Veterinary Microbiology 206 (2017) 29–34

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Strategies to broaden the cross-protective efficacy of vaccines against porcine reproductive and respiratory syndrome virus

Hiep L.X. Vu*, Asit K. Pattnaik, Fernando A. Osorio

Nebraska Center for Virology, and School of Veterinary Medicine and Biomedical Sciences, University of Nebraska-Lincoln, Lincoln, Nebraska, United States

ARTICLE INFO

Article history:
Received 26 August 2016
Received in revised form 19 September 2016
Accepted 20 September 2016

Keywords:
Heterologous protection
Immune evasion
Genetic diversity
DNA shuffling
Consensus immunogens
Chimeric virus

ABSTRACT

Porcine reproductive and respiratory syndrome virus (PRRSV) is one of the most economically important viral pathogens currently affecting swine production worldwide. Although PRRS vaccines have been commercially available for over 20 years, the available vaccines are considered inadequately effective for control and eradication of the virus. Major obstacles for the development of a highly effective PRRS vaccine include the highly variable nature of the viral genome, the viral ability to subvert the host immune system, and the incomplete understanding of the immune protection against PRRSV infection. This article summarizes the impediments for the development of a highly protective PRRS vaccine and reviews the vaccinology approaches that have been attempted to overcome one of the most formidable challenges, which is the substantial genetic variation among PRRSV isolates, to broaden the antigenic coverage of PRRS vaccines.

1. Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is endemic in most swine producing countries, causing significant losses to swine producers worldwide. The virus can infect pigs of all ages. However, clinical signs of infection are more severe in pregnant sows and young pigs [reviewed in (Rossow, 1998)]. Infection with PRRSV during the last trimester of gestation may result in premature farrowing with stillborn, partially autolyzed, and mummified fetuses (Terpstra et al., 1991). Neonatal pigs infected with PRRSV often display fever, severe dyspnea, anorexia, lethargy, edema of the eyelids, and blue or red discoloration of the ears or hindquarters (Rossow et al., 1994). The clinical signs of PRRSV infection in finishing pigs, boars and unbred gilts are less obvious (Gradil et al., 1996). The clinical symptoms are more severe when PRRSV-infected pigs are co-infected with other pathogens.

PRRSV belongs to Order *Nidovirales*, Family *Arteriviridae*. Other members of the *Arteriviridae* include equine arteritis virus (EAV), mouse lactate dehydrogenase elevating virus (LDV), and simian hemorrhagic fever virus (SHFV) (Cavanagh, 1997). The PRRSV genome is a linear, positive sense and single stranded RNA molecule of approximately 15 kb in length that is flanked by a methyl-cap and a poly (A) tail at its 5′ and 3′ end, respectively (reviewed in Snijder et al., 2013). The genome contains at least 12 open reading frames (ORFs). ORFs 1a and 1b comprise about 75% of the viral genome and encode two polyproteins pp1a and pp1ab that are cleaved into at least 14 non-structural proteins (nsp) responsible for replication and transcription of the viral genome (Li et al., 2015). Additionally, the nsps are involved in modulating the host innate immunity (reviewed in Fang and Snijder, 2010). The remaining 8 ORFs encode 8 structural proteins. ORFs 2a, 3 and 4 encode the minor envelope glycoproteins GP2, GP3 and GP4, respectively. The minor GPs are dispensable for viral particle formation but are required for viral infectivity, presumably due to their binding to the cellular receptor CD163 (Das et al., 2010; Wissink et al., 2005). ORFs 5 and 6 encode GP5 and membrane (M) protein which interact with each other to form the hetero-dimers that are indispensable for the viral particle formation (Wissink et al., 2005). ORF7 encodes the viral nucleocapsid protein. ORF2b and ORF5a encode the non-glycosylated envelope protein E and ORF5a protein, respectively. E protein has ion channel activity which seems to be important for viral un-coating (Lee and Yoo, 2006). The ORF5a-encoded protein is essential for virus viability but its function is not known (Sun et al., 2013).

Vaccines for protection against PRRSV have been commercially available since 1994. Currently, two types of PRRSV vaccines are commercially available including killed-virus (KV) vaccines and modified-live virus (MLV) vaccines. There is a large volume of literature describing the development of subunit vaccines against PRRSV. However, the experimental subunit vaccines are not

* Corresponding author.
E-mail address: hiepvu@unl.edu (H.L.X. Vu).

http://dx.doi.org/10.1016/j.vetmic.2016.09.014
0378-1135/

capable of conferring adequate levels of protection, even against homologous PRRSV strains (reviewed in Renukaradhya et al., 2015). Under experimental conditions, the current KV vaccines provide very limited efficacy. In the young pig model, KV vaccines failed to prevent or reduce viremia after the vaccinated pigs were challenged with a virulent PRRSV strain (Zuckermann et al., 2007). In the sow model, KV vaccines did not prevent reproductive failure or congenital transmission of the challenge virus to their offspring (Scortti et al., 2007). Administration of KV vaccines in boars did not change the duration or magnitude of viremia and viral shedding in semen following challenge infection (Nielsen et al., 1997).

MLV vaccines have proven to be much more effective than KV vaccines (Zuckermann et al., 2007). Current MLV vaccines can provide nearly complete homologous protection, partial protection against heterologous PRRSV strains of the same genotype as the vaccine strains, and minimal protection against PRRSV strains of different genotypes. When tested against heterologous PRRSV strains of the same genotypes, the MLV vaccines protect pigs from clinical diseases (*e.g.* reducing fever, and lung pathology and improving weight gain) but often do not to protect pigs from being infected with the virus. The use of MLV vaccines can significantly reduce the duration and magnitude of viremia and viral shedding after the vaccinated pigs are challenged with virulent PRRSV strains (Nodelijk et al., 2001; Pileri et al., 2015; Rose et al., 2015). Although experimental data demonstrate the beneficial effects of MLV vaccines, the efficacy of MLV vaccines under field conditions is not optimal. Severe PRRS outbreaks occur in farms that are well vaccinated with MLV vaccines (Wang et al., 2015). It is generally accepted that currently available MLV vaccines are not adequately effective for control and eradication of PRRSV (Rock, 2007). In this article, we first discuss the major hurdles for the development of broadly protective PRRSV vaccines. We then review the approaches that have been explored to overcome one of the most formidable challenges, which is the extensively variable nature the viral genome, to broaden the antigenic coverage of PRRS vaccines.

2. Obstacles for the development of effective vaccines against PRRSV

2.1. Highly variable nature of the PRRSV genome

PRRSV is classified into 2 major genotypes: type 1 (European) and type 2 (North American). These 2 genotypes share approximately 60% sequence similarity at the full-genome level. Considerable levels of genetic variation exist among PRRSV isolates within each of these genotypes. Genetic relatedness among PRRSV isolates is commonly studied by analyzing viral ORF5 because it is the most variable envelop protein. Over 13,000 ORF5 sequences of type 2 PRRSV are publicly available. Based on phylogenetic analysis of the ORF5 sequences, type 2 PRRSVs are classified into 9 lineages, with the inter-lineage genetic distances varying from 11 to 18% (Shi et al., 2010b). Importantly, the genetic diversity of type 2 ORF5 sequences seems to increase over time. While the pairwise sequence distances among PRRSV isolates collected before 2000 tend to fall below the 10% threshold, the genetic distances among PRRSV isolates collected in 2008–2010 have expanded to be greater than 10% (Brar et al., 2015). Also based on phylogenetic analysis of ORF5 sequences, type 1 PRRSVs are classified into 4 subtypes (Shi et al., 2010a). Subtype 1 contains the majority of the sequences which originate in many countries worldwide. Subtype 1 is further divided into 12 clades where the inter-clade genetic distances are greater than 11% (Shi et al., 2010a).

The PRRSV genome is constantly evolving, leading to the emergence of new PRRSV strains with increased levels of virulence. For instance, a highly pathogenic form of type 2 PRRSV (so-called HP-PRRSV) emerged in China in 2006, causing death in pigs of all ages (Tian et al., 2007). Recently, a new strain of PRRSV (designated PRRSV 1-7-4) emerged in the U.S., causing unusually severe outbreaks, even in farms that are well vaccinated with PRRS vaccines (personal communication with field veterinarians).

2.2. Immune evasion

Pigs infected with PRRSV usually display extended periods of acute and persistent infection (Allende et al., 2000; Wills et al., 2003). This observation leads to the notion that the pigs do not mount effective immune responses to rapidly clear the infection. Most PRRSV strains can modulate the innate immune response by actively suppressing the induction of type-I interferons (IFNs) (reviewed in Sun et al., 2012). Additionally, the virus can selectively subvert the induction of virus-neutralizing (VN) antibodies. Although PRRSV specific antibodies appear as early as 7 days post-infection (p.i.), these antibodies are not capable of neutralizing the virus. VN antibodies usually do not appear until a month after infection (reviewed in Lopez and Osorio, 2004). Moreover, VN antibodies are highly strain-specific. PRRSV can also evade the induction of cell-mediated immunity (CMI). The frequency of IFN-γ secreting cells (IFN-γ-SC) in peripheral blood has been widely used as an indicator of CMI against PRRSV infection. The onset and magnitude of IFN-γ-SC responses are variable depending on the PRRSV strain involved in a particular infection. Meier et al. reported that IFN-γ-SC were not detected until 8–10 weeks p.i., then gradually increased until 48 weeks p.i., and remained stable until 690 days p.i. (Meier et al., 2003). Conversely, several studies had reported that PRRSV-specific IFN-γ-SC appeared in peripheral blood of infected pigs at about 14 days p.i., increased to the maximum levels at 28 days p.i., and declined thereafter (Diaz et al., 2005, 2006; Zuckermann et al., 2007).

2.3. Incomplete understanding of the correlates of protection

Contrary to other models of RNA virus persistence, PRRSV does not persist in the infected animals for life. Instead, the virus is eventually eliminated from the infected pigs at approximately 5–6 months after infection (Allende et al., 2000; Wills et al., 2003). Importantly, convalescent pigs are fully protected against subsequent exposure to the same virus strain that they were previously infected with (Lager et al., 1997). These observations clearly indicate that the pigs can develop protective immunity against PRRSV and that it requires a long time for this process to happen. Both VN antibody and CMI are believed to be the components of the protective immunity against PRRSV infection. Passive immunization studies demonstrated that VN antibodies can protect pigs against infection with a virulent PRRSV strain, providing that sufficient amounts of VN antibodies are present in the pigs prior to challenge infection (Lopez et al., 2007; Osorio et al., 2002). However, most of the MLV vaccines do not elicit robust levels of VN antibody responses (reviewed in Lopez and Osorio, 2004). Moreover, it has been frequently observed that vaccinated pigs are protected from challenge infection in the absence of VN antibodies (Roca et al., 2012; Trus et al., 2014; Zuckermann et al., 2007). These observations lead to the notion that CMI plays an important role in protection against PRRSV infection. Nonetheless, the correlation between the frequencies of virus-specific IFN-γ-SC and the levels of protection are greatly variable (Charerntantanakul et al., 2006; Meier et al., 2004). It has been hypothesized that not the quantity but the quality of T cell responses after vaccination may affect the levels of protection (Zuckermann et al., 2007). At the present, the phenotypic and functional characteristics of T cell responses after vaccination with a PRRS vaccine have not been well studied.

Additionally, the identification and characterization of the viral proteins that are involved in induction of protective immunity remain incomplete. Multiple viral structural proteins including GP2, GP3, GP4 and GP5 and M are reported to be able to elicit VN antibodies (Vanhee et al., 2011). Likewise, several structural and nonstructural proteins are able to elicit virus-specific IFN-γ-SC (Mokhtar et al., 2014). However, none of the viral proteins alone is able to elicit complete protection.

2.4. Lack of reliable parameters to predict vaccine protection

Classification of PRRSV isolates is of cardinal importance for deciding which viral strain and/or how many strains should be represented in a vaccine in order to achieve a broad spectrum of protection. Phylogenetic analysis of ORF5 sequence has been used extensively to classify and study genetic relationships among PRRSV strains (Shi et al., 2010b). Early studies suggested that the protective efficacy of MLV vaccines seems to correlate with the degree of ORF5 sequence similarity between the viral strain used for vaccine formulation and the strain to which the vaccinated animals are exposed (Labarque et al., 2004). However, it is not clear how similar the ORF5 sequence between the vaccine strains and the field strains must be in order to warrant complete protection. Although type 2 PRRSV is classified into 9 different lineages based on the phylogenetic analysis of ORF5 sequences, the levels of intra – and inter – lineage protection remain unknown. It appears that PRRSV isolates belonging to the same lineage do not always provide complete protection against one another. For instance, the MLV vaccine Fostera® PRRS belongs to lineage 8, which includes HP-PRRSV. However, vaccination of pigs with Fostera® PRRS only resulted in partial protection against challenge with HP-PRRSV (Do et al., 2015). Moreover, the protective efficacy of Fostera® PRRS against HP-PRRSV was not significantly different from that of Ingelvac PRRS® MLV even though Ingelvac PRRS® MLV belongs to lineage 5 (Do et al., 2015).

Restriction fragment length polymorphism (RFLP) has been frequently used by veterinary diagnostic laboratories in the U.S. for typing type 2 PRRSV. This method is based on the digestion patterns of ORF5, using three restriction enzymes MluI, HincII and SacII. One single nucleotide change in the enzyme recognition site could result in a change in the RFLP pattern (Cha et al., 2004). Thus, the RFLP analysis does not accurately reflect the genetic relatedness among PRRSV isolates.

Several research groups have attempted to assess the antigenic relationship among PRRSV strains by serological reactions with different monoclonal antibodies (MAbs) (Yang et al., 2000). It is noteworthy that most of the MAbs used to define the sero-groups of PRRSV were incapable of neutralizing viral infection. Thus far, the correlation between the sero-groups defined by MAbs and the cross-protection has not been analyzed.

Due to the lack of a reliable surrogate marker of protection, the protective efficacy of PRRS vaccine candidates can only be measured through vaccination/challenge experiments in pigs, the target species. Such experiments are expensive and cumbersome; therefore, severely hampering the progress in vaccine development.

3. Approaches to expand the antigenic coverage of PRRS vaccines

3.1. Multi-strain vaccines

One common approach to expand antigenic coverage of a vaccine is to incorporate multiple strains of the pathogen into the vaccine make-up. One example of a multi-strain vaccine against a viral pathogen would be the tri-valent vaccine against seasonal influenza in human which is comprised of 2 strains of influenza A and 1 strain of influenza B. Thus far, there are only a few studies testing the efficacy of multi-valent vaccines against PRRSV. In one study, the safety and efficacy of a multi-valent vaccine comprising 5 live-attenuated PRRSV type 2 strains was compared with that of a single-strain live-attenuated vaccine (Mengeling et al., 2003). Under the experimental condition of that study, the multi-valent vaccine did not provide any better heterologous protection than the single-strain vaccine. It was observed that pigs vaccinated with the multi-strain vaccine displayed lymphoid hyperplasia, raising the concern regarding the safety of a multi-valent MLV vaccine (Mengeling et al., 2003). It was not known if lymphoid hyperplasia observed in the multi-strain vaccine was due to the co-vaccination or due to the effects of a strain that was not sufficiently attenuated.

In another study, Park et al. evaluated the effects of co-vaccination of pigs with both type 1 and type 2 MLV vaccines against dual or individual challenge with type 1 and type 2 PRRSV strains (Park et al., 2015). The authors reported that co-vaccination with type 1 and 2 MLV vaccines can only protect against challenge infection with type 1 but not type 2 PRRSV strains. Interestingly, the protective efficacy of co-vaccination against type 2 challenge was reduced as compared to single vaccination with type 2 vaccine. It is not clear why co-vaccination failed to protect against challenge infection with type 2 virus. In this study, VN antibodies were not detected in any of the vaccinated pigs. Co-vaccination elicited equal levels of T cell responses against type 1 and type 2 PRRSVs, suggesting that co-vaccination did not affect the levels of T cell responses against each component of the vaccine.

Recently, an experimental trivalent KV vaccine comprised of one type 1 and two genetically distinct type 2 PRRSV strains was developed and tested in pigs (Yeom et al., 2015). In this study, pigs were vaccinated twice with the trivalent vaccine at 4 and 6 weeks of age, respectively. Two weeks after the second vaccination, pigs were separately challenged with one of the 3 PRRSV strains used to formulate the vaccine. The trivalent vaccine was able to confer equal levels of partial protection against challenge infection with these 3 PRRSV strains. The results of this study would be encouraging. However, it remains to be seen how this trivalent KV vaccine can protect against other PRRSV strains that are genetically different from the 3 viral strains used to formulate the vaccine.

3.2. Chimeric virus

Multiple structural proteins of PRRSV can elicit neutralizing antibody (Vanhee et al., 2011) and confer partial protection (reviewed in Renukaradhya et al., 2015). Thus, one approach to broaden the heterologous protection is to generate chimeric PRRSV strains that carry structural proteins from genetically distinct PRRSV strains. Several chimeric type 2 PRRSVs have been generated by replacing structural genes of PRRSV strain VR-2332 with the corresponding genes of PRRSV strain JA-142 (Kim and Yoon, 2008). The VR-2332 chimeric viruses carrying ORFs 2–6 (designated JAP23456) or ORFs 3–6 (designated JAP3456) of JA-142 were totally resistant to neutralization by anti-VR-2332 antiserum while gaining susceptibility to neutralization by anti-JA-142 antiserum, suggesting that ORFs 3–6 of PRRSV are the main determinants of virus neutralization. In contrast, the VR-2332 chimera carrying ORFs 5 and 6 of JA-142 (designated JAP56) became susceptible to neutralization by both anti-VR-2332 and anti JA-142 antisera. Interestingly, immunization by infection of pigs with the chimeric JAP56 resulted in protection against both VR-2332 and JA-142 (Sun et al., 2016). It remains to be determined how this JAP56 chimera can protect against PRRSV strains genetically distinct from its parental strains. Theoretically, the cross-protective capability of the JAP56 could be improved further

by incorporating more genes from other heterologous PRRSV strains. However, one technical constraint resides determining which gene of the heterologous PRRSV strains should be incorporated into the JAP56 to expand its heterologous protection.

3.3. DNA shuffling

DNA shuffling is a molecular method that allows randomly recombining DNA from multiple parental genes to create a large library of mosaic sequences displaying DNA fragments from the various parental sequences. The library is then screened to identify recombinant sequences encoding a desirable characteristic. DNA shuffling has proven to be a powerful technique to expand the antigenic coverage of vaccine immunogens as it allows generating vaccine immunogens containing mosaic DNA sequences from diverse viral strains (reviewed in Locher et al., 2005).

DNA shuffling was employed to recombine sequences of individual genes encoding envelope proteins (ORF3, ORF4 and ORF6), using donor sequences from 6 genetically different parental type 2 PRRSV strains (Zhou et al., 2012, 2013). The individually shuffled envelope genes were then separately cloned into an cDNA clone of PRRSV strain VR-2385 to generate infectious PRRSV chimeras. A number of chimeras simultaneously displaying cDNA fragments of all 6 parental PRRSV strains have been successfully generated. Some chimeras can elicit better levels of cross-neutralizing antibodies against one or two parental PRRSV strains from which ORF3, ORF4 and ORF6 sequences were used for DNA shuffling. However, so far none of these chimeras could elicit improved levels of cross-neutralizing antibodies against all parental PRRSV strains (Zhou et al., 2012, 2013). To further improve the ability of inducing cross-neutralizing antibodies, individually-shuffled envelope genes were incorporated in different configurations, using the Fostera® PRRS MLV vaccine strain as a backbone (Tian et al., 2015). The resulting chimeric viruses carrying multiple shuffled envelope proteins still can elicit cross-neutralizing antibodies against only one of the heterologous strains tested. A subsequent vaccination/challenge study revealed that chimeric virus did not confer significantly better heterologous protection than the parental Fostera® PRRS MLV vaccine strain (Tian et al., 2015).

3.4. Centralized immunogen

Centralized immunogens have proven to be an effective approach to expand the antigen coverage for vaccines against genetically variable RNA viruses such as human immunodeficiency virus type 1 (HIV-1) and influenza. This approach relies on the use of computational algorithms to create artificial immunogen sequences in a way that the immunogen sequences are located at the center of the phylogenetic tree (reviewed in Gao et al., 2004). Consequently, the centralized vaccine immunogens display shorter average genetic distances to wild-type sequences than the average genetic distances among wild-type sequences. Three different computational methods have been developed to generate a centralized immunogen sequence: consensus, common ancestor, and center of the tree (Gao et al., 2004). A consensus sequence that carries the most common amino acid found at each position of the alignment is the simplest method for the construction of a centralized immunogen (Gaschen et al., 2002). Studies on HIV-1 and influenza virus have clearly demonstrated that vaccines based on the consensus sequences elicit broader immune responses than vaccines based on naturally occurring sequences (Chen et al., 2008; Santra et al., 2008).

We recently employed the centralized immunogen approach to generate a candidate vaccine against type 2 PRRSV. Since no viral proteins are known to be able to elicit complete immune protection, we aimed to generate a fully infectious PRRSV strain based on a centralized whole genome sequence (Vu et al., 2015). By using a set of 59 full-genome sequences of type 2 PRRSVs originating in the U.S., a consensus genome (designated PRRSV-CON) was generated by aligning these 59 PRRSV full-genome sequences, followed by selecting the most common nucleotide found at each position of the alignment. Next, the synthetic PRRSV-CON strain was generated through the use of reverse genetics. The synthetic PRRSV-CON replicates as efficiently as our prototype PRRSV strain FL12, both *in vitro* and *in vivo*. Importantly, when tested in pigs, the synthetic PRRSV-CON confers significantly broader levels of heterologous protection than the reference PRRSV strain FL12. At the presence, the mechanisms by which the PRRSV-CON confers heterologous protection remain unknown. Additionally, it remains to be determined if the PRRSV-CON virus still maintains its cross-protective phenotype after the virus is attenuated to be use as a MLV vaccine.

4. Summary

The availability of highly effective vaccines against PRRSV is highly needed for the control of this devastating virus. Multiple types of vaccines have been tested, of which MLV vaccine appears to be the most effective one. A major drawback of the current MLV vaccines is that they do not provide sufficient levels of heterologous protection, mainly due to the pronounced genetic diversity of the PRRSV isolates circulating in the field. Different approaches have been pursued to broaden the antigenic coverage of PRRS vaccines. Particularly worthwhile to mention are the approaches that rely on the use of molecular techniques to manipulate the viral genome such as DNA shuffling and centralized antigens. The data from the immunization/challenge experiments conducted with the synthetic PRRSV-CON strain provide compelling evidence of heterologous protection and open a promising route to the improvement of the elusive broadly protective PRRS vaccine.

Conflict of interest statement

The authors declare no conflict of interest.

Acknowledgments

This work was supported by the US National Pork Board grant no 15-159 (to HLXV) and by Agriculture and Food Research Initiative Competitive Grant no. 2016-67015-24922 (to HLXV) and 2013-01035 (to FAO) from the USDA National Institute of Food and Agriculture.

References

Allende, R., Laegreid, W.W., Kutish, G.F., Galeota, J.A., Wills, R.W., Osorio, F.A., 2000. Porcine reproductive and respiratory syndrome virus: description of persistence in individual pigs upon experimental infection. J. Virol. 74, 10834–10837.

Brar, M.S., Shi, M., Murtaugh, M.P., Leung, F.C., 2015. Evolutionary diversification of type 2 porcine reproductive and respiratory syndrome virus. J. Gen. Virol. 96, 1570–1580.

Cavanagh, D., 1997. Nidovirales: a new order comprising Coronaviridae and Arteriviridae. Arch. Virol. 142, 629–633.

Cha, S.H., Chang, C.C., Yoon, K.J., 2004. Instability of the restriction fragment length polymorphism pattern of open reading frame 5 of porcine reproductive and respiratory syndrome virus during sequential pig-to-pig passages. J. Clin. Microbiol. 42, 4462–4467.

Charerntantanakul, W., Platt, R., Johnson, W., Roof, M., Vaughn, E., Roth, J.A., 2006. Immune responses and protection by vaccine and various vaccine adjuvant candidates to virulent porcine reproductive and respiratory syndrome virus. Vet. Immunol. Immunopathol. 109, 99–115.

Chen, M.W., Cheng, T.J., Huang, Y., Jan, J.T., Ma, S.H., Yu, A.L., Wong, C.H., Ho, D.D., 2008. A consensus-hemagglutinin-based DNA vaccine that protects mice against divergent H5N1 influenza viruses. Proc. Natl. Acad. Sci. U. S. A. 105, 13538–13543.
Das, P.B., Dinh, P.X., Ansari, I.H., de Lima, M., Osorio, F.A., Pattnaik, A.K., 2010. The minor envelope glycoproteins GP2a and GP4 of porcine reproductive and respiratory syndrome virus interact with the receptor CD163. J. Virol. 84, 1731–1740.
Diaz, I., Darwich, L., Pappaterra, G., Pujols, J., Mateu, E., 2005. Immune responses of pigs after experimental infection with a European strain of Porcine reproductive and respiratory syndrome virus. J. Gen. Virol. 86, 1943–1951.
Diaz, I., Darwich, L., Pappaterra, G., Pujols, J., Mateu, E., 2006. Different European-type vaccines against porcine reproductive and respiratory syndrome virus have different immunological properties and confer different protection to pigs. Virology 351, 249–259.
Do, D.T., Park, C., Choi, K., Jeong, J., Nguyen, T.T., Nguyen, K.D., Vo, D.T., Chae, C., 2015. Comparison of two genetically distant type 2 porcine reproductive and respiratory syndrome virus (PRRSV) modified live vaccines against Vietnamese highly pathogenic PRRSV. Vet. Microbiol. 179, 233–241.
Fang, Y., Snijder, E.J., 2010. The PRRSV replicase: exploring the multifunctionality of an intriguing set of nonstructural proteins. Virus Res. 154, 61–76.
Gao, F., Korber, B.T., Weaver, E., Liao, H.X., Hahn, B.H., Haynes, B.F., 2004. Centralized immunogens as a vaccine strategy to overcome HIV-1 diversity. Expert Rev. Vaccines 3, S161–168.
Gaschen, B., Taylor, J., Yusim, K., Foley, B., Gao, F., Lang, D., Novitsky, V., Haynes, B., Hahn, B.H., Bhattacharya, T., Korber, B., 2002. Diversity considerations in HIV-1 vaccine selection. Science 296, 2354–2360.
Gradil, C., Dubuc, C., Eaglesome, M.D., 1996. Porcine reproductive and respiratory syndrome virus: seminal transmission. Vet. Rec. 138, 521–522.
Kim, W.I., Yoon, K.J., 2008. Molecular assessment of the role of envelope-associated structural proteins in cross neutralization among different PRRS viruses. Virus Genes 37, 380–391.
Labarque, G., Reeth, K.V., Nauwynck, H., Drexler, C., Van Gucht, S., Pensaert, M., 2004. Impact of genetic diversity of European-type porcine reproductive and respiratory syndrome virus strains on vaccine efficacy. Vaccine 22, 4183–4190.
Lager, K.M., Mengeling, W.L., Brockmeier, S.L., 1997. Duration of homologous porcine reproductive and respiratory syndrome virus immunity in pregnant swine. Vet. Microbiol. 58, 127–133.
Lee, C., Yoo, D., 2006. The small envelope protein of porcine reproductive and respiratory syndrome virus possesses ion channel protein-like properties. Virology 355, 30–43.
Li, Y., Tas, A., Sun, Z., Snijder, E.J., Fang, Y., 2015. Proteolytic processing of the porcine reproductive and respiratory syndrome virus replicase. Virus Res. 202, 48–59.
Locher, C.P., Paidhungat, M., Whalen, R.G., Punnonen, J., 2005. DNA shuffling and screening strategies for improving vaccine efficacy. DNA Cell Biol. 24, 256–263.
Lopez, O.J., Osorio, F.A., 2004. Role of neutralizing antibodies in PRRSV protective immunity. Vet. Immunol. Immunopathol. 102, 155–163.
Lopez, O.J., Oliveira, M.F., Garcia, E.A., Kwon, B.J., Doster, A., Osorio, F.A., 2007. Protection against porcine reproductive and respiratory syndrome virus (PRRSV) infection through passive transfer of PRRSV-neutralizing antibodies is dose dependent. Clin. Vaccine Immunol. 14, 269–275.
Meier, W.A., Galeota, J., Osorio, F.A., Husmann, R.J., Schnitzlein, W.M., Zuckermann, F. A., 2003. Gradual development of the interferon-gamma response of swine to porcine reproductive and respiratory syndrome virus infection or vaccination. Virology 309, 18–31.
Meier, W.A., Husmann, R.J., Schnitzlein, W.M., Osorio, F.A., Lunney, J.K., Zuckermann, F.A., 2004. Cytokines and synthetic double-stranded RNA augment the T helper 1 immune response of swine to porcine reproductive and respiratory syndrome virus. Vet. Immunol. Immunopathol. 102, 299–314.
Mengeling, W.L., Lager, K.M., Vorwald, A.C., Clouser, D.F., 2003. Comparative safety and efficacy of attenuated single-strain and multi-strain vaccines for porcine reproductive and respiratory syndrome. Vet. Microbiol. 93, 25–38.
Mokhtar, H., Eck, M., Morgan, S.B., Essler, S.E., Frossard, J.P., Ruggli, N., Graham, S.P., 2014. Proteome-wide screening of the European porcine reproductive and respiratory syndrome virus reveals a broad range of T cell antigen reactivity. Vaccine 32, 6828–6837.
Nielsen, T.L., Nielsen, J., Have, P., Baekbo, P., Hoff-Jorgensen, R., Botner, A., 1997. Examination of virus shedding in semen from vaccinated and from previously infected boars after experimental challenge with porcine reproductive and respiratory syndrome virus. Vet. Microbiol. 54, 101–112.
Nodelijk, G., de Jong, M.C., van Leengoed, L.A., Wensvoort, G., Pol, J.M., Steverink, P.J., Verheijden, J.H., 2001. A quantitative assessment of the effectiveness of PRRSV vaccination in pigs under experimental conditions. Vaccine 19, 3636–3644.
Osorio, F.A., Galeota, J.A., Nelson, E., Brodersen, B., Doster, A., Wills, R., Zuckermann, F., Laegreid, W.W., 2002. Passive transfer of virus-Specific antibodies confers protection against reproductive failure induced by a virulent strain of porcine reproductive and respiratory syndrome virus and establishes sterilizing immunity. Virology 302, 9–20.
Park, C., Choi, K., Jeong, J., Kang, I., Park, S.J., Chae, C., 2015. Concurrent vaccination of pigs with type 1 and type 2 porcine reproductive and respiratory syndrome virus (PRRSV) protects against type 1 PRRSV but not against type 2 PRRSV on dually challenged pigs. Res. Vet. Sci. 103, 193–200.
Pileri, E., Gibert, E., Soldevila, F., Garcia-Saenz, A., Pujols, J., Diaz, I., Darwich, L., Casal, J., Martin, M., Mateu, E., 2015. Vaccination with a genotype 1 modified live vaccine against porcine reproductive and respiratory syndrome virus significantly reduces viremia, viral shedding and transmission of the virus in a quasi-natural experimental model. Vet. Microbiol. 175, 7–16.
Renukaradhya, G.J., Meng, X.J., Calvert, J.G., Roof, M., Lager, K.M., 2015. Inactivated and subunit vaccines against porcine reproductive and respiratory syndrome: current status and future direction. Vaccine 33, 3065–3072.
Roca, M., Gimeno, M., Bruguera, S., Segales, J., Diaz, I., Galindo-Cardiel, I.J., Martinez, E., Darwich, L., Fang, Y., Maldonado, J., March, R., Mateu, E., 2012. Effects of challenge with a virulent genotype II strain of porcine reproductive and respiratory syndrome virus on piglets vaccinated with an attenuated genotype I strain vaccine. Vet. J. 193, 92–96.
Rock, D.L., 2007. Colloquium on prospects for development of an effective PRRS virus vaccine. AASV Newsletter. http://www.aasp.org/news/story.php?id=2527.
Rose, N., Renson, P., Andraud, M., Paboeuf, F., Le Potier, M.F., Bourry, O., 2015. Porcine reproductive and respiratory syndrome virus (PRRSv) modified-live vaccine reduces virus transmission in experimental conditions. Vaccine 33, 2493–2499.
Rossow, K.D., Bautista, E.M., Goyal, S.M., Molitor, T.W., Murtaugh, M.P., Morrison, R. B., Benfield, D.A., Collins, J.E., 1994. Experimental porcine reproductive and respiratory syndrome virus infection in one-, four-, and 10-week-old pigs. J. Vet. Diagn. Invest. 6, 3–12.
Rossow, K.D., 1998. Porcine reproductive and respiratory syndrome. Vet. Pathol. 35, 1–20.
Santra, S., Korber, B.T., Muldoon, M., Barouch, D.H., Nabel, G.J., Gao, F., Hahn, B.H., Haynes, B.F., Letvin, N.L., 2008. A centralized gene-based HIV-1 vaccine elicits broad cross-clade cellular immune responses in rhesus monkeys. Proc. Natl. Acad. Sci. U. S. A. 105, 10489–10494.
Scortti, M., Prieto, C., Alvarez, E., Simarro, I., Castro, J.M., 2007. Failure of an inactivated vaccine against porcine reproductive and respiratory syndrome to protect gilts against a heterologous challenge with PRRSV. Vet. Rec. 161, 809–813.
Shi, M., Lam, T.T., Hon, C.C., Hui, R.K., Faaberg, K.S., Wennblom, T., Murtaugh, M.P., Stadejek, T., Leung, F.C., 2010a. Molecular epidemiology of PRRSV: a phylogenetic perspective. Virus Res. 154, 7–17.
Shi, M., Lam, T.T., Hon, C.C., Murtaugh, M.P., Davies, P.R., Hui, R.K., Li, J., Wong, L.T., Yip, C.W., Jiang, J.W., Leung, F.C., 2010b. Phylogeny-based evolutionary, demographical, and geographical dissection of North American type 2 porcine reproductive and respiratory syndrome viruses. J. Virol. 84, 8700–8711.
Snijder, E.J., Kikkert, M., Fang, Y., 2013. Arterivirus molecular biology and pathogenesis. J. Gen. Virol. 94, 2141–2163.
Sun, Y., Han, M., Kim, C., Calvert, J.G., Yoo, D., 2012. Interplay between interferon-mediated innate immunity and porcine reproductive and respiratory syndrome virus. Viruses 4, 424–446.
Sun, L., Li, Y., Liu, R., Wang, X., Gao, F., Lin, T., Huang, T., Yao, H., Tong, G., Fan, H., Wei, Z., Yuan, S., 2013. Porcine reproductive and respiratory syndrome virus ORF5a protein is essential for virus viability. Virus Res. 171, 178–185.
Sun, D., Khatun, A., Kim, W.I., Cooper, V., Cho, Y.I., Wang, C., Choi, E.J., Yoon, K.J., 2016. Attempts to enhance cross-protection against porcine reproductive and respiratory syndrome viruses using chimeric viruses containing structural genes from two antigenically distinct strains. Vaccine 34, 4335–4342.
Terpstra, C., Wensvoort, G., Pol, J.M., 1991. Experimental reproduction of porcine epidemic abortion and respiratory syndrome (mystery swine disease) by infection with Lelystad virus: koch's postulates fulfilled. Vet. Q. 13, 131–136.
Tian, K., Yu, X., Zhao, T., Feng, Y., Cao, Z., Wang, C., Hu, Y., Chen, X., Hu, D., Tian, X., Liu, D., Zhang, S., Deng, X., Ding, Y., Yang, L., Zhang, Y., Xiao, H., Qiao, M., Wang, B., Hou, L., Wang, X., Yang, X., Kang, L., Sun, M., Jin, P., Wang, S., Kitamura, Y., Yan, J., Gao, G.F., 2007. Emergence of fatal PRRSV variants: unparalleled outbreaks of atypical PRRS in China and molecular dissection of the unique hallmark. PLoS One 2, e526.
Tian, D., Ni, Y.Y., Zhou, L., Opriessnig, T., Cao, D., Pineyro, P., Yugo, D.M., Overend, C., Cao, Q., Lynn Heffron, C., Halbur, P.G., Pearce, D.S., Calvert, J.G., Meng, X.J., 2015. Chimeric porcine reproductive and respiratory syndrome virus containing shuffled multiple envelope genes confers cross-protection in pigs. Virology 485, 402–413.
Trus, I., Bonckaert, C., van der Meulen, K., Nauwynck, H.J., 2014. Efficacy of an attenuated European subtype 1 porcine reproductive and respiratory syndrome virus (PRRSV) vaccine in pigs upon challenge with the East European subtype 3 PRRSV strain Lena. Vaccine 32, 2995–3003.
Vanhee, M., Van Breedam, W., Costers, S., Geldhof, M., Noppe, Y., Nauwynck, H., 2011. Characterization of antigenic regions in the porcine reproductive and respiratory syndrome virus by the use of peptide-specific serum antibodies. Vaccine 29, 4794–4804.
Vu, H.L., Ma, F., Laegreid, W.W., Pattnaik, A.K., Steffen, D., Doster, A.R., Osorio, F.A., 2015. A synthetic porcine reproductive and respiratory syndrome virus strain confers unprecedented levels of heterologous protection. J. Virol. 89, 12070–12083.
Wang, X., Marthaler, D., Rovira, A., Rossow, S., Murtaugh, M.P., 2015. Emergence of a virulent porcine reproductive and respiratory syndrome virus in vaccinated herds in the United States. Virus. Res. 210, 34–41.
Wills, R.W., Doster, A.R., Galeota, J.A., Sur, J.H., Osorio, F.A., 2003. Duration of infection and proportion of pigs persistently infected with porcine reproductive and respiratory syndrome virus. J. Clin. Microbiol. 41, 58–62.
Wissink, E.H., Kroese, M.V., van Wijk, H.A., Rijsewijk, F.A., Meulenberg, J.J., Rottier, P. J., 2005. Envelope protein requirements for the assembly of infectious virions of porcine reproductive and respiratory syndrome virus. J. Virol. 79, 12495–12506.
Yang, L., Frey, M.L., Yoon, K.J., Zimmerman, J.J., Platt, K.B., 2000. Categorization of North American porcine reproductive and respiratory syndrome viruses:

epitopic profiles of the N, M, GP5 and GP3 proteins and susceptibility to neutralization. Arch. Virol. 145, 1599–1619.
Yeom, M., Lyoo, K.S., Kang, B.K., Song, D., Park, B., 2015. Efficacy of a combined inactivated porcine reproductive and respiratory syndrome virus vaccine using North American and European strains in specific pathogen free pigs. Vet. J. 204, 157–161.
Zhou, L., Ni, Y.-Y., Pineyro, P., Sanford, B.J., Cossaboom, C.M., Dryman, B.A., Huang, Y.-W., Cao, D.-J., Meng, X.-J., 2012. DNA shuffling of the GP3 genes of porcine reproductive and respiratory syndrome virus (PRRSV) produces a chimeric virus with an improved cross-neutralizing ability against a heterologous PRRSV strain. Virology 434, 96–109.
Zhou, L., Ni, Y.Y., Pineyro, P., Cossaboom, C.M., Subramaniam, S., Sanford, B.J., Dryman, B.A., Huang, Y.W., Meng, X.J., 2013. Broadening the heterologous cross-neutralizing antibody inducing ability of porcine reproductive and respiratory syndrome virus by breeding the GP4 or M genes. PLoS One 8.
Zuckermann, F.A., Garcia, E.A., Luque, I.D., Christopher-Hennings, J., Doster, A., Brito, M., Osorio, F., 2007. Assessment of the efficacy of commercial porcine reproductive and respiratory syndrome virus (PRRSV) vaccines based on measurement of serologic response, frequency of gamma-IFN-producing cells and virological parameters of protection upon challenge. Vet. Microbiol. 123, 69–85.