion in Nsp2 coding region was compatible to highly pathogenic strain (HP-PRRSV)[20]. Another study showed that genetic modifications in Nsp2 region do not concern with virulence of PRRSV[21].

It's been almost 25 years until the first report about new disease in swine, there are additional hypotheses about the highly genetic diversity and rapid expansion of PRRSV (Figure 2)[22-25]. Murtaugh *et al*. (2010) demonstrated an increasing genetic variation among two geographical genotypes by their phylogenetic analysis of PRRSV, immunological selection and cellular modification processes. Stadejek *et al*. (2008) suggested the new division of PRRSV genotype 1 into 3 subtypes basing on extensive ORF7 size: subtype 1; subtype 2 (Bor) strains and subtype 3 (Lena) with different length in nucleocapsid protein from 124 to 132 amino acids[26]. Additionally, the new evidence of extensive size polymorphism might be discussed as a fourth subtype of European genotype[27] (Stadejek, 2013). According to Shi *et al*. (2010), the classification study of 9 genetically distinct lineages of genotype 2 PRRSV showed no overall genetic diversity compared to genotype 1[28]. In the other hand, the phylogeny-based evolutionary of PRRSV genotype 2 was investigated from 8,624 ORF5 sequences that the average diversity reaching 12.5% was a rapid virus evolution for future researches in virus diagnosis and vaccination. Karniychuk *et al*. (2010) concluded that the PRRSV subtypes 3 (Lena) has high pathogenesis by testing sets of polyclonal antisera in immunoperoxidase monolayer assay (IPMA)[29].

The domestic or wild swine (*Sus crofa*) is the only species naturally infected by PRRSV. Within 12 hours of infection, PRRSVs tendentiously invade the macrophage of lymphoid node, lungs and tonsil tissue, as well as muscle tissue. The virus primarily replicate in macrophage damaging the cellular immune system, leading to the acute phage of infection with serious clinical signs: reduced appetite, fever, premature farrowing and abortion in adults; stillborn pigs, high preweaning mortality and mummified pigs in affected litters; loss of appetite and lethargy, obvious failure to thrive in weaned pigs. The PRRSV is a severe pathogenic strain that quickly spreads through the herd over 4-11 days. The virus transmission was not only direct contact with infected pigs but also contaminated sources such as feces; aerosol; fomites (even boots and coveralls)[30-32], and insect vectors (mosquitoes or houseflies)[33,34].

PRRSV Diagnostic Techniques

Virus Identification

According to the Ausvet plan of Animal Health Australia (2006), there is no specific treatment for PRRSV-infected pigs[35]. In OIE (World Organization for Animal Health) reference laboratory for Porcine Reproductive and Respiratory Syndrome 2010, they mentioned that it is difficult to identify virological diagnostic PRRSV infection but there are researches focusing on isolating the virus from affected pig samples such as serum, fluids, organ of lungs, tonsil, lymph nodes and spleen[36]. Virus isolates can easily infect the MA-104 cell line and two subpopulations (embryonic monkey kidney cell): CL-2621 and Marc-145, but the isolation of PRRSV can be problematic because of genetically distinguishable strains[37].

The PRRSV could infect a subpopulation of macrophages *in vivo* and porcine alveolar macrophage (PAM) or susceptible cell lines *in vitro* such as Marc-145, MA-104, CL 2126 cells (derived from Africa green monkey kidneys that are identified by two main macrophage receptors: heparan sulphate and sialoadhesin), and the vimetin molecules as a putative PRRSV receptor on Marc-145 cell line[37,38]. Costers *et al*. reported that the PRRSV glycoprotein are not combined to the plasma membrane or primary macrophages both *in vivo* and *in vitro* thus the PRRSV-infected cells conquer the natural immune system and are protected against the PRRSV specific antibodies[39]. As the PRRSV tendentiously infect and mostly replicate in porcine alveolar macrophage (PAM) so PAM is the recommended cells for viral protein identification. However, the PAM collection is not an easy procedure because its source needs to be strictly selected from pigs not over 8 week ages and in good health status, pathogen-free pigs[1]. Since there are more cell lines with genetic modification, it is possible to replicate PRRSV such as porcine, feline and baby hamster kidney cells expressing CD163; PK-15 expressing CD163; immortalized PAM cell line expressing CD163; immortalized porcine mono-myeloid cells[40,41].

Identification of PRRSV based on nucleic acid can be developed with molecule strategy such as RT-PCR (reverse-transcription PCR); real-time RT-PCR and nested RT-PCR which are commonly useful in detecting PRRSV nucleic acid from serum and tissue samples[16,18,42-48]. Additional analysis of restriction fragment length polymorphism (RFLP) of open reading frame (ORF) is necessary, such as ORF5 to differentiate a PRRSV vaccine strain from the North American (NA) field strain[49]; ORF6 and ORF7 to compare genetic analysis between live attenuated vaccine strain with six field viruses and three PRRSV local field isolates in Czech and Slovak swine[50] and to identify two PRRSV full-length genome of newly virulent isolates MN184 share approximately 87% and 59% nucleotide identity with the PRRSV prototype database with a nucleotide

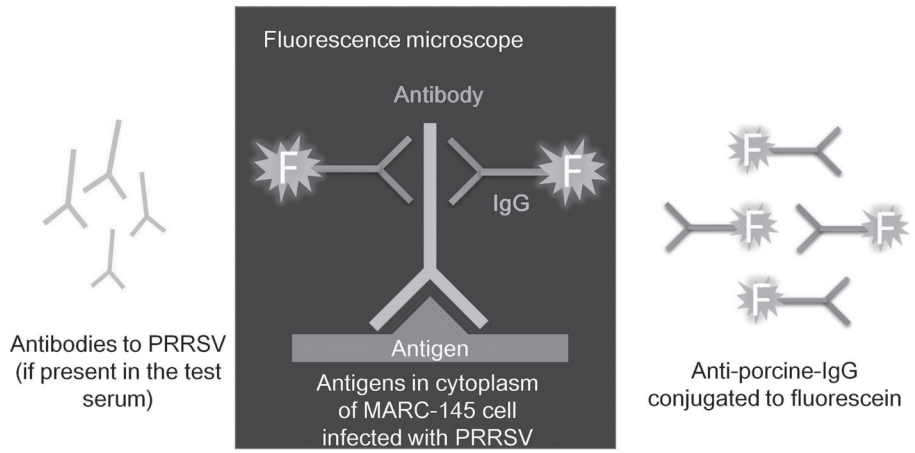

Figure 3. Schematic diagram of the indirect immunofluorescence assay (IFA) for PRRSV antibody detection.

length of 15019 bases[51]. Larochelle and Magar reported the non-radioactive in situ hydridization (ISH) method, using a 245 bps cDNA with digoxigenin label for detection PRRSV-specific nucleic acid. It is more rapid and sensitive than the immunohistochemical method known as the immunogold silver staining (IGSS), using monoclonal antibody SDOW17 for detection of PRRSV antigen[16]. Both methods have processed in formalin-fixed paraffin-embedded tissue which allows the visualization and localization of virus within cells and tissues, could be applied in PRRSV diagnosis.

The sensitivity of RT PCR was evaluated between the pooled and individual sample of swine showed 6% and 8% decrease in sensitivity of pooled sample sizes of 3 and 5, respectively. Besides, Rovira *et al*. (2007) inferred that serum and blood swab sample was the best to detect PRRSV in acute phase while semen from 18 boars was false to detect the disease agent in most of RT PCR tests[52]. All nucleic acid-based tests showed more rapid, specific and sensitive PRRSV detection than virus isolation in cell culture of PAMs[52].

Serological Test

Various immunogenic tests have been developed to detect the serum antibodies to PRRSV with low-cost, high specificity and high sensitivity. The tests are widely commercial for diagnosis of PRRSV such as the indirect immunofluorescence assay (IFA) (Figure 3), the immunoperoxidase monolayer assay (IPMA), the enzyme-linked immunosorbent assay, virus neutralization assay (VN) and immunochromographic test strip. According to the report of OIE, serological test could be early used to detect for IgM within 7 days and IgG within 14 days of post-infection from the pig sera. Initial researches suggested that the earliest antibodies were detected around first week of post-infection, against the nucleocapsid N-protein and were non-neutralizing[53,54].

The large quantity, easy collection and ready availability of serological sample such as serum, oral fluid and muscle transudate are first advantages of serology to researchers. N protein is an abundant target antigen for surveying the humoral immune response by ELISA, IFA, VN and immunoblotting assays (IB). Nelson *et al*. (1994) employed IFA, VN and IB with 3 structural proteins to detect antibody-to-PRRSV[55]. The IFA and IB test showed the same time to recognize serum antibody but later in VN test, that could be explained by circulating immune affection and less antigenic epitope which interacted to VN assay. Additionally, the poor sensitivity of VN was the reason to lack of correlation between these tests.

The VN assays are capable of detecting antibody neutralizing infectious virus in cell culture[56]. A given amount of PRRSV were incubated with series of sample serum dilution, after that the mixture was added to host cells (recommended PAMs and cell lines Marc-145 or MA-104) to allow visible cytopathogenic effect (CPE). The presence of neutralizing antibody allowed no apparent CPE at lower serum dilution and at concentration which CPE become positive is a titer for virus. Despite great specificity, VN assay is only utilized for research because of poor sensitivity; no standardized protocol; long time-consuming and high cost.

Cho *et al*. (1996) processed the ELISA and IFA test for the same late-infection sera. The results showed that the ELISA was more sensitive than IFA test because all IFA-positive sera were the same with ELISA-positive reaction but almost 50% IFA-negative sera were also ELISA reactors[57]. Besides, ELISA technique is cost-effective and suitable for testing of a large number of sample volumes. Denac *et al*. modified the commercial ELISA, termed rnPRRS ELISA, using a single

recombinant viral nucleocapsid protein (rNC) as antigen instead of whole virus antigen[58]. The rnPRRS ELISA is suitable for detecting antibodies in large number of sera within a short period time, resulting in 95.8 % specificity and 100% sensitivity.

Previous serological techniques focused on detecting not only IgG antibodies but also other immunoglobulins (Ig), especially IgMs to optimize detection of many viral pathogens in early state[59-61]. The ability of double recognition ELISA (DR-ELISA) is highly sensitive by double detecting of antigen by antibody in a sandwich format[62]. The principle of this assay is based on a recombinant N protein of PRRSV (EU genotype 1) used both as a capture antigen and a signal antigen with HRP-conjugate, which was simultaneously bound by antibody molecules (Figure 4)[63]. The result from Venteo *et al*. (2012) showed short time verified detection of infected pigs at day 7 pi to IgMs even IgGs at day 14 pi, confirming capability to recognize major Igs and to detect EU PRRSV at early post infection.

Coster *et al*. (2006) suggested that the viral protein were not observed on the plasma membrane of PRRSV-infected macrophage and might be retained within the cell on the Golgi apparatus and/or ER (endoplasmic reticulum). The absence of viral protein from the surface helped the infected-cell against the antibody-dependent, complement-mediated cell lysis (ADCML) assay *in vitro* as well as from the natural immune response *in vivo*[39]. This could explain for the virus persistence and the clearance of antibody-mediated immune system that the role of PRRSV-specific antibodies for clearing free virus is potential but limited for infected-cells. Although the sensitivity and specificity of these serological tests was generally good, the false positive reactions may happen. Nevertheless, the ELISA assays may not determine low levels of antibodies and the specific epitope of the antibodies must be known.

Vaccination

Vaccination for PRRSV is one of traditional and needed methods to treat and control virus infection in swine. Recently, PRRS vaccine products are commercially available in many countries all over the world[64]. However, the highly genetic diversity as well as rapid formation of virus sequence deposition makes vaccines not efficient for all PRRSV genotype. In other words, the immune response in vaccinated swine is not completely cross-protective because of high-rate evolution. Commercial PRRS vaccines are often derived from modified-lived virus (MLV); inactivated virus (with preparation of multiple virulent isolates or enriched viral antigen) and subunit components expressing selected protein[65-69].

Trus *et al*. (2014) tested 2 vaccination experiments

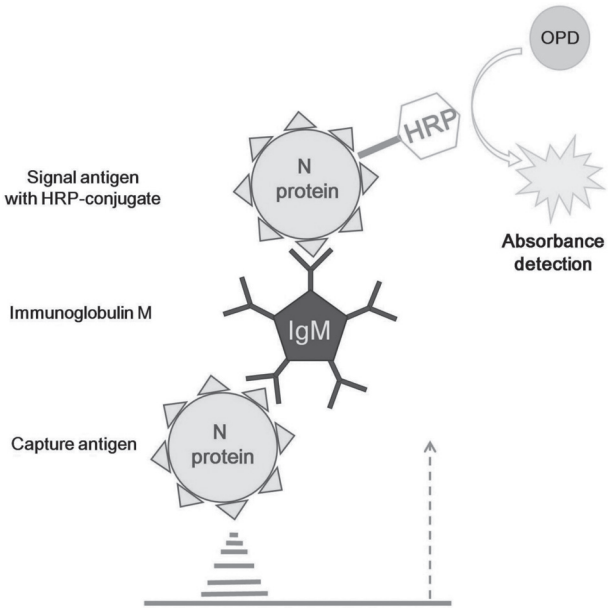

Figure 4. Schematic diagram of double-antigen ELISA method. The principle of this assay is based on a recombinant N protein of PRRSV (EU genotype 1) used both as a capture antigen and a signal antigen with HRP-conjugate, which was simultaneously bound by antibody molecules.

with different age pigs by specific IPMA antibodies. These pigs, which were both vaccinated with the MLV based on the European subtype 1 strain DV and infected with the East European PRRS virus subtype 3 strain Lena, were partially protected from severe syndrome (long-lasting fever, viremia, nasal shedding), nevertheless they still died because of second infection by *Trueperella pyogenes* and *Streptococcus suis* in lung[70]. Ellingson *et al*. (2010) suggested that the most effective vaccine were determined not only on percent similarity but also on specific gene regions that concerned genome replication and viral interaction, and the utilization of chimeric viruses were adapted as vaccine formula[71].

DNA vaccines and recombinant DNA vector vaccines are favored approaches to generate a more effective vaccine. The ORF5-encoded glycoprotein GP5, which is a major structural protein of PRRSV, induced neutralizing antibodies in swine. Pirzadeh *et al*. (1998) immunized pigs with anti-GP5-specific neutralizing antibody production by a plasmid encoding GP5 of PRRSV. However, the *E. coli*-expressed recombinant GST-ORF5 protein might not effectively trigger the immune response to induce neutralizing antibodies because of different polypeptide formation or post-translational modification but they proposed that GP5 is a proper candidate for subunit recombinant vaccines[72].

Table 1. PRRSV identification techniques focus on predicted time[53,57,83].

Test	Specimen sample required	Detection	Predictable time	Advantage	Disadvantage
Virus isolation	Buffy coat, serum, lung, lymph node spleen, tonsils. Purified alveolar macrophages or MARC-14 cell line.	Virus	Minimum 3 daysrequired for cytopathic effects (CPE)	Confirmation of PRRS virus infection. Early detection even with no clear syndromes or Characteristic lesions.	Special material and specific cell lines are Required. Virus isolation takes long time.
Pathologic test	Lung, brain or Heart tissues.	Clinical signals and Infected tissue	1 day	Quick identification of the disease signal.	Complicated by secondary bacterial infections and Subsequent inflammation. Lack of specificity and general inconsistency in field cases. Must be linked to some virological procedure to confirm that PRRSV is involved.
Serological test	ELISA: serum of EDTA blood Immunohistochemistry: monolayers of infected cell	Antibody and viral antigen	The IgM and IgG can be detected within 7 and 14 days respectively. Serological tests take 1-2 days.	ELISA is a time-efficient and cost effective technique with high sensitivity and suitability for testing a large number of samples inshort time. Fluorescent antibody tissue test (FA) is fast and can detect viral antigen even if the virus could not be isolated.	Difficult in herds that have not experienced the classic clinical syndrome and do not verify disease. Non-specific reactions or individual false positive reactions may occur in the ELISA test limit of objectivity and the confidence level of FA Depends on the variable amount of antigen in the tissue samples.
PCR, RT-PCR	Lung tissue or serum.	Viral virus (RNA)	2 days	Rapid and do not require Cell culture. Useful when disease samples are autolysed and degraded by heat during Virus isolation.	Expensive

Rompato *et al*. (2005) studied the affection of PRRSV-ORF7 DNA vaccine (phCMV-ORK7) to the immune response with correlation to some adjuvant. There was a positive inductive influence on the activation of vaccine-induced cellular immunity of specific virus by adding interleukin 2 (IL-2), whilst a suppressive effect by adding interleukin 4 (IL-4) in the context of an ORF7 DNA vaccine-induced immune response[73]. These results demonstrated the ability of DNA vaccine adjuvant to enhance and promote cellular immune system upon vaccination for PRRSV DNA vaccines in particular and for animal vaccines in general[74-79].

The limitation of the innate and adaptive mechanism of anti-PRRS virus immunity in boars, with no knowledge about the relationship between this syndrome to other diseases in swine and the highly genetic differences among PRRSV keep many efforts to generate novel vaccines for the porcine reproductive and respiratory syndrome frustrated[64,80-82]. The various diagnostic techniques as listed in Table 1 are complex and time consuming, and acquire accurate results depend on the quality and suitability of the samples[53,57,83].

Perspective

The desired diagnostic techniques not only should be robust, fast, but also cost-effective and capable of working for large number of samples. Virus isolation is capable of identifying the causative agent and confirming the clinical case in cell cultures but long time-consuming and complicated process. Molecular techniques can be useful to detect viral RNA with high sensitivity as well as rapid evaluation of high number of samples but not effective for a highly genetic diversity of PRRSV and not distinguish inactivated virus from the live one. Serological diagnosis is usually utilized to detect serum antibodies to PRRSV in spite of positive nonspecific reaction.

Although many available methods and commercial vaccines have significantly improved nowadays, these tests are not effective and perfect for all PRRSV subtypes. The requirement for additional tests should be considered because the negative and false positive re-

sults are high. More importantly, it has been imperative researches for sophisticated knowledge in (1) PRRS viral target of host immunity; (2) immunological correlation of PRRS to other diseases in swine and (3) challenge experiments that interpret the highly genetic diversity of PRRSV to predict cross-protective immune mechanism. This mini review aimed to demonstrate extensive discussion about the global PRRS situation with up-to-date methods and its contribution towards effective control of PRRS in swine all over the world.

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