RESEARCHREPORT

SWINE HEALTH

pork checkoff

Title: PRRS Virology Literature Review – **NPB #13-245**

Investigator: Dongwan Yoo (Section Chair; dyoo@illinois.edu)

Institution: University of Illinois at Urbana-Champaign, Urbana IL

Date Submitted: July 1, 2014

Virology Team Members:

Dr. Ying Fang:
Associate Professor of Virology (yfang@vet.k-state.edu)
Department of Diagnostic Medicine and Pathobiology
College of Veterinary Medicine
Kansas State University, Manhattan KS

Dr. Frederick Leung:
Professor (fcleung@hkucc.hku.hk)
School of Biological Sciences
The Hong Kong University
Hong Kong, China

Dr. Joan Lunney:
Research Scientist (Joan.Lunney@ars.usda.gov)
US Department of Agriculture Agricultural Research Center,
Beltsville Agriculture Agricultural Research Center (BARC)
Beltsville MD

Dr. Raymond R. Rowland:
Professor of Virology (browland@vet.k-state.edu)
Department of Diagnostic Medicine and Pathobiology
College of Veterinary Medicine
Kansas State University, Manhattan KS

INDUSTRY SUMMARY

Pork producers face many different challenges to the health of their pigs, and PRRS (porcine reproductive and respiratory syndrome) is one of such challenges. PRRS emerged in the US for the first time 25 years ago and has since become the most costly disease of pigs with estimated annual losses of $664 million. The Swine Health Committee of the National Pork Board has committed to more than $11 million to research on PRRS to help better control and manage this disease and to reduce producer losses. To assist

These research results were submitted in fulfillment of checkoff-funded research projects. This report is published directly as submitted by the project's principal investigator. This report has not been peer-reviewed.

For more information contact:
National Pork Board • PO Box 9114 • Des Moines, IA 50306 USA • 800-456-7675 • Fax: 515-223-2646 • pork.org

the Swine Health Committee to determine the value of PRRS research and the return on investment of Checkoff dollars, a comprehensive literature review has been conducted to update current knowledge for 'PRRS Virology' and to apply this information to future research priority and goals that may be funded by National Pork Board. Of more than 2,000 scientific articles published on PRRS as of June 2014, approximately 400 articles relevant to 'PRRS Virology' have been referred to prepare this report. This review is organized to describe five main topics on PRRS virology; 1) virion proteins and viral replication, 2) cellular receptors for PRRSV infection, 3) viral modulation of host immunity, 4) PRRSV reverse genetics and application of infectious clones, and 5) evolution and diversification of PRRSV. The viral capacity for innate immune suppression may be linked to inadequate elimination of the virus and persistence in infected pigs. The genetic technology to modify viral genome has become available for PRRSV and has been used for development of better vaccine candidates and also for study of the virus. The cellular receptor is known for PRRSV and tremendous advances have been made on the cell virus interactions. Some key knowledge gaps have been identified and this report should be useful not only for the National Pork Board but for the swine research community and other stakeholders.

Contact information: Dr. Dongwan Yoo, Professor of Virology, Department of Pathobiology, College of Veterinary Medicine, University of Illinois at Urbana-Champaign, Urbana IL; Email: dyoo@illinois.edu; Phone: 217-244-9120.

KEYWORDS

PRRS (porcine reproductive and respiratory syndrome); PRRSV; Arterivirus; Receptors; Reverse genetics; Infectious clones; Evolution, Immune evasion; Innate immune modulation; Type I interferon; Structural proteins; Non-structural proteins; CD163; sialoadhesin; Genetic heterogeneity

SCIENTIFIC ABSTRACT

Prevention and control of PRRS require an in-depth understanding of the biology of virus. During the past two decades, much has been learned about the disease and the virus, and our knowledge on the molecular and cellular aspects of PRRS Virology has been advanced. This review summarizes the current knowledge and understanding of the structure and function of PRRSV proteins, cellular receptors for PRRSV and entry into cells, viral modulation of cellular processes and host immune responses, development and application of infectious clones, and evolutionary diversification of PRRSV. Some important perspectives, key knowledge gaps, and future directions toward PRRSV research are suggested. This review is timely and necessary to fill the research gaps and to develop strategies for better control of the disease caused by this virus.

INTRODUCTION:

This research has been conducted to perform a comprehensive and critical review of research relevant to 'PRRS Virology' with a focus on North American PRRS strains. This review summarizes the current knowledge for PRRS virus and identifies the knowledge gaps.

OBJECTIVES

1) Identify the key gaps in knowledge for the areas of virology
2) Summarize key research gaps and develop a future research roadmap

3) Identify what/where are the discrepancies between laboratory and field results/responses to PRRSV.

MATERIALS & METHODS:

This review has been prepared by analyzing the published articles on PRRSV. PubMed is a search engine accessing the MEDLINE database of references and abstracts on life sciences and biomedical topics and is maintained by the US National Library of Medicine at the National Institutes of Health (NIH). PubMed allows us to search health sciences database at the National Center for Biotechnology Information (NCBI) of NIH, and the keyword 'porcine reproductive and respiratory syndrome virus' displays a total of 2,021 citations as of June 2014. These citations were filtered using combinations of different keywords related to chosen topics on PRRSV, which formed the basis to prepare the current review. Approximately 400 articles have been cited in this report. This review is organized to contain the following topics for 'PRRS Virology'.

1. INTRODUCTION
2. THE VIRUS, PRRSV
    - 2.1. The virion structure and viral proteins
    - 2.2. Polyprotein processing and generation of non-structural proteins
3. RECEPTORS AND VIRUS ENTRY TO CELL
    - 3.1. Cellular receptors and entry to cell
    - 3.2. Sialoadhesin (CD169; siglec-1)
    - 3.3. CD163 (cysteine-rich scavenger receptor)
    - 3.4. Other entry mediators
    - 3.5. CD163 or Sialoadhesin or both?
    - 3.6. Concluding remarks
4. INNATE IMMUNE MODULATION BY PRRSV
    - 4.1. PRRSV-mediated type I interferon modulation
    - 4.2. Regulation of other cytokines by PRRSV
    - 4.3. Immune modulation by PRRSV
5. INFECTIOUS CLONES AND APPLICATIONS
    - 5.1. Development of infectious clones
    - 5.2. Engineering PRRSV infectious clones
    - 5.3. Application of PRRSV infectious clone
6. EVOLUTIONARY DIVERSIFICATION OF PRRSV
    - 6.1. Recapitulating PRRS
    - 6.2. Molecular mechanisms underlying PRRS evolution
    - 6.3. Molecular epidemiology of PRRSV
    - 6.4. Beyond PRRS diversity
    - 6.5. Concluding remarks
7. DISCUSSION AND SUGGESTIONS:
8. ACKNOWLEDGEMENTS
9. REFERENCES
10. TABLES AND FIGURES

RESULTS:

*(**IX. DISCUSSION:** is found in VIII. RESULTS, Section 7, Discussion and Suggestions)*

INTRODUCTION

The Arteriviridae family of viruses was first established by the International Committee on the Taxonomy of Viruses (ICTV) in 1996, and it now includes equine arteritis virus (EAV), lactate dehydrogenase-elevating virus (LDV) of mice, porcine reproductive and respiratory syndrome (PRRS) virus (PRRSV), and simian hemorrhagic fever virus (SHFV). Wobbly possum disease virus has recently identified and newly added to this family. The Arteriviridae family forms the order Nidovirales along with three other families; Coronaviridae, Roniviridae, and Mesoniviridae. Nidoviruses include a very large group of single-strand, positive-sense RNA viruses infecting humans, mammals, and birds (de Vries et al., 1997; Cavanagh, 1997; Snijder and Meulenberg, 1998; ICTV, http://www.ictvonline.org/virusTaxonomy.asp?version=2014).

PRRSV emerged in the USA pigs first time in the late 1980s, and subsequently in Germany and other European countries almost simultaneously and independently (Benfield *et al.*, 1992; Collins et al., 1992; Keffaber, 1989; Wensvoort et al., 1991). PRRS viruses from two continents are similar but strikingly different in their genomic sequences by only 55-70% sequence identity (Allende et al., 1999; Nelsen et al., 1999), and hence they form two distinct genogroups; genotype I for European PRRSV and genotype II for North American PRRSV. The genetic distance between the two genotypes leads to the hypothesis that they have evolved independently from a common ancestor (Plagemann, 2003).

PRRSV has caused significant economic losses to the pork industry worldwide since its emergence. The clinical symptoms include mild to severe respiratory disease in young pigs whereas in pregnant pigs, abortion and reproductive failures are common. The virus changes rapidly and continues to evolve (Epperson and Holler, 1997; Halbur and Bush, 1997; Bush et al., 1999; Murtaugh, 2009; Faaberg et al,). A highly virulent PRRSV has appeared in China with its mortality of up to 100% in all age of animals (Tian et al., 2007). This highly pathogenic PRRS has spread to neighbor countries including Myanmar, Viet Nam, and Russia.

Prevention and control of PRRS require an in-depth understanding of the biology of virus. During the past two decades, much has been learned about the disease and the virus, and our knowledge on the molecular and cellular biology of PRRSV has been advanced. In this review, we will summarize the current knowledge on the structure and function of PRRSV proteins, viral modulation of cellular processes and host immune responses, development and application of viral reverse genetics systems, and viral evolution to some extent. We will suggest some important perspectives and future directions toward PRRSV research, which will be necessary to fill the knowledge gaps and thus to develop better strategies for controlling the disease caused by PRRSV.

1. THE VIRUS, PRRSV

1.1. The virion structure and viral proteins:

PRRSV virion is a spherical- or oval-shaped particle of 50-60 nm in diameter with a relatively smooth external appearance. The viral genome is enclosed in the capsid structure, surrounded by lipid-bilayered envelope of which viral glycoproteins and membrane proteins are inserted. The nucleocapsid structure has been assumed to be icosahedrally symmetric, but a recent cryo-electron tomographic study suggests rather a helical coil-like, or a more loosely organized filamentous structure (Dokland, 2010). The capsid structure is formed by the nucleocapsid (N) protein and is proposed to form a two-layered structure with protein dimers linked into a twisted chain with the RNA in the middle. The N-terminal domain of the nucleocapsid protein interacts with the RNA genome, while the C-terminal domain forms the basis for the capsid folding (Doan, 2003; Dokland, 2010). The N protein is a phosphorylated protein which may play a role for RNA binding or protein-protein interactions (Wootton et al., 2002).

The PRRSV genome is a positive-sense, single-strand RNA molecule of 15 kb in length, which is enclosed in the capsid structure. The PRRSV genome codes for two large replicases in the ORF1a and ORF1b genes that occupy the

5'-three quarters of the genome and 8 structural proteins in the 3'-one quarter of the genome (Figure 1). While the length of ORF1b is consistent, ORF1a is variable due to the presence of hypervariable regions. The genes in the 3'-one quarter of the genome code for structural proteins that are translated from the 3' co-terminal nested set of subgenomic (sg) mRNAs, which is the hallmark of the PRRSV gene expression. The sg mRNAs are structurally polycistronic but most of them are functionally monocistronic. Notable exceptions are mRNA2 and mRNA5; they are functionally bicistronic from which E and GP2, and ORF5a and GP5 are expressed, respectively (Snijder et al., 1999; Firth et al., 2011; Johnson et al., 2011).

In PRRSV, each sg mRNA contains a common leader sequence at their 5' end, which is derived from the 5' end of the genomic RNA (Figure 1). This property is shared with all arteriviruses and coronaviruses. It is postulated that the leader-body fusion during sg mRNA synthesis uses a mechanism of discontinuous RNA synthesis (den Boon et al., 1995; Gorbalenya et al., 2006; Pasternak et al., 2006; Snijder and Spaan, 2006; Sawicki et al., 2007). The detection of a nested set of sg-length minus-strand RNAs complementary to the sg-length plus-strand sg RNAs in coronavirus- and arterivirus-infected cells has resulted in a model in which a discontinuous extension of minus-strand RNA yields sg-length minus-strand templates for sg mRNA synthesis (Sawicki et al., 1995). A short-conserved, transcription-regulatory sequence (TRS) is critical in this process, and a TRS is present at the 5' end of the genomic RNA (leader TRS) and at the immediate upstream of each transcription unit (body TRS).

During infection, the PRRSV genome is first translated into two large polyproteins; pp1a and pp1ab. The genome translation begins by ribosomal scanning of the 5' UTR which is followed by two ribosomal frame-shifting events. In a recent study (Fang et al., 2012), a novel ORF has been identified within the nsp2 gene, and this ORF is translated via a -2 ribosomal frame-shift mechanism. This frame-shifting with an efficiency of ~ 20% occurs at the conserved G_GUU_UUU sequence in the central region of ORF1a and results in the expression of a trans-frame protein nsp2TF which comprises of the N-terminal two thirds of nsp2 fused to a 169-aa C-terminal region encoded by the TF ORF. The study reveals the occurrence of -1 frame-shifting at the same site with an estimated efficiency of ~7%, yielding a truncated nsp2 protein (nsp2N). The ORF1b translation requires a -1 ribosomal frame-shift with an efficiency of 15-20% in the ORF1a/ORF1b overlapping region where a slippery sequence and the downstream RNA pseudoknot structure are required to promote this event (den Boon, 1991).

Once translated, the pp1a and pp1ab polyproteins are further processed into non-structural proteins (nsps). These nsps form a replication complex for viral RNA synthesis to generate a genome-length minus-RNA which serves as the template for genomic RNA synthesis. The signals involved in the genome replication have been explored, and a "kissing" interaction between the loop sequences of RNA hairpins in the 3' UTR and N gene is crucial (Verheije et al., 2002).

The lipid bilayer surrounding the viral capsid contains 7 membrane proteins; two major and five minor proteins in their abundance. The major proteins represent the non-glycosylated membrane (M) protein and glycoprotein 5 (GP5) and they form a disulfide-linked heterodimer on the surface of virion. The M protein is conserved with its N-terminal half traversing the membrane three times and results in a short ectodomain of 10-18 residues exposed on the virion surface (Meulenberg, 1993; Faaberg, 1995). GP5 is the most variable protein in PRRSV. GP5 is composed of an ectodomain with N-linked glycans, a central hydrophobic region of triple-spanning through the membrane, followed by a relatively long cytoplasmic tail (Faaberg et al., 1995; Meulenberg et al., 1993). The N-terminal signal sequence is assumed to be cleaved from the ectodomain of GP5.

GP2, GP3, and GP4 are less abundant minor glycoproteins and form a GP2-GP4 hetero-dimer and a GP2-GP3-GP4 hetero-trimer on the virion. A recent study confirms the importance of the heteromultimeric formation for viral infectivity (de Lima et al., 2009). The GP2 protein encoded by ORF2a (PRRSV) or ORF2b (EAV/LDV) is a typical class I integral membrane protein, containing a signal peptide, an ectodomain with potential N-glycosylation, and a C-terminal transmembrane segment. GP3 is also a glycosylated integral membrane protein with an uncleaved N-terminal signal sequence and a hydrophobic C-terminal domain, suggesting the protein is

anchored in the membrane with both termini (Hedges, 1999; Wieringa, 2002). GP4 is predicted as a class I membrane protein (van Nieuwstadt, 1996; Meulenberg, 1997; Wieringa, 2002). A recent study shows that GP4 is lipid-anchored protein (Du et al, 2010) and is responsible for interaction of the GP2-GP3-GP4 heterotrimer with GP5 (Das et al., 2010). GP4 is colocalized in the lipid-rafts with CD163 which is considered a cellular receptor for PRRSV (Du et al., 2010).

The E protein is a small envelop protein incorporated in the virion of PRRSV and is essential for viral infectivity. The E protein has been proposed to form an ion channel that may play a key role during the entry process (Lee et al., 2006). The membrane topology of this protein is unknown but the N-terminus contains a myristoylation signal conserved across the arteriviruses (Du et al, 2010). A small hydrophobic ORF5a protein has recently been identified for PRRSV as a structural component (Johnson et al., 2011). The function of ORF5a protein and its biological significance remain to be determined.

During infection, the N protein localizes to both the nucleus and the cytosol where it mediates the packaging of viral RNA. The function of N in the nucleus is involved in the viral virulence and pathogenesis but needs to be elaborated (reviewed in Yoo et al., 2010). Cellular proteins interacting with N have been identified and some of these proteins may be related to immune evasion of PRRSV. The N protein up-regulates the interlukin-10 production in pigs through induction of specific regulatory T cell populations (Thanawongnuwech, et al., 2010).

2.2 Polyprotein processing and generation of non-structural proteins

The post-translational processing of pp1a and pp1ab involves a complex proteolytic cascade directed by four proteinase domains encoded by ORF1a. The poly-protein processing generates nsp1 through nsp12. Nsp1 is further cleaved to nsp1α and nsp1ß, and similarly nsp7 is further processed to nsp7α and nsp7β. Thus, a total of 14 nsps are generated. The nsp3 through nsp8 region is subject to two alternative processing cascades, in which nsp2 acts as a co-factor to mediate cleavage of the nsp4/5 site. This is thought to constitute the major processing pathway. When nsp2 does not associate with nsp3 through nsp8 region, a minor pathway is chosen in which the nsp4/5 site remains uncleaved and the nsp5/6 and nsp6/7 sites are processed instead. The -1/-2 frame-shifting products, nsp2N/nsp2TF, represent additional nonstructural proteins of PRRSV (Fang et al., 2012).

Proteinase activities reside in nsp1α, nsp1β, nsp2, and nsp4 (Fang & Snijder, 2010; Snijder et al., 2013). Each of nsp1α, nsp1β, and nsp2 contains a papain-like proteinase domain (PLP), whereas nsp4 incorporates a chymotrypsin-like serine proteinase (SP). Recent studies by X-ray crystallography have determined the tertiary structure of nsp1α (Sun et al., 2009) and nsp1β (Xue et al., 2010), and confirm that both PLPα and PLPβ employ a Cys-His tandem as catalytic residues, which occurs to act exclusively *in cis.* The two structures reveal the presence of C-terminal region in the PLP's substrate-binding pocket, suggesting an intra-molecular cleavage mechanism that would preclude further proteolytic reactions. The PLP2 proteinase domain appears to possess both *cis* and *trans* cleavage activities (Han et al., 2009). The crystallography study has also been conducted for nsp4 (Tian et al., 2009). The nsp4 protein contains three domains. Domain I and domain II form a typical chymotrypsin-like two-ß-barrel fold of the SP, while domain III at the C-terminus is dispensable for proteolytic activity and may involve in fine-tuning the polyprotein processing.

PRRSV infection induces both the production of an early and high level of non-protective antibodies and a late and low level of neutralizing antibodies. The neutralizing antibodies remain low. In infected animals, antibodies against the GP2, GP3, GP4, GP5, M, and N proteins are detected, among which the anti-N antibody response is the earliest and most abundant (Lopez & Osorio, 2004; Darwich et al., 2010). Recent studies showed that nsp1α, nsp1β, nsp2, and nsp7 play an important role in the induction of humoral immune response (Johnson, 2007; Brown et al., 2009), and antibodies specific to these proteins can be detected as early as 14 dpi (days post-infection) and last more than 202 dpi (Brown et al., 2009). Neutralizing antibodies produced in PRRSV-infected animals are predominantly directed to GP3 and GP5 proteins. Neutralizing epitopes of PRRSV are mapped to the

ectodomain of GP5 in which a linear conserved neutralizing epitope is identified (Plagemann, 2002, 2004). A neutralizing epitope on GP5 has also been identified in the ectodomain (Meulenberg et al., 1997). However, synthetic peptides representing the neutralizing epitope did not elicit neutralizing antibodies, suggesting additional residues and/or structures are critical to maintain the neutralizing epitope (Lopez & Osorio, 20 et al., 2001; Lopez & Osorio, 2004). Heterodimeric structure of GP5 and M may be critical for the configuration of neutralizing epitopes (Balasuriya et al., 2004; Lopez & Osorio, 2004). One of the common explanations for delayed and weak neutralizing antibody production is the presence of glycan in the GP5 ectodomain (Plagmann, 2001; Lopez & Osorio, 2004). Expression of a hypoglycosylated form of GP5 induces a significantly higher level of neutralizing antibody in pigs (Vu et al., 2011), and an immuno-dominant decoy epitope was identified immediate upstream of the GP5 neutralizating epitope (Ostrowski et al., 2002). The decoy epitope diminishes the immune response against the adjacent neutralizing epitope. An alternative explanation for the delayed neutralizing antibody production is deduced from observation that PRRSV manipulates the development of B cell repertoire in pigs (Butler et al., 2008).

The PRRSV nsp2 protein contains PLP2 in its N-terminal domain, which cleaves the nsp2/nsp3 junction in pp1a and pp1ab, and PLP2 is found to belong to the OTU family of deubiquitinating enzymes (DUB) (Makarova, 2000). Overexpression PLP2 showed the deubiquitinating activity towards cellular ubiquitin conjugates. This activity also cleaves the interferon-induced ubiquitin homolog ISG15 which is an antiviral conjugate (Frias-Staheli, 2007; Sun et al., 2012). The DUB activity is functional for suppression of innate immune response. PLP2 in PRRSV nsp2 is also shown to inhibit the NF-kB signaling pathway as well as the IRF3-dependent IFN-β pathway (Sun, 2010). PLP2 appears to remove K48-linked polyubiquitin from IkBα in order to prevent its proteasomal degradation and thereby downstream signaling towards NF-kB activation is inhibited. Besides PLP2, nsp2 also contains variable regions in the central part of nsp2 which may influence the antiviral responses (Chen, 2010). PLP2 is active for DUB in all arteriviruses and suppresses RLR-mediated IFN-β induction. Upon over-expression, nsp2 is able to remove K63-linked polyubiquitin from RIG-I and inhibits downstream IFN signaling (van Kasteren, 2011). The endoribonuclease actvity in the NendoU domain of PRRSV nsp11 also participates in suppression of innate immunity (Shi, 2011; Yoo, 2010). It is unclear whether nsp11 confers specific activity targeting certain innate immune responses or attacks the overall mRNA population of cell due to the RNAse activity.

2. RECEPTORS AND VIRUS ENTRY

2.1. Cellular receptors and entry to cell:

The PRRSV genome codes for at least 14 non-structural proteins (nsps) and 8 structural proteins (Firth et al., 2011; Johnson et al., 2011; Snijder, 1998; Snijder and Meulenberg, 1998). Among ateriviruses, EAV has a relatively broad tropism of cells and infects various cell types including BHK-21 (baby hamster kidney), Vero (African green monkey kidney), RK-13 (rabbit kidney), LLC-MK2 (rhesus monkey kidney), HmLu (hamster lung), MA-104 (African green monkey kidney) and MARC-145 (a derivative of MA-104) (Hedges et al., 2001; Hyllseth, 1969; Konishi et al., 1975; Maess et al., 1970; Radwan and Burger, 1973; Kim et al., 1993), while other member viruses in the family exhibit a limited cell tropism. LDV infection is restricted to a subset of murine primary peritoneal macrophages (Plagemann and Moennig, 1992; Stueckemann et al., 1982), and SHFV infection is limited to MA-104 cells and primary macrophages (Plagemann and Moennig, 1992). PRRSV also has a restricted tropism of cells and host. PRRSV infection is limited to differentiated monocytes and a subset of porcine alveolar macrophages (PAMs) (Mardassi et al., 1994; Snijder and Meulenberg, 1998). PAMs and blood monocytes are only porcine cells that are known to be susceptible for infection of PRRSV. CL2621, MA-104, and MARC-145 cells support PRRSV infection and thus are used for research and propagation of PRRSV but these cells are of monkey kidney origin and the biological relevance is often asked (Bautista et al., 1993; Benfield et al., 1992; Kim et al., 1993). SJPL (St. Jude lung epithelial cell line; ATCC PTA-3256) cells, which were thought to be of the swine respiratory tract, were identified permissive for PRRSV (Provost et al., 2012; Silversides et al.,

2010). However, after extensive genetic studies, SJPL cells appeared to be not of porcine but of monkey as with MARC-145 cells.

Six molecules have been described as potential cellular receptors for PRRSV, which includes heparan sulphate, CD151, vimentin, sialoadhesin (CD169; SIGLEC1), CD163, and DC-SIGN (Calvert et al., 2007; Duan et al., 1998; Huang et al., 2009; Jusa et al., 1997; Kim et al., 2006; Shanmukhappa et al., 2007). Among these, sialoadhesin and CD163 have been studied most extensively as possible receptors for PRRSV. Accumulating data unveil the interaction of these proteins with PRRSV.

2.2. Sialoadhesin (CD169; siglec-1)

Structure and function of sialoadhesin: Sialoadhesin was first identified as a sialic acid-dependent sheep erythrocyte receptor on bone marrow macrophages (Crocker and Gordon, 1986). Sialoadhesin is a macrophage-restricted lectin that binds sialic acid and referred to as CD169 or SIGLEC1. Sialoadhesin is a type I transmembrane glycoprotein of the immunoglobulin (Ig) superfamily and consists of 17 repeats of extracellular Ig-like domains and a short cytoplasmic tail (Williams and Barclay, 1988). The extracellular domain is subdivided into 16 C2-set domains and a single N-terminal V-set domain, both of which are necessary and sufficient for the sialic acid binding (Nath et al., 1995). Expression of sialoadhesin is highly regulated and restricted to tissue macrophages particularly in secondary lymphoid tissues (Munday et al., 1999). Unlike other siglecs, sialoadhesin contains a poorly conserved cytoplasmic tail and lacks tyrosine-based signaling motifs, suggesting that it is the major sialic acid binding receptor for cell-cell interactions rather than for cell signaling. IFN-α and IFN-γ have been shown to induce sialoadhesin expression in monocytes and macrophages, which may play a role in potentiating inflammatory diseases (Rempel et al., 2008; York et al., 2007). Increasing evidence supports that sialoadhesin modulates immune responses, erythropoiesis (Fraser and Gordon, 1994), and inflammatory conditions (Crocker et al., 1997; Jiang et al., 2006). Sialoadhesin expression in macrophages also facilitates pathogen interactions, thereby promotes uptaking sialylated pathogens such as PRRSV (Delputte et al., 2007b), HIV-1 (Rempel et al., 2008; Zou et al., 2011), *Nigeria meningitides* (Jones et al., 2003), *Campylobacter jejuni* (Heikema et al., 2010), and *Trypanosoma cruzi* (Monteiro et al., 2005).

Sialoadhesin as a cellular receptor for PRRSV infection: A 210 kDa protein was initially identified as a putative receptor for PRRSV on PAMs (Duan et al., 1998), which was later determined as sialoadhesin. PK-15 cells are PRRSV non-permissive and do not express a detectable level of sialoadhesin. When these cells are made to express sialoadhesin, they confer internalization and infection of both type I and type II PRRSVs (Vanderheijden et al., 2003). Sialoadhesin mediates PRRSV endocytosis in PAMs, and has been indicated as a potential entry mediator for PRRSV (Vanderheijden et al., 2003). Sialoadhesin binds to sialic acids on the PRRSV virion, and this interaction is blocked by sialoadhesin-specific mAb, suggesting the requirement of sialoadhesin for PRRSV infection in PAM cells (Delputte and Nauwynck, 2004). Heparan sulphate has also been identified as a receptor mediator in PAMs for PRRSV, and the attachment kinetics shows that heparan sulphate alone is sufficient to establish an early attachment of PRRSV and enhance a subsequent interaction of PRRSV with sialoadhesin (Delputte et al., 2005). Phagocytosis by PAMs was inhibited in vitro by Type I PRRSV, and sialoadhesin was responsible for inhibition at the early stage of virus entry (De Baere et al., 2012). Sialoadhesin colocalizes with PRRSV at the cell surface and beneath the plasma membrane after internalization (Van Gorp et al., 2009), which provides further evidence for sialoadhesin as an internalization receptor.

Interaction of sialoadhesin with viral ligands: R116E (mutation of arginine at position 116 to glutamic acid) of sialoadhesin blocks the binding to sialic acids and thus R116 is predicted as the sialic acid-binding domain. The sialic acid-binding of sialoadhesin is essential for PRRSV attachment to cells and the N-terminal domain is responsible for the binding (Delputte et al., 2007b). A truncated sialoadhesin composed of only N-terminal domain directly coupled with the transmembrane domain and cytoplasmic tail still retains the PRRSV binding activity, suggesting that the N-terminal 17-150 residues are necessary and sufficient to mediate PRRSV

attachment to PAMs (An et al., 2010). The N-terminal structure of sialoadhesin has been predicted by the bioinformatics and homology modeling and the data show that S107 may be crucial in forming a special cavity and hydrogen bond, thereby facilitates PRRSV binding during infection (Jiang et al., 2013b). Taken together, the data show that the N-terminal domain of sialoadhesin is indispensable for the PRRSV binding. For PRRSV, the GP5 and M heterodimer complex has been identified as the viral ligand for sialoadhesin, and this interaction is dependent on the binding capacity of sialoadhesin and sialic acids on GP5 (Van Breedam et al., 2010b).

3.3 CD163 (Cysteine-rich scavenger receptor)

Structure and cellular function of CD163: Macrophages play a pivotal role in host immune system. Mature macrophages and their progenitor monocytes constitute highly heterogeneous subsets of cells. The macrophage subset is specialized in function and reflected in expression of complement receptors, Fc-receptors, scavenger receptors, growth factors, adhesion molecules, and receptors for various soluble mediators (Akila et al., 2012). Among these, scavenger receptors are consisted of eight different classes (A-H), and CD163 belongs to class B of the cysteine-rich scavenger receptor superfamily (SRCR-SF) (Areschoug and Gordon, 2009). CD163 is a 130-kDa protein consisting of nine tandem repeats of SRCR domain (SRCR 1 through SRCR 9), a transmembrane domain, and an intracellular cytoplasmic tail. CD163 is initially identified as an adhesion receptor for erythroblasts and plays a regulatory role in erythropoiesis (Van den Heuvel et al., 1999). The best characterized function of CD163 is to clear the cell-free form of hemoglobin (Hb) and participates in anti-inflammation as a soluble factor. When Hb is released from erythrocytes during hemolysis, it binds to haptoglobin (Hp) and instantly forms an Hb-Hp complex. By ligand-affinity, SRCR domain 3 of CD163, which is exposed on the surface of macrophages, binds to the Hb-Hp complex in calcium- and pH-dependent manners (Madsen et al., 2004; Kristiansen et al., 2001; Madsen et al., 2004), which then mediates the endocytosis of Hb-Hp complexes thereby protecting tissues from Hb-mediated oxidative damages (Graversen et al., 2002; Kristiansen et al., 2001; Madsen et al., 2001). The Hb-Hp complex is delivered to early endosomes where CD163 is dissociated and recycled to the plasma membrane of macrophages, while Hb-Hp undergoes intracellular metabolism in the lysosomes (Schaer et al., 2006). The interaction between CD163 and Hb-Hp complex also triggers the release of anti-inflammatory mediators such as IL-10, Fe^{2+}, CO, and bilirubin (Nielsen et al., 2010; Philippidis et al., 2004). Alternative splicing in the cytoplasmic tail generates CD163 variants. CD163 expression is restricted to the monocyte/macrophage lineage and has been studied for humans (Van den Heuvel et al., 1999) and rats (Dijkstra et al., 1985). Low level expression of CD163 in undifferentiated monocytes increases the maturation of tissue macrophages, indicating that CD163 can be a differentiation marker along with the macrophage differentiation pathway (Sanchez et al., 1999). CD163 expression is closely regulated by pro-inflammatory and anti-inflammatory signals, suggesting a critical role in immune responses. It may also function as an innate immune sensor for bacteria rather than as a phagocytic receptor (Fabriek et al., 2009). Some viruses exploit CD163 to enter the target cell, and African swine fever virus (ASFV) (Sanchez-Torres et al., 2003) and PRRSV (Van Gorp et al., 2008) are among such viruses. Soluble CD163 which contains most of the extracellular domain of membrane-bound CD163 are detected in the plasma and body fluids including synovial fluids (Van Gorp et al., 2010a). It is considered a biomarker related to some clinical conditions. Both soluble CD163 and membrane-bound CD163 exert strong anti-inflammatory effects but only soluble CD163 can directly inhibit T cell proliferation (Frings et al., 2002; Kowal et al., 2011).

Role of CD163 for PRRSV entry: For the entry of PRRSV into MARC-145 cells, a low pH is required and thus the receptor-mediated endocytosis was postulated (Kreutz and Ackermann, 1996). This hypothesis was supported by the involvement of specific receptors in the entry to PAMs and the clathrin-dependent pathway in the vesicles (Nauwynck et al., 1999). PRRSV replication can be achieved in non-susceptible cells such as BHK (baby hamster kidney), CRFK (Crandall feline kidney), and Vero (African green monkey kidney) cells by transfection of viral genomic RNA, indicating that the binding of PRRSV to certain factors on the cellular membrane determines the cell tropism (Kreutz, 1998). CD163 was identified as an important receptor for PRRSV infection by screening a cDNA expression library of PAM cells (Calvert et al., 2007). Several lines of PRRSV non-susceptible cells (BHK-21, PK-0809, and NLFK) have been gene-transfected to express CD163, and these cells become fully susceptible

for PRRSV and yielded high titers of virus (Calvert et al., 2007). Independently, primary PAMs have been immortalized by expressing SV40 large T antigen but unexpectedly, these cells do not support PRRSV infection (Weingartl et al., 2002). CD163 expression in these cells (3D4/21; ATCC CRL-2843) was undetectable for both mRNA and protein. When these cells are transfected with the CD163 gene and constitutively express CD163, they become fully susceptible for both Type I and Type II PRRSV (Lee et al., 2010). These cells express a higher level of CD163 and produce a higher titer of PRRSV than those in MARC-145 cells (Lee et al., 2010). Another cell line (LLC-PK) non-susceptible for PRRSV has become susceptible for PRRSV replication when a full-length porcine CD163 is expressed (Patton et al., 2009). Dulac cells (a subline of PK-15) and PK-15 cells are naturally non-susceptible for PRRSV but become susceptible upon expression of CD163 (unpublished data; Wang et al., 2013). MARC-145 cells, which are fully susceptible for PRRSV, do not express sialoadhesion but express CD163.

It seems evident that CD163 plays an essential role in PRRSV infection in many types of cells including PAMs since the expression of CD163 confers a full range of PRRSV infection in cells (Patton et al., 2009; Van Gorp et al., 2008; Patton et al., 2009; Lee et al., 2010; Calvert et al., 2007; Wang et al., 2013). CD163 expression correlates with PRRSV infection in PAMs and cells of monocyte origin. Surface expression of CD163 on PAMs and CD14-positive monocyte-derived macrophages (MDMs) may be modulated following treatment with IL-10, LPS, or TPA (Patton et al., 2009). In contrast, no correlation has been reported between IL-10 and CD163 expression in other report (Gimeno et al., 2011). The data clearly demonstrate that CD163 alone can confer non-permissive cells to permissive for PRRSV and establish a productive replication cycle. Together, it seems clear that CD163 is likely the essential and sufficient receptor for PRRSV.

***Interaction of CD163 with viral ligands**:* A series of CD163 deletion mutants have been constructed to determine an essential region for binding to PRRSV. CD163-L1 of human does not confer PRRSV infectivity, and thus chimeric constructs using porcine CD163 and human CD163-L1 have been constructed to identify the essential region of porcine CD163 (Van Gorp et al., 2010b). The study shows that the SRCR5 domain of CD163 is essential for PRRSV whereas the first four N-terminal SRCR domains and cytoplasmic tail are not. The loop 5-6 region of SRCR5 is believed to be responsible for primary ligand binding (Graversen et al., 2002). Multiple sequence alignments show that the loop 5-6 region from different animal species is conserved, which may explain that CD163 is a receptor from divergent mammalian species (Welch and Calvert, 2010). Remaining domains are necessary but replaceable by corresponding domains of CD163-L1, and the replacement leads to reduced or unchanged efficacy of infection (Van Gorp et al., 2010b). The CD163 cytoplasmic tail varies in length and the long-tail isoforms have no distinct biological functions (Nielsen et al., 2006). The transfection study shows that the short-tail variants of CD163 render cells wit a higher capacity for endocytosis, and it may be due to the increased deviations in subcellular distribution. The high endocytic activity of the short-tail variants and its high mRNA expression level indicate that it accounts for the majority of Hb-Hp uptake from circulation (Nielsen et al., 2006). A tailless-CD163 is also sufficient to confer the susceptibility of BHK-21 cells to PRRSV infection (Lee and Lee, 2010; Van Gorp et al., 2010b). Interestingly, the tailless-CD163 notably enhanced PRRSV replication, suggesting an unidentified function of the intracellular domain for PRRSV infection (Lee and Lee, 2010). A high degree of homology is found in the CD163 transmembrane (TM) domain among different species, and the TM domain is necessary for CD163 to confer PRRSV permissiveness (Welch and Calvert, 2010). The TM domain is required for CD163 in conferring PRRSV susceptibility (Das et al., 2010), but does not affect the virus infectivity if replaced with a heterologous domain (Van Gorp et al., 2010b). This suggest that CD163 TM domain is required only for the purpose of anchoring the receptor to cell membrane (Welch and Calvert, 2010).

The GP5 and M proteins of PRRSV were initially hypothesized as viral ligands for receptor interaction. To study this aspect and cell tropism, chimeric viruses have been made. The studies using chimeric viruses show that the GP5 ectodomain is not a determinant for cell tropism for EAV (Dobbe et al., 2001), and the heterodimeric nature of GP5 and M do not determine the viral tropism (Verheije et al., 2002). Using an EAV infectious clone, ectodomains of GP5 and M have been substituted with those of PRRSV, and three PRRSV/EAV chimeric constructs have been made (Lu et al., 2012). These chimeric constructs are viable and show that neither GP5 nor

M is involved in the cell tropism (Lu et al., 2012), which is consistent with previous studies (Dobbe et al., 2001; Verheije et al., 2002). The M protein is shown to bind to a heparin-like receptor on PAM cells (Delputte et al., 2002), suggesting an additional role of GP5/M for nonspecific interactions. The M/GP5 ectodomain chimeric protein binds to heparan sulfate and blocks PRRSV infection of susceptible cells (Hu et al., 2012). In addition, the E protein has been described as a putative ion channel protein facilitating the uncoating process and release of the genome to the cytoplasm. The low pH in the endosome may trigger conformational changes of E protein on the virion envelop and allows ion influx to the inner cavity of PRRS virion through pores, and causes structural changes of the nucleocapsids for successful uncoating (Lee and Yoo, 2006).

Previous reports show that the GP2a and GP4 proteins interact with CD163 (Das et al., 2010). Truncated CD163 lacking the C-terminal 223 residues still interacts with the GP2a and GP4 proteins, indicating that the C-terminal region of CD163 is not required for ligand interactions. The results for the first time indicated that GP4 and GP2a function as viral attachment proteins and allow PRRSV binding to cell surface receptor for infection (Das et al., 2010). Lately, another chimeric virus (vAPRRS-EAV2ab34) was constructed using a PRRSV infectious clone by replacing ORFs 2a through 4 with those of EAV (Tian et al., 2012). This construct was also viable and infectious virus was obtained. This chimeric virus was unable to infect PAM cells and instead acquired a broad cell tropism similar to as for EAV. This indicates that the GP2, GP3 and GP4 minor glycoproteins and the E protein in combination play a critical role for attachment and entry and thus are responsible for PRRSV cell tropism (Tian et al., 2012).

The glycans of the minor glycoproteins have been studied for their role in interaction with CD163 (Das et al., 2011). N184 is one of the glycosylation sites of GP2 and is required for infectivity in type II PRRSV, and glycosylation of GP2a and GP4 appears to be necessary for efficient interaction with CD163. A previous report using type I PRRSV shows that the loss of a glycosylation site in GP2 did not affect the virus infectivity, suggesting a possibility that the role of glycans in GP2a and GP4 may be genotype-dependent. In another study, GP4 but GP2 was involved in the interaction with CD163 and also showed that glycosylation of GP4 did not play a vital role for this interaction (Wei et al., 2012). The C-terminal domain of GP4 functions as a GPI (glycosylphosphatydyl inositol)-anchor to associate GP4 to the membrane and co-localize it with CD163 in the lipid rafts, suggesting that the GP4 and CD163 interaction may occur in the lipid rafts of the membrane and this interaction is involved in the virus entry (Du et al., 2012).

3.4 Other entry mediators

Heparan sulphate is a member of the glycosaminoglycan family of carbohydrates. Haparan sulfate is widely expressed on the cell surface and in the extracellular matrix (ECM) of almost all types of mammalian cells. It regulates a wide variety of biological activities such as blood coagulation, angiogenesis, tumor metastasis, and developmental process. Heparan sulphate was identified as a possible mediator for PRRSV entry (Jusa et al., 1997). Heparan sulfate proteoglycans and heparin-like molecules bind to the M protein or GP5/M heterodimer of PRRSV (Delputte et al., 2002). Rhe role of heparan on PRRSV infectivity is highly virus-dependent (Delputte et al., 2002; Vanderheijden et al., 2001). It is proposed that heparan sulphate and sialoadhesin cooperate to promote the attachment of PRRSV to cellular receptors. In this proposal, PRRSV first binds to heparan sulphate on the cell and this interaction facilitates the binding of PRRSV to sialoadhesin for internalization. However, heparan sulphate is not absolutely required for PRRSV entry to PAMs (Delputte et al., 2002; Vanderheijden et al., 2001).

CD151 belongs to the tetraspanin superfamily and exhibits various cellular functions including cell signaling, cell activation, and platelet aggregation (Fitter et al., 1999; Roberts et al., 1995; Sincock et al., 1999). CD151 was identified as an RNA-binding protein to the 3' UTR of PRRSV genome during RNA-ligand screening of a MARC-145 cell expression library (Shanmukhappa et al., 2007). BHK-21 cells that are not susceptible for PRRSV infection became susceptible after expression of CD151 by gene transfection. The efficacy of PRRSV infection in MARC-145 cells decreased significantly after gene silencing against CD151 and the infection was completely

blocked by anti-CD151 antibody. This study proposes that CD151 is one of the key molecules facilitating PRRSV infection.

Recently, SJPL cells, that are of the swine respiratory tract and phenotypically distinct from MARC-145 cells, have been identified to be permissive to PRRSV infection (Provost et al., 2012; Silversides et al., 2010). Interestingly, SJPL cells express CD151, but neither CD163 nor sialoadhesin. Initially, the PRRSV and SJPL cell interaction was suggested as a new model for PRRSV pathogenesis. However, the percentage of acrosomic and telocentric chromosomes of the SJPL cell line obtained from ATCC was determined, and the findings were compared to what would be expected for a normal pig karyotype as well as for a normal green monkey karyotype. Therefore, it was concluded that SJPL cell line was not of porcine origin but was more likely of monkey origin, as with MARC-145 cells.

Simian vimentin (Kim et al., 2006) and DC-SIGN (Huang et al., 2009) also interact with PRRSV. Vimentin is expressed on the surface of MARC-145 cells and binds to PRRSV N protein. Anti-vimentin mAb blocks PRRSV infection. BHK-21 and CRFK cells that are non-susceptible for PRRSV became susceptible upon delivery of the recombinant simian vimentin. Thus, it may play a role along with the other cytoskeletal filaments for PRRSV infection. BHK-21 cells expressing DC-SIGN (also known as CD209) on cell surface enhance PRRSV transmission to target cells *in trans* (Huang et al., 2009).

3.5. CD163 or Sialoadhesin or both?

CD163 and sialoadhesin have both been identified as important receptors for PRRSV and a model of the entry process has been proposed (Van Breedam et al., 2010a). However, there still exist controversies and debates regarding the receptor utilization. This section will focus mainly on the importance of CD163 and sialoadhesin. CD163 and sialoadhesin genes are assigned to chromosome 5 and chromosome 17 in pigs, respectively, according to the recent studies. Besides macrophages, CD163 is highly expressed in liver muscles and fats, whereas sialoadhesin is expressed in heart, liver, spleen, lung fats and lymph glands, moderately in stomach and small intestine, and expressed slightly in kidney and muscle tissues. CD163 is also associated with the blood IgG contents, whereas sialoadhesin is closely associated with white blood cells in peripheral blood (Wang et al., 2012). Gene expression patterns of CD163 and sialoadhesin have been investigated in the lung tissues of PRRSV-resistant and -susceptible pigs and show that sialoadhesin is more important than CD163 underlying PRRSV-resistance (Jiang et al., 2013a). The transcription of sialoadhesin mRNA is clearly upregulated in endometrial and placental macrophages during PPRSV infection (Karniychuk et al., 2013). Although IFN-α is a potent antiviral cytokine, it stimulates the sialoadhesin expression in cultivated monocytes in vitro and leads to enhanced infection of monocytes, suggesting a possible viral strategy for immune evasion (Delputte et al., 2007a). Two subsets of macrophages are found in endometrium, placentas, and lungs: $Sn^{+}CD163^{+}$ and $Sn^{-}CD163^{+}$. Most $CD163^{+}$ macrophages are sialoadhesin-negative in mid-gestation fetal placentas, which may explain the difficulty for PRRSV to spread transplacentally at this stage of gestation (Karniychuk and Nauwynck, 2009). Role of sialoadhesin as a putative receptor for PRRSV attachment and internalization has been studied further. In cells expressing porcine CD163, co-expression of sialoadhesin promotes PRRSV infection, and this synergistic effect is equal for porcine, murine, and human sialoadhesin (Van Breedam et al., 2013).

However, the role of sialoadhesin for PRRSV infection of pigs has recently been challenged by the study using gene-knockout pigs (Prather et al., 2013). In that study, sialoadhesin gene-knockout pigs were generated by removing part of exon 1 and all of exons 2 and 3 of the sialoadhesin gene, and these pigs were infected at 3-weeks of age. To avoid a possibility of altered tropism which might have been introduced during cell culture adaptation, a low-passage virus of type II genotype (isolate KS-06) was used at the dose of 10^5 $TCID_{50}$, first intramuscularly then intranasally. In the pig infection study, the absence of sialoadhesin surface expression did not alter the CD163 expression and did not significantly alter the maturation of PRRSV-permissive macrophages. Furthermore, both PRRSV-specific viremia and antibody production in the gene-knock-out pigs were similar to those of control pigs

and no difference was observed. This study clearly demonstrates that sialoadhesin is not required for infection of pigs with PRRSV and the absence of sialoadhesin gene does not contribute to acute PRRS and PRRSV pathogenesis (Prather et al., 2013).

CD163 colocalizes with PRRSV in early endosomes, but neither in late endosomes nor plasma membrane, whereas sialoadhesin colocalizes with PRRSV at the cell surface just underneath the plasma membrane upon internalization (Van Gorp et al., 2009), showing that sialoadhesin is the primary molecule mediating the PRRSV internalization from the cell surface, while CD163 only interacts with PRRSV in early endosomes. This data further suggests that CD163 plays a role for uncoating during the later stage of virus entry, and thus is not a receptor for virus attachment. However, anti-CD163 antibody inhibited PRRSV infection of PAMs and this inhibition was dose-dependent (Van Gorp et al., 2008), indicating that CD163 is not an endosome-restricted protein and may interact with PRRSV on the cell surface of PAMs. Furthermore, CD163 is released in a soluble form and taken back up into cells, thus it is possible that CD163 may also bind and internalize the virus (Van Gorp et al., 2010a). In addition, CD163 cycles between the plasma membrane and early endosomes (Schaer et al., 2006), and thus low level expression of CD163 on the cell membrane may still be responsible for PRRSV recognition and infection. The level of CD163 expression correlates with PRRSV infection. Since PRRSV receptors remain to be further clarified, viral ligands mediating PRRSV-receptor interaction also remain to be determined. However, studies show that the GP2 and GP4 minor glycoproteins are viral ligands interacting with CD163 (Tian et al., 2012), whereas M and GP5 are the envelop proteins interacting with sialoadhesin. Taken together, accumulating data suggest that CD163 is most likely the primary/core receptor and determines the primary susceptibility of cells for PRRSV. Accordingly, the GP2 and GP4 proteins may likely be the viral attachment proteins mediating the initial binding to the cellular receptor.

Since monocytes differentiate to macrophages and macrophages are primary target cells for PRRSV, relationships between blood monocytes (BMo) differentiation/aging, receptor expression, and PRRSV susceptibility have been studied in detail (Wang et al., 2011). Blood monocytes (BMo) do not express CD163 and sialoadhesin, or express it at extremely low levels, and thus are resistant to PRRSV infection. When BMo were cultivated with pig serum plus L929 cell culture supernatant, sialoadhesin levels were significantly increased after 5 days of cultivation, while CD163-positive monocytes reached up to 50% after 4 days. The susceptibility of these cells to PRRSV was enhanced, and the enhanced susceptibility was correlated with increased CD163 expression on the cultured BMo. No correlation was observed for enhanced susceptibility with delayed expression of sialoadhesin (Wang et al., 2011). These results are in contrast with previous reports on the role of cultivated BMo (Delputte et al., 2007a; Duan et al., 1997) and sialoadhesin expression (Van Gorp et al., 2008) during PRRSV infection. A sialoadhesin or its homolog is detectable in MARC-145 cells (Duan et al., 1998). Also, mAbs specific for the 150 kDa or 210 kDa CD163 proteins did not block the Lelystad virus (type I PRRSV) infection of MARC-145 cells, while did block the infection of PAMs (Wissink et al., 2003). Cells expressing recombinant sialoadhesin do not support the Lelystad virus infection (Delputte et al., 2005), showing again that sialoadhesin is not a critical protein for PRRSV, at least type I PRRSV infection of MARC-145 cells.

In the Yoo laboratory, expression of CD163 and sialoadhesin were examined in various cell types using RT-PCR. Primers were designed to amplify a conserved region of CD163 gene among different animal species, and for sialoadhesin expresison, species-specific primers were used for better detection. The susceptibility of these cells for PRRSV was also determined using type I PRRSV (Lelystad strain) and type II PRRSV (PA8 strain). Cytopathogenic effects were monitored daily. In addition, virus infection was determined by detecting N gene from culture supernatants and cell lysates at 5 to 9 days post infection. MARC-145 and Dulac-CD163 cells, a stable cell line expressing CD163, were able to establish PRRSV infection. Vero cells did not express sialoadhesin but showed a limited expression of CD163. Vero cells were susceptible for PA8 virus infection to a certain extent as the PRRSV N gene was detectable by RT-PCR, but these cells were not susceptible for Lelystad virus. A previous study suggested that Vero cells can bind and internalize PRRSV but do not support PRRSV replication thereby resulting in abortive infection. This resistance was eliminated by using polyethylene glycol (PEG)

(Kreutz, 1998). RT-PCR is a sensitive method; the N gene was weakly but clearly detectable from PA8 virus-infected Vero cells. It indicates that Vero cells support PRRSV infection and suggest that the infection was weak due to a low level expression of CD163 in these cells. Vero cells do not express sialoadhesin and, in our study, these cells were not susceptible for Lelystad virus. Sialoadhesin expression only does not confer PRRSV infection, as shown in 3D4/21 (SV 40 immortalized PAMs), Dulac (porcine kidney cell line), LLCMK2 (rhesus monkey kidney cell line), HeLa (human cervical cancer cell line), and RAW (mouse macrophage lineage cell line) cells. This data further supports that CD163 is likely a core receptor molecule for PRRSV infection, and that sialoadhesin is not a necessary protein to establish PRRSV infection.

Not all cells endogenously expressing CD163 are permissive for PRRSV infection (Welch and Calvert, 2010), suggesting a requirement for high levels of constitutive expression. It is also possible that PRRSV may utilize a yet-identified alternative receptor or co-receptor for infection. Binding assays showed that many non-permissive cells to PRRSV, such as RK-13, BHK-21, PK-15, PT, U937, and 293A, can bind the virus, suggesting that more than one receptor may be required for PRRSV internalization (Therrien et al., 2000). MARC-145 cells express vimentin on the surface and are susceptible to PRRSV infection; simian vimentin renders BHK-21 and CRFK susceptible to PRRSV infection. Recently, immortalized PAM cells have been established using the telomerase gene. These cells express both CD163 and sialoadhesin and are fully permissive for both type 1 and type 2 PRRSVs (Sagong et al., 2012). In another study, however, a line of porcine endometrial endothelial (PEE) cells showed no CD163 expression but sialoadhesin and CD151. These cells were susceptible to PRRSV infection, for which the reason remains unclear (Feng et al., 2013). Previous reports show that CD151 renders BHK21 and SJPL cells permissive to PRRSV infection (Provost et al., 2012; Shanmukhappa et al., 2007), suggesting CD151 as a possible alternative receptor for PRRSV in some cells. Porcine FcRIIb mediates the enhancement of PRRSV Infection (Wang et al., 2011). Cholesterol depletion does not alter CD163 expression in MARC-145 cells, and thus cholesterol is likely to mediate PRRSV entry by a lipid raft-dependent pathway (Huang et al., 2011).

Highly virulent variants of PRRSV have recently emerged in China (highly pathogenic PRRSV) and Eastern Europe (Lena PRRSV) (Karniychuk et al., 2010; Tian et al., 2007). Different PRRSV may have different capacities of infection. A study using the polarized nasal mucosa explant system shows that monocytic subtypes of $CD163^{+}Sn^{+}$, $CD163^{+}Sn^{-}$, and to a lesser extent $CD163^{-}Sn^{-}$ were all involved in Lena-infected nasal mucosa, whereas almost all cells positive for viral antigen were $CD163^{+}Sn^{+}$ during Lelystad virus infection (Frydas et al., 2013). This study suggests that Lena PRRSV may utilize diverse alternative receptors in order to gain wider cell tropism (Frydas et al., 2013).

3.6. Concluding Remarks

Virus infection is initiated by the entry of virus into cells; the success of entry depends on the utilization of a specific receptors on the cell surface. For PRRSV, increasing amounts of data have been accumulating on receptors and receptor-virus interactions, but conflicting data exists and the entire process for virus entry remains to be fully understood. Overall, the data indicate that CD163 is most likely the primary/core receptor for PRRSV and determine the susceptibility of cells to the virus, whereas sialoadhesin is either unnecessary or functions as an accessory protein for infection. Two important unanswered questions for virus entry include: 1) whether each genotype of PRRSV utilizes different receptors; for example genotype I PRRSV uses sialoadhesin and genotype II uses CD163 and 2) how is the nucleocapsid of PRRSV released from the endosome to the cytoplasm.

4. IMMUNE MODULATION BY PRRSV

The host range of arteriviruses is restricted to suids, mice, equids, and non-human primates for PRRSV, LDV, EAV and SHFV, respectively, and macrophages appear to be primary target cell for their infection (Snijder and Meulenberg, 1998). The arterivirus infections may cause persistence in some of infected animals. PRRS may persist up to 6 months and EAV may persist for life-long in some horses. LDV typically causes asymptomatic,

life long, persistent infection in mice (Anderson et al., 1995; Plagemann et al., 1995). For SHFV, a fatal hemorrhagic fever occurs in its infected macaques but asymptomatic persistent infections appear in infected baboons (Vatter and Brinton, 2014). Arteriviruses seem to have developed an ability to manipulate a variety of host cell processes related to the innate immunity to facilitate the virus survival. PRRSV infection is characterized by poor induction of proinflammatory cytokines and interferons (IFNs). Viral proteins modulating the innate immune responses have been identified. Among these, nsp1 has been regarded as the most potent protein affecting the innate immune responses (Sun et al., 2012a; Yoo et al., 2010). This review summarizes the host innate immune modulation by PRRSV.

4.1. PRRSV-mediated type I interferon modulation

The type I IFN system is a key component of the innate immunity and represents one of the first lines of defense against virus infection (Samuel, 2001). PRRSV has a fairly good sensitivity to the anti-viral effects of type I IFNs according to previous studies. Treatment with porcine IFN-α could significantly impede the growth of PRRSV *in vivo* and *in vitro* (Albina et al., 1998; Buddaert et al., 1998). The sensitivity of PRRSV isolates to IFN-α is variable and dose-dependent (Lee et al., 2004). Arterivirus infections usually induce poor innate immune responses which may explain the weak adaptive response and viral persistence. Unlike other respiratory infections such as swine influenza and porcine respiratory coronavirus infections whose infections induce high concentrations of IFN-α, a minimal level of IFN-α is expressed during PRRSV infection in vivo and in vitro (Van Reeth et al., 1999). However, considerable variations are observed for IFN-α induction by different isolates (Lee et al., 2004; Nan et al., 2012). In addition, the IFN induction by PRRSV is variable and inconsistent in different cell types. Even though the IFN production is limited in MARC-145 cells and PAMs, the transcription of IFN is increased (Lee et al., 2004; Miller et al., 2004). Dendritic cells (DCs) play a role for anti-viral immunity by providing early innate protection against viral replication and by presenting antigens to T cells for initiation of the adaptive immune response (Clark et al., 2000); PRRSV-induced IFN response has been extended to DCs. Monocyte-derived DCs are susceptible for PRRSV infection, and expression of IFN-α/β mRNA is elevated in a time-dependent and transient manners. However, a little or no detectable levels of IFN are found in the supernatants and cell lysates (Loving et al., 2007; Zhang et al., 2012). A significant level of IFN is observed in plasmacytoid dendritic cells (pDCs) after infection (Baumann et al., 2013). pDC is the primary cell type that produces IFN-α during LDV infection (Ammann et al., 2009). EAV infection barely induces IFN production in pulmonary endothelial cells (EECs) (Go et al., 2014). The suppression of IFN-α is observed in PRRSV-infected macrophages (Albina et al., 1998; Lee et al., 2004), and the inhibition occurs likely at the post-transcriptional level since IFN-α mRNA is increased after stimulation (Lee et al., 2004; Miller et al., 2009). pDCs are non-susceptible for PRRSV, but in these cells, IFN expression is impeded by PRRSV. The inhibition of IFN in pDC suggests this inhibition on the cell surface since UV-treated PRRSV still blocks the IFN-α production (Calzada-Nova et al., 2011). It is possible that PRRSV prevents pDCs from the IFN paracrine loop to shut off the IFN-boosted IRF7 expression but still induces the IFN production at the early stage of infection.

Despite minimal IFN production during infection, Pattern Recognition Receptors (PRRs) still sense invading viruses. In PRRSV-infected PAMs and DCs, differential expression of TLRs has been observed and the expression of TLR3 and TLR7 are inhibited at early infection with later restoration (Chaung et al., 2010; Liu et al., 2009). In PRRSV-infected MARC-145 cells, the IRF3 nuclear localization is inhibited and thus IFNs production is inhibited. Also, the NF-κB suppression seems essential even though PRRSV activates NF-κB at a later stage (Lee and Kleiboeker, 2005; Song et al., 2013; Sun et al., 2010). In the nucleus, CREB-binding protein (CBP) is reduced by nsp1, resulting in the inhibition of enhanceosome formation (Kim et al., 2010). PRRSV also inhibits the IFN signaling pathway by blocking the nuclear localization of IFN-stimulated gene factor 3 (ISGF3) (Patel et al., 2010). Viral proteins modulating the IFN production have been identified, which includes nsp1α, nsp1β, nsp2, nsp4, nsp11, and N protein. It appears that arteriviruses tend to employ a combination of functions to modulate the host innate immunity.

4.2. Regulation of other cytokines by PRRSV

Besides type I IFNs, PRRSV stimulates the production of other cytokines such as TNF-α, IL-6 and IL-10. A study also indicates IL-8 stimulation by PRRSV (Darwich et al., 2010) and association of three serum cytokines (IL-8, IL-1β, IFN-γ) was significantly correlated with virus persistence (Lunney et al., 2010),. TNF-α is a pleiotropic cytokine mainly produced by monocytes/macrophages that plays important roles in the induction and regulation of inflammatory response (Hawiger, 2001). Addition of TNF-α to PAMs inhibits PRRSV replication, and a high level accumulation of TNF-α mRNA is linked with a reduction of viral growth (Ait-Ali et al., 2007; Lopez-Fuertes et al., 2000). TNF-α induction by PRRSV is controversial. An early study showed impaired production of TNF-α in PRRSV-infected PAMs, and this was consistent with the findings in bronchoalveolar fluids after infection (Thanawongnuwech et al., 2001; Van Reeth et al., 1999). TNF-α was detectable however in PAMs, peripheral blood mononuclear cells (PBMCs), and bronchoalveolar cells (Aasted et al., 2002; Ait-Ali et al., 2007; Johnsen et al., 2002), along with the virus in the lesion of interstitial pneumonia, lungs, lymph nodes, and serum of infected pigs (Choi et al., 2002; Miguel et al., 2010; Rowland et al., 2001). Different breeds and ages of pigs also lead to the differential expression of TNF-α (Ait-Ali et al., 2007; Johnsen et al., 2002). In the study using 39 different isolates of PRRSV, distinct patterns of TNF-α expression are observed (Gimeno et al., 2011). In another study using highly pathogenic (HP)-PRRSV, strain-dependent TNF-α expression was also observed; a lower level of TNF-α expression by HP-PRRSV compared to a conventional isolate (Hou et al., 2012). Activation of the extracellular signal-regulated kinase (ERK), p38 mitogen-activated protein kinase (MAPK), and NF-κB signaling pathways are responsible for TNF-α induction (Mathur et al., 2004; Saccani et al., 2002), and PRRSV has been shown to induce a robust but transient activation of ERK (Lee and Lee, 2010). A later study shows that activation of the ERK pathway, rather than p38 MAPK and NF-κB pathways, is associated with differential expression of TNF-α in macrophages by different isolates. HP-PRRSV suppresses LPS- and poly(I:C)-stimulated TNF-α release by impairing the ERK activation (Hou et al., 2012). This may explain the reduction of TNF-α after phorbol myristate acetate (PMA) stimulation (Lopez-Fuertes et al., 2000).

IL-10 is a pleiotropic cytokine with immuno-modulatory functions important for PRRSV immunopathogenesis (Conti et al., 2003; Darwich et al., 2010). Since IL-10 functions as a potent immunosuppressive cytokine, extensive studies have been conducted to investigate IL-10 expression during infection. A minimal IL-10 response has been reported in some studies (Thanawongnuwech et al., 2001; Wang et al., 2007), but other studies show the up-regulation of IL-10 expression by PRRSV. Increased expression of IL-10 has been observed in PBMCs, bronchoalveolar cells, and tissues including lung and lymph nodes in virus-infected pigs at different ages (Feng et al., 2003; Johnsen et al., 2002; Rowland et al., 2001; Suradhat et al., 2003). A significant increase of IL-10 is also noted in PRRSV-infected bone marrow-derived immature DCs (BM-imDCs) at 48 h following infection (Chang et al., 2008). In PAMs, the induction of IL10 expression is time-dependent and dose-dependent (Genini et al., 2008; Song et al., 2013). During PRRSV infection, stress-activated protein kinases (SAPKs) including p38 MAPK and c-Jun N-terminal kinases (JNK) is activated probably through a post-entry process leading to activation of transcription factors such as activator protein-1 (AP-1) (Lee and Lee, 2012). Later studies show that the activated p38 MAPK and NF-κB pathways are responsible for IL-10 up-regulation in PAMs (Song et al., 2013). For NF-κB activation, MyD88 has been shown to be essential and the TLR-MyD88-NF-κB signaling cascade is speculated to be involved in PRRSV-induced IL-10 expression. The nucleocapsid (N) protein is able to trigger NF-κB activation and is demonstrated to up-regulate IL-10 expression in PAMs, and thus, PRRSV N protein-mediated IL-10 induction may rely on NF-κB activation (Luo et al., 2011; Wongyanin et al., 2012).

4.3 Immune modulation by PRRSV

PRRSV nsp1 is the first viral protein synthesized during infection and functions in viral genome replication and innate immune modulation. Domains including papain-like proteinase (PLP), two zinc finger (ZF) motifs, and a nuclease motif are found in the nsp1 protein (Fang and Snijder, 2010; Sun et al., 2009; Xue et al., 2010). The PLP domain usually appears in the most N-terminal region of the viral polyprotein of single-stranded positive-sense

RNA viruses including picornavirus, coronavirus, arterivirus, and pestivirus, and those PLP activities are used for polyprotein processing (Chen et al., 1996; den Boon et al., 1995; Gorbalenya et al., 1989; Gorbalenya et al., 1991; Guarne et al., 2000; Harcourt et al., 2004; Karpe and Lole, 2011; Lim et al., 2000; Mielech et al., 2014a; Snijder et al., 1994). The existence of PLP domains has been proved in each of arterivirus nsp1 and responsible for cleaving off nsp1 from the polypeptide or for internal cleavages within nsp1. Depending on the number of PLP domains present in the nsp1 region, PLP1 domains are designated as PLP1α, PLP1β, or PLP1γ (Fig.1). Correct processing of the polyproteins by PLP1 is essential for viral genome replication and RNA synthesis, and impaired PLP1α and PLP1β activities are lethal for PRRSV replication (Kroese et al., 2008). Unlike the leader proteinase (L^{pro}) of foot-and-mouth disease virus (FMDV) which cleaves the host cellular eukaryotic initiation factor 4G (eIF4G) as well as itself from the nascent viral polyprotein, PLPs in arteriviruses hardly maintain their protease activities after self-cleavages (Guarne et al., 1998; Sun et al., 2009; Xue et al., 2010). Tertiary structural studies of PRRSV nsp1α and PRRSV nsp1β indicate stable interactions between the C-terminal extension (CTE) and PLP1α and PLP1β domains, hence further proteolytic processing is barely conducted by inhibiting the access of other potential substrates (Sun et al., 2009; Xue et al., 2010). In comparison with L^{pro}, a large interaction surface with PRRSV PLP1α for CTE binding is observed, which enables to stabilize the intramolecular complex in PRRSV nsp1α (Steinberger et al., 2013).

Besides acting as protease, PLP2 of nsp2 functions as a deubiquitinating (DUB) enzyme which removes ubiquitin modifications from a target protein, and this viral protease/DUB activity is conserved in other viruses. The DUB activity has been identified in the PLP of Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV), Middle East Respiratory Syndrome coronavirus (MERS-CoV), PLP2 of mouse hepatitis virus (MHV), porcine epidemic diarrhea virus (PEDV), human coronavirus (HCoV)-NL63, and transmissible gastroenteritis coronavirus (TGEV), together with the de-ISGylating activity in some of these protease domains (Chen et al., 2007; Clementz et al., 2010; Lindner et al., 2005; Mielech et al., 2014b; Wojdyla et al., 2010; Xing et al., 2013; Zheng et al., 2008). For PRRSV and EAV, DUB and deISGylating activities are identified in PLP2 domain, and as with coronaviruses, these activities are essential for viral modulation on innate immunity (Frias-Staheli et al., 2007; Sun et al., 2010; Sun et al., 2012b; van Kasteren et al., 2013; van Kasteren et al., 2012). No DUB and deISGylating activities are associated with PLP1 in arteriviruses.

Computer-based sequence alignments of nsp1 from different arteriviruses reveal a conserved zinc finger (ZF) motif in the N-terminal region, and the crystal structure of PRRSV nsp1α suggests that the topology of the N-terminal ZF domain is generally similar to that of the ββα ZF family of over 1,000 known transcription factors (Sun et al., 2009; Tijms et al., 2001). The zinc ion coordinating the C-terminal zinc motif is identified in PRRSV nsp1α but the biological function of this motif is unknown. Mutations in the N-terminal zinc finger domain of nsp1α selectively abolish the viral transcription whereas the genome replication is not affected (Tijms et al., 2007; Tijms et al., 2001). For PRRSV nsp1β, no ZF is found according to the crystal structure. Instead, a nuclease activity is identified to degrade either double-stranded (ds) DNA or ssRNA (Xue et al., 2010). Recent studies show the involvement of arterivirus nsp1 in viral gene transcription and translation by interacting with the genome RNA (Li et al., 2014; Nedialkova et al., 2010). EAV nsp1 involves in controlling the accumulation of genome-length and subgenome-length minus-strand RNA for mRNA synthesis (Nedialkova et al., 2010), whereas PRRSV nsp1β regulates the trasactivation of both -2 and -1 frame-shifting for nsp2 (Li et al., 2014).

The motif for PLP in the N-terminal region of pp1a and pp1a/b was initially postulated on the basis of sequence similarities to somewhere between amino acid positions 158 and 178 (den Boon et al., 1991). Later by in vitro translation and mutagenesis, this activity was found to mediate an autoproteolytic cleavage to produce the nsp1 protein accommodating the PLP domain (Snijder et al., 1993). Amino acid residues Cys164 and His230 of the EAV polyprotein are the PLP catalytic residues, and Gly260↓Gly261 is the PLP-mediated cleavage site (Snijder et al., 1993).

In contrast to EAV-nsp1, two adjacent PLP domains, PLP1α and PLP1β, are found in nsp1 of PRRSV and LDV. PLP1α mediates the internal cleavage of nsp1 to release nsp1α and, PLP1β mediate the cleavage to release nsp1β from nsp2. Their active sites are predicted to Cys76 and His146 for PRRSV PLP1α and Cys76 and His147 for LDV PLP1α (Fig. 1) (den Boon et al., 1995). Sequence comparisons show that PLP1α in PRRSV and LDV is similar to the inactive PLP motif in EAV-nsp1, but PLP1β can be aligned with EAV PLP (den Boon et al., 1995). The presence of two PLP motifs may reflect an ancient duplication during viral evolution (Snijder et al., 2013). The cleavage site between nsp1α and nsp1β was initially predicted to the region of 164 to 168 (den Boon et al., 1995), and subsequently specified to Gln166↓Arg167 (Allende et al., 1999). Recent studies however show that the authentic cleavage occurs at Met180↓Ala181 (Chen et al., 2010; Sun et al., 2009). For the cleavage between nsp1 and nsp2, Gly203↓Ala204 are shown according to the nsp1β crystal structure (Xue et al., 2010).

Cellular localization of nsp1 has been investigated in both virus-infected cells and gene-transfected cells. Deviating from other nsps, EAV nsp1 is found in the nucleus in addition to partial appearance in the cytoplasm during infection (Tijms et al., 2002). For PRRSV infection, both nsp1α and nsp1β localize in the nucleus and the cytoplasm with distinct localization patterns (Song et al., 2010). PRRSV-nsp1β has two different intracellular localization patterns; punctate perinuclear localization during early infection and predominantly nuclear localization later in infection (Li et al., 2012). In gene-transfected cells, both EAV-nsp1 and PRRSV-nsp1β appear in the nucleus predominantly, while PRRSV nsp1α is retained in the cytoplasm combined with nuclear distribution (Chen et al., 2010; Han et al., 2013; Song et al., 2010; Tijms et al., 2002).

Among the IFN antagonists identified for PRRSV, nsp1 and its two subunits are the potent modulators for IFN production and signaling (Fang and Snijder, 2010; Sun et al., 2012a). The nsp1α and nsp1β subunits suppress IFN-β activation following stimulation of innate immune responses (Beura et al., 2010; Chen et al., 2010; Kim et al., 2010; Song et al., 2010). IFN-β production in inhibited by nsp1α or nsp1β; individual elements in the IFN production pathway including RIG-1, IPS-1, MDA-5, TBK1, IKKε and IRF3 are all inhibited, suggesting that both subunits block the processes downstream of IRF-3 possibly in the nucleus (Chen et al., 2010). The total amount of IRF3 and its nuclear localization are not affected by nsp1, CBP was degraded in the proteasome-dependent manner by nsp1 (Kim et al., 2010). Further studies show that nsp1α is responsible for CBP degradation (Han et al., 2013). PRRSV-nsp1α also reduces the NF-κB activation (Song et al., 2010). CBP degradation is also mediated LDV-nsp1α but not found in cells expressing other nsp1 subunits of arteriviruses, and the molecular basis for IFN suppression by other nsp1 subunits are unknown (Han et al., 2014). Besides, all arterivirus nsp1 subunits possess the suppressive activity of ISRE promoter. The ISRE activity was suppressed by nsp1β, and nsp1β interrupts the STAT1 phosphorylation and nuclear translocation of ISGF3 in the JAK (Janus kinase)-STAT (signal transducer and activator of transcription) pathway (Chen et al., 2010; Patel et al., 2010). A study shows that PRRSV-nsp1β blocks the nuclear localization of ISGF3 by degrading karyopherin-α1 (KPNA1) (Wang et al., 2013). Both nsp1α and nsp1β involve in the suppression of TNF-α promoter activity through inhibiting the NF-κB activation and Sp1 transactivation (Subramaniam et al., 2010).

Functional domains of PRRSV nsp1 for IFN regulation have been determine. The PLP1α and the C-terminal ZF motif are not required for IFN suppression, and the N-terminal ZF motif is shown to be critical for IFN regulation (Han et al., 2013). Critical residues in nsp1α and nsp1β for IFN modulation have been identified by mutational analyses (Beura et al., 2012; Subramaniam et al., 2012), and a highly conserved motif in nsp1β has been implicated to be imporant for IFN suppression (Li et al., 2013).

5. INFECTIOUS CLONES AND THEIR APPLICATION

The reverse genetics system has been developed for many RNA viruses, and infectious clones have been utilized for the study of biology and vaccinology of viruses. The availability of such a powerful molecular tool has revolutionized the structure function studies for viral genomic RNA and viral proteins and facilitated the studies for virulence, pathogenesis, immune responses, and vaccine development. The first full-length genomic cDNA

clone was constructed for poliovirus in 1981 and its infectivity was demonstrated (Racaniello and Baltimore, 1981). Infectious clones have since been constructed for many viruses and their infectivity has been demonstrated for picornaviruses, caliciviruses, flaviviruses, togaviruses, influenza viruses, paramyxoviruses, rhabdoviruses, and coronavirus to name a few (Almazan et al., 2000; Boyer and Haenni, 1994; Pu et al., 2011; Scobey et al., 2013; Sosnovtsev and Green, 1995; Yount et al., 2003). For arterivirus, EAV and PRRSV are the first nidoviruses for which reverse genetics systems became available (Meulenberg et al., 1998; van Dinten et al., 1997). This review will discuss the current knowledge on the design of PRRSV infectious clones, engineering strategies for genome modification, and application to chimeric virus constructions, foreign gene expression, and functional study for viral proteins. We will summarize (1) our current knowledge of the principle of PRRSV infectious clone construction and the composition of each individual PRRSV infectious clone; (2) the common methods used for infectious clone engineering and the identified nonessential regions in the viral genome; and (3) applications of PRRSV infectious clones to the studies for viral virulence, pathogenesis, immune responses. We also discuss future perspectives on the use of PRRSV infectious clones.

5.1. Construction of infectious clones

Given the demonstrated infectivity of positive-sense RNA genomes, an arterivirus infectious clone was first made for EAV. The full-length clone pEAV030 which contains a 12.7-kb cDNA copy of the EAV genome was infectious (van Dinten et al., 1997), and the first PRRSV infectious clone pABV437 was developed for the European type PRRSV Lelystad virus (Meulenberg et al., 1998). Subsequently, infectious clones for VR-2332, which is the prototype North American PRRSV, and SD01-08, an European-like type I PPRSV circulating in the US have been developed (Fang et al., 2006a; Fang et al., 2006b; Nielsen et al., 2003). Since, numerous clones have been developed including the high-pathogenic PRRS emerged in China in 2006, and others (Guo et al., 2013; Lv et al., 2008; Zhou et al., 2009). To date, PRRSV infectious clones have been developed at least for 14 isolates (Table. 1).

Most PRRSV infectious clones have been developed based on the RNA transcription strategy and either T7 or SP6 promoter is placed at the 5’ upstream of the viral sequence to produce full-length genome RNA by *in vitro* transcription. To rescue the virus, RNA transcripts are synthesized in vitro off the full-length genomic cDNA using T7 RNA polymerase and transfected to cells. BHK-21 cells, MA-104 cells, and MARC-145 cells have been selected for producing progeny virus. Although BHK-21 is a non-susceptible cell line for PRRSV infection, these cells may provide a high transfection efficiency, and the production and release of infectious virus particle have been proven in BHK-21 cells (Meulenberg et al., 1998). To eliminate the need for in vitro transcription and the variability associated with transfection of cells with RNA, a eukaryotic promoter has been used for PRRSV P129. P129 virus was isolated from an outbreak of highly virulent atypical PRRS in the mid-Western USA in 1995 (Lee et al., 2005; Yoo et al., 2004). The use of eukaryotic promoter is easy and simple and provides a consistent efficiency of transfection and recovery of progeny virus (Lee et al., 2005).

Progeny virus generated from an infectious clone should retain the biological properties of parental virus, such as growth rate, virulence, and transmissibility (Kwon et al., 2008; Lee et al., 2005; Meulenberg et al., 1998; Nielsen et al., 2003; Truong et al., 2004; Yuan and Wei, 2008). To differentiate from the parental virus, genetic markers, either restricted enzyme sites or certain nucleotide mutations, have been introduced to the infectious clone, and those artificial modifications should be non-lethal and stable during the virus passages.

5.2. Engineering PRRSV infectious clones

Like most RNA viruses, PRRSV genome has evolved to optimal fitness and most of the genetic information is expected to be essential (Verheije et al., 2001). Engineering PRRS genome is complex and the extremely compact viral genome is a challenge. In addition, minor alternations in conserved regions or functional domains in the

genome will almost inevitably lead to the production of a non-viable virus (Ansari et al., 2006; Kroese et al., 2008; Lee et al., 2005). Despite such difficulties, genetic manipulations for PRRSV have been successful.

Mutation, deletion, insertion, and substitution are major approaches to viral genome manipulation. Due to the large genome of PRRSV, shuttle plasmids have been used as an intermediate platform to contain the target viral genomic sequence with a pair of unique enzyme sites at each ends. Mutations are introduced to target sites or sequences in the shuttle plasmid. Biological functions of PLP1α and PLP1β in nsp1, conserved cysteine residues (C49 and C54) in E protein, N-linked glycosylation sites in GP3 (N131) and GP5(N34, N44, N51), residues (C23, C75, C90) for homo-dimerization of N protein, and the nuclear localization signal (NLS) of N protein have been mutated to produce PRRSV mutants (Ansari et al., 2006; Kroese et al., 2008; Lee et al., 2005; Lee et al., 2006; Lee and Yoo, 2005; Pei et al., 2009; Vu et al., 2011). Alanine scanning and protein surface accessibility predictions were conducted for identification of residues for type I IFNs or TNF-α antagonism of nsp1, and specific residues have been substituted in the PRRSV infectious clones (Beura et al., 2012; Li et al., 2013; Subramaniam et al., 2012). Mutations are also been introduced to knockout genes by changing the translation initiation codon, and this approach destroys the expression of nsp1 and E proteins (Lee and Yoo, 2006; Tijms et al., 2001).

Deletion of genomic sequences has been applied to identifying non-essential regions for PRRSV replication or to obtaining attenuated live vaccine candidates (Verheije et al., 2001). Inter-genotypic sequence alignments between genotype 1 and genotype 2 reveal regions of sequence heterogeneity which have a potential to tolerate the deletions, and several non-essential regions have been identified in the N gene and 3'-UTR (Table 2; Sun et al., 2010b; Tan et al., 2011). The existence of hyper-variable regions of insertions and deletions have been observed in nsp2 gene of various isolates (Fang et al., 2004; Gao et al., 2004; Ni et al., 2013; Shen et al., 2000; Tian et al., 2007), suggesting a non-essential region in nsp2 (Chen et al., 2010b; Han et al., 2007; Ran et al., 2008; Xu et al., 2012b). Deletion of ORF2 or ORF4 results in the absence of infectivity, suggesting the requirement of structural proteins for PRRSV infectivity (Welch et al., 2004).

Insertion of additional nucleotides to the viral genome expands the scope of modifications. An attempt was made to separate overlapping regions of PRRSV structural protein genes, and three restriction enzyme sites were inserted between ORFs 5/6 and ORFs 6/7 (Yu et al., 2009), which produced viral viruses. A possibility for expressing foreign genes has been explored; nsp2 was considered as an insertion site for expression of green fluorescent protein (GFP) and FLAG tag using PRRSV infectious clones (Fang et al., 2006b; Kim et al., 2007). An alternate approach aimed at insertion of foreign genes within structural genes, for example, the gene for influenza virus hemagglutinin (HA) into the 5' or 3' end of ORF7 of PRRSV (Bramel-Verheije et al., 2000). However, the insertion of HA-N fusion protein resulted in nonviable virus. A strategy utilizing the mechanism of transcription in PRRSV is of interest. Using an infectious clone, two unique enzyme sites have been introduced between ORF1b and ORF2, and a copy of the TRS6 sequence was inserted to drive the synthesis of mRNA for foreign gene expression (Lee et al., 2005; Pei et al., 2009; Yoo et al., 2004). The foreign genes including GFP, capsid protein of porcine circovirus type 2 (PCV2), *Discosoma* sp. red fluorescent protein (DsRED), *Renilla* luciferase (Rluc), IFN-α1, IFN-β, IFN-δ3, and IFN-ω5 have all been expressed using this approach (Table 3; Pei et al., 2009; Sang et al., 2012).

Multiple genes, a single gene, or partial viral genome sequences have been substituted with corresponding sequences from other arteriviruses and chimeric arteriviruses have been constructed. The first chimeric arterivirus was generated using an EAV infectious clone as a backbone and the ectodomains of two membrane proteins, GP5 and M, were substituted with the corresponding sequences from PRRSV or LDV (Dobbe et al., 2001). Other chimeric arteriviruses have also been constructed (Table 4). The production of intra- or inter-genotypic PRRSV chimeras is more maneuverable, and viral regions including 5'-UTR, nsp coding sequences, and structural genes were replaced (Gao et al., 2013; Lu et al., 2012; Tian et al., 2012; Tian et al., 2011; Vu et al., 2011; Zhou et al., 2009). To facilitate intra-genotypic substitution, a technique named gene-swapping mutagenesis was developed

to substitute structural genes. Briefly, the ORF from substituent genes is amplified with RT-PCR and purified as primers, and a shuttle plasmid containing the counterpart sequence is used as a template. The PCR reaction generates shuttle plasmid with the sequence from substituent for assembly (Kim and Yoon, 2008). Using this technique, individual replacement of ORFa, ORF2 through 6 in the V2332 infectious clone was successfully carried out with corresponding ORFs from other isolates of PRRSV including JA142, SDSU73, PRRS124, and 2M11715 (Kim and Yoon, 2008).

PRRSV nsp2 is a multifunctional protein that undergoes remarkable genetic variations. The nsp2 protein consists of five regions: hypervariable region I (HV-I), PLP2 cysteine protease core, hypervariable region II (HV-II), transmembrane regions, and a C-terminal tail (Figure 2; Han et al., 2009). The PLP2 domain in the N-terminal region posseses cis-acting and trans-acting cleavage activities and mediates its rapid release from pp1a and pp1ab (Han et al., 2009; Snijder et al., 1995). Two sites were intially predicted for PRRSV nsp2/3 cleavage (981G/G and 1196G/G/G) by comparative sequece analysis, but recent studies show the actual cleavage site to 1196G/G for VR2332 PRRSV (Allende et al., 1999; Han et al., 2009; Nelsen et al., 1999). The corresponding cleavage site for EuroPRRSV nsp2/3 in the US (SD01-08) likely occurs at 1445GG/A (Fang and Snijder, 2010). PLP2 has also been identified as a member of the ovarian tumor domain (OTU) family of deubiquitinating enzyme, and subsequently shown to deconjugate ubiquitin (Ub) and IFN-stimulated gene (ISG) 15 from cellular targets. This is an important PRRS strategy inhibiting the Ub-dependent and ISG15-dependent host innate immune responses (Frias-Staheli et al., 2007; Sun et al., 2010a; Sun et al., 2012b; van Kasteren et al., 2012)

Heterogeneity and deletions in nsp2: Besides the proteinase and deubiquitinase functions, nsp2 contributes to the major genetic variation between genotypes I and II, sharing only less than 40% similarity at the amino acid level (Allende et al., 1999; Nelsen et al., 1999). The nsp2 gene also contains insertions and deletions in the the middle hypervariable region (Figure 2a, white areas) (Fang et al., 2004; Gao et al., 2004; Ni et al., 2011; Shen et al., 2000; Tian et al., 2007). Two discontuguous deletions of 12 amino acids in nsp2 are found in the highly pathogenic PRRSV in China in comparison with other North American isolates (Gao et al., 2004; Tian et al., 2006). Sequence analysis of PRRSV MN184 isolate reveals three discontinuous deletions of 111, 1, and 19 amino acids at corresponding VR2332 positions at 324-434, 486, and 505-523, respectively (Han et al., 2006). The 30 amino acids dicontinous deletion consists of 1 aa deletion at residue 482 and 29 aa deletion at 534-562, and the deletion region contains B-cell epitopes (de Lima et al., 2006) and T-cell epitopes (Chen et al., 2010b). Strikingly, cell culture passaging of PRRSV may generate a deletion in nsp2, and a study shows generation of a large deletion of 135 aa at 581-725 in nsp2 during passages (Ni et al., 2011). Deletion in nsp2 is also found in genotype I PRRSV, and SD-01-08 in the US shows a 17 aa deletion at position 349-365 of nsp2 when compared to Lelystad virus (Fang et al., 2004). The biological significance of genetic deletion in nsp2 remains to be determined.

Foreign gene insertion into nsp2: Given the tolerance of deletions and insertions in the middle hypervirable region of nsp2, this region has been considered as a site for foreign gene insertion (Figure 2). The GFP gene was inserted into nsp2 of the SD01-08 strain and fully infectious virus was rescued (Fang et al., 2006b). The GFP insertion did not affect the growth of the virus and infectivity was comparable to parental virus. The capacity of deletion in nsp2 has been determined by introducing a series of in-frame deletions (Han et al., 2007). The PLP2 domain, the PLP2 downstream flanking region, and the transmembrane domain were found to be crucial for virus replication but deletions aa 13 to 35 from N-terminal hypervariable region and aa 324 to 813 from the middle hypervariable region appeared to be tolerable. In the middle hypervariable region, the largest deletion that can be achieved is about 400 aa (324-726), although a deletion of upto 200 aa is preferable. Insertion of GFP or other foreign genes such as Newcastle disease virus nucleoprotein (NP) gene has been successful (Kim et al., 2007; Xu et al., 2012a). Immunodominant linear B-cell epitopes (ES2-ES7) deletion were attempted; deletion of ES3, ES4, or ES7 allowed the generation of infectious virus (Chen et al., 2010b; Oleksiewicz et al., 2001).

N gene: N protein is mutilfunctional. Domains and residues critical for virus replication have been identified. The N protein is comprised of 123 or 128 aa for the North American and European genotypes, respectively (Music

and Gagnon, 2010). N is consisted of the N-terminal RNA-binding domain (RBD) at 41-47 and the C-terminal dimerization domain comprising a four-stranded antiparallel β-sheet floor flanked by α-helices (Doan and Dokland, 2003; Yoo et al., 2003). As the sole component of viral capsid, N interacts with itself via covalent or noncovalent interactions (Doan and Dokland, 2003; Wootton and Yoo, 2003). The cysteine at position 23 is responsible for the formation of intermolecular disulfide bond, and aa 30-37 are essential for mediating noncovalent homodimers (Wootton and Yoo, 2003). A crystallographic study on N shows the imprtance of the C-terminal dimerization domain for N (Doan and Dokland, 2003; Spilman et al., 2009). N is a phosphoprotein, which is a common property for N of EAV and coronaviruses (Music and Gagnon, 2010; Wootton et al., 2002). The phosphorylation site of N is at position 120, but its biological significance is still unknown. N contains NLS in a stretch of basic amino acids 41-PGKKNKK-47 which is overlapping with the RNA-binding domain (Rowland et al., 1999; Rowland et al., 2003). The nuclear export signal (NES) is found at positions 106-117, and is responsible for the nucleolar-cytoplasmic shuttling of PRRSV N protein (Rowland and Yoo, 2003) .

The structure of N is functionally compact, and thus N is sensitive to structural modifications. The secondary structure in the C-terminal residues 112-123 is an important determinant for conformational epitopes, and mutations in this region change the monoclonal antibody (MAb) reactivity (Wootton et al., 2001). Insertion of a foreign sequence into N gene has been attemped using the HA epitope either at the N-terminus or C-terminus. Despite the initial rescue of infectious virus, HA expression was unsuccessful (Bramel-Verheije et al., 2000). The GFP tag was inserted between ORF6 and ORF7 to moniter the ORF7 mRNA synthesis, but no mRNA was made, indicating the 5' end of ORF7 gene is essential for mRNA synthesis (Yoo et al., 2004). The N protein is inter-genotypically conserved but shares only 60% identity between LV and VR2332 (Dea et al., 2000). The C-terminus of N is heterogenous and truncation of up to 6 aa is tolerable (Verheije et al., 2001). In another study, serial deletions were made at the inter-genotypic variable region or conserved region of N, and 4 regions at 5-13, 39-42, 48-52, and 120-123, were found to be dispensible for viability (Tan et al., 2011). No foreign gene can be incorporated in these rgions.

Non-conding regions**:** The PRRSV genome is flanked by 5'- and 3'-UTR, and the UTR sequences play a vital role fpr genomic replication, mRNA transcription, and protein translation (Pasternak et al., 2006; Snijder et al., 2013). Non-essential regions in the UTR have been investigated. By serial deletions, the first 3 nucleotides in 5'-UTR appears to be dispensible for viability (Gao et al., 2012). For 3'-UTR, the first 11 nucleotides are unique for each genotype, and a stretch of 38 nucleotides present in VR2332 is absent in LV (Allende et al., 1999; Verheije et al., 2001). A deletion study shows that 7 nucleotides at the 5' end of the 3'-UTR is tolerable for genotype I PRRSV (Verheije et al., 2001). The 3'-UTR of genotype II has also been studied, and at least 40 nucleotides immediately following the ORF7 gene is dispensable for virus viability (Sun et al., 2010b).

Structural region: The genetic information in the structural regions of arteriviruses is organized in an extremely efficient manner. The genes for GP2/3/4 overlap each other, and similarly the genes for GP5/M/N overlap each other for PRRSV. The importance of the overlapping gene arrangement for the life-cycle of virus has been studied (Verheije et al., 2002; Yu et al., 2009). genetic manipulation may be hampered by gene overlaps. A series of engineered full-length clones were made to separate the overlapping genes for EAV ORFs4/5 or ORF5/6 by inserting small additional sequences containing a termination codon for the upstream gene, a unique restriction site, and a translation initiation codon for the downstream gene. The insertions result in the functional separation of overlapping ORFs, and do not impair infectivity (de Vries et al., 2000). The ORFs5/6 separation is possible and progeny virus is produced (Verheije et al., 2002). For North American PRRSV, restriction sites were inserted between ORFs1/2, ORFs4/5, ORFs5/6, ORFs6/7, and ORFs7/3′-NTR, and progeny viruses are generated from these modifications. This indicates that gene overlap is dispensable for infectivity, and that separation of each gene does not interrupt mRNA synthesis (Yu et al., 2009).

5.3. Application of PRRSV infectious clone

Chimeric viruses and cell tropism: The development of infectious clones allows the construction of chimeric arteriviruses. An attempt was made to swab the ectodomains of GP5 and M. In the engineered chimeras using the EAV clone as a backbone, the ectodomains were replaced by corresponding sequences from related and unrelated RNA viruses. The chimeric viruses containing the GP5 ectodomain from LDV and PRRSV were infectious. These chimeric viruses however retain their cell tropism for BHK-21 cells, which are susceptible for EAV but non-susceptible for LDV or PRRSV (Dobbe et al., 2001). Replacement of the M ectodomain of EAV with the corresponding sequence from other arteriviruses does not produce infectious virus, but replacement of the M ectodomain of PRRSV with the corresponding sequence from LDV, EAV, and genotype II PRRSV produced infectious virus. Substitutions with the EAV M ectodomain or VR2332 M ectodomain is impossible, but removal of the gene overlap between the M and GP5 genes is required before the swapping, indicating that the VR2332 M ectodomain and EAV M ectodomain are incompatible with the remaining part of LV M. It is also possible that unintended mutations may have been introduced to GP5 during the ectodomain swap (Verheije et al., 2002). Substitution of structural genes between arteriviruses has been extremely useful to identify viral factors for viral tropism. The substitution of GP5 or/and M do not alter their cell tropism (Dobbe et al., 2001; Lu et al., 2012; Verheije et al., 2002). In contrast, the substitution of minor envelop proteins and E using the PRRSV infectious clone as a backbone allows the chimeric PRRSV to acquire a broad cell tropism but lose the ability to infect PAMs. It indicates that GP2/GP3/GP4 minor proteins are the determinants for cell entry and tropism (Tian et al., 2011).

***Chimeric viruses and virulence immunogenicity*:** Intra-genotypic or inter-genotypic gene-swapping have been done between EAV and PRRSV to study the genetic compatibility and viral-specific phenotypes including neutralization, virulence, and pathogenesis. For neutralization phenotypes, 9 chimeric EAV were generated in which each contains individual ORF5 from different isolates (Balasuriya et al., 2004). Also, the role of individual envelope proteins of GP2 through M for cross neutralization was studied using the VR2332 as a backbone (Kim and Yoon, 2008). The PRRSV-01 strain is highly susceptible to neutralization and induces atypically rapid and robust neutralizing antibodies in pigs. Analysis of structural genes of PRRSV-01 reveals the absence of two N-linked glycosylation sites in GP3 and GP5, and subsequently the significance of the missing glycans for neutralization was determined by replacing the structural genes from PRRSV-01 (Vu et al., 2011). The major virulence determinants have also been identified to locate in nsp3 through nsp8 and GP5 by gene swapping experiments (Kwon et al., 2008). Highly pathogenic PRRSV contains the 30 aa deletion in nsp2 sequence (Tian et al., 2007), However, by gene swapping studies using the corresponding sequence from an avirulent PRRSV, the deletion in nsp2 was shown to be irrelevant to virulence and pathogenicity (Zhou et al., 2009). The inter-genotypic 5'-UTR swap between genotypes I and II was investigated and show that the 5'-UTR of genotype II PRRSV may be substituted with the corresponding sequence from genotype I, while substitution of 5'- UTR with its corresponding sequence from genotype II is lethal (Gao et al., 2013). Using the approach, the envelope proteins representing GP2 through GP5 of genotype I PRRSV are shown to be fully functional for genotype II PRRSV using genotype II as a backbone (Tian et al., 2011).

Molecular breeding and shuffling: Sequence shuffling has been employed to generate immunologic variants of PRRSV (Ni et al., 2013; Zhou et al., 2013; Zhou et al., 2012). The GP3 sequence representing immunologically diverse PRRSV are randomly shuffled and incorporated in the infectious clone to generate a new virus that may improve the cross neutralization (Zhou et al., 2012). The breeding of GP4 or M have also been achieved and the rescued virus induces a broad spectrum of cross-neutralizing antibodies (Zhou et al., 2013). The GP5 sequence from 7 genetically diverse strains of PRRSV and the GP5-M sequence from 6 different strains were subjected to molecular breeding and the shuffled genes were cloned in an infectious clone for generation of a new virus. Two representative chimeric viruses, DS722 with GP5 shuffling and DS5M3 with GP5-M shuffling, are attenuated (Ni et al., 2013). This approach allows rapid generation of attenuated virus and may be useful for vaccine development for antigenetically variable viruses (Ni et al., 2013; Zhou et al., 2012). Another approach has been employed to rapidly generate attenuated PRRSV; this approach is referred to as SAVE (synthetic attenuated virus engineering). Codon-pair bias is a phenomenon that certain codon pairs appear in a higher frequency in comparison to other

synonymous codon pairs for the same amino acid, and the codon-pair bias is species-dependent related to the efficiency of protein synthesis (Coleman et al., 2008; Moura et al., 2007; Mueller et al., 2010). By de-optimizing the codon pair of a virulence gene, the expression level of this protein decreases. The computer-aided de-optimization of codon-pairs modifies only naturally optimized pairs of codons and do not change the amino acid sequence (Mueller et al., 2010). Using this approach, the GP5 gene was codon-pair de-optimized, and the modified GP5 sequence did not affect the generation of PRRSV which was attenuated in pigs (Ni et al., 2014).

PRRSV as a foreign gene expression vector: Foreign gene expression may serve as a vaccine vector. PRRSV infectious clones have been developed as a gene delivery vector for foreign gene expression. Identification of gene insertion sites in the viral genome and viral infectivity are critical for gene delivery. GFP and B-cell epitope of the Newcastle disease virus (NDV) nucleoprotein have been inserted into the non-essential regions of PRRSV nsp2 (Fang et al., 2008; Fang et al., 2006b; Kim et al., 2007; Xu et al., 2012a). In this approach however, stability of the inserted gene was a concern. When PRRSV expressing GFP in nsp2 was cell-culture passaged, a population of GFP-expression negative-virus appears by 7th passage. Sequencing shows a deletion of GFP at the N-terminal half (1 to 159), leading to the loss of GFP expression. Insertion of 2 amino acids at position of 160 of GFP was also observed in some viral clones (Fang et al., 2006b). The stability of GFP gene in this recombinant virus was improved by deleting the ES4 epitope located downstream of GFP gene, and the GFP expression in this virus was stable for 10 passages. However, R97C mutation was found in GFP and this mutation caused the loss of florescence (Fang et al., 2008). The loss of fluorescence was also observed in two other GFP recombinant viruses during serial passages. In another study, the GFP coding sequence remained intact but point mutations within EGFP were identified and these mutations caused amino acid changes to R96C and N106Y (Kim et al., 2007). The expression of 49 aa-B-cell epitope from NDV NP in PRRSV nsp2 remained stable up to 20 passages (Xu et al., 2012a; Zhang et al., 2011). The instability of foreign gene insertion in nsp2 is not fully understood. The length of insertion may be important for stability.

An attempt was made to produce an additional mRNA for foreign gene expression. The GFP gene was inserted between ORF1b and ORF2a for PRRSV along with a copy of TRS6 plus two unique enzyme sites (Lee et al., 2005; Pei et al., 2009; Sang et al., 2012; Yoo et al., 2004). Compared to insertion in nsp2, this site is suitable for foreign gene insertion since the recombinant virus was stable for up to at least 37 passages without the loss of gene or fluorescence (Pei et al., 2009). The genetic stability has been shown by expressing other genes including DsRED, Rluc, IFNα1, IFNβ, IFNδ3, and IFNω5 (Sang et al., 2012). This approach has a particular advantage by eliminating the need to alter the coding sequence of a viral gene, and also minimizing the effects on expression and post-translational modification of viral proteins (Pei et al., 2009).

Application of infectious clones to structure function studies: Infectious clones are important molecular tools to study structure function relationships of proteins and genomic sequences using infectious viruses in vivo. Specific sequence motifs may be mutated or deleted from the virus and their phenotypes may be examined to determine their functions. The removal of N-linked glycosylation at N34 and N51 of GP5 results in mutant PRRSV with a phenotype of enhanced sensitivity to neutralization and induction of higher levels of neutralizing antibodies (Ansari et al., 2006). The E gene knock-out mutation allows the genome replication and transcription but does not produce infectious progeny, indicating that the E protein is essential for virion assembly (Lee and Yoo, 2006). PRRSV nsp1 is a multifunctional protein regulating the accumulation of genomic RNA and mRNAs. It also has the ability to modulate the host innate immunity by suppressing the type I IFN production (Nedialkova et al., 2010; Sun et al., 2012a; Yoo et al., 2010). Motifs for zinc fingers, PLPs, and nuclease have been identified in nsp1 (Fang and Snijder, 2010; Snijder et al., 2013; Xue et al., 2010). By deleting nsp1 from the genome, nsp1 is shown to be dispensable for genome replication but crucial for mRNA transcription. Mutation in the catalytic sites of PLP1 impairs both viral genome and mRNA synthesis as well as the cleavage between nsp1 and nsp2 is impaired. Mutations in the zinc finger motif abolish the mRNA transcription, whereas replication was not affected (Tijms et al., 2007; Tijms et al., 2001). When the catalytic sites of PLP1α are mutated using the PRRSV infectious clone, the proteinase activity disappears and mRNA synthesis is completely blocked. In contrast, mutations at the

PLP1β catalytic sites result in no mRNA synthesis and no viral infectivity, indicating that the normal cleavage of nsp1 and nsp2 is critical for viral replication (Kroese et al., 2008).

To design effective vaccine candidates that may be useful to overcome antigenic heterogeneity of PRRS, extensive studies have been conducted to eliminate the IFN antagonistic function from the virus (see reviews Snijder et al., 2013; Sun et al., 2012a; Yoo et al., 2010). Among viral proteins, nsp1α and nsp1β have been identified as potent IFN analogists (Beura et al., 2010; Chen et al., 2010a; Han et al., 2013; Kim et al., 2010; Song et al., 2010). The subsequent studies have identified specific residues regulating the IFN antagonism, and a mutant virus with a stretch of alanine substitution at positions 16-20 of nsp1β showed the loss of IFN suppression (Beura et al., 2012). In another study, K124 and R128 were mutated to release the surface accessibility of nsp1β, and mutant PRRSV impaired the IFN antagonism (Li et al., 2013).

Motifs in the N protein have broadly been studied using mutant viruses. The importance of N protein dimerization has been examined by mutating C23S which is responsible for the covalent interaction between N proteins. Mutant viruses of C23S, C75S, and C90S were constructed, and with the exception of C75S, both C23S and C90S completely lost the infectivity. Genome replication and mRNA transcription were normal for both mutants, suggesting the dimerization of N may be important for particle assembly or maturation (Lee et al., 2005). The nuclear localization signal (NLS) of N was also mutated to examine the biological consequence of N in the nucleus in PRRSV-infected cells. Compared to the wild-type PRRSV-infected pigs, NLS-null mutant PRRSV was attenuated in pigs and produced significantly shorter mean duration of viremia and higher titers of neutralizing antibodies in pigs (Lee et al., 2006; Pei et al., 2008), demostarting that the N protein nuclear localization is a virulence factor.

6. EVOLUTIONARY DIVERSIFICATION OF PRRSV

6.1. Recapitulating PRRS

The late 1980s marked the emergence of a "Mystery Swine Disease" causing reproductive complications in sows and respiratory disease in growing pigs in the United States (Hill, 1990). In near synchrony, reports began trickling in from swine rearing countries across Europe about a swine reproductive disease matching clinical presentations seen in North America (Hopper et al., 1992; Plana et al., 1992; Wensvoort et al., 1991). After ruling out known bacterial and viral pathogens and the isolation of a novel virus, the mystery disease was formally recognized as a novel swine illness and named porcine reproductive and respiratory syndrome (PRRS) along with the etiological agent as PRRS virus (PRRSV). The initial lack of knowledge on many aspects of PRRSV biology including its transmission modes allowed the virus to quickly spread globally from its original epicenters. Today, far from eradication, PRRSV is endemic to virtually all swine rearing countries and presents substantial challenges in its management.
Despite the commonality in clinical symptoms and timing of emergence, comparative sequence analysis of prototype strains from Europe (Lelystad Virus; LV) and North America (ATCC VR2332) surprisingly revealed significant divergence (Murtaugh et al., 1995; Nelsen et al., 1999) which was evident at the antigenic level as well (Magar et al., 1995; Nelson et al., 1993). All subsequent isolates characterized till now have been found to be either closely related to LV or VR2332 establishing two genotypes. Those that group with the former are referred to as type 1 or EU while the latter is categorized as type 2 or NA. PRRSV was found to share similarities with equine arteritis virus (EAV), simian hemorrhagic fever virus (SHFV), and lactate dehydrogenase elevating virus (LDV); all of which now collectively comprise the *Arteriviridae* family under the *Nidovirales* order (Conzelmann et al., 1993; Meulenberg et al., 1993). LDV, being the nearest evolutionary relative, has been speculated in one origins of PRRSV hypothesis to have undergone one or more cross-species event(s) and intercontinental migration via intermediate hosts before eventual establishment in regional swine herds as PRRSV (Plagemann, 2003). More recently, the plausibility of such a scenario has been posited in a more refined manner under the historical backdrop of large scale changes in the swine production industry and erstwhile global pig

movement (Murtaugh et al., 2010). However, the failure to detect ancestral PRRSV strains or LDV-like isolates in suspected hosts or reservoirs together with other reasons discussed at length elsewhere (Shi et al., 2010a) raise reservations about this pre-emergence account.

Unlike the uncertainty surrounding the origins, there is unanimity regarding the immense genetic diversity not only between genotypes but within each genotype as well. This genetic variability, while a natural survival mechanism to host immunity responses, simultaneously has been the primary reason for the inability to develop a vaccine offering broad protection against PRRS challenge. Hefty economic loss is incurred from PRRS annually in the United States alone (Neumann et al., 2005; Holtkamp et al., 2013). As a result, the surveillance and monitoring of PRRSV evolution is fundamental to all stakeholders in the management of PRRS. Herein, with a specific focus on type 2 PRRSV, we describe the mechanisms which drive PRRSV radiation, discuss an approach to organizing and tracking viral diversity, highlight temporal changes in regional strain diversity and their implications. We close by looking at the shortcomings of current diversity related studies and how next to utilize sequence information beyond simple inter-strain relatedness investigations.

6.2. Molecular mechanisms underlying PRRSV evolution

Replication traits: As is the case with other RNA viruses, PRRSV replication is an error prone process resulting from a lack of proofreading capability of the viral polymerase. Although, no empirical estimate of mutation rate in PRRSV is presently available, it is widely accepted RNA viruses display the highest mutation rates of any other organism, approximately one mutation per replication (Drake, 1993). Secondly, replication kinetics enable the production of thousands of viral particles within hours of a single successful infection event. Extrapolating to the individual host level, even by conservative estimates many millions of viral particles are present at its peak. Such large populations enhance the effectiveness of natural selection wherein novel mutants with increased fitness supersede competitively inferior alleles. In addition, there are a number host cellular enzymes which bolster the viral mutation rate partly as innate antiviral defense mechanisms in an attempt to push RNA viruses over the tolerable error threshold so that the accumulation of deleterious mutations either reduce fitness or render inability to reproduce. Cellular activities, in this respect, possibly impacting PRRSV replication fidelity have been discussed elsewhere (Murtaugh et al., 2010).

Quasispecies: The poor fidelity with which RNA viruses replicate inevitably quickly gives rise to a mutational swarm of variants. Very frequently, including for PRRSV (Brar et al., 2014; Goldberg et al., 2003; Rowland et al., 1999), this intra-host variation is collectively attributed to the quasispecies phenomenon. The quasispecies theory (Eigen and Schuster, 1977), if formally applied to viruses, mandates natural selection to act on the quasispecies population as a single unit envisioning mutational coupling of individual variants, a consequence of the mutation rate bordering the error threshold. With this as the bedrock, some doubt has been expressed as to the relevance of the quasispecies model in shaping the evolution of RNA viruses since evidence of natural selection operating on the population as a unit rather than favouring the "fittest" variant(s) remains to be presented especially under natural conditions (Holmes, 2010). This is not to dismiss the recognition or significance of generating and evolving intra-host genetic heterogeneity to cope with various pressures like host immune responses, vaccination, or transmission bottlenecks among others. Rather, caution is advixed against solely basing it on the quasispecies model.

Recombination: Recombination is an integral activity of the PRRSV life cycle enabling the generation of subgenomic (sg) mRNAs through discontinuous RNA synthesis of a nested set of subgenomic negative strands serving as templates. The process mirrors the "copy choice" recombination (Lai, 1992) model in which the RNA-dependent RNA polymerase switches from the donor template to the acceptor template, a process mediated by transcription regulatory sequences in PRRSV, while remaining attached to the nascent strand. A similar role is played by recombination in the production of heteroclite RNAs, the functional relevance of which remains to be elucidated, at a scale of comparable prominence as sgmRNAs. The process occurs at variable short stretches of

sequence similarity independent of TRS sites (Yuan et al., 2000; Yuan et al., 2004). In this sense, it would appear recombination is simply a by-product of genome organization and a means to control gene expression.

From an evolutionary standpoint, in cases of co-infection with more than one strain, recombination can hasten the pace at which combinations of advantageous mutations are obtained or deleterious mutations are purged. However, experimental data demonstrating the importance of PRRSV recombination in these aspects or to what extent is still lacking since it may be argued that the mutation rate of RNA viruses is sufficiently high along with large population sizes which enable the generation of beneficial mutations or compensatory ones for deleterious alleles at a frequency rendering recombination inessential for such purposes. Regardless, there is adequate evidence establishing the emergence of recombinants from multiple non-identical parental strains and even becoming a successful part of circulating PRRSV diversity. Cell culture studies have shown this in both EU (van Vugt et al., 2001) and NA (Murtaugh et al., 2002; Yuan et al., 1999) genotypes. Observations have been confirmed under experimental conditions in animals (Liu et al., 2011). At the population level, bioinformatics analyses screening for recombination in varying lengths and regions of sequence datasets have revealed the prominent role played by recombination in shaping global PRRSV diversity (Fang et al., 2007; Forsberg et al., 2002; Shi et al., 2013a). What remains to be determined is what, if any, phenotypic changes can recombination bring about in PRRSV. For example, can it alter virulence or inter-host transmission? A question of added importance now since cases have been presented wherein recombinants have been either associated with outbreaks (Shi et al., 2013a) or identified in significant circulating numbers over wide geographical areas originating from common ancestors (Shi et al., 2013b) when in the past only sporadic instances were detected (Li et al., 2009a; Li et al., 2009b; Yoshii et al., 2008).

6.3. Molecular epidemiology of PRRSV

Over the years, the study of viral sequence diversity in conjunction with epidemiological accounts has taken root because of the valuable insights that can be gained into questions of origins, changes in population demographics, dating historical points of interest, selective pressures at individual nucleotide sites, and many more. This area is only set to gain further prominence spurred by the advent and widespread adoption of next generation sequencing technologies which offer unparalleled throughput while bringing down cost per nucleotide sequenced. While this will undoubtedly increase the quantity of PRRSV related sequence data that will become available to researchers in future, at present there is already a significant volume of such data, mostly from structural genes like ORF5 and ORF7, spanning two and half decades and broad geographical regions. Making sense of any new PRRSV sequence in terms of what is already known becomes a daunting task. Naturally, this underscores the fundamental need for organizing and establishing a system of contextualizing any new sequence enabling meaningful answers to basic questions. Since PRRSV is a rapidly evolving virus with its sequence diversifying over short periods of time, phylogenetics represents, perhaps, the most appropriate means of accomplishing the task in contrast to other methods (Murtaugh et al., 2010).

Phylogenetic classification of Type 2 PRRSV: The relatively high sequence variability observed in ORF5 early on (Kapur et al., 1996; Meng et al., 1995) popularized its use in discerning field isolates and epidemiological studies (Goldberg et al., 2000; Mondaca-Fernández et al., 2006). As a result, to date, the primary source of PRRSV sequence data in public databases pertains to ORF5, making it a popular choice for phylogenetic studies. In 2010, Shi and co-workers performed analysis on the largest collection ORF5 sequences, until then, amassed from public databases and private contributors (Shi et al., 2010b). This represented the first, and the only attempt so far, to introduce a framework for utilizing all known PRRSV genome diversity, though limited to a single gene, and putting forth a high resolution typing system (Figure 1a). The devised system categorized Type 2 isolates into nine well-supported lineages with any one inter-lineage genetic distance being greater than 10%. With this cutoff, a number of the lineages appeared to have diverged well before the first recognized outbreak of PRRS. As such, it not very likely that the accommodation of any subsequent new diversity related updates would radically change the lineage system put forth (bearing in mind the query sequences conform to assumptions of evolution in

phylogenetic analysis). In fact, this has now been demonstrated with the typing of large sequence data sets not available to the authors at the time of initial analysis (Brar et al., 2011; Kvisgaard et al., 2013; Shi et al., 2013b) and numerous other typing queries [Frederick Leung, HKU, personal communication]. The availability of this system in a user-friendly setting performing typing tasks in a time efficient manner without the need to manually perform individual steps (alignment, recombination screening, and phylogeny reconstruction) every time would be a logical and important advancement. Such a prospect which would help standardize diversity or phylogenetic studies on PRRSV allowing meaningful comparisons across studies.

Type 2 PRRSV diversity dynamics - what's new?: Aside from establishing a typing system, the analysis of global ORF5 sequences revealed important insights into the evolving diversity of PRRSV in terms origins, outbreaks, dating historically important events, changes in population demographics, and patterns of geographic migration (Shi et al., 2010b). This was recapitulated and reviewed shortly thereafter (Murtaugh et al., 2010; Shi et al., 2010a) and hence will not be repeated here. Since then, the collection of ORF5 sequences has expanded from approximately 8,000 to well over 13,000. This begs questions like: what's new in terms of diversity? Or, what are the implications?
As mentioned, while the stark divergence between type 1 and type 2 PRRSV was recognized very early on (Murtaugh et al., 1995; Nelsen et al., 1999), over time the considerable diversity within each genotype is now equally appreciated. Aside from intrinsic causes discussed earlier, epidemiology of viral diversity can also be shaped by extrinsic factors like migration, transmission, swine production practices, vectors, vaccination, and biosecurity standards. As a result, despite PRRS being a virtually ubiquitous issue for swine industries globally, the diversity dynamics are not equally homogeneous as the mix of factors dominating at a particular region can vary. Accordingly, we discuss PRRSV diversity at two levels: (1) global, akin to viewing a forest; (2) regional, akin to viewing the trees in that forest.

Diversity: looking at the "forest": The increase in sequence data since the initial analysis is partly the result of the subsequent availability of Canadian and Mexican sequences. Input from these countries is an important step in better gauging global type 2 diversity especially since retrospective serologic screening indicated Canada to have the earliest history of type 2 PRRSV circulation (Carman et al., 1995) while significant diversity has been reported in the case of Mexico based on ORF7 (Batista et al., 2004). Incorporation of all subsequent sequences since 2010 has still maintained the nine lineages. Seven of these lineages are still dominated by North American isolates while the remaining two comprise Asian isolates primarily. Based on the topology of overall phylogenetic tree and ancestral state reconstruction, it is now fairly rational to state that the origins of lineages 1 and 2 lie in Canada while those of 6 to 9 in USA (Shi et al., 2013b). Of the nine lineages, global type 2 diversity is dominated mainly by isolates of four lineages (L1, L5, L8, and L9) which collectively constitute over 97% of all ORF5 sequences (Figure 4a). While some lineages remain more or less static in terms of diversity over an extended temporal scale, there are clear instances where longitudinal sample analysis reveals a gradual rise in sequence divergence amongst field isolates. Such a trend is most apparent in lineages 1 and 9 (Figure 4b). In these cases, over the last decade to decade and a half, there has been a demonstrable shift wherein most pairwise sequence comparisons of variability from samples earlier on fell under or topped around 10% to now peaking well over 10% in more recent sampling.

Aside from the circulation of wild-type strains in the field, global type 2 genetic diversity has also been shaped by the use of modified live vaccines that are still capable of replication in hosts and with the possibility of reversion to virulence based on past accounts (Nielsen et al., 2001; Opriessnig et al., 2002). However, due to the high similarity between vaccine and parental strains, it can be difficult to discern whether closely-related isolates are vaccine or prototype derivatives. Nonetheless, in order to a get a conservative estimate, we looked at two sub-lineages – 5.1 (VR2332 / RespPRRS MLV) and 8.9 (JA142 / PRRS ATP). Within each sub-lineage, the genetic distance between the prototype and vaccine strains was determined and used as a cutoff for the subsequent step. Next, field isolates of each sub-lineage with a divergence from the prototype and / or vaccine being less than the cutoff were extracted (sub-lineage 5.1: n=451; sub-lineage 8.9: n=550). Counts for being wild-type or vaccine-

related were incremented depending on whether greater similarity was shared with the parental or vaccine strain. Based on the above method, of the isolates analyzed, 80% could be deemed vaccine-related in sub-lineage 5.1 while the same figure would be 60% for sub-lineage 8.9. Overall, such vaccine-associated sequences would represent approximately 5% of global type 2 viral sampling.

Diversity: looking at the "trees": The extent of genetic diversity in different countries or regions is shaped by a host of factors as mentioned earlier. Unsurprisingly, Canada and USA have a high degree of circulating diversity based on available sequence data (Figure 5a). Since the type 2 genotype is generally accepted to have originated in North America, the diversity observed in these countries is primarily the result of radiation over two decades. Within Canada, PRRSV diversity is highly skewed. Ontario and Quebec field isolates exhibit significant divergence comparable to that observed within the USA (Figure 5b). Manitoba, Saskatchewan, and Alberta have much less divergent circulating strains. Within USA, there is not a large discrepancy in terms of field diversity when looking at different regions. The median divergence, based on pairwise comparisons, in most USA regions is quite similar. Mexico bordering USA on the south habours strains with a median divergence only slightly less than that seen in the States. However, there seem to be an indication of a small share of isolates (pairwise comparisons outside the upper whisker, Figure 5b) that are significantly more diverse from the dominant field isolates.
In Europe, since type 1 PRRSV is the more prevalent genotype, the impact of type 2 PRRSV is limited. Not too much diversity is seen in Danish isolates spanning a period of over a decade. All of which have been typed to a single lineage (L5) and even speculated to have possibly been introduced by the use of a modified live vaccine typed to the same (sub-) lineage (Bøtner et al., 1997; Kvisgaard et al., 2013; Nielsen et al., 2001). Very limited sequence data is available from the rest of Europe and Russia. What limited data that is available (from Germany, Lithuania, Poland, and Slovakia) upon classification indicates several independent introduction events from different lineages (L1, L5 and L8) which explains the significant diversity seen in pairwise comparisons.

Within Asia, there is a wide spectrum in the variability of isolates within various countries. In pairwise comparisons, China and Vietnam are at the lower end. In the case of the former, the bulk of the sequence data available pertains to isolates sampled from sequential large-scale outbreaks that were closely-related (Tian et al., 2007; Zhou et al., 2011). Since these viruses spread very quickly across the country within a short span, they show little heterogeneity. Isolates from Vietnam from 2008-2012 are very similar to each other and closely-related to the "high fever" PRRS outbreaks in China (Thi Dieu Thuy et al., 2013) and represent a spillover event from there. The much larger intra-country divergence amongst isolates of other Asian nations is the result of co-circulation of isolates distinct to the region that diverged early in the evolution of type 2 and the introduction of isolates from lineages originating in North America. Thailand and South Korea have been particularly vulnerable to multiple introductions of varied non-domestic evolutionary lineages.

Implications: The primary concern in relation to the diversity of PRRSV pertains to control measures. Vaccination is one key component in this respect. It has long been recognized that available vaccines fail to provide complete protection against heterogeneous infection even amongst strains of the same genotype (Kimman et al., 2009; Labarque et al., 2004). The efficacy of a vaccine depends significantly on the degree of genetic and antigenic relatedness of the vaccine and the infecting strains. Since presently available modified live vaccines are derived from single parental strains, their ability to offer broad protection against the immense circulating diversity of type 2 PRRSV (Figure 4a) is very limited. With diversity shown to appreciate over time in proportionately prominent lineages (Figure 4b), the problem of ineffective heterologous protection will only be exacerbated. An examination of genetic distances between longitudinally stratified isolates and commercially available vaccines shows a clear pattern of widening divergence over time (Figure 4c). However, vaccines based on singular strains may prove more useful in places of limited diversity like China. Although China has suffered huge economic losses due to PRRS especially during bouts of highly pathogenic PRRSV (HP-PRRSV), the country has not experienced many introduction events of PRRSV from elsewhere unlike its neighbouring

countries. The sporadic emergence of HP-PRRSV has been from endemic diversity. In this case, vaccines modeled over local strains could prove more effective than those used in North America.

Another issue linked to vaccination is safety. The potential of reversion to virulence by modified live vaccines has been discussed already. Although, a conservative estimate of vaccine-associated sequences has been provided here, it is not possible to infer what share might exhibit virulence traits based on sequences of a single ORF and a lack of experimental data as produced elsewhere (Opriessnig et al., 2002). Presently, attempts are underway to develop vaccines using novel strategies that are safer and provide better protection (Hu and Zhang, 2012; Huang and Meng, 2010).

Comparisons of pairwise genetic distances of different regions in Canada (specifically Ontario and Quebec) and USA revealing similar divergence is partly reflective of the intensive viral flow between the countries as revealed by phylogeographic analyses (Shi et al., 2010b; Shi et al., 2013b). This continuous viral flow, especially in recent times, is gradually displacing previously endemic USA PRRSVs in many regions like the Midwest, Corn Belt, Lake States, and Northern Plains with those that originate in Canada. The largely unidirectional cross-border viral flow is supported by patterns of hog movement in which a substantial importation of Canadian growing pigs into low cost feeder sites as those in the Corn Belt and Lake States takes place (Shields and Mathews, 2003). Additionally, the robust viral traffic network within the USA (Shi et al., 2013b) enabled by the now established system of multisite swine production facilitates the rapid spread of locally emerged or introduced diversity. Collectively, this highlights the need for strengthening surveillance or screening of animals transported from one site to another to break the viral chains of transmission.

Diversity - what are we missing?: All of the insights into the continually evolving PRRSV diversity presented here are primarily based on sequence data deposited into public databases or volunteered by private holders. Despite the voluminous nature, the question of representativeness of this collection of actual field diversity does arise. Farm surveillance practices, a veterinarian's recommendations, a diagnostic lab's protocols, and disclosure policy are just some of the factors that influence if, what, how much, or when sequence data from a particular site, region, or country makes it into the public domain. Despite most of these countries being impacted by PRRS, it is quite clear that the magnitude of production (if assumed to roughly indicate the ranking of PRRS burden in those countries as well) is not directly proportional to PRRSV sequence deposition. While this subjectivity inevitably distorts the accuracy of diversity reflected, the extent of disparity between perceived and actual PRRSV diversity is difficult to ascertain. There is some indication of the consequences of this as in the case of the MN184-related outbreak wherein the sudden emergence of novel variants could not be immediately traced (Brar et al., 2011; Han et al., 2006). Although the standardization of practices pertaining to PRRSV sample collection, screening, and decision to sequence is a longer term target, the choice to release privately held sequence data, perhaps after a short moratorium if need be, would help abridge in part the problem for the time being.

The other major obstacle is the highly skewed availability of sequence data dominated by a select few ORFs or genomic regions. This hinders a systematic monitoring of PRRSV evolution at the genome level. A possible added drawback is that genetic determinants in deciphering phenotypes of interest like virulence may lie in less studied regions. It is hoped that with the advent of next generation sequencing technologies, that deliver unparalleled throughput and are bringing down cost per base, will spur the switch from looking at restricted viral regions to the entire genome as reported in some recent PRRSV studies (Brar et al., 2014; Guo et al., 2011; Kvisgaard et al., 2013; Lu et al., 2014). Moreover, the high depth at which each nucleotide is covered allows the study of intra-sample (or intra-host) viral heterogeneity with the detection of low(er) frequency variants that would traditionally escape discovery by Sanger sequencing.

6.4. Beyond PRRSV diversity

The direct link between genotype and phenotype is a fundamental cornerstone of genetics. Inductively, it would make sense to leverage the substantial amount of sequence data available to establish associations or relationships to various phenotypes - an outcome that could assist in more focused strategies of PRRSV control or neutralization. Key to this process is the availability of at least basic, if not rich, descriptive metadata accompanying each sequence deposited into any public database. To explore the practicality of this at present, we downloaded all nucleotide submissions pertaining to PRRSV (excluding entries related to patents) in GenBank [largest public database of PRRSV sequences as of April, 2014]. A total of 16,143 sequences (UTRs, ORFs, partial and complete genomes) were obtained. Of these, 16% did not have any information on country or region of isolation and 22% did not have any indication of isolation date (not even a year), basic information critical to the study of PRRSV evolution. Next, attempts were made to ascertain what proportion of sequence data had matching phenotypic information available. Since such metadata is not fed into GenBank directly, the source for phenotypic data is limited to GenBank submissions linked to published works which, in this case, accounted for only 25% of all submissions. Of these, in turn, most works on characterizing select ORFs or complete genomes were largely devoid of phenotypic traits of the reported isolate(s) or used subjective descriptors like "highly pathogenic" or "moderately pathogenic" without any qualifying statements on quantitative measures of morbidity, mortality, or immunological aspects.

While works wherein the genetic makeup of isolates was studied in relation to the variability in different phenotypes have certainly been carried out (Brockmeier et al., 2012; Diaz et al., 2006; Gauger et al., 2012; Kim et al., 2007; Lee et al., 2004; Li et al., 2010; Martínez-Lobo et al., 2011a; Martínez-Lobo et al., 2011b; Mateu, 2011; Yu et al., 2013), sequence data from such studies forms only a small fraction of that found in GenBank. Moreover, since the phenotypes examined can vary among genotype-phenotype studies, comparisons between isolates can still be cumbersome. Progress in elucidating genetic determinants of phenotypes of interest like virulence, immunological response, and clinical presentation would be assisted by the coming into existence of a public database dedicated to housing PRRSV related sequences along with pertinent phenotypic metadata supplied by submitters. The buildup of such a resource would enable the design of more focused experiments involving chimeric infectious clones and/or site-directed mutagenesis to examine regions of difference potentially linked to phenotypic outcomes and not just the result of genetic drift and/or random mutation.

6.5. Concluding remarks

The initial cross-over of PRRSV as a novel swine disease may have been an entirely natural phenomenon. However, the study of PRRS over the past two and a half decades has revealed the establishment, expansion in diversity, and geographic spread of PRRSV has been anything but a solely natural phenomenon. Instead, these aspects have been largely shaped by modern standards of multisite swine production, breeding practices, extensive transportation, international trade in live animals, and management and control practices (vaccination, air filtration, etc) which, in one way or another, proved advantageous to the intrinsic ability of PRRSV to generate and promulgate diversification. In order for management and control strategies to keep pace with rapidly evolving virus, it is important to keep abreast of field diversity and to do so using high resolution tools like phylogenetics rather than less informative or established methods. At the same time, tools allowing such analyses in an easy and simple manner are also needed so that data analysis is integrated for public sequence deposition and downloading along with phenotypic metadata. Such resources could help abridge the disparity between known and actual diversity as well as serve as informative starting points in designing better focused lab experiments in the context of genotype-phenotype hypotheses and vaccination design.

7. GENERAL DISCUSSION

Since its emergence, PRRSV has been a major pathogen damaging the US pork industry for more than 2 and a half decades. Two commercial vaccines are available in the US market, but their protective efficacies are less

than satisfactory. Importantly, the virus continues to evolve and causes economic losses. A large body of information is available for PRRS through research and as of June 2014, more than 2,000 research articles have been published. We gain a tremendous understanding about the disease through research. Based on those research citations, a comprehensive literature review has been prepared on the virus. PRRSV-associated ongoing problems are two fold: one for the virus and the other for the host.

On the virus, key issues include antigenic heterogeneity, rapid evolution and quasi-species nature, viral persistence, and viral capacity to modulate host immunity. These topics have been covered and reviewed in this report.

On the host, inadequate innate immunity, poor induction of adaptive response, and host genetics are of concern. Of these concerns, both innate and adaptive immunities play key roles for protection and clearance from infection. However, both innate and adaptive responses of host are affected by antigenicity and immune modulatory function of PRRSV, and thus these factors lead us back to the biological function of the virus. By studying the viral proteins, much of these concerns may be resolved.

The following research gaps have been identified from the review. The identified issues may be utilized as guides for future research emphases and prioritization of funding.

1) Viral evasion of host innate immunity: Type I Interferons (IFNs) are the most potent antiviral cytokines required for both innate immunity and adaptive response, and PRRSV suppresses the IFN production in pigs. This is probably the most important mechanism as to how PRRSV survives in pigs and persists for a long time. At least 5 viral proteins have been identified as IFN antagonist for PRRSV, and their mechanisms of action for suppression have been studied to some extent. A better understanding on the viral strategy for immune modulation and viral evasion from host immune system is cricual. Balancing in vitro and in vivo analyses will be needed to affirm each effect. Molecular mapping of functional sites on each viral protein is necceary so that such functions can be removed from the virus.

2) Removal of immune suppressive function from PRRSV and application to vaccine development: All RNA viruses appear to have a capavity to suppress IFN production of host during infection. Dissecting viral immune modulation and applying the information to vaccine development are current research trends in all animal and human virology. This approach has a great potential for developing a new vaccine candidate for PRRS. IFN suppression is a hallmark during PRRSV infection and this research area should be expanded to identify specific residues and motifs on viral proteins. The immune suppressive function may be removed from PRRSV using infectious clones, and such knockout viruses may be examined in pigs for virulence, attenuation, immunogenecity, protection, and viral persistence to evaluate as a vaccine candidate.

3) Viral receptor for cell entry: The receptor studies for PRRSV have been confusing and controvertial. Of 6 cellular proteins identified as putative receptors, CD163 and sialoadhesin are two molecules most extensively studied. We suggest CD163 as the likely receptor for PRRSV, at least for the North American genotype, if not for both genotypes. Studies for interaction of PRRSV proteins and CD163 will provide insights into identification and stucture of neutralizing epitopes. Suceptibility and permissiveness studies for PRRSV in cells expressing CD163 from other animal species may provide some insights into possible future cross-species infection of PRRSV. Production of more well-characterized swine cell lines that support PRRSV replication would provide an important tool for thesse studies.

4) Effective utilization of infectious clones: Infectious clones are the most powerful molecular tool to study viruses in modern virology. It took more than 5 years to develop an infectious clone for PRRSV by at least 5 independent researchers at one time point. As a result, more than 10 infectious clones are now available for North American type II PRRSV (Table 1). These clones however are mostly under-utilized, and researchers

should be encouraged to use this tool more effectively and efficiently to study PRRSV replication, diversification and vaccination strategies..

5) Identification of viral proteins inducing neutralizing antibodies: It is still uncertain which viral proteins are able to induce neutralizing antibodies in pigs, and to our knowledge, no single neutralizng monoclonal antibody is available for PRRSV. This hampers PRRSV reseach greatly.

6) Biological function of viral structural proteins and non-structural proteins: When virus infects the host, every response of the host is caused by individual proteins of the infecting viruses. Thus, biological functions of each viral protein determine the celluar processes and consequences of infection. For each PRRSV protein, very little is known for their biological function other than their structural function for particle assemly. As an example, PRRSV replicates in the cytoplasm of the cell, but at least 3 viral proteins are specifically localized in the nucleus, implicating important regulatory function of those protein in the nucleus. ORF5a is a newly identified viral protein but its function is unknown. PRRSV structural proteins are poorly understood for their cellular function, and the viral proteins are needed to be studied for better understandning of the virus.

7) Probe viral RNA polymerase to understand the high frequency of infidelity and mutation rates: PRRSV undergoes extremly high rates of mutations during its RNA synthesis, which contributes to rapid evolution of the virus. For influenza virus, antigenic drift is a major reason for sequence variation and heterogeneity, and viral RNA polymerases have been determined as virulence factors. No such study has been conducted for PRRSV. For PRRSV, the viral RNA synthesis is largely catalysed by nsps released from pp1b including RNA polymerase, helicase, NendoU RNase, and nsp12, and the understanding of such enzyme activities for RNA fidelity may help us understand unsually high rates of mutations and antigenic heterogeneity of PRRSV.

8. ACKNOWLEDGMENTS

This work was supported by funding from National Pork Board (NPB 13-245) and Agriculture and Food Research Initiative (AFRI) (2013-67015-21243) of the US Department of Agriculture-National Institute of Food and Agriculture (USDA-NIFA). The authors thank Mingyuan Han and Qingzhan Zhang for their writing assistance and Joan Lunney at USDA BARC for proofing the final draft.

9. REFERENCES:

1. Aasted, B., Bach, P., Nielsen, J., Lind, P., 2002. Cytokine profiles in peripheral blood mononuclear cells and lymph node cells from piglets infected in utero with porcine reproductive and respiratory syndrome virus. Clinical and diagnostic laboratory immunology 9(6), 1229-1234.
2. Ait-Ali, T., Wilson, A.D., Westcott, D.G., Clapperton, M., Waterfall, M., Mellencamp, M.A., Drew, T.W., Bishop, S.C., Archibald, A.L., 2007. Innate immune responses to replication of porcine reproductive and respiratory syndrome virus in isolated Swine alveolar macrophages. Viral immunology 20(1), 105-118.
3. Akila, P., Prashant, V., Suma, M.N., Prashant, S.N., Chaitra, T.R., 2012. CD163 and its expanding functional repertoire. Clinica chimica acta; international journal of clinical chemistry 413(7-8), 669-674.
4. Albina, E., Carrat, C., Charley, B., 1998. Interferon-alpha response to swine arterivirus (PoAV), the porcine reproductive and respiratory syndrome virus. J Interf Cytok Res 18(7), 485-490.
5. Allende, R., Lewis, T.L., Lu, Z., Rock, D.L., Kutish, G.F., Ali, A., Doster, A.R., Osorio, F.A., 1999. North American and European porcine reproductive and respiratory syndrome viruses differ in non-structural protein coding regions. Journal of General Virology 80, 307-315.
6. Almazan, F., Gonzalez, J.M., Penzes, Z., Izeta, A., Calvo, E., Plana-Duran, J., Enjuanes, L., 2000. Engineering the largest RNA virus genome as an infectious bacterial artificial chromosome. P Natl Acad Sci USA 97, 5516-5521.
7. Ammann, C.G., Messer, R.J., Peterson, K.E., Hasenkrug, K.J., 2009. Lactate dehydrogenase-elevating virus induces systemic lymphocyte activation via TLR7-dependent IFNalpha responses by plasmacytoid dendritic cells. PloS one 4(7), e6105.
8. An, T.Q., Tian, Z.J., He, Y.X., Xiao, Y., Jiang, Y.F., Peng, J.M., Zhou, Y.J., Liu, D., Tong, G.Z., 2010. Porcine reproductive and respiratory syndrome virus attachment is mediated by the N-terminal domain of the sialoadhesin receptor. Veterinary microbiology 143(2-4), 371-378.
9. Anderson, G.W., Rowland, R.R.R., Palmer, G.A., Even, C., Plagemann, P.G.W., 1995. Lactate Dehydrogenase-Elevating Virus-Replication Persists in Liver, Spleen, Lymph-Node, and Testis Tissues and Results in Accumulation of Viral-Rna in Germinal-Centers, Concomitant with Polyclonal Activation of B-Cells. J Virol 69(8), 5177-5185.
10. Ansari, I.H., Kwon, B., Osorio, F.A., Pattnaik, A.K., 2006. Influence of N-linked glycosylation of porcine reproductive and respiratory syndrome virus GP5 on virus infectivity, antigenicity, and ability to induce neutralizing antibodies. J Virol 80, 3994-4004.
11. Areschoug, T., Gordon, S., 2009. Scavenger receptors: role in innate immunity and microbial pathogenesis. Cellular microbiology 11(8), 1160-1169.
12. Baccala, R., Hoebe, K., Kono, D.H., Beutler, B., Theofilopoulos, A.N., 2007. TLR-dependent and TLR-independent pathways of type I interferon induction in systemic autoimmunity. Nat Med 13(5), 543-551.
13. Balasuriya, U.B.R., Dobbe, J.C., Heidner, H.W., Smalley, V.L., Navarrette, A., Snijder, E.J., MacLachlan, N.J., 2004. Characterization of the neutralization determinants of equine arteritis virus using recombinant chimeric viruses and site-specific mutagenesis of an infectious cDNA clone (vol 321, pg 235, 2004). Virology 327, 318-319.
14. Batista, L., Pijoan, C., Lwamba, H., Johnson, C.R., Murtaugh, M.P., 2004. Genetic diversity and possible avenues of dissemination of porcine reproductive and respiratory syndrome virus in two geographic regions of Mexico. Journal of swine health and production 12, 170-175.
15. Baumann, A., Mateu, E., Murtaugh, M.P., Summerfield, A., 2013. Impact of genotype 1 and 2 of porcine reproductive and respiratory syndrome viruses on interferon-alpha responses by plasmacytoid dendritic cells. Veterinary research 44.
16. Bautista, E.M., Goyal, S.M., Yoon, I.J., Joo, H.S., Collins, J.E., 1993. Comparison of porcine alveolar macrophages and CL 2621 for the detection of porcine reproductive and respiratory syndrome (PRRS) virus and anti-PRRS antibody. Journal of veterinary diagnostic investigation : official publication of the American Association of Veterinary Laboratory Diagnosticians, Inc 5(2), 163-165.

17. Benfield, D.A., Nelson, E., Collins, J.E., Harris, L., Goyal, S.M., Robison, D., Christianson, W.T., Morrison, R.B., Gorcyca, D., Chladek, D., 1992. Characterization of swine infertility and respiratory syndrome (SIRS) virus (isolate ATCC VR-2332). Journal of veterinary diagnostic investigation : official publication of the American Association of Veterinary Laboratory Diagnosticians, Inc 4(2), 127-133.
18. Beura, L.K., Sarkar, S.N., Kwon, B., Subramaniam, S., Jones, C., Pattnaik, A.K., Osorio, F.A., 2010. Porcine reproductive and respiratory syndrome virus nonstructural protein 1beta modulates host innate immune response by antagonizing IRF3 activation. J Virol 84, 1574-1584.
19. Beura, L.K., Subramaniam, S., Vu, H.L., Kwon, B., Pattnaik, A.K., Osorio, F.A., 2012. Identification of amino acid residues important for anti-IFN activity of porcine reproductive and respiratory syndrome virus non-structural protein 1. Virology 433(2), 431-439.
20. Bøtner, A., Strandbygaard, B., Sørensen, K., Have, P., Madsen, K., Madsen, E.S., Alexandersen, S., 1997. Appearance of acute PRRS-like symptoms in sow herds after vaccination with a modified live PRRS vaccine. Veterinary Record 141, 497-499.
21. Boyer, J.C., Haenni, A.L., 1994. Infectious Transcripts and Cdna Clones of Rna Viruses. Virology 198, 415-426.
22. Bramel-Verheije, M.H.G., Rottier, P.J.M., Meulenberg, J.J.M., 2000. Expression of a foreign epitope by porcine reproductive and respiratory syndrome virus. Virology 278, 380-389.
23. Brar, M.S., Shi, M., Ge, L., Carman, S., Murtaugh, M.P., Leung, F.C.-C., 2011. Porcine reproductive and respiratory syndrome virus in Ontario, Canada 1999 to 2010: genetic diversity and restriction fragment length polymorphisms. Journal of general virology 92, 1391-1397.
24. Brar, M.S., Shi, M., Hui, R.K.-H., Leung, F.C.-C., 2014. Genomic Evolution of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) Isolates Revealed by Deep Sequencing. PLoS ONE 9, e88807.
25. Brockmeier, S.L., Loving, C.L., Vorwald, A.C., Kehrli Jr, M.E., Baker, R.B., Nicholson, T.L., Lager, K.M., Miller, L.C., Faaberg, K.S., 2012. Genomic sequence and virulence comparison of four Type 2 porcine reproductive and respiratory syndrome virus strains. Virus research 169, 212-221.
26. Buddaert, W., Van Reeth, K., Pensaert, M., 1998. In vivo and in vitro interferon (IFN) studies with the porcine reproductive and respiratory syndrome virus (PRRSV). Coronaviruses and Arteriviruses 440, 461-467.
27. Calvert, J.G., Slade, D.E., Shields, S.L., Jolie, R., Mannan, R.M., Ankenbauer, R.G., Welch, S.K., 2007. CD163 expression confers susceptibility to porcine reproductive and respiratory syndrome viruses. Journal of virology 81(14), 7371-7379.
28. Calzada-Nova, G., Schnitzlein, W.M., Husmann, R.J., Zuckermann, F.A., 2011. North American Porcine Reproductive and Respiratory Syndrome Viruses Inhibit Type I Interferon Production by Plasmacytoid Dendritic Cells. J Virol 85(6), 2703-2713.
29. Carman, S., Sanford, S., Dea, S., 1995. Assessment of seropositivity to porcine reproductive and respiratory syndrome (PRRS) virus in swine herds in Ontario--1978 to 1982. The Canadian Veterinary Journal 36, 776.
30. Chand, R.J., Trible, B.R., Rowland, R.R.R., 2012. Pathogenesis of porcine reproductive and respiratory syndrome virus. Curr Opin Virol 2, 256-263.
31. Chang, H.C., Peng, Y.T., Chang, H.L., Chaung, H.C., Chung, W.B., 2008. Phenotypic and functional modulation of bone marrow-derived dendritic cells by porcine reproductive and respiratory syndrome virus. Veterinary microbiology 129(3-4), 281-293.
32. Chaung, H.C., Chen, C.W., Hsieh, B.L., Chung, W.B., 2010. Toll-Like Receptor expressions in porcine alveolar macrophages and Dendritic Cells in responding to poly IC stimulation and porcine reproductive and respiratory syndrome virus (PRRSV) infection. Comparative immunology, microbiology and infectious diseases 33(3), 197-213.
33. Chen, J.P., Strauss, J.H., Strauss, E.G., Frey, T.K., 1996. Characterization of the rubella virus nonstructural protease domain and its cleavage site. J Virol 70(7), 4707-4713.
34. Chen, Y., Guo, R., He, S., Zhang, X., Xia, X., Sun, H., 2014. Additive inhibition of porcine reproductive and respiratory syndrome virus infection with the soluble sialoadhesin and CD163 receptors. Virus research 179, 85-92.

35. Chen, Z., Lawson, S., Sun, Z., Zhou, X., Guan, X., Christopher-Hennings, J., Nelson, E.A., Fang, Y., 2010a. Identification of two auto-cleavage products of nonstructural protein 1 (nsp1) in porcine reproductive and respiratory syndrome virus infected cells: nsp1 function as interferon antagonist. Virology 398, 87-97.
36. Chen, Z.B., Wang, Y.H., Ratia, K., Mesecar, A.D., Wilkinson, K.D., Baker, S.C., 2007. Proteolytic processing and deubiquitinating activity of papain-like proteases of human coronavirus NL63. J Virol 81(11), 6007-6018.
37. Chen, Z.H., Zhou, X.X., Lunney, J.K., Lawson, S., Sun, Z., Brown, E., Christopher-Hennings, J., Knudsen, D., Nelson, E., Fang, Y., 2010b. Immunodominant epitopes in nsp2 of porcine reproductive and respiratory syndrome virus are dispensable for replication, but play an important role in modulation of the host immune response. J Gen Virol 91, 1047-1057.
38. Choi, C., Cho, W.S., Kim, B., Chae, C., 2002. Expression of Interferon-gamma and tumour necrosis factor-alpha in pigs experimentally infected with Porcine Reproductive and Respiratory Syndrome Virus (PRRSV). Journal of comparative pathology 127(2-3), 106-113.
39. Clark, G.J., Angel, N., Kato, M., Lopez, J.A., MacDonald, K., Vuckovic, S., Hart, D.N., 2000. The role of dendritic cells in the innate immune system. Microbes and infection / Institut Pasteur 2(3), 257-272.
40. Clementz, M.A., Chen, Z.B., Banach, B.S., Wang, Y.H., Sun, L., Ratia, K., Baez-Santos, Y.M., Wang, J., Takayama, J., Ghosh, A.K., Li, K., Mesecar, A.D., Baker, S.C., 2010. Deubiquitinating and Interferon Antagonism Activities of Coronavirus Papain-Like Proteases. J Virol 84(9), 4619-4629.
41. Coleman, J.R., Papamichail, D., Skiena, S., Futcher, B., Wimmer, E., Mueller, S., 2008. Virus attenuation by genome-scale changes in codon pair bias. Science 320, 1784-1787.
42. Conti, P., Kempuraj, D., Kandere, K., Di Gioacchino, M., Barbacane, R.C., Castellani, M.L., Felaco, M., Boucher, W., Letourneau, R., Theoharides, T.C., 2003. IL-10, an inflammatory/inhibitory cytokine, but not always. Immunology letters 86(2), 123-129.
43. Conzelmann, K.-K., Visser, N., Van Woensel, P., Thiel, H.-J., 1993. Molecular characterization of porcine reproductive and respiratory syndrome virus, a member of the arterivirus group. Virology 193, 329-339.
44. Crocker, P.R., Gordon, S., 1986. Properties and distribution of a lectin-like hemagglutinin differentially expressed by murine stromal tissue macrophages. The Journal of experimental medicine 164(6), 1862-1875.
45. Crocker, P.R., Hartnell, A., Munday, J., Nath, D., 1997. The potential role of sialoadhesin as a macrophage recognition molecule in health and disease. Glycoconjugate journal 14(5), 601-609.
46. Darwich, L., Diaz, I., Mateu, E., 2010. Certainties, doubts and hypotheses in porcine reproductive and respiratory syndrome virus immunobiology. Virus Res 154(1-2), 123-132.
47. 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. Journal of virology 84(4), 1731-1740.
48. Das, P.B., Vu, H.L., Dinh, P.X., Cooney, J.L., Kwon, B., Osorio, F.A., Pattnaik, A.K., 2011. Glycosylation of minor envelope glycoproteins of porcine reproductive and respiratory syndrome virus in infectious virus recovery, receptor interaction, and immune response. Virology 410(2), 385-394.
49. De Baere, M.I., Van Gorp, H., Delputte, P.L., Nauwynck, H.J., 2012. Interaction of the European genotype porcine reproductive and respiratory syndrome virus (PRRSV) with sialoadhesin (CD169/Siglec-1) inhibits alveolar macrophage phagocytosis. Veterinary research 43, 47.
50. de Lima, M., Pattnaik, A.K., Flores, E.F., Osorio, F.A., 2006. Serologic marker candidates identified among B-cell linear epitopes of Nsp2 and structural proteins of a North American strain of porcine reproductive and respiratory syndrome virus. Virology 353, 410-421.
51. de Vries, A.A., Glaser, A.L., Raamsman, M.J., de Haan, C.A., Sarnataro, S., Godeke, G.J., Rottier, P.J., 2000. Genetic manipulation of equine arteritis virus using full-length cDNA clones: separation of overlapping genes and expression of a foreign epitope. Virology 270, 84-97.
52. Dea, S., Bilodeau, R., Athanaseous, R., Sauvageau, R.A., Martineau, G.P., 1992. PRRS syndrome in Quebec: isolation of a virus serologically related to Lelystad virus. The Veterinary record 130(8), 167.

53. Dea, S., Gagnon, C.A., Mardassi, H., Pirzadeh, B., Rogan, D., 2000. Current knowledge on the structural proteins of porcine reproductive and respiratory syndrome (PRRS) virus: comparison of the North American and European isolates. Arch Virol 145, 659-688.
54. Delputte, P.L., Costers, S., Nauwynck, H.J., 2005. Analysis of porcine reproductive and respiratory syndrome virus attachment and internalization: distinctive roles for heparan sulphate and sialoadhesin. The Journal of general virology 86(Pt 5), 1441-1445.
55. Delputte, P.L., Nauwynck, H.J., 2004. Porcine arterivirus infection of alveolar macrophages is mediated by sialic acid on the virus. Journal of virology 78(15), 8094-8101.
56. Delputte, P.L., Van Breedam, W., Barbe, F., Van Reeth, K., Nauwynck, H.J., 2007a. IFN-alpha treatment enhances porcine Arterivirus infection of monocytes via upregulation of the porcine Arterivirus receptor sialoadhesin. Journal of interferon & cytokine research : the official journal of the International Society for Interferon and Cytokine Research 27(9), 757-766.
57. Delputte, P.L., Van Breedam, W., Delrue, I., Oetke, C., Crocker, P.R., Nauwynck, H.J., 2007b. Porcine arterivirus attachment to the macrophage-specific receptor sialoadhesin is dependent on the sialic acid-binding activity of the N-terminal immunoglobulin domain of sialoadhesin. Journal of virology 81(17), 9546-9550.
58. Delputte, P.L., Vanderheijden, N., Nauwynck, H.J., Pensaert, M.B., 2002. Involvement of the matrix protein in attachment of porcine reproductive and respiratory syndrome virus to a heparinlike receptor on porcine alveolar macrophages. Journal of virology 76(9), 4312-4320.
59. Delrue, I., Van Gorp, H., Van Doorsselaere, J., Delputte, P.L., Nauwynck, H.J., 2010. Susceptible cell lines for the production of porcine reproductive and respiratory syndrome virus by stable transfection of sialoadhesin and CD163. BMC biotechnology 10, 48.
60. den Boon, J.A., Faaberg, K.S., Meulenberg, J.J., Wassenaar, A.L., Plagemann, P.G., Gorbalenya, A.E., Snijder, E.J., 1995. Processing and evolution of the N-terminal region of the arterivirus replicase ORF1a protein: identification of two papainlike cysteine proteases. J Virol 69(7), 4500-4505.
61. den Boon, J.A., Snijder, E.J., Chirnside, E.D., de Vries, A.A., Horzinek, M.C., Spaan, W.J., 1991. Equine arteritis virus is not a togavirus but belongs to the coronaviruslike superfamily. J Virol 65(6), 2910-2920.
62. Denboon, J.A., Faaberg, K.S., Meulenberg, J.J.M., Wassenaar, A.L.M., Plagemann, P.G.W., Gorbalenya, A.E., Snijder, E.J., 1995. Processing and Evolution of the N-Terminal Region of the Arterivirus Replicase Orf1a Protein - Identification of 2 Papainlike Cysteine Proteases. J Virol 69, 4500-4505.
63. Denboon, J.A., Snijder, E.J., Chirnside, E.D., Devries, A.A.F., Horzinek, M.C., Spaan, W.J.M., 1991. Equine Arteritis Virus Is Not a Togavirus but Belongs to the Coronaviruslike Superfamily. J Virol 65, 2910-2920.
64. 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.
65. Dijkstra, C.D., Dopp, E.A., Joling, P., Kraal, G., 1985. The heterogeneity of mononuclear phagocytes in lymphoid organs: distinct macrophage subpopulations in the rat recognized by monoclonal antibodies ED1, ED2 and ED3. Immunology 54(3), 589-599.
66. Doan, D.N., Dokland, T., 2003. Structure of the nucleocapsid protein of porcine reproductive and respiratory syndrome virus. Structure 11, 1445-1451.
67. Dobbe, J.C., van der Meer, Y., Spaan, W.J.M., Snijder, E.J., 2001. Construction of chimeric arteriviruses reveals that the ectodomain of the major glycoprotein is not the main determinant of equine arteritis virus tropism in cell culture. Virology 288, 283-294.
68. Drake, J.W., 1993. Rates of spontaneous mutation among RNA viruses. Proceedings of the National Academy of Sciences 90, 4171-4175.
69. Du, Y., Pattnaik, A.K., Song, C., Yoo, D., Li, G., 2012. Glycosyl-phosphatidylinositol (GPI)-anchored membrane association of the porcine reproductive and respiratory syndrome virus GP4 glycoprotein and its co-localization with CD163 in lipid rafts. Virology 424(1), 18-32.

70. Duan, X., Nauwynck, H.J., Favoreel, H.W., Pensaert, M.B., 1998. Identification of a putative receptor for porcine reproductive and respiratory syndrome virus on porcine alveolar macrophages. Journal of virology 72(5), 4520-4523.
71. Duan, X., Nauwynck, H.J., Pensaert, M.B., 1997. Effects of origin and state of differentiation and activation of monocytes/macrophages on their susceptibility to porcine reproductive and respiratory syndrome virus (PRRSV). Archives of virology 142(12), 2483-2497.
72. Dunowska, M., Biggs, P.J., Zheng, T., Perrott, M.R., 2012. Identification of a novel nidovirus associated with a neurological disease of the Australian brushtail possum (Trichosurus vulpecula). Veterinary microbiology 156(3-4), 418-424.
73. Eigen, M., Schuster, P., 1977. A principle of natural self-organization. Naturwissenschaften 64, 541-565.
74. Fabriek, B.O., van Bruggen, R., Deng, D.M., Ligtenberg, A.J., Nazmi, K., Schornagel, K., Vloet, R.P., Dijkstra, C.D., van den Berg, T.K., 2009. The macrophage scavenger receptor CD163 functions as an innate immune sensor for bacteria. Blood 113(4), 887-892.
75. Fang, Y., Christopher-Hennings, J., Brown, E., Liu, H., Chen, Z., Lawson, S.R., Breen, R., Clement, T., Gao, X., Bao, J., Knudsen, D., Daly, R., Nelson, E., 2008. Development of genetic markers in the non-structural protein 2 region of a US type 1 porcine reproductive and respiratory syndrome virus: implications for future recombinant marker vaccine development. J Gen Virol 89, 3086-3096.
76. Fang, Y., Faaberg, K.S., Rowland, R.R., Christopher-Hennings, J., Pattnaik, A.K., Osorio, F., Nelson, E.A., 2006a. Construction of a full-length cDNA infectious clone of a European-like Type 1 PRRSV isolated in the U.S. Advances in experimental medicine and biology 581, 605-608.
77. Fang, Y., Kim, D.Y., Ropp, S., Steen, P., Christopher-Hennings, J., Nelson, E.A., Rowland, R.R., 2004. Heterogeneity in Nsp2 of European-like porcine reproductive and respiratory syndrome viruses isolated in the United States. Virus Res 100, 229-235.
78. Fang, Y., Rowland, R.R., Roof, M., Lunney, J.K., Christopher-Hennings, J., Nelson, E.A., 2006b. A full-length cDNA infectious clone of North American type 1 porcine reproductive and respiratory syndrome virus: expression of green fluorescent protein in the Nsp2 region. J Virol 80, 11447-11455.
79. Fang, Y., Schneider, P., Zhang, W., Faaberg, K., Nelson, E., Rowland, R., 2007. Diversity and evolution of a newly emerged North American Type 1 porcine arterivirus: analysis of isolates collected between 1999 and 2004. Archives of virology 152, 1009-1017.
80. Fang, Y., Snijder, E.J., 2010. The PRRSV replicase: exploring the multifunctionality of an intriguing set of nonstructural proteins. Virus Res 154, 61-76.
81. Fang, Y., Treffers, E.E., Li, Y.H., Tas, A., Sun, Z., van der Meer, Y., de Ru, A.H., van Veelen, P.A., Atkins, J.F., Snijder, E.J., Firth, A.E., 2012. Efficient-2 frameshifting by mammalian ribosomes to synthesize an additional arterivirus protein. P Natl Acad Sci USA 109, E2920-E2928.
82. Feng, L., Zhang, X., Xia, X., Li, Y., He, S., Sun, H., 2013. Generation and characterization of a porcine endometrial endothelial cell line susceptible to porcine reproductive and respiratory syndrome virus. Virus research 171(1), 209-215.
83. Feng, W.H., Tompkins, M.B., Xu, J.S., Zhang, H.X., McCaw, M.B., 2003. Analysis of constitutive cytokine expression by pigs infected in-utero with porcine reproductive and respiratory syndrome virus. Veterinary immunology and immunopathology 94(1-2), 35-45.
84. Firth, A.E., Zevenhoven-Dobbe, J.C., Wills, N.M., Go, Y.Y., Balasuriya, U.B.R., Atkins, J.F., Snijder, E.J., Posthuma, C.C., 2011. Discovery of a small arterivirus gene that overlaps the GP5 coding sequence and is important for virus production. J Gen Virol 92, 1097-1106.
85. Fitter, S., Sincock, P.M., Jolliffe, C.N., Ashman, L.K., 1999. Transmembrane 4 superfamily protein CD151 (PETA-3) associates with beta 1 and alpha IIb beta 3 integrins in haemopoietic cell lines and modulates cell-cell adhesion. The Biochemical journal 338 (Pt 1), 61-70.
86. Forsberg, R., Storgaard, T., Nielsen, H.S., Oleksiewicz, M.B., Cordioli, P., Sala, G., Hein, J., Bøtner, A., 2002. The genetic diversity of European type PRRSV is similar to that of the North American type but is geographically skewed within Europe. Virology 299, 38-47.

87. Fraser, I.P., Gordon, S., 1994. Murine erythroleukemia (MEL) cells bear ligands for the sialoadhesin and erythroblast receptor macrophage hemagglutinins. European journal of cell biology 64(2), 217-221.
88. Frias-Staheli, N., Giannakopoulos, N.V., Kikkert, M., Taylor, S.L., Bridgen, A., Paragas, J., Richt, J.A., Rowland, R.R., Schmaljohn, C.S., Lenschow, D.J., Snijder, E.J., Garcia-Sastre, A., Virgin, H.W., 2007. Ovarian tumor domain-containing viral proteases evade ubiquitin- and ISG15-dependent innate immune responses. Cell Host Microbe 2, 404-416.
89. Frings, W., Dreier, J., Sorg, C., 2002. Only the soluble form of the scavenger receptor CD163 acts inhibitory on phorbol ester-activated T-lymphocytes, whereas membrane-bound protein has no effect. FEBS letters 526(1-3), 93-96.
90. Frydas, I.S., Verbeeck, M., Cao, J., Nauwynck, H.J., 2013. Replication characteristics of porcine reproductive and respiratory syndrome virus (PRRSV) European subtype 1 (Lelystad) and subtype 3 (Lena) strains in nasal mucosa and cells of the monocytic lineage: indications for the use of new receptors of PRRSV (Lena). Veterinary research 44, 73.
91. Gao, F., Lu, J.Q., Yao, H.C., Wei, Z.Z., Yang, Q., Yuan, S.S., 2012. Cis-acting structural element in 5 ' UTR is essential for infectivity of porcine reproductive and respiratory syndrome virus. Virus Res 163, 108-119.
92. Gao, F., Yao, H., Lu, J., Wei, Z., Zheng, H., Zhuang, J., Tong, G., Yuan, S., 2013. Replacement of the heterologous 5' untranslated region allows preservation of the fully functional activities of type 2 porcine reproductive and respiratory syndrome virus. Virology 439, 1-12.
93. Gao, L., Guo, X.K., Wang, L., Zhang, Q., Li, N., Chen, X.X., Wang, Y., Feng, W.H., 2013. MicroRNA 181 suppresses porcine reproductive and respiratory syndrome virus (PRRSV) infection by targeting PRRSV receptor CD163. Journal of virology 87(15), 8808-8812.
94. Gao, Z.Q., Guo, X., Yang, H.C., 2004. Genomic characterization of two Chinese isolates of porcine respiratory and reproductive syndrome virus. Arch Virol 149, 1341-1351.
95. Gauger, P.C., Faaberg, K.S., Guo, B., Kappes, M.A., Opriessnig, T., 2012. Genetic and phenotypic characterization of a 2006 United States porcine reproductive and respiratory virus isolate associated with high morbidity and mortality in the field. Virus research 163, 98-107.
96. Genini, S., Delputte, P.L., Malinverni, R., Cecere, M., Stella, A., Nauwynck, H.J., Giuffra, E., 2008. Genome-wide transcriptional response of primary alveolar macrophages following infection with porcine reproductive and respiratory syndrome virus. The Journal of general virology 89(Pt 10), 2550-2564.
97. Gimeno, M., Darwich, L., Diaz, I., de la Torre, E., Pujols, J., Martin, M., Inumaru, S., Cano, E., Domingo, M., Montoya, M., Mateu, E., 2011. Cytokine profiles and phenotype regulation of antigen presenting cells by genotype-I porcine reproductive and respiratory syndrome virus isolates. Veterinary research 42.
98. Go,Y.Y., Li, Y., Chen, Z., Han, M., Yoo, D., Fang, Y., Balasuriya, U.B.R., 2014. Equine arteritis virus does not induce interferon production in equine endothelial cells: identification of nonstructural protein 1 as a main interferon antagonist. Biomed Res Int.
99. Goldberg, T.L., Hahn, E.C., Weigel, R.M., Scherba, G., 2000. Genetic, geographical and temporal variation of porcine reproductive and respiratory syndrome virus in Illinois. Journal of General Virology 81, 171-179.
100. Goldberg, T.L., Lowe, J.F., Milburn, S.M., Firkins, L.D., 2003. Quasispecies variation of porcine reproductive and respiratory syndrome virus during natural infection☆. Virology 317, 197-207.
101. Gorbalenya, A.E., Koonin, E.V., Donchenko, A.P., Blinov, V.M., 1989. Coronavirus genome: prediction of putative functional domains in the non-structural polyprotein by comparative amino acid sequence analysis. Nucleic acids research 17(12), 4847-4861.
102. Gorbalenya, A.E., Koonin, E.V., Lai, M.M., 1991. Putative papain-related thiol proteases of positive-strand RNA viruses. Identification of rubi- and aphthovirus proteases and delineation of a novel conserved domain associated with proteases of rubi-, alpha- and coronaviruses. FEBS letters 288(1-2), 201-205.
103. Graversen, J.H., Madsen, M., Moestrup, S.K., 2002. CD163: a signal receptor scavenging haptoglobin-hemoglobin complexes from plasma. The international journal of biochemistry & cell biology 34(4), 309-314.

104. Guarne, A., Hampoelz, B., Glaser, W., Carpena, X., Tormo, J., Fita, I., Skern, T., 2000. Structural and biochemical features distinguish the foot-and-mouth disease virus leader proteinase from other papain-like enzymes. Journal of molecular biology 302(5), 1227-1240.
105. Guarne, A., Tormo, J., Kirchweger, R., Pfistermueller, D., Fita, I., Skern, T., 1998. Structure of the foot-and-mouth disease virus leader protease: a papain-like fold adapted for self-processing and eIF4G recognition. The EMBO journal 17(24), 7469-7479.
106. Guo, B., Vorwald, A.C., Alt, D.P., Lager, K.M., Bayles, D.O., Faaberg, K.S., 2011. Large scale parallel pyrosequencing technology: PRRSV strain VR-2332 nsp2 deletion mutant stability in swine. Virus research 161, 162-169.
107. Guo, B.Q., Lager, K.M., Henningson, J.N., Miller, L.C., Schlink, S.N., Kappes, M.A., Kehrli, M.E., Brockmeier, S.L., Nicholson, T.L., Yang, H.C., Faaberg, K.S., 2013. Experimental infection of United States swine with a Chinese highly pathogenic strain of porcine reproductive and respiratory syndrome virus. Virology 435, 372-384.
108. Guo, X.K., Zhang, Q., Gao, L., Li, N., Chen, X.X., Feng, W.H., 2013. Increasing expression of microRNA 181 inhibits porcine reproductive and respiratory syndrome virus replication and has implications for controlling virus infection. Journal of virology 87(2), 1159-1171.
109. Han, J., Liu, G., Wang, Y., Faaberg, K.S., 2007. Identification of nonessential regions of the nsp2 replicase protein of porcine reproductive and respiratory syndrome virus strain VR-2332 for replication in cell culture. J Virol 81, 9878-9890.
110. Han, J., Rutherford, M.S., Faaberg, K.S., 2009. The porcine reproductive and respiratory syndrome virus nsp2 cysteine protease domain possesses both trans- and cis-cleavage activities. J Virol 83, 9449-9463.
111. Han, J., Wang, Y., Faaberg, K.S., 2006. Complete genome analysis of RFLP 184 isolates of porcine reproductive and respiratory syndrome virus. Virus Res 122, 175-182.
112. Han, M., Kim, C.Y., Rowland, R.R., Fang, Y., Kim, D., Yoo, D., 2014. Biogenesis of non-structural protein 1 (nsp1) and nsp1-mediated type I interferon modulation in arteriviruses. Virology 458-459C, 136-150.
113. Han, M.Y., Du, Y.J., Song, C., Yoo, D.W., 2013. Degradation of CREB-binding protein and modulation of type I interferon induction by the zinc finger motif of the porcine reproductive and respiratory syndrome virus nsp1 alpha subunit. Virus Res 172, 54-65.
114. Harcourt, B.H., Jukneliene, D., Kanjanahaluethai, A., Bechill, J., Severson, K.M., Smith, C.M., Rota, P.A., Baker, S.C., 2004. Identification of severe acute respiratory syndrome coronavirus replicase products and characterization of papain-like protease activity. J Virol 78(24), 13600-13612.
115. Hawiger, J., 2001. Innate immunity and inflammation: A transcriptional paradigm. Immunol Res 23(2-3), 99-109.
116. Hedges, J.F., Demaula, C.D., Moore, B.D., McLaughlin, B.E., Simon, S.I., MacLachlan, N.J., 2001. Characterization of equine E-selectin. Immunology 103(4), 498-504.
117. Heikema, A.P., Bergman, M.P., Richards, H., Crocker, P.R., Gilbert, M., Samsom, J.N., van Wamel, W.J., Endtz, H.P., van Belkum, A., 2010. Characterization of the specific interaction between sialoadhesin and sialylated Campylobacter jejuni lipooligosaccharides. Infection and immunity 78(7), 3237-3246.
118. Hill, H., 1990. Overview and history of mystery swine disease (swine infertility respiratory syndrome), Proceedings of the Mystery Swine Disease Committee Meeting, pp. 29-30.
119. Holtkamp D.J., Kliebenstein, J.B, Neumann, E.J., Zimmerman, J.J., Rotto, H.F., Yoder, T.K., Wang, C., Yeske, P.E., Mowrer, C.L., Haley, C.A. 2013. Assessment of the economic impact of porcine reproductive and respiratory syndrome virus on United States pork producers. J. Swine Health Prod. 21:72-84.
120. Holmes, E.C., 2010. The RNA virus quasispecies: fact or fiction? Journal of molecular biology 400, 271-273.
121. Hopper, S., White, M., Twiddy, N., 1992. An outbreak of blue-eared pig disease (porcine reproductive and respiratory syndrome) in four pig herds in Great Britain. Veterinary Record 131, 140-144.
122. Hou, J., Wang, L., He, W., Zhang, H., Feng, W.H., 2012. Highly pathogenic porcine reproductive and respiratory syndrome virus impairs LPS- and poly(I:C)-stimulated tumor necrosis factor-alpha release by inhibiting ERK signaling pathway. Virus Res 167(1), 106-111.

123. Hu, J., Ni, Y., Meng, X.J., Zhang, C., 2012. Expression and purification of a chimeric protein consisting of the ectodomains of M and GP5 proteins of porcine reproductive and respiratory syndrome virus (PRRSV). Journal of chromatography. B, Analytical technologies in the biomedical and life sciences 911, 43-48.
124. Hu, J., Zhang, C., 2012. Porcine reproductive and respiratory syndrome virus vaccines: current status and strategies to a universal vaccine. Transboundary and emerging diseases.
125. Huang, L., Zhang, Y.P., Yu, Y.L., Sun, M.X., Li, C., Chen, P.Y., Mao, X., 2011. Role of lipid rafts in porcine reproductive and respiratory syndrome virus infection in MARC-145 cells. Biochemical and biophysical research communications 414(3), 545-550.
126. Huang, Y., Meng, X., 2010. Novel strategies and approaches to develop the next generation of vaccines against porcine reproductive and respiratory syndrome virus (PRRSV). Virus research 154, 141-149.
127. Huang, Y.W., Dryman, B.A., Li, W., Meng, X.J., 2009. Porcine DC-SIGN: molecular cloning, gene structure, tissue distribution and binding characteristics. Developmental and comparative immunology 33(4), 464-480.
128. Hyllseth, B., 1969. A plaque assay of equine arteritis virus in BHK-21 cells. Archiv fur die gesamte Virusforschung 28(1), 26-33.
129. Jiang, C., Xing, F., Xing, J., Jiang, Y., Zhou, E., 2013a. Different expression patterns of PRRSV mediator genes in the lung tissues of PRRSV resistant and susceptible pigs. Developmental and comparative immunology 39(1-2), 127-131.
130. Jiang, H.R., Hwenda, L., Makinen, K., Oetke, C., Crocker, P.R., Forrester, J.V., 2006. Sialoadhesin promotes the inflammatory response in experimental autoimmune uveoretinitis. Journal of immunology 177(4), 2258-2264.
131. Jiang, Y., Khan, F.A., Pandupuspitasari, N.S., Kadariya, I., Cheng, Z., Ren, Y., Chen, X., Zhou, A., Yang, L., Kong, D., Zhang, S., 2013b. Analysis of the binding sites of porcine sialoadhesin receptor with PRRSV. International journal of molecular sciences 14(12), 23955-23979.
132. Johnsen, C.K., Botner, A., Kamstrup, S., Lind, P., Nielsen, J., 2002. Cytokine mRNA profiles in bronchoalveolar cells of piglets experimentally infected in utero with porcine reproductive and respiratory syndrome virus: association of sustained expression of IFN-gamma and IL-10 after viral clearance. Viral immunology 15(4), 549-556.
133. Johnson, C.R., Griggs, T.F., Gnanandarajah, J., Murtaugh, M.P., 2011. Novel structural protein in porcine reproductive and respiratory syndrome virus encoded by an alternative ORF5 present in all arteriviruses. J Gen Virol 92, 1107-1116.
134. Jones, C., Virji, M., Crocker, P.R., 2003. Recognition of sialylated meningococcal lipopolysaccharide by siglecs expressed on myeloid cells leads to enhanced bacterial uptake. Molecular microbiology 49(5), 1213-1225.
135. Jusa, E.R., Inaba, Y., Kouno, M., Hirose, O., 1997. Effect of heparin on infection of cells by porcine reproductive and respiratory syndrome virus. American journal of veterinary research 58(5), 488-491.
136. Kapur, V., Elam, M.R., Pawlovich, T.M., Murtaugh, M.P., 1996. Genetic variation in porcine reproductive and respiratory syndrome virus isolates in the midwestern United States. J Gen Virol 77, 1271-1276.
137. Karniychuk, U.U., De Spiegelaere, W., Nauwynck, H.J., 2013. Porcine reproductive and respiratory syndrome virus infection is associated with an increased number of Sn-positive and CD8-positive cells in the maternal-fetal interface. Virus research 176(1-2), 285-291.
138. Karniychuk, U.U., Geldhof, M., Vanhee, M., Van Doorsselaere, J., Saveleva, T.A., Nauwynck, H.J., 2010. Pathogenesis and antigenic characterization of a new East European subtype 3 porcine reproductive and respiratory syndrome virus isolate. BMC veterinary research 6, 30.
139. Karniychuk, U.U., Nauwynck, H.J., 2009. Quantitative changes of sialoadhesin and CD163 positive macrophages in the implantation sites and organs of porcine embryos/fetuses during gestation. Placenta 30(6), 497-500.
140. Karpe, Y.A., Lole, K.S., 2011. Deubiquitination activity associated with hepatitis E virus putative papain-like cysteine protease. The Journal of general virology 92(Pt 9), 2088-2092.

141. Kim, D.Y., Calvert, J.G., Chang, K.O., Horlen, K., Kerrigan, M., Rowland, R.R.R., 2007. Expression and stability of foreign tags inserted into nsp2 of porcine reproductive and respiratory syndrome virus (PRRSV). Virus Res 128, 106-114.
142. Kim, H.S., Kwang, J., Yoon, I.J., Joo, H.S., Frey, M.L., 1993. Enhanced replication of porcine reproductive and respiratory syndrome (PRRS) virus in a homogeneous subpopulation of MA-104 cell line. Archives of virology 133(3-4), 477-483.
143. Kim, J.K., Fahad, A.M., Shanmukhappa, K., Kapil, S., 2006. Defining the cellular target(s) of porcine reproductive and respiratory syndrome virus blocking monoclonal antibody 7G10. Journal of virology 80(2), 689-696.
144. Kim, O., Sun, Y., Lai, F.W., Song, C., Yoo, D., 2010. Modulation of type I interferon induction by porcine reproductive and respiratory syndrome virus and degradation of CREB-binding protein by non-structural protein 1 in MARC-145 and HeLa cells. Virology 402, 315-326.
145. Kim, W.I., Lee, D.S., Johnson, W., Roof, M., Cha, S.H., Yoon, K.J., 2007. Effect of genotypic and biotypic differences among PRRS viruses on the serologic assessment of pigs for virus infection. Veterinary microbiology 123, 1-14.
146. 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.
147. Kimman, T.G., Cornelissen, L.A., Moormann, R.J., Rebel, J.M., Stockhofe-Zurwieden, N., 2009. Challenges for porcine reproductive and respiratory syndrome virus (PRRSV) vaccinology. Vaccine 27, 3704-3718.
148. Konishi, S., Akashi, H., Sentsui, H., Ogata, M., 1975. Studies on equine viral arteritis. I. Characterization of the virus and trial survey on antibody with vero cell cultures. Nihon juigaku zasshi. The Japanese journal of veterinary science 37(5), 259-267.
149. Kowal, K., Silver, R., Slawinska, E., Bielecki, M., Chyczewski, L., Kowal-Bielecka, O., 2011. CD163 and its role in inflammation. Folia histochemica et cytobiologica / Polish Academy of Sciences, Polish Histochemical and Cytochemical Society 49(3), 365-374.
150. Kreutz, L.C., 1998. Cellular membrane factors are the major determinants of porcine reproductive and respiratory syndrome virus tropism. Virus research 53(2), 121-128.
151. Kreutz, L.C., Ackermann, M.R., 1996. Porcine reproductive and respiratory syndrome virus enters cells through a low pH-dependent endocytic pathway. Virus research 42(1-2), 137-147.
152. Kristiansen, M., Graversen, J.H., Jacobsen, C., Sonne, O., Hoffman, H.J., Law, S.K., Moestrup, S.K., 2001. Identification of the haemoglobin scavenger receptor. Nature 409(6817), 198-201.
153. Kroese, M.V., Zevenhoven-Dobbe, J.C., Ruijter, J.N.A.B.D., Peeters, B.P.H., Meulenberg, J.J.M., Cornelissen, L.A.H.M., Snijder, E.J., 2008. The nsp1 alpha and nsp1 beta papain-like autoproteinases are essential for porcine reproductive and respiratory syndrome virus RNA synthesis. J Gen Virol 89, 494-499.
154. Kuzemtseva, L., de la Torre, E., Martin, G., Soldevila, F., Ait-Ali, T., Mateu, E., Darwich, L., 2014. Regulation of toll-like receptors 3, 7 and 9 in porcine alveolar macrophages by different genotype 1 strains of porcine reproductive and respiratory syndrome virus. Veterinary immunology and immunopathology 158(3-4), 189-198.
155. Kvisgaard, L.K., Hjulsager, C.K., Brar, M.S., Leung, F.C., Larsen, L.E., 2013. Genetic dissection of complete genomes of Type 2 PRRS viruses isolated in Denmark over a period of 15 years. Veterinary microbiology 167, 334-344.
156. Kwon, B., Ansari, I.H., Osorio, F.A., Pattnaik, A.K., 2006. Infectious clone-derived viruses from virulent and vaccine strains of porcine reproductive and respiratory syndrome virus mimic biological properties of their parental viruses in a pregnant sow model. Vaccine 24, 7071-7080.
157. Kwon, B., Ansari, I.H., Pattnaik, A.K., Osorio, F.A., 2008. Identification of virulence determinants of porcine reproductive and respiratory syndrome virus through construction of chimeric clones. Virology 380, 371-378.

158. Labarque, G., Reeth, K.V., Nauwynck, H., Drexler, C., Gucht, S.V., Pensaert, M., 2004. Impact of genetic diversity of European-type porcine reproductive and respiratory syndrome virus strains on vaccine efficacy. Vaccine 22, 4183-4190.
159. Lai, M., 1992. RNA recombination in animal and plant viruses. Microbiological reviews 56, 61-79.
160. Lauck, M., Hyeroba, D., Tumukunde, A., Weny, G., Lank, S.M., Chapman, C.A., O'Connor, D.H., Friedrich, T.C., Goldberg, T.L., 2011. Novel, Divergent Simian Hemorrhagic Fever Viruses in a Wild Ugandan Red Colobus Monkey Discovered Using Direct Pyrosequencing. PloS one 6(4).
161. Lauck, M., Sibley, S.D., Hyeroba, D., Tumukunde, A., Weny, G., Chapman, C.A., Ting, N., Switzer, W.M., Kuhn, J.H., Friedrich, T.C., O'Connor, D.H., Goldberg, T.L., 2013. Exceptional Simian Hemorrhagic Fever Virus Diversity in a Wild African Primate Community. J Virol 87(1), 688-691.
162. Lee, A., Chick, J.M., Kolarich, D., Haynes, P.A., Robertson, G.R., Tsoli, M., Jankova, L., Clarke, S.J., Packer, N.H., Baker, M.S., 2011. Liver membrane proteome glycosylation changes in mice bearing an extra-hepatic tumor. Molecular & cellular proteomics: MCP 10(9), M900538MCP900200.
163. Lee, C., Calvert, J.G., Welich, S.K.W., Yoo, D., 2005. A DNA-launched reverse genetics system for porcine reproductive and respiratory syndrome virus reveals that homodimerization of the nucleocapsid protein is essential for virus infectivity. Virology 331, 47-62.
164. Lee, C., Hodgins, D., Calvert, J.G., Welch, S.K.W., Jolie, R., Yoo, D., 2006. Mutations within the nuclear localization signal of the porcine reproductive and respiratory syndrome virus nucleocapsid protein attenuate virus replication. Virology 346, 238-250.
165. Lee, C., Yoo, D., 2005. Cysteine residues of the porcine reproductive and respiratory syndrome virus small envelope protein are non-essential for virus infectivity. J Gen Virol 86, 3091-3096.
166. 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.
167. Lee, S.M., Kleiboeker, S.B., 2005. Porcine arterivirus activates the NF-kappaB pathway through IkappaB degradation. Virology 342(1), 47-59.
168. Lee, S.M., Schommer, S.K., Kleiboeker, S.B., 2004. Porcine reproductive and respiratory syndrome virus field isolates differ in in vitro interferon phenotypes. Veterinary immunology and immunopathology 102, 217-231.
169. Lee, S.M., Schommer, S.K., Kleiboeker, S.B., 2004. Porcine reproductive and respiratory syndrome virus field isolates differ in in vitro interferon phenotypes. Veterinary immunology and immunopathology 102(3), 217-231.
170. Lee, Y.J., Lee, C., 2010. Deletion of the cytoplasmic domain of CD163 enhances porcine reproductive and respiratory syndrome virus replication. Archives of virology 155(8), 1319-1323.
171. Lee, Y.J., Lee, C., 2010. Porcine reproductive and respiratory syndrome virus replication is suppressed by inhibition of the extracellular signal-regulated kinase (ERK) signaling pathway. Virus Res 152(1-2), 50-58.
172. Lee, Y.J., Lee, C., 2012. Stress-activated protein kinases are involved in porcine reproductive and respiratory syndrome virus infection and modulate virus-induced cytokine production. Virology 427(2), 80-89.
173. Lee, Y.J., Park, C.K., Nam, E., Kim, S.H., Lee, O.S., Lee du, S., Lee, C., 2010. Generation of a porcine alveolar macrophage cell line for the growth of porcine reproductive and respiratory syndrome virus. Journal of virological methods 163(2), 410-415.
174. Li, B., Fang, L., Xu, Z., Liu, S., Gao, J., Jiang, Y., Chen, H., Xiao, S., 2009. Recombination in vaccine and circulating strains of porcine reproductive and respiratory syndrome viruses. Emerging infectious diseases 15.
175. Li, Y, Tas, A., Snijder, E.J., Fang, Y., 2012. Identification of porcine reproductive and respiratory syndrome virus ORF1a-encoded non-structural proteins in virus-infected cells. Journal of General Virology 93, 829-839.
176. Li, Y., Treffers, E.E., Napthine, S., Tas, A., Zhu, L., Sun, Z., Bell, S., Mark, B.L., van Veelen, P.A., van Hemert, M.J., Firth, A.E., Brierley, I., Snijder, E.J., Fang, Y., 2014. Transactivation of programmed

ribosomal frameshifting by a viral protein. Proceedings of the National Academy of Sciences of the United States of America 111(21), E2172-2181.
177. Li, Y., Wang, X., Jiang, P., Wang, X., Chen, W., Wang, X., Wang, K., 2009. Genetic variation analysis of porcine reproductive and respiratory syndrome virus isolated in China from 2002 to 2007 based on ORF5. Veterinary microbiology 138, 150-155.
178. Li, Y., Xue, C., Wang, L., Chen, X., Chen, F., Cao, Y., 2010. Genomic analysis of two Chinese strains of porcine reproductive and respiratory syndrome viruses with different virulence. Virus genes 40, 374-381.
179. Li, Y., Zhu, L., Lawson, S.R., Fang, Y., 2013. Targeted mutations in a highly conserved motif of the nsp1beta protein impair the interferon antagonizing activity of porcine reproductive and respiratory syndrome virus. J Gen Virol 94, 1972-1983.
180. Lim, K.P., Ng, L.F., Liu, D.X., 2000. Identification of a novel cleavage activity of the first papain-like proteinase domain encoded by open reading frame 1a of the coronavirus Avian infectious bronchitis virus and characterization of the cleavage products. J Virol 74(4), 1674-1685.
181. Lindner, H.A., Fotouhi-Ardakani, N., Lytvyn, V., Lachance, P., Sulea, T., Menard, R., 2005. The papain-like protease from the severe acute respiratory syndrome coronavirus is a deubiquitinating enzyme. J Virol 79(24), 15199-15208.
182. Liu, C.H., Chaung, H.C., Chang, H.L., Peng, Y.T., Chung, W.B., 2009. Expression of Toll-like receptor mRNA and cytokines in pigs infected with porcine reproductive and respiratory syndrome virus. Veterinary microbiology 136(3-4), 266-276.
183. Liu, D., Zhou, R., Zhang, J., Zhou, L., Jiang, Q., Guo, X., Ge, X., Yang, H., 2011. Recombination analyses between two strains of porcine reproductive and respiratory syndrome virus in vivo. Virus research 155, 473-486.
184. Lopez-Fuertes, L., Campos, E., Domenech, N., Ezquerra, A., Castro, J.M., Dominguez, J., Alonso, F., 2000. Porcine reproductive and respiratory syndrome (PRRS) virus down-modulates TNF-alpha production in infected macrophages. Virus Res 69(1), 41-46.
185. Loving, C.L., Brockmeier, S.L., Sacco, R.E., 2007. Differential type I interferon activation and susceptibility of dendritic cell populations to porcine arterivirus. Immunology 120(2), 217-229.
186. Lu, Z., Zhang, J., Huang, C.M., Go, Y.Y., Faaberg, K.S., Rowland, R.R., Timoney, P.J., Balasuriya, U.B., 2012. Chimeric viruses containing the N-terminal ectodomains of GP5 and M proteins of porcine reproductive and respiratory syndrome virus do not change the cellular tropism of equine arteritis virus. Virology 432, 99-109.
187. Lu, Z.H., Brown, A., Wilson, A.D., Calvert, J.G., Balasch, M., Fuentes-Utrilla, P., Loecherbach, J., Turner, F., Talbot, R., Archibald, A.L., 2014. Genomic variation in macrophage-cultured European porcine reproductive and respiratory syndrome virus Olot/91 revealed using ultra-deep next generation sequencing. Virology journal 11, 42.
188. Lunney JK, Fritz ER, Reecy JM, Kuhar D, Prucnal E, Molina R, Christopher-Hennings J, Zimmerman J, Rowland RR. 2010. Interleukin-8, interleukin-1beta, and interferon-gamma levels are linked to PRRS virus clearance. Viral Immunol. 23:127-34.
189. Luo, R., Fang, L., Jiang, Y., Jin, H., Wang, Y., Wang, D., Chen, H., Xiao, S., 2011. Activation of NF-kappaB by nucleocapsid protein of the porcine reproductive and respiratory syndrome virus. Virus Genes 42(1), 76-81.
190. Luo, R., Xiao, S., Jiang, Y., Jin, H., Wang, D., Liu, M., Chen, H., Fang, L., 2008. Porcine reproductive and respiratory syndrome virus (PRRSV) suppresses interferon-beta production by interfering with the RIG-I signaling pathway. Molecular immunology 45(10), 2839-2846.
191. Lv, J., Zhan, J.W., Sun, Z., Liu, W.Q., Yuan, S.S., 2008. An infectious cDNA clone of a highly pathogenic porcine reproductive and respiratory syndrome virus variant associated with porcine high fever syndrome. J Gen Virol 89, 2075-2079.
192. Madsen, M., Graversen, J.H., Moestrup, S.K., 2001. Haptoglobin and CD163: captor and receptor gating hemoglobin to macrophage lysosomes. Redox report : communications in free radical research 6(6), 386-388.

193. Madsen, M., Moller, H.J., Nielsen, M.J., Jacobsen, C., Graversen, J.H., van den Berg, T., Moestrup, S.K., 2004. Molecular characterization of the haptoglobin.hemoglobin receptor CD163. Ligand binding properties of the scavenger receptor cysteine-rich domain region. The Journal of biological chemistry 279(49), 51561-51567.
194. Maess, J., Reczko, E., Bohm, H.O., 1970. [Equine arteritis virus: multiplication in BHK 21-cells buoyant density and electron microscopical demonstration]. Archiv fur die gesamte Virusforschung 30(1), 47-58.
195. Magar, R., Larochelle, R., Dea, S., Gagnon, C., Nelson, E., Christopher-Hennings, J., Benfield, D., 1995. Antigenic comparison of Canadian and US isolates of porcine reproductive and respiratory syndrome virus using monoclonal antibodies to the nucleocapsid protein. Canadian Journal of Veterinary Research 59, 232.
196. Magar, R., Robinson, Y., Dubuc, C., Larochelle, R., 1995. Isolation and experimental oral transmission in pigs of a porcine reproductive and respiratory syndrome virus isolate. Advances in experimental medicine and biology 380, 139-144.
197. Mardassi, H., Athanassious, R., Mounir, S., Dea, S., 1994. Porcine reproductive and respiratory syndrome virus: morphological, biochemical and serological characteristics of Quebec isolates associated with acute and chronic outbreaks of porcine reproductive and respiratory syndrome. Canadian journal of veterinary research = Revue canadienne de recherche veterinaire 58(1), 55-64.
198. Martínez-Lobo, F., Díez-Fuertes, F., Segalés, J., García-Artiga, C., Simarro, I., Castro, J., Prieto, C., 2011a. Comparative pathogenicity of type 1 and type 2 isolates of porcine reproductive and respiratory syndrome virus (PRRSV) in a young pig infection model. Veterinary microbiology 154, 58-68.
199. Martínez-Lobo, F.J., Díez-Fuertes, F., Simarro, I., Castro, J.M., Prieto, C., 2011b. Porcine Reproductive and Respiratory Syndrome Virus isolates differ in their susceptibility to neutralization. Vaccine 29, 6928-6940.
200. Mateu, E., 2011. Genetic and immunobiological diversity of porcine reproductive and respiratory syndrome genotype I strains. Veterinary Microbiology.
201. Mathur, R.K., Awasthi, A., Wadhone, P., Ramanamurthy, B., Saha, B., 2004. Reciprocal CD40 signals through p38MAPK and ERK-1/2 induce counteracting immune responses. Nat Med 10(5), 540-544.
202. Meng, X.-J., Paul, P.S., Halbur, P.G., Morozov, I., 1995. Sequence comparison of open reading frames 2 to 5 of low and high virulence United States isolates of porcine reproductive and respiratory syndrome virus. Journal of General Virology 76, 3181-3188.
203. Meulenberg, J.J., Hulst, M.M., de Meijer, E.J., Moonen, P.L., den Besten, A., de Kluyver, E.P., Wensvoort, G., Moormann, R.J., 1993. Lelystad virus, the causative agent of porcine epidemic abortion and respiratory syndrome (PEARS), is related to LDV and EAV. Virology 192, 62-72.
204. Meulenberg, J.J.M., BosDeRuijter, J.N.A., vandeGraaf, R., Wensvoort, G., Moormann, R.J.M., 1998. Infectious transcripts from cloned genome-length cDNA of porcine reproductive and respiratory syndrome virus. J Virol 72, 380-387.
205. Meyers, G., Tautz, N., Becher, P., Thiel, H.J., Kummerer, B.M., 1997. Recovery of cytopathogenic and noncytopathogenic bovine viral diarrhea viruses from cDNA constructs. J Virol 71, 1735.
206. Mielech, A.M., Chen, Y., Mesecar, A.D., Baker, S.C., 2014a. Nidovirus papain-like proteases: Multifunctional enzymes with protease, deubiquitinating and deISGylating activities. Virus Res.
207. Mielech, A.M., Kilianski, A., Baez-Santos, Y.M., Mesecar, A.D., Baker, S.C., 2014b. MERS-CoV papain-like protease has deISGylating and deubiquitinating activities. Virology 450, 64-70.
208. Miguel, J.C., Chen, J., Van Alstine, W.G., Johnson, R.W., 2010. Expression of inflammatory cytokines and Toll-like receptors in the brain and respiratory tract of pigs infected with porcine reproductive and respiratory syndrome virus. Veterinary immunology and immunopathology 135(3-4), 314-319.
209. Mildner, A., Jung, S., 2014. Development and Function of Dendritic Cell Subsets. Immunity 40(5), 642-656.
210. Miller, L.C., Laegreid, W.W., Bono, J.L., Chitko-McKown, C.G., Fox, J.M., 2004. Interferon type I response in porcine reproductive and respiratory syndrome virus-infected MARC-145 cells. Arch Virol 149(12), 2453-2463.

211. Miller, L.C., Lager, K.M., Kehrli, M.E., Jr., 2009. Role of Toll-like receptors in activation of porcine alveolar macrophages by porcine reproductive and respiratory syndrome virus. Clinical and vaccine immunology: CVI 16(3), 360-365.
212. Mondaca-Fernández, E., Murtaugh, M.P., Morrison, R.B., 2006. Association between genetic sequence homology of porcine reproductive and respiratory syndrome virus and geographic distance between pig sites. Canadian journal of veterinary research 70, 237.
213. Monteiro, V.G., Lobato, C.S., Silva, A.R., Medina, D.V., de Oliveira, M.A., Seabra, S.H., de Souza, W., DaMatta, R.A., 2005. Increased association of Trypanosoma cruzi with sialoadhesin positive mice macrophages. Parasitology research 97(5), 380-385.
214. Moore, B.D., Balasuriya, U.B., Watson, J.L., Bosio, C.M., MacKay, R.J., MacLachlan, N.J., 2003. Virulent and avirulent strains of equine arteritis virus induce different quantities of TNF-alpha and other proinflammatory cytokines in alveolar and blood-derived equine macrophages. Virology 314(2), 662-670.
215. Moormann, R.J., van Gennip, H.G., Miedema, G.K., Hulst, M.M., van Rijn, P.A., 1996. Infectious RNA transcribed from an engineered full-length cDNA template of the genome of a pestivirus. J Virol 70, 763-770.
216. Moura, G., Pinheiro, M., Arrais, J., Gomes, A.C., Carreto, L., Freitas, A., Oliveira, J.L., Santos, M.A., 2007. Large scale comparative codon-pair context analysis unveils general rules that fine-tune evolution of mRNA primary structure. PloS one 2, e847.
217. Mueller, S., Coleman, J.R., Papamichail, D., Ward, C.B., Nimnual, A., Futcher, B., Skiena, S., Wimmer, E., 2010. Live attenuated influenza virus vaccines by computer-aided rational design. Nature biotechnology 28, 723-726.
218. Munday, J., Floyd, H., Crocker, P.R., 1999. Sialic acid binding receptors (siglecs) expressed by macrophages. Journal of leukocyte biology 66(5), 705-711.
219. Murakami, Y., Kato, A., Tsuda, T., Morozumi, T., Miura, Y., Sugimura, T., 1994. Isolation and Serological Characterization of Porcine Reproductive and Respiratory Syndrome (Prrs) Viruses from Pigs with Reproductive and Respiratory Disorders in Japan. J Vet Med Sci 56, 891-894.
220. Murtaugh, M.P., Elam, M.R., Kakach, L.T., 1995. Comparison of the Structural Protein-Coding Sequences of the Vr-2332 and Lelystad Virus-Strains of the Prrs Virus. Arch Virol 140, 1451-1460.
221. Murtaugh, M.P., Stadejek, T., Abrahante, J.E., Lam, T.T., Leung, F.C.-C., 2010. The ever-expanding diversity of porcine reproductive and respiratory syndrome virus. Virus research 154, 18-30.
222. Murtaugh, M.P., Yuan, S., Nelson, E.A., Faaberg, K.S., 2002. Genetic interaction between porcine reproductive and respiratory syndrome virus (PRRSV) strains in cell culture and in animals. Journal of Swine Health and Production 10, 15-22.
223. Music, N., Gagnon, C.A., 2010. The role of porcine reproductive and respiratory syndrome (PRRS) virus structural and non-structural proteins in virus pathogenesis. Animal health research reviews / Conference of Research Workers in Animal Diseases 11, 135-163.
224. Nan, Y., Wang, R., Shen, M., Faaberg, K.S., Samal, S.K., Zhang, Y.J., 2012. Induction of type I interferons by a novel porcine reproductive and respiratory syndrome virus isolate. Virology 432(2), 261-270.
225. Nath, D., van der Merwe, P.A., Kelm, S., Bradfield, P., Crocker, P.R., 1995. The amino-terminal immunoglobulin-like domain of sialoadhesin contains the sialic acid binding site. Comparison with CD22. The Journal of biological chemistry 270(44), 26184-26191.
226. Nauwynck, H.J., Duan, X., Favoreel, H.W., Van Oostveldt, P., Pensaert, M.B., 1999. Entry of porcine reproductive and respiratory syndrome virus into porcine alveolar macrophages via receptor-mediated endocytosis. The Journal of general virology 80 (Pt 2), 297-305.
227. Nedialkova, D.D., Gorbalenya, A.E., Snijder, E.J., 2010. Arterivirus Nsp1 Modulates the Accumulation of Minus-Strand Templates to Control the Relative Abundance of Viral mRNAs. Plos Pathog 6.
228. Nelsen, C.J., Murtaugh, M.P., Faaberg, K.S., 1999. Porcine reproductive and respiratory syndrome virus comparison: divergent evolution on two continents. J Virol 73, 270-280.

229. Nelson, E., Christopher-Hennings, J., Drew, T., Wensvoort, G., Collins, J., Benfield, D., 1993. Differentiation of US and European isolates of porcine reproductive and respiratory syndrome virus by monoclonal antibodies. Journal of clinical microbiology 31, 3184-3189.
230. Neumann, E.J., Kliebenstein, J.B., Johnson, C.D., Mabry, J.W., Bush, E.J., Seitzinger, A.H., Green, A.L., Zimmerman, J.J., 2005. Assessment of the economic impact of porcine reproductive and respiratory syndrome on swine production in the United States. Journal of the American Veterinary Medical Association 227(3), 385-392.
231. Nga, P.T., Parquet, M.D., Lauber, C., Parida, M., Nabeshima, T., Yu, F.X., Thuy, N.T., Inoue, S., Ito, T., Okamoto, K., Ichinose, A., Snijder, E.J., Morita, K., Gorbalenya, A.E., 2011. Discovery of the first insect nidovirus, a missing evolutionary link in the emergence of the largest RNA virus genomes. Plos Pathog 7(9).
232. Ni, Y.Y., Huang, Y.W., Cao, D.J., Opriessnig, T., Meng, X.J., 2011. Establishment of a DNA-launched infectious clone for a highly pneumovirulent strain of type 2 porcine reproductive and respiratory syndrome virus: Identification and in vitro and in vivo characterization of a large spontaneous deletion in the nsp2 region. Virus Res 160, 264-273.
233. Ni, Y.Y., Opriessnig, T., Zhou, L., Cao, D.J., Huang, Y.W., Halbur, P.G., Meng, X.J., 2013. Attenuation of Porcine Reproductive and Respiratory Syndrome Virus by Molecular Breeding of Virus Envelope Genes from Genetically Divergent Strains. J Virol 87, 304-313.
234. Ni, Y.Y., Zhao, Z., Opriessnig, T., Subramaniam, S., Zhou, L., Cao, D., Cao, Q., Yang, H., Meng, X.J., 2014. Computer-aided codon-pairs deoptimization of the major envelope GP5 gene attenuates porcine reproductive and respiratory syndrome virus. Virology 450-451, 132-139.
235. Nielsen, H.S., Liu, G., Nielsen, J., Oleksiewicz, M.B., Botner, A., Storgaard, T., Faaberg, K.S., 2003. Generation of an infectious clone of VR-2332, a highly virulent North American type isolate of porcine reproductive and respiratory syndrome virus. J Virol 77, 3702-3711.
236. Nielsen, H.S., Oleksiewicz, M.B., Forsberg, R., Stadejek, T., Bøtner, A., Storgaard, T., 2001. Reversion of a live porcine reproductive and respiratory syndrome virus vaccine investigated by parallel mutations. Journal of General Virology 82, 1263-1272.
237. Nielsen, M.J., Madsen, M., Moller, H.J., Moestrup, S.K., 2006. The macrophage scavenger receptor CD163: endocytic properties of cytoplasmic tail variants. Journal of leukocyte biology 79(4), 837-845.
238. Nielsen, M.J., Moller, H.J., Moestrup, S.K., 2010. Hemoglobin and heme scavenger receptors. Antioxidants & redox signaling 12(2), 261-273.
239. Oleksiewicz, M.B., Botner, A., Toft, P., Normann, P., Storgaard, T., 2001. Epitope mapping porcine reproductive and respiratory syndrome virus by phage display: the nsp2 fragment of the replicase polyprotein contains a cluster of B-cell epitopes. J Virol 75, 3277-3290.
240. Ooms, K., Van Gorp, H., Botti, S., Van Gaever, T., Delputte, P.L., Nauwynck, H.J., 2013a. Evaluation of viral peptide targeting to porcine sialoadhesin using a porcine reproductive and respiratory syndrome virus vaccination-challenge model. Virus research 177(2), 147-155.
241. Ooms, K., Van Gorp, H., Van Gaever, T., Nauwynck, H.J., Delputte, P.L., 2013b. Development of a recombinant antibody to target peptides and proteins to sialoadhesin-expressing macrophages. BMC biotechnology 13, 33.
242. Opriessnig, T., Halbur, P., Yoon, K.-J., Pogranichniy, R., Harmon, K., Evans, R., Key, K., Pallares, F., Thomas, P., Meng, X., 2002. Comparison of molecular and biological characteristics of a modified live porcine reproductive and respiratory syndrome virus (PRRSV) vaccine (ingelvac PRRS MLV), the parent strain of the vaccine (ATCC VR2332), ATCC VR2385, and two recent field isolates of PRRSV. Journal of virology 76, 11837-11844.
243. Pasternak, A.O., Spaan, W.J.M., Snijder, E.J., 2006. Nidovirus transcription: how to make sense ... ? J Gen Virol 87, 1403-1421.
244. Patel, D., Nan, Y.C., Shen, M.Y., Ritthipichai, K., Zhu, X.P., Zhang, Y.J., 2010. Porcine Reproductive and Respiratory Syndrome Virus Inhibits Type I Interferon Signaling by Blocking STAT1/STAT2 Nuclear Translocation. J Virol 84(21), 11045-11055.

245. Patton, J.B., Rowland, R.R., Yoo, D., Chang, K.O., 2009. Modulation of CD163 receptor expression and replication of porcine reproductive and respiratory syndrome virus in porcine macrophages. Virus research 140(1-2), 161-171.
246. Pei, Y., Hodgins, D.C., Lee, C., Calvert, J.G., Welch, S.K.W., Jolie, R., Keith, M., Yoo, D., 2008. Functional mapping of the porcine reproductive and respiratory syndrome virus capsid protein nuclear localization signal and its pathogenic association. Virus Res 135, 107-114.
247. Pei, Y.L., Hodgins, D.C., Wu, J.Q., Welch, S.K.W., Calvert, J.G., Li, G., Du, Y.J., Song, C., Yoo, D.W., 2009. Porcine reproductive and respiratory syndrome virus as a vector: Immunogenicity of green fluorescent protein and porcine circovirus type 2 capsid expressed from dedicated subgenomic RNAs. Virology 389, 91-99.
248. Philippidis, P., Mason, J.C., Evans, B.J., Nadra, I., Taylor, K.M., Haskard, D.O., Landis, R.C., 2004. Hemoglobin scavenger receptor CD163 mediates interleukin-10 release and heme oxygenase-1 synthesis: antiinflammatory monocyte-macrophage responses in vitro, in resolving skin blisters in vivo, and after cardiopulmonary bypass surgery. Circulation research 94(1), 119-126.
249. Plagemann, P.G., 2003. Porcine reproductive and respiratory syndrome virus: origin hypothesis. Emerging infectious diseases 9, 903.
250. Plagemann, P.G., Moennig, V., 1992. Lactate dehydrogenase-elevating virus, equine arteritis virus, and simian hemorrhagic fever virus: a new group of positive-strand RNA viruses. Advances in virus research 41, 99-192.
251. Plagemann, P.G.W., Rowland, R.R.R., Even, C., Faaberg, K.S., 1995. Lactate Dehydrogenase-Elevating Virus - an Ideal Persistent Virus. Springer Semin Immun 17(2-3), 167-186.
252. Plana, J., Vayreda, M., Vilarrasa, J., Bastons, M., Rosell, R., Martinez, M., San Gabriel, A., Pujols, J., Badiola, J.L., Ramos, J.A., 1992. Porcine epidemic abortion and respiratory syndrome (mystery swine disease). Isolation in Spain of the causative agent and experimental reproduction of the disease. Veterinary microbiology 33, 203-211.
253. Prather, R.S., Rowland, R.R., Ewen, C., Trible, B., Kerrigan, M., Bawa, B., Teson, J.M., Mao, J., Lee, K., Samuel, M.S., Whitworth, K.M., Murphy, C.N., Egen, T., Green, J.A., 2013. An intact sialoadhesin (Sn/SIGLEC1/CD169) is not required for attachment/internalization of the porcine reproductive and respiratory syndrome virus. Journal of virology 87(17), 9538-9546.
254. Provost, C., Jia, J.J., Music, N., Levesque, C., Lebel, M.E., del Castillo, J.R., Jacques, M., Gagnon, C.A., 2012. Identification of a new cell line permissive to porcine reproductive and respiratory syndrome virus infection and replication which is phenotypically distinct from MARC-145 cell line. Virology journal 9, 267.
255. Pu, S.Y., Wu, R.H., Yang, C.C., Jao, T.M., Tsai, M.H., Wang, J.C., Lin, H.M., Chao, Y.S., Yueh, A., 2011. Successful propagation of flavivirus infectious cDNAs by a novel method to reduce the cryptic bacterial promoter activity of virus genomes. J Virol 85, 2927-2941.
256. Racaniello, V.R., Baltimore, D., 1981. Cloned Poliovirus Complementary-DNA Is Infectious in Mammalian-Cells. Science 214, 916-919.
257. Radwan, A.I., Burger, D., 1973. The complement-requiring neutralization of equine arteritis virus by late antisera. Virology 51(1), 71-77.
258. Ran, Z.G., Chen, X.Y., Guo, X., Ge, X.N., Yoon, K.J., Yang, H.C., 2008. Recovery of viable porcine reproductive and respiratory syndrome virus from an infectious clone containing a partial deletion within the Nsp2-encoding region. Arch Virol 153, 899-907.
259. Randall, R.E., Goodbourn, S., 2008. Interferons and viruses: an interplay between induction, signalling, antiviral responses and virus countermeasures. Journal of General Virology 89, 1-47.
260. Rempel, H., Calosing, C., Sun, B., Pulliam, L., 2008. Sialoadhesin expressed on IFN-induced monocytes binds HIV-1 and enhances infectivity. PloS one 3(4), e1967.
261. Roberts, J.J., Rodgers, S.E., Drury, J., Ashman, L.K., Lloyd, J.V., 1995. Platelet activation induced by a murine monoclonal antibody directed against a novel tetra-span antigen. British journal of haematology 89(4), 853-860.

262. Rowland, R.R., Kervin, R., Kuckleburg, C., Sperlich, A., Benfield, D.A., 1999. The localization of porcine reproductive and respiratory syndrome virus nucleocapsid protein to the nucleolus of infected cells and identification of a potential nucleolar localization signal sequence. Virus Res 64, 1-12.
263. Rowland, R.R., Robinson, B., Stefanick, J., Kim, T.S., Guanghua, L., Lawson, S.R., Benfield, D.A., 2001. Inhibition of porcine reproductive and respiratory syndrome virus by interferon-gamma and recovery of virus replication with 2-aminopurine. Arch Virol 146(3), 539-555.
264. Rowland, R.R., Steffen, M., Ackerman, T., Benfield, D.A., 1999. The evolution of porcine reproductive and respiratory syndrome virus: quasispecies and emergence of a virus subpopulation during infection of pigs with VR-2332. Virology 259, 262-266.
265. Rowland, R.R.R., Schneider, P., Fang, Y., Wootton, S., Yoo, D., Benfield, D.A., 2003. Peptide domains involved in the localization of the porcine reproductive and respiratory syndrome virus nucleocapsid protein to the nucleolus. Virology 316, 135-145.
266. Rowland, R.R.R., Yoo, D., 2003. Nucleolar-cytoplasmic shuttling of PRRSV nucleocapsid protein: a simple case of molecular mimicry or the complex regulation by nuclear import, nucleolar localization and nuclear export signal sequences. Virus Res 95, 23-33.
267. Saccani, S., Pantano, S., Natoli, G., 2002. p38-Dependent marking of inflammatory genes for increased NF-kappa B recruitment. Nature immunology 3(1), 69-75.
268. Sagong, M., Lee, C., 2011. Porcine reproductive and respiratory syndrome virus nucleocapsid protein modulates interferon-beta production by inhibiting IRF3 activation in immortalized porcine alveolar macrophages. Arch Virol 156(12), 2187-2195.
269. Sagong, M., Park, C.K., Kim, S.H., Lee, K.K., Lee, O.S., Lee du, S., Cha, S.Y., Lee, C., 2012. Human telomerase reverse transcriptase-immortalized porcine monomyeloid cell lines for the production of porcine reproductive and respiratory syndrome virus. Journal of virological methods 179(1), 26-32.
270. Samuel, C.E., 2001. Antiviral actions of interferons. Clin Microbiol Rev 14(4), 778-809.
271. Sanchez, C., Domenech, N., Vazquez, J., Alonso, F., Ezquerra, A., Dominguez, J., 1999. The porcine 2A10 antigen is homologous to human CD163 and related to macrophage differentiation. Journal of immunology 162(9), 5230-5237.
272. Sanchez-Torres, C., Gomez-Puertas, P., Gomez-del-Moral, M., Alonso, F., Escribano, J.M., Ezquerra, A., Dominguez, J., 2003. Expression of porcine CD163 on monocytes/macrophages correlates with permissiveness to African swine fever infection. Archives of virology 148(12), 2307-2323.
273. Sang, Y.M., Shi, J.S., Sang, W.J., Rowland, R.R.R., Blecha, F., 2012. Replication-Competent Recombinant Porcine Reproductive and Respiratory Syndrome (PRRS) Viruses Expressing Indicator Proteins and Antiviral Cytokines. Viruses-Basel 4, 102-116.
274. Sawicki, S.G., Sawicki, D.L., Siddell, S.G., 2007. A contemporary view of coronavirus transcription. J Virol 81, 20-29.
275. Schaer, C.A., Schoedon, G., Imhof, A., Kurrer, M.O., Schaer, D.J., 2006. Constitutive endocytosis of CD163 mediates hemoglobin-heme uptake and determines the noninflammatory and protective transcriptional response of macrophages to hemoglobin. Circulation research 99(9), 943-950.
276. Scobey, T., Yount, B.L., Sims, A.C., Donaldson, E.F., Agnihothram, S.S., Menachery, V.D., Graham, R.L., Swanstrom, J., Bove, P.F., Kim, J.D., Grego, S., Randell, S.H., Baric, R.S., 2013. Reverse genetics with a full-length infectious cDNA of the Middle East respiratory syndrome coronavirus. P Natl Acad Sci USA 110, 16157-16162.
277. Shanmukhappa, K., Kim, J.K., Kapil, S., 2007. Role of CD151, A tetraspanin, in porcine reproductive and respiratory syndrome virus infection. Virology journal 4, 62.
278. Shen, S., Kwang, J., Liu, W., Liu, D.X., 2000. Determination of the complete nucleotide sequence of a vaccine strain of porcine reproductive and respiratory syndrome virus and identification of the Nsp2 gene with a unique insertion. Arch Virol 145, 871-883.
279. Shi, M., Holmes, E.C., Brar, M.S., Leung, F.C.-C., 2013a. Recombination is Associated with an Outbreak of Novel Highly Pathogenic Porcine Reproductive and Respiratory Syndrome Viruses in China. Journal of virology 87, 10904-10907.

280. Shi, M., Lam, T.T.-Y., Hon, C.-C., Hui, R.K.-H., Faaberg, K.S., Wennblom, T., Murtaugh, M.P., Stadejek, T., Leung, F.C.-C., 2010a. Molecular epidemiology of PRRSV: a phylogenetic perspective. Virus research 154, 7-17.
281. Shi, M., Lam, T.T.-Y., Hon, C.-C., Murtaugh, M.P., Davies, P.R., Hui, R.K.-H., Li, J., Wong, L.T.-W., Yip, C.-W., Jiang, J.-W., 2010b. Phylogeny-based evolutionary, demographical, and geographical dissection of North American type 2 porcine reproductive and respiratory syndrome viruses. Journal of virology 84, 8700-8711.
282. Shi, M., Lemey, P., Singh Brar, M., Suchard, M.A., Murtaugh, M.P., Carman, S., D'Allaire, S., Delisle, B., Lambert, M.-È., Gagnon, C.A., 2013b. The spread of type 2 Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) in North America: a phylogeographic approach. Virology 447, 146-154.
283. Shi, X., Wang, L., Zhi, Y., Xing, G., Zhao, D., Deng, R., Zhang, G., 2010. Porcine reproductive and respiratory syndrome virus (PRRSV) could be sensed by professional beta interferon-producing system and had mechanisms to inhibit this action in MARC-145 cells. Virus Res 153(1), 151-156.
284. Shields, D., Mathews, K., 2003. Interstate livestock movements. Electronic outlook report from the economic research service United States Department of Agriculture.
285. Shimizu, M., Yamada, S., Murakami, Y., Morozumi, T., Kobayashi, H., Mitani, K., Ito, N., Kubo, M., Kimura, K., Kobayashi, M., et al., 1994. Isolation of porcine reproductive and respiratory syndrome (PRRS) virus from Heko-Heko disease of pigs. J Vet Med Sci 56, 389-391.
286. Silversides, D.W., Music, N., Jacques, M., Gagnon, C. A. 2010. Investigation of the species origin of the St. Jude porcine lung epithelial cell line (SJPL) made available to researchers. J. Virol. 84, 5454–5455.
287. Sincock, P.M., Fitter, S., Parton, R.G., Berndt, M.C., Gamble, J.R., Ashman, L.K., 1999. PETA-3/CD151, a member of the transmembrane 4 superfamily, is localised to the plasma membrane and endocytic system of endothelial cells, associates with multiple integrins and modulates cell function. Journal of cell science 112 (Pt 6), 833-844.
288. Snijder, E.J., 1998. The arterivirus replicase. The road from RNA to protein(s), and back again. Advances in experimental medicine and biology 440, 97-108.
289. Snijder, E.J., Kikkert, M., Fang, Y., 2013. Arterivirus molecular biology and pathogenesis. Journal of General Virology 94, 2141-2163.
290. Snijder, E.J., Meulenberg, J.J.M., 1998. The molecular biology of arteriviruses. J Gen Virol 79, 961-979.
291. Snijder, E.J., van Tol, H., Pedersen, K.W., Raamsman, M.J.B., de Vries, A.A.F., 1999. Identification of a novel structural protein of arteriviruses. J Virol 73(8), 6335-6345.
292. Snijder, E.J., Wassenaar, A.L., Spaan, W.J., 1993. Proteolytic processing of the N-terminal region of the equine arteritis virus replicase. Advances in experimental medicine and biology 342, 227-232.
293. Snijder, E.J., Wassenaar, A.L., Spaan, W.J., Gorbalenya, A.E., 1995. The arterivirus Nsp2 protease. An unusual cysteine protease with primary structure similarities to both papain-like and chymotrypsin-like proteases. The Journal of biological chemistry 270, 16671-16676.
294. Snijder, E.J., Wassenaar, A.L.M., Spaan, W.J.M., 1994. Proteolytic Processing of the Replicase Orf1a Protein of Equine Arteritis Virus. J Virol 68(9), 5755-5764.
295. Sola, I., Mateos-Gomez, P.A., Almazan, F., Zuniga, S., Enjuanes, L., 2011. RNA-RNA and RNA-protein interactions in coronavirus replication and transcription. Rna Biol 8, 237-248.
296. Song, C., Krell, P., Yoo, D., 2010. Nonstructural protein 1alpha subunit-based inhibition of NF-kappaB activation and suppression of interferon-beta production by porcine reproductive and respiratory syndrome virus. Virology 407, 268-280.
297. Song, S., Bi, J., Wang, D., Fang, L., Zhang, L., Li, F., Chen, H., Xiao, S., 2013. Porcine reproductive and respiratory syndrome virus infection activates IL-10 production through NF-kappaB and p38 MAPK pathways in porcine alveolar macrophages. Developmental and comparative immunology 39(3), 265-272.
298. Sosnovtsev, S., Green, K.Y., 1995. RNA transcripts derived from a cloned full-length copy of the feline calicivirus genome do not require VpG for infectivity. Virology 210, 383-390.
299. Spilman, M.S., Welbon, C., Nelson, E., Dokland, T., 2009. Cryo-electron tomography of porcine reproductive and respiratory syndrome virus: organization of the nucleocapsid. J Gen Virol 90, 527-535.

300. Steinberger, J., Kontaxis, G., Rancan, C., Skern, T., 2013. Comparison of self-processing of foot-and-mouth disease virus leader proteinase and porcine reproductive and respiratory syndrome virus leader proteinase nsp1alpha. Virology 443(2), 271-277.
301. Stueckemann, J.A., Holth, M., Swart, W.J., Kowalchyk, K., Smith, M.S., Wolstenholme, A.J., Cafruny, W.A., Plagemann, P.G., 1982. Replication of lactate dehydrogenase-elevating virus in macrophages. 2. Mechanism of persistent infection in mice and cell culture. The Journal of general virology 59(Pt 2), 263-272.
302. Subramaniam, S., Beura, L.K., Kwon, B., Pattnaik, A.K., Osorio, F.A., 2012. Amino acid residues in the non-structural protein 1 of porcine reproductive and respiratory syndrome virus involved in down-regulation of TNF-alpha expression in vitro and attenuation in vivo. Virology 432, 241-249.
303. Subramaniam, S., Kwon, B., Beura, L.K., Kuszynski, C.A., Pattnaik, A.K., Osorio, F.A., 2010. Porcine reproductive and respiratory syndrome virus non-structural protein 1 suppresses tumor necrosis factor-alpha promoter activation by inhibiting NF-kappaB and Sp1. Virology 406(2), 270-279.
304. Sumiyoshi, H., Hoke, C.H., Trent, D.W., 1992. Infectious Japanese Encephalitis-Virus Rna Can Be Synthesized from Invitro-Ligated Cdna Templates. J Virol 66, 5425-5431.
305. Sun, Y., Han, M.Y., Kim, C., Calvert, J.G., Yoo, D., 2012a. Interplay between Interferon-Mediated Innate Immunity and Porcine Reproductive and Respiratory Syndrome Virus. Viruses-Basel 4, 424-446.
306. Sun, Y.N., Xue, F., Guo, Y., Ma, M., Hao, N., Zhang, X.J.C., Lou, Z.Y., Li, X.M., Rao, Z.H., 2009. Crystal Structure of Porcine Reproductive and Respiratory Syndrome Virus Leader Protease Nsp1 alpha. J Virol 83(21), 10931-10940.
307. Sun, Z., Chen, Z., Lawson, S.R., Fang, Y., 2010. The cysteine protease domain of porcine reproductive and respiratory syndrome virus nonstructural protein 2 possesses deubiquitinating and interferon antagonism functions. J Virol 84(15), 7832-7846.
308. Sun, Z., Li, Y., Ransburgh, R., Snijder, E.J., Fang, Y., 2012. Nonstructural protein 2 of porcine reproductive and respiratory syndrome virus inhibits the antiviral function of interferon-stimulated gene 15. J Virol 86(7), 3839-3850.
309. Sun, Z., Liu, C.L., Tan, F.F., Gao, F., Liu, P., Qin, A.J., Yuan, S.S., 2010. Identification of dispensable nucleotide sequence in 3 ' untranslated region of porcine reproductive and respiratory syndrome virus. Virus Res 154, 38-47.
310. Suradhat, S., Thanawongnuwech, R., Poovorawan, Y., 2003. Upregulation of IL-10 gene expression in porcine peripheral blood mononuclear cells by porcine reproductive and respiratory syndrome virus. The Journal of general virology 84(Pt 2), 453-459.
311. Tan, F.F., Wei, Z.Z., Li, Y.H., Zhang, R., Zhuang, J.S., Sun, Z., Yuan, S.S., 2011. Identification of non-essential regions in nucleocapsid protein of porcine reproductive and respiratory syndrome virus for replication in cell culture. Virus Res 158, 62-71.
312. Thanawongnuwech, R., Young, T.F., Thacker, B.J., Thacker, E.L., 2001. Differential production of proinflammatory cytokines: in vitro PRRSV and Mycoplasma hyopneumoniae co-infection model. Veterinary immunology and immunopathology 79(1-2), 115-127.
313. Therrien, D., St-Pierre, Y., Dea, S., 2000. Preliminary characterization of protein binding factor for porcine reproductive and respiratory syndrome virus on the surface of permissive and non-permissive cells. Archives of virology 145(6), 1099-1116.
314. Thi Dieu Thuy, N., Thi Thu, N., Son, N.G., Ha, L.T.T., Hung, V.K., Nguyen, N.T., Khoa, D.V.A., 2013. Genetic analysis of ORF5 porcine reproductive and respiratory syndrome virus isolated in Vietnam. Microbiology and immunology 57, 518-526.
315. Tian, D., Wei, Z., Zevenhoven-Dobbe, J.C., Liu, R., Tong, G., Snijder, E.J., Yuan, S., 2012. Arterivirus minor envelope proteins are a major determinant of viral tropism in cell culture. Journal of virology 86(7), 3701-3712.
316. Tian, D.B., Zheng, H.H., Zhang, R., Zhuang, J.S., Yuan, S.S., 2011. Chimeric porcine reproductive and respiratory syndrome viruses reveal full function of genotype 1 envelope proteins in the backbone of genotype 2. Virology 412, 1-8.

317. 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(6), e526.
318. Tijms, M.A., Nedialkova, D.D., Zevenhoven-Dobbe, J.C., Gorbalenya, A.E., Snijder, E.J., 2007. Arterivirus subgenomic mRNA synthesis and virion biogenesis depend on the multifunctional nsp1 autoprotease. J Virol 81(19), 10496-10505.
319. Tijms, M.A., van der Meer, Y., Snijder, E.J., 2002. Nuclear localization of non-structural protein 1 and nucleocapsid protein of equine arteritis virus. Journal of General Virology 83, 795-800.
320. Tijms, M.A., van Dinten, L.C., Gorbalenya, A.E., Snijder, E.J., 2001. A zinc finger-containing papain-like protease couples subgenomic mRNA synthesis to genome translation in a positive-stranded RNA virus. Proceedings of the National Academy of Sciences of the United States of America 98(4), 1889-1894.
321. Truong, H.M., Lu, Z., Kutish, G.F., Galeota, J., Osorio, F.A., Pattnaik, A.K., 2004. A highly pathogenic porcine reproductive and respiratory syndrome virus generated from an infectious cDNA clone retains the in vivo virulence and transmissibility properties of the parental virus. Virology 325, 308-319.
322. van Aken, D., Zevenhoven-Dobbe, J., Gorbalenya, A.E., Snijder, E.J., 2006. Proteolytic maturation of replicase polyprotein pp1a by the nsp4 main proteinase is essential for equine arteritis virus replication and includes internal cleavage of nsp7. J Gen Virol 87, 3473-3482.
323. Van Breedam, W., Delputte, P.L., Van Gorp, H., Misinzo, G., Vanderheijden, N., Duan, X., Nauwynck, H.J., 2010a. Porcine reproductive and respiratory syndrome virus entry into the porcine macrophage. The Journal of general virology 91(Pt 7), 1659-1667.
324. Van Breedam, W., Van Gorp, H., Zhang, J.Q., Crocker, P.R., Delputte, P.L., Nauwynck, H.J., 2010b. The M/GP(5) glycoprotein complex of porcine reproductive and respiratory syndrome virus binds the sialoadhesin receptor in a sialic acid-dependent manner. PLoS pathogens 6(1), e1000730.
325. Van Breedam, W., Verbeeck, M., Christiaens, I., Van Gorp, H., Nauwynck, H.J., 2013. Porcine, murine and human sialoadhesin (Sn/Siglec-1/CD169): portals for porcine reproductive and respiratory syndrome virus entry into target cells. The Journal of general virology 94(Pt 9), 1955-1960.
326. Van den Heuvel, M.M., Tensen, C.P., van As, J.H., Van den Berg, T.K., Fluitsma, D.M., Dijkstra, C.D., Dopp, E.A., Droste, A., Van Gaalen, F.A., Sorg, C., Hogger, P., Beelen, R.H., 1999. Regulation of CD 163 on human macrophages: cross-linking of CD163 induces signaling and activation. Journal of leukocyte biology 66(5), 858-866.
327. Van Gorp, H., Delputte, P.L., Nauwynck, H.J., 2010a. Scavenger receptor CD163, a Jack-of-all-trades and potential target for cell-directed therapy. Molecular immunology 47(7-8), 1650-1660.
328. Van Gorp, H., Van Breedam, W., Delputte, P.L., Nauwynck, H.J., 2008. Sialoadhesin and CD163 join forces during entry of the porcine reproductive and respiratory syndrome virus. The Journal of general virology 89(Pt 12), 2943-2953.
329. Van Gorp, H., Van Breedam, W., Delputte, P.L., Nauwynck, H.J., 2009. The porcine reproductive and respiratory syndrome virus requires trafficking through CD163-positive early endosomes, but not late endosomes, for productive infection. Archives of virology 154(12), 1939-1943.
330. Van Gorp, H., Van Breedam, W., Van Doorsselaere, J., Delputte, P.L., Nauwynck, H.J., 2010b. Identification of the CD163 protein domains involved in infection of the porcine reproductive and respiratory syndrome virus. Journal of virology 84(6), 3101-3105.
331. van Kasteren, P.B., Bailey-Elkin, B.A., James, T.W., Ninaber, D.K., Beugeling, C., Khajehpour, M., Snijder, E.J., Mark, B.L., Kikkert, M., 2013. Deubiquitinase function of arterivirus papain-like protease 2 suppresses the innate immune response in infected host cells. Proceedings of the National Academy of Sciences of the United States of America 110(9), E838-847.
332. van Kasteren, P.B., Beugeling, C., Ninaber, D.K., Frias-Staheli, N., van Boheemen, S., Garcia-Sastre, A., Snijder, E.J., Kikkert, M., 2012. Arterivirus and Nairovirus Ovarian Tumor Domain-Containing Deubiquitinases Target Activated RIG-I To Control Innate Immune Signaling. J Virol 86(2), 773-785.

333. Van Reeth, K., Labarque, G., Nauwynck, H., Pensaert, M., 1999. Differential production of proinflammatory cytokines in the pig lung during different respiratory virus infections: correlations with pathogenicity. Research in veterinary science 67(1), 47-52.
334. van Vugt, J.J., Storgaard, T., Oleksiewicz, M.B., Bøtner, A., 2001. High frequency RNA recombination in porcine reproductive and respiratory syndrome virus occurs preferentially between parental sequences with high similarity. Journal of General Virology 82, 2615-2620.
335. Vanderheijden, N., Delputte, P., Nauwynck, H., Pensaert, M., 2001. Effects of heparin on the entry of porcine reproductive and respiratory syndrome virus into alveolar macrophages. Advances in experimental medicine and biology 494, 683-689.
336. Vanderheijden, N., Delputte, P.L., Favoreel, H.W., Vandekerckhove, J., Van Damme, J., van Woensel, P.A., Nauwynck, H.J., 2003. Involvement of sialoadhesin in entry of porcine reproductive and respiratory syndrome virus into porcine alveolar macrophages. Journal of Virology 77(15), 8207-8215.
337. vanDinten, L.C., denBoon, J.A., Wassenaar, A.L.M., Spaan, W.J.M., Snijder, E.J., 1997. An infectious arterivirus cDNA clone: Identification of a replicase point mutation that abolishes discontinuous mRNA transcription. P Natl Acad Sci USA 94, 991-996.
338. Vatter, H.A., Brinton, M.A., 2014. Differential Responses of Disease-Resistant and Disease-Susceptible Primate Macrophages and Myeloid Dendritic Cells to Simian Hemorrhagic Fever Virus Infection. J Virol 88(4), 2095-2106.
339. Vatter, H.A., Di, H., Donaldson, E.F., Radu, G.U., Maines, T.R., Brinton, M.A., 2014. Functional analyses of the three simian hemorrhagic fever virus nonstructural protein 1 papain-like proteases. J Virol.
340. Verheije, M.H., Kroese, M.V., Rottier, P.J.M., Meulenberg, J.J.M., 2001. Viable porcine arteriviruses with deletions proximal to the 3 ' end of the genome. J Gen Virol 82, 2607-2614.
341. Verheije, M.H., Welting, T.J., Jansen, H.T., Rottier, P.J., Meulenberg, J.J., 2002. Chimeric arteriviruses generated by swapping of the M protein ectodomain rule out a role of this domain in viral targeting. Virology 303(2), 364-373.
342. Vu, H.L.X., Kwon, B., Yoon, K.J., Laegreid, W.W., Pattnaik, A.K., Osorio, F.A., 2011. Immune Evasion of Porcine Reproductive and Respiratory Syndrome Virus through Glycan Shielding Involves both Glycoprotein 5 as Well as Glycoprotein 3. J Virol 85, 5555-5564.
343. Wang, F., Qiu, H., Zhang, Q., Peng, Z., Liu, B., 2012. Association of two porcine reproductive and respiratory syndrome virus (PRRSV) receptor genes, CD163 and SN with immune traits. Molecular biology reports 39(4), 3971-3976.
344. Wang, L., Zhang, H., Suo, X., Zheng, S., Feng, W.H., 2011. Increase of CD163 but not sialoadhesin on cultured peripheral blood monocytes is coordinated with enhanced susceptibility to porcine reproductive and respiratory syndrome virus infection. Veterinary immunology and immunopathology 141(3-4), 209-220.
345. Wang, R., Nan, Y., Yu, Y., Zhang, Y.J., 2013. Porcine reproductive and respiratory syndrome virus Nsp1beta inhibits interferon-activated JAK/STAT signal transduction by inducing karyopherin-alpha1 degradation. J Virol 87(9), 5219-5228.
346. Wang, X., Eaton, M., Mayer, M., Li, H., He, D., Nelson, E., Christopher-Hennings, J., 2007. Porcine reproductive and respiratory syndrome virus productively infects monocyte-derived dendritic cells and compromises their antigen-presenting ability. Arch Virol 152(2), 289-303.
347. Wang, X., Wei, R., Li, Q., Liu, H., Huang, B., Gao, J., Mu, Y., Wang, C., Hsu, W.H., Hiscox, J.A., Zhou, E.M., 2013. PK-15 cells transfected with porcine CD163 by PiggyBac transposon system are susceptible to porcine reproductive and respiratory syndrome virus. Journal of virological methods 193(2), 383-390.
348. Wei, Z., Tian, D., Sun, L., Lin, T., Gao, F., Liu, R., Tong, G., Yuan, S., 2012. Influence of N-linked glycosylation of minor proteins of porcine reproductive and respiratory syndrome virus on infectious virus recovery and receptor interaction. Virology 429(1), 1-11.
349. Weingartl, H.M., Sabara, M., Pasick, J., van Moorlehem, E., Babiuk, L., 2002. Continuous porcine cell lines developed from alveolar macrophages: partial characterization and virus susceptibility. Journal of virological methods 104(2), 203-216.

350. Welch, S.K., Calvert, J.G., 2010. A brief review of CD163 and its role in PRRSV infection. Virus research 154(1-2), 98-103.
351. Welch, S.K.W., Jolie, R., Pearce, D.S., Koertje, W.D., Fuog, E., Shields, S.L., Yoo, D., Calvert, J.G., 2004. Construction and evaluation of genetically engineered replication-defective porcine reproductive and respiratory syndrome virus vaccine candidates. Vet Immunol Immunop 102, 277-290.
352. Wensvoort, G., Terpstra, C., Pol, J.M., ter Laak, E.A., Bloemraad, M., de Kluyver, E.P., Kragten, C., van Buiten, L., den Besten, A., Wagenaar, F., et al., 1991. Mystery swine disease in The Netherlands: the isolation of Lelystad virus. The Veterinary quarterly 13(3), 121-130.
353. Williams, A.F., Barclay, A.N., 1988. The immunoglobulin superfamily--domains for cell surface recognition. Annual review of immunology 6, 381-405.
354. Wissink, E.H., Kroese, M.V., Maneschijn-Bonsing, J.G., Meulenberg, J.J., van Rijn, P.A., Rijsewijk, F.A., Rottier, P.J., 2004. Significance of the oligosaccharides of the porcine reproductive and respiratory syndrome virus glycoproteins GP2a and GP5 for infectious virus production. The Journal of general virology 85(Pt 12), 3715-3723.
355. Wissink, E.H., van Wijk, H.A., Pol, J.M., Godeke, G.J., van Rijn, P.A., Rottier, P.J., Meulenberg, J.J., 2003. Identification of porcine alveolar macrophage glycoproteins involved in infection of porcine respiratory and reproductive syndrome virus. Archives of virology 148(1), 177-187.
356. Wojdyla, J.A., Manolaridis, I., van Kasteren, P.B., Kikkert, M., Snijder, E.J., Gorbalenya, A.E., Tucker, P.A., 2010. Papain-Like Protease 1 from Transmissible Gastroenteritis Virus: Crystal Structure and Enzymatic Activity toward Viral and Cellular Substrates. J Virol 84(19), 10063-10073.
357. Wongyanin, P., Buranapraditkul, S., Yoo, D., Thanawongnuwech, R., Roth, J.A., Suradhat, S., 2012. Role of porcine reproductive and respiratory syndrome virus nucleocapsid protein in induction of interleukin-10 and regulatory T-lymphocytes (Treg). The Journal of general virology 93(Pt 6), 1236-1246.
358. Wootton, S., Koljesar, G., Yang, L.Z., Yoon, K.J., Yoo, D., 2001. Antigenic importance of the carboxy-terminal beta-strand of the porcine reproductive and respiratory syndrome virus nucleocapsid protein. Clinical and diagnostic laboratory immunology 8, 598-603.
359. Wootton, S., Yoo, D., Rogan, D., 2000. Full-length sequence of a Canadian porcine reproductive and respiratory syndrome virus (PRRSV) isolate. Arch Virol 145, 2297-2323.
360. Wootton, S.K., Rowland, R.R., Yoo, D., 2002. Phosphorylation of the porcine reproductive and respiratory syndrome virus nucleocapsid protein. J Virol 76, 10569-10576.
361. Wootton, S.K., Yoo, D., 2003. Homo-oligomerization of the porcine reproductive and respiratory syndrome virus nucleocapsid protein and the role of disulfide linkages. J Virol 77, 4546-4557.
362. Xing, Y.L., Chen, J.F., Tu, J., Zhang, B.L., Chen, X.J., Shi, H.Y., Baker, S.C., Feng, L., Chen, Z.B., 2013. The papain-like protease of porcine epidemic diarrhea virus negatively regulates type I interferon pathway by acting as a viral deubiquitinase. Journal of General Virology 94, 1554-1567.
363. Xu, Y.Z., Zhou, Y.J., Zhang, S.R., Jiang, Y.F., Tong, W., Yu, H., Tong, G.Z., 2012a. Stable expression of foreign gene in nonessential region of nonstructural protein 2 (nsp2) of porcine reproductive and respiratory syndrome virus: Applications for marker vaccine design. Vet Microbiol 159, 1-10.
364. Xu, Y.Z., Zhou, Y.J., Zhang, S.R., Tong, W., Li, L., Jiang, Y.F., Tong, G.Z., 2012b. Identification of nonessential regions of the nsp2 protein of an attenuated vaccine strain (HuN4-F112) of highly pathogenic porcine reproductive and respiratory syndrome virus for replication in marc-145 cell. Virol J 9.
365. Xue, F., Sun, Y.N., Yan, L.M., Zhao, C., Chen, J., Bartlam, M., Li, X.M., Lou, Z.Y., Rao, Z.H., 2010. The Crystal Structure of Porcine Reproductive and Respiratory Syndrome Virus Nonstructural Protein Nsp1 beta Reveals a Novel Metal-Dependent Nuclease. Journal of virology 84(13), 6461-6471.
366. Yoo, D., Song, C., Sun, Y., Du, Y.J., Kim, O., Liu, H.C., 2010. Modulation of host cell responses and evasion strategies for porcine reproductive and respiratory syndrome virus. Virus Res 154(1-2), 48-60.
367. Yoo, D., Wootton, S.K., Li, G., Song, C., Rowland, R.R., 2003. Colocalization and interaction of the porcine arterivirus nucleocapsid protein with the small nucleolar RNA-associated protein fibrillarin. J Virol 77, 12173-12183.

368. Yoo, D.W., Welch, S.K.W., Lee, C.H., Calvert, J.G., 2004. Infectious cDNA clones of porcine reproductive and respiratory syndrome virus and their potential as vaccine vectors. Vet Immunol Immunop 102, 143-154.
369. York, M.R., Nagai, T., Mangini, A.J., Lemaire, R., van Seventer, J.M., Lafyatis, R., 2007. A macrophage marker, Siglec-1, is increased on circulating monocytes in patients with systemic sclerosis and induced by type I interferons and toll-like receptor agonists. Arthritis and rheumatism 56(3), 1010-1020.
370. Yoshii, M., Okinaga, T., Miyazaki, A., Kato, K., Ikeda, H., Tsunemitsu, H., 2008. Genetic polymorphism of the nsp2 gene in North American type-porcine reproductive and respiratory syndrome virus. Archives of virology 153, 1323-1334.
371. Yount, B., Curtis, K.M., Fritz, E.A., Hensley, L.E., Jahrling, P.B., Prentice, E., Denison, M.R., Geisbert, T.W., Baric, R.S., 2003. Reverse genetics with a full-length infectious cDNA of severe acute respiratory syndrome coronavirus. P Natl Acad Sci USA 100, 12995-13000.
372. Yu, D.D., Lv, J., Sun, Z., Zheng, H.H., Lu, J.Q., Yuan, S.S., 2009. Reverse genetic manipulation of the overlapping coding regions for structural proteins of the type II porcine reproductive and respiratory syndrome virus. Virology 383, 22-31.
373. Yu, X., Chen, N., Deng, X., Cao, Z., Han, W., Hu, D., Wu, J., Zhang, S., Wang, B., Gu, X., Tian, K., 2013. Genomic sequencing reveals mutations potentially related to the overattenuation of a highly pathogenic porcine reproductive and respiratory syndrome virus. Clinical and vaccine immunology : CVI 20, 613-619.
374. Yuan, S., Murtaugh, M.P., Faaberg, K.S., 2000. Heteroclite subgenomic RNAs are produced in porcine reproductive and respiratory syndrome virus infection. Virology 275, 158-169.
375. Yuan, S., Murtaugh, M.P., Schumann, F.A., Mickelson, D., Faaberg, K.S., 2004. Characterization of heteroclite subgenomic RNAs associated with PRRSV infection. Virus research 105, 75-87.
376. Yuan, S., Nelsen, C.J., Murtaugh, M.P., Schmitt, B.J., Faaberg, K.S., 1999. Recombination between North American strains of porcine reproductive and respiratory syndrome virus. Virus research 61, 87-98.
377. Yuan, S., Wei, Z., 2008. Construction of infectious cDNA clones of PRRSV: separation of coding regions for nonstructural and structural proteins. Science in China. Series C, Life sciences / Chinese Academy of Sciences 51, 271-279.
378. Zhang, H., Guo, X., Nelson, E., Christopher-Hennings, J., Wang, X., 2012. Porcine reproductive and respiratory syndrome virus activates the transcription of interferon alpha/beta (IFN-alpha/beta) in monocyte-derived dendritic cells (Mo-DC). Veterinary microbiology 159(3-4), 494-498.
379. Zhang, S.R., Zhou, Y.J., Jiang, Y.F., Li, G.X., Yan, L.P., Yu, H., Tong, G.Z., 2011. Generation of an infectious clone of HuN4-F112, an attenuated live vaccine strain of porcine reproductive and respiratory syndrome virus. Virol J 8.
380. Zheng, D.H., Chen, G., Guo, B.C., Cheng, G.H., Tang, H., 2008. PLP2, a potent deubiquitinase from murine hepatitis virus, strongly inhibits cellular type I interferon production. Cell Res 18(11), 1105-1113.
381. 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, e66645.
382. 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.
383. Zhou, L., Zhang, J., Zeng, J., Yin, S., Li, Y., Zheng, L., Guo, X., Ge, X., Yang, H., 2009. The 30-amino-acid deletion in the Nsp2 of highly pathogenic porcine reproductive and respiratory syndrome virus emerging in China is not related to its virulence. J Virol 83, 5156-5167.
384. Zhou, Z., Ni, J., Cao, Z., Han, X., Xia, Y., Zi, Z., Ning, K., Liu, Q., Cai, L., Qiu, P., 2011. The epidemic status and genetic diversity of 14 highly pathogenic porcine reproductive and respiratory syndrome virus (HP-PRRSV) isolates from China in 2009. Veterinary microbiology 150, 257-269.

385. Ziebuhr, J., Snijder, E.J., Gorbalenya, A.E., 2000. Virus-encoded proteinases and proteolytic processing in the Nidovirales. J Gen Virol 81, 853-879.
386. Zirkel, F., Kurth, A., Quan, P.L., Briese, T., Ellerbrok, H., Pauli, G., Leendertz, F.H., Lipkin, W.I., Ziebuhr, J., Drosten, C., Junglen, S., 2011. An Insect Nidovirus Emerging from a Primary Tropical Rainforest. Mbio 2(3).
387. Zou, Z., Chastain, A., Moir, S., Ford, J., Trandem, K., Martinelli, E., Cicala, C., Crocker, P., Arthos, J., Sun, P.D., 2011. Siglecs facilitate HIV-1 infection of macrophages through adhesion with viral sialic acids. PloS one 6(9), e24559.

10. TABLES AND FIGURES

Table 1. Construction of PRRSV infectious clone

Name	Year[a]	Genotype[b]	Isolate	GenBank #	Cell type for		Vector	Promoter	Genetic marker[d]	Reference
					Transfection	Passage				
pABV414[e]	1998	I	*Ter Huurne* (TH)	N/A	BHK-21	PAM/ CL2621	pOK12	T7	N/A	(Meulenberg et al., 1998)
pABV416[e]	1998	I	*Ter Huurne* (TH)	N/A	BHK-21	PAM/ CL2621	pOK12	T7	N/A	(Meulenberg et al., 1998)
pABV437	1998	I	*Ter Huurne* (TH)	N/A	BHK-21	PAM/ CL2621	pOK12	T7	pacI(3'UTR)	(Meulenberg et al., 1998)
N/A	2003	II	VR-2332	AY150564	BHK-21C	PAM/ MARC-145	pOK12	T7	BstZ17I (ORF1a)/ HpaI(3'UTR)	(Nielsen et al., 2003)
pFL12	2004	II	NVSL#97-7895	AY545985	MARC-145	PAM/ MARC-145	pBR322	T7	BsrGI(ORF1a)	(Truong et al., 2004)
pT7-P129	2005	II	P129	AF494042	MARC-145	MARC-145	pCR2.1	T7	C1559T/ A12622G	(Lee et al., 2005)
pCMV-S-P129	2005	II	P129	AF494042	MARC-145	MARC-145	pCMV	hCMV	C1559T/ A12622G	(Lee et al., 2005)
pSD01-08	2006	NA I[f]	SD 01-08 (P34)	DQ489311	BHK-21	PAM/ MARC-145	pACYC177	T7	ScaI(ORF7)	(Fang et al., 2006a; Fang et al., 2006b)
pPP18	2006	II	Prime Pac (PP)	DQ779791	MARC-145	PAM/ MARC-145	pOK12	T7	SpeI(ORF1a)	(Kwon et al., 2006)
pVR-V7	2007	II	VR2332	DQ217415	MA-104/ MARC-145	MA-104/ MARC-145	pOK12HDV-PacI	T7	G7329A/ T7554 C	(Han et al., 2007)
pWSK-DCBA	2007	II	BJ-4	EU360128	MARC-145	PAM/ MARC-145	pWSK29	SP6	VspI(ORF1b)	(Ran et al., 2008)
pAPRRS	2008	II	APRRS	N/A	MA-104	MA-104	pBluescript SK(+)	T7	N/A	(Yuan and Wei, 2008)
pORF5M	2008	II	APRRS	N/A	MA-104	MA-104	pBluescript SK(+)	T7	MluI (ORF5)	(Yuan and Wei, 2008)
pJX143	2008	II	JX143	EF488048	MA-104	MA-104	pBlueScript II SK (+)	T7	N/A	(Lv et al., 2008)
pJX143M	2008	II	JX143	N/A	MA-104	MA-104	pBlueScript II SK (+)	T7	MluI (ORF6)	(Lv et al., 2008)
pWSK-JXwn	2009	II	JXwn06	N/A	BHK-21	MARC-145	pWSK29	SP6	BstBI(ORF1a)	(Zhou et al., 2009)
pWSKHB-1/3.9	2009	II	HB-1/3.9	N/A	BHK-21	MARC-145	pWSK29M	SP6	MluI (ORF1a) SifI (ORF1b)	(Zhou et al., 2009)
pHuN4-F112	2011	II	HuN4-F112	N/A	BHK-21	MARC-145	pBlueScript II SK (+)	SP6	MluI (ORF6)	(Zhang et al., 2011)
pACYC-VR2385-CA	2011	II	VR2385-CA	N/A	BHK-21	MARC-145	pACYC177	T7	Sph I(ORF1a)	(Ni et al., 2011)
pIR-VR2385-CA	2011	II	VR2385-CA	N/A	BHK-21	MARC-145	pIRES-EGFP2	CMV	Sph I(ORF1a)	(Ni et al., 2011)
pSHE	2013	I	SHE(AMER-VAC-PRRS/A3)	GQ461593	BHK-21	MARC-145	pB-ZJS	CMV	N/A	(Gao et al., 2013)
pCMV-SD95-21	2013	II	SD95-21	KC469618	BHK-21	MARC-145	pACYC177	CMV	N/A	(Li et al., 2013)

Table 2. Identification of non-essential regions of PRRSV genome by deletion

Genotype and gene		Mutation/deletion (nts or aa)	Motif	Infectious clone	Growth	GenBank
II	5'UTR	1-3 nts		pAPRRS	N/A	GQ330474.2
II	nsp2	13-35 aa	hypervariable	pVR-V7	↓	DQ217415
II	nsp2	324-726 aa	hypervariable	pVR-V7	↓	DQ217415
II	nsp2	727-813 aa	hypervariable	pVR-V7	↓	DQ217415
II	nsp2	480-667 aa	hypervariable	pHuN4-F112	N/A	EF635006
I	nsp2	691-722 aa	ES3[a]	pSD01-08	↑	DQ489311
I	nsp2	736–790 aa	ES4[a]	pSD01-08	nc	DQ489311
I	nsp2	1015–1040 aa	ES7[a]	pSD01-08	↓	DQ489311
II	ORF7	5-13 aa		pAPRRS	↓	GQ330474.2
II	ORF7	39-42 aa		pAPRRS	↓	GQ330474.2
II	ORF7	48-52 aa		pAPRRS	nc	GQ330474.2
II	ORF7	120-123 aa		pAPRRS	nc	GQ330474.2
II	ORF7	43,44 aa	NLS[b]	pCMV-S-P129	↓	AF494042
II	ORF7	43,44,46 aa	NLS[b]	pCMV-S-P129	↓	AF494042
II	ORF7	46,47 aa	NLS[b]	pCMV-S-P129	↓	AF494042
I	ORF7	123-128 aa		pABV437	nc	N/A
I	3'UTR	14989-14995 nts		pABV437	nc	N/A
II	3'UTR	15370-15409 nts		pAPRRS	N/A	GQ330474.2

Table 3. Insertion-tolerable regions in PRRSV genome

Genotype	Genomic region		Position		foreign sequence	Infectious clone	Growth rate	GenBank
			nt	aa				
II	ORF1a	nsp2	3219/3220	N/A	GFP	pCMV-S-P129	nc	AF494042
II	ORF1a	nsp2	3219/3220	N/A	FLAG-tag	pCMV-S-P129	↓	AF494042
II	ORF1a	nsp2	3614/3615	N/A	GFP	pCMV-S-P129	↓	AF494042
I	ORF1a	nsp2	N/A	348/349	GFP	pSD01-08	↓	DQ489311
II	ORF1a	nsp2	N/A	507/508	B-cell epitope in NDV NP	pSK-F112-D508–532	nc	N/A
II	ORF1b/ORF2		N/A	N/A	TRS6+GFP	pCMV-S-P129	nc	AF494042
II	ORF1b/ORF2		N/A	N/A	TRS6+PCV2 C	pCMV-S-P129	nc	AF494042
II	ORF1b/ORF2		N/A	N/A	TRS6+DsRED	pCMV-S-P129	nc	AF494042
II	ORF1b/ORF2		N/A	N/A	TRS6+Rluc	pCMV-S-P129	nc	AF494042
II	ORF1b/ORF2		N/A	N/A	IFNα1	pCMV-S-P129	↓	AF494042
II	ORF1b/ORF2		N/A	N/A	IFNβ	pCMV-S-P129	↓	AF494042
II	ORF1b/ORF2		N/A	N/A	IFNδ3	pCMV-S-P129	nc	AF494042
II	ORF1b/ORF2		N/A	N/A	IFNω5	pCMV-S-P129	↓	AF494042
II	ORF1b/ORF2		N/A	N/A	AscI ,SwaI, PacI	pAPRRS	nc	GQ330474.2
II	ORF4/ORF5		N/A	N/A	NdeI	pAPRRS	nc	GQ330474.2
II	ORF5/ORF6		N/A	N/A	AscI ,SwaI, PacI	pAPRRS	↓	GQ330474.2
II	ORF6/ORF7		N/A	N/A	AscI ,SwaI, PacI	pAPRRS	nc	GQ330474.2
II	ORF7/3'UTR		N/A	N/A	NdeI	pAPRRS	nc	GQ330474.2

Table 4. Construction of chimeric PRRSV

Swapped region [a]					Substituent [b]				Viability
Virus	strain	Infectious clone [c]	Genome region	Position aa	Virus	Strain	Genome region	Position aa	
EAV	Bucyrus	pA45	ORF5	1-114	LDV	P	GP5	1-64	+
					PRRSV	IAF-Klop	GP5	1-64	+
					SHFV	LVR 42-0/M6941	GP7	1-138	-
					SinV	San Juan	E1	1-428	-
					VSV	HR	G	1-402	-
				Whole	EAV	A45-80.4	GP5	Whole	+
						5rUCD	GP5	Whole	+
						5r6D10	GP5	Whole	+
						5rVAC	GP5	Whole	+
						5rKY84	GP5	Whole	+
						5rIL93	GP5	Whole	+
						5rCA95	GP5	Whole	+
						5rWA97	GP5	Whole	+
						5rATCC	GP5	Whole	+
						5r10B4	GP5	Whole	+
			ORF6	17-162	PRRSV	IAF-Klop	M	1-16	-
					LDV	P	M	1-14	-
	ARVAC	prMLVB4/5	ORF5	115-255	PRRSV	IA-1107	ORF5	1-64	+
		prMLVB4/5	ORF5	N/A[d]	PRRSV	IA-1107	ORF5	Whole	-
		prMLVB5/6	ORF6	N/A[d]	PRRSV	IA-1107	ORF6	Whole	-
		prMLVB4/5/6	ORF6	17-162	PRRSV	IA-1107	ORF6	1-17	+
PRRSV	LV	pABV437	ORF6	1-16	PRRSV	V2332	M	1-16	-
					LDV	P	M	1-14	+
					EAV	Bucyrus	M	1-17	-
		pABV871	ORF6	1-16	PRRSV	V2332	M	1-16	+
					EAV	Bucyrus	M	1-17	+
	VR2332	N/A	ORF2	Whole	PRRSV	JA142	ORF2	Whole	+
			ORF3	Whole	PRRSV	JA142	ORF3	Whole	+
				1-194	PRRSV	JA142	ORF3	1-194	+
				183-255	PRRSV	JA142	ORF3	183-255	+
			ORF4	Whole	PRRSV	JA142	ORF4	Whole	+
			ORF5	Whole	PRRSV	JA142	ORF5	Whole	+
						SDSU73	ORF5	Whole	+
						2M11715	ORF5	Whole	+
						PRRS124	ORF5	Whole	+
			ORF6	Whole	PRRSV	JA142	ORF6	Whole	+
			ORFs5-6	Whole	PRRSV	JA142	ORFs5-6	Whole	+
			ORFs4-6	Whole	PRRSV	JA142	ORFs4-6	Whole	+
			ORFs3-6	Whole	PRRSV	JA142	ORFs3-6	Whole	+
			ORFs2-6	Whole	PRRSV	JA142	ORFs2-6	Whole	+
	APRRS	pAPRRS asc	ORFs2a-4	Whole	PRRSV	SHE	ORFs2a-4	Whole	+
					EAV	vEAV030	ORFs2a-4	Whole	+
			ORFs2a-5	Whole	PRRSV	SHE	ORFs2a-5	Whole	+
			ORF5	Whole	PRRSV	SHE	ORF5	Whole	+
	NVSL# 97-7895	FL12	ORFs2a-7	Whole	PRRSV	PRRSV01	ORFs2a-7	Whole	+

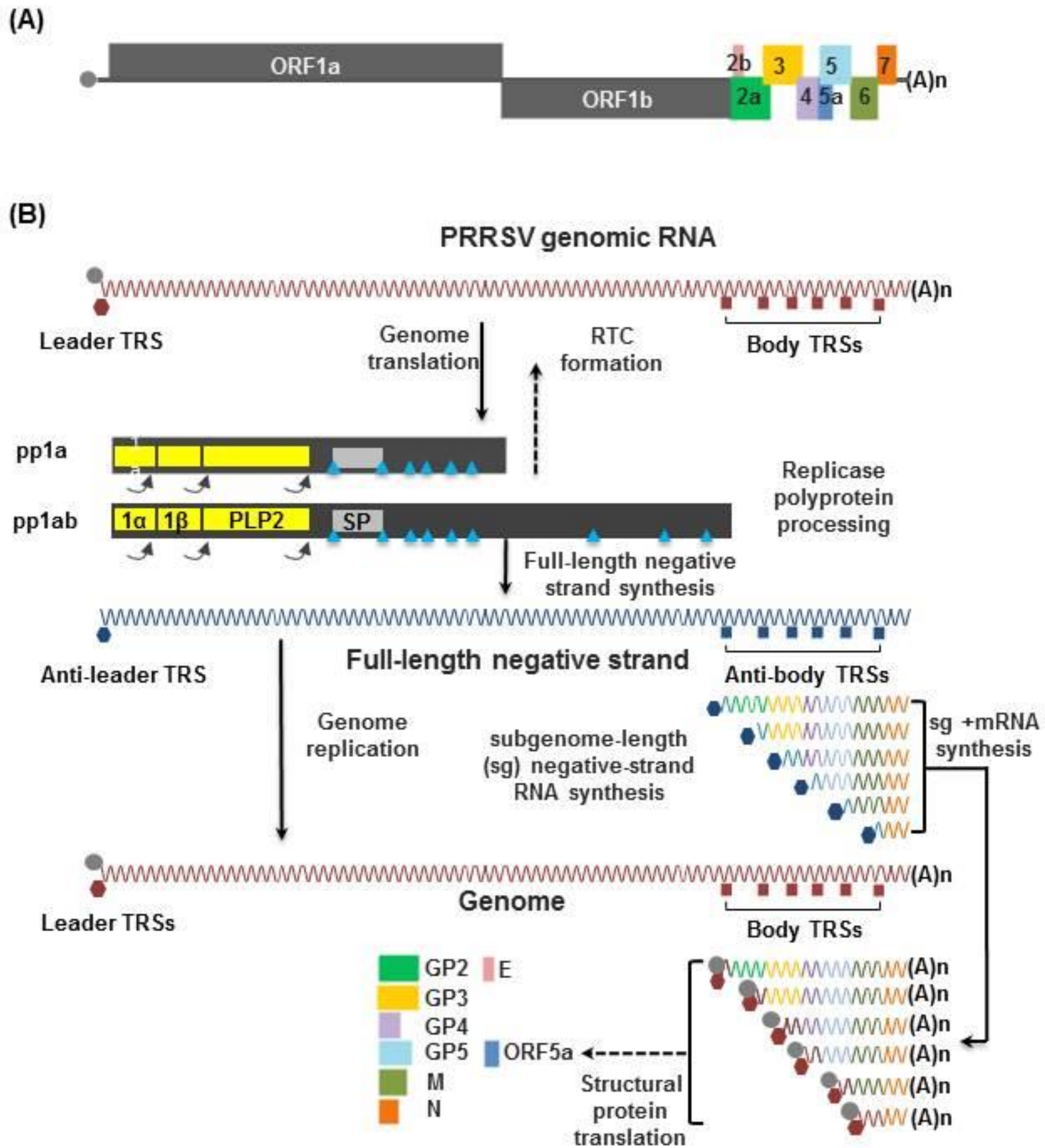

Figure 1. (A) PRRSV genome organization. (B) viral gene expression. Non-structural proteins (in black) are expressed from pp1a and pp1ab after proteolytic processing. Structural proteins (color-coded) are expressed from the subset of sg mRNA. TRS, transcription regulatory sequence.

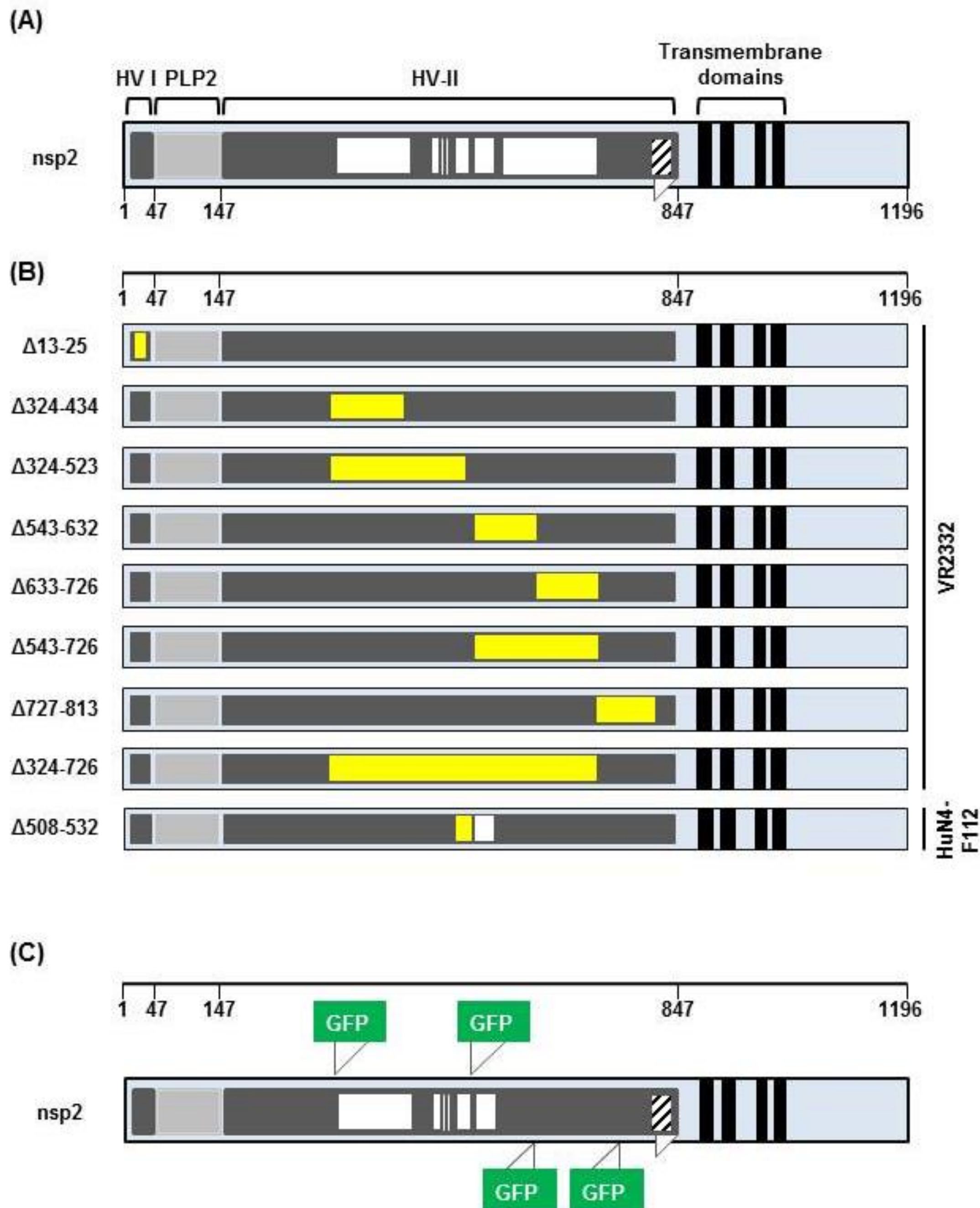

Figure 2. (A) Schematic presentation of the nsp2 protein. White areas indicate naturally occurring deletion in some isolates of PRRSV. (B) Location of sequence deletion (yellow). (C) Foreign gene insertion sites. GFP, green fluorescent protein; HV, hypervariable region; PLP, papain-like proteinase; numbers indicate amino acid positions of nsp2.

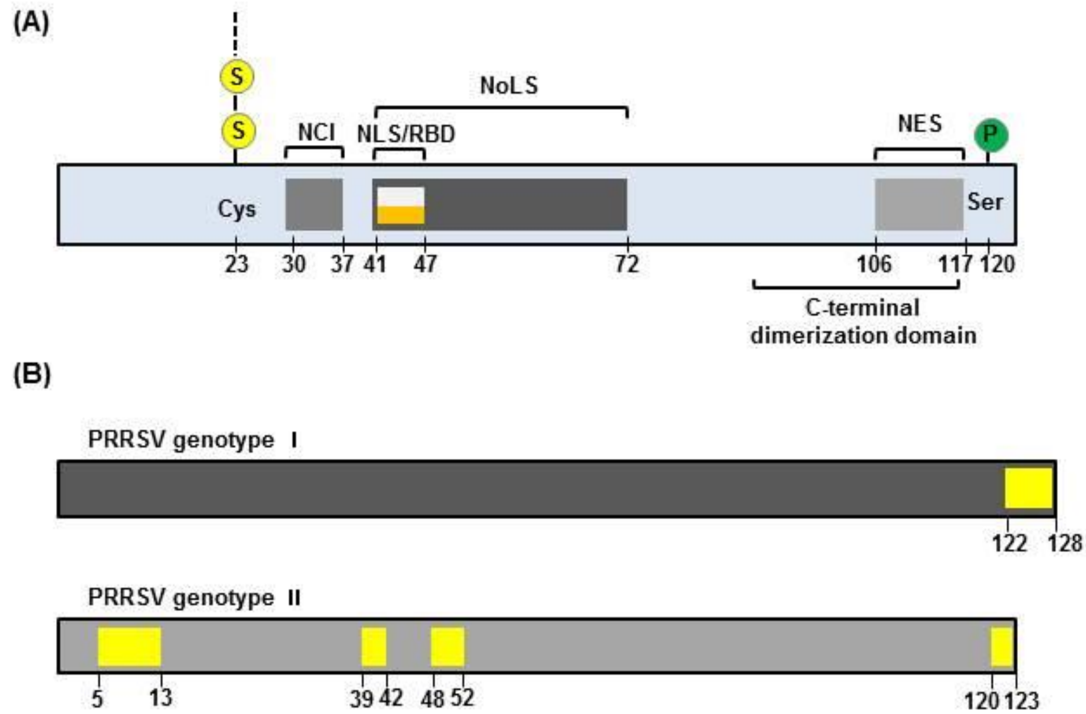

Figure 3. (A) Schematic presentation of the nucleocapsid (N) protein. (B) Several regions of N (yellow areas) have been attempted for deletion but none of those regions can be deleted. Some amino acids can be mutated or deleted (orange area). NLS, nuclear localization signal; NES, nuclear export signal; P, phophorylation site; S, disulfide bridge, RBD, RNA-binding domain; NoLS, nucleolar localization signal. NCI, non-covalent interaction motif.

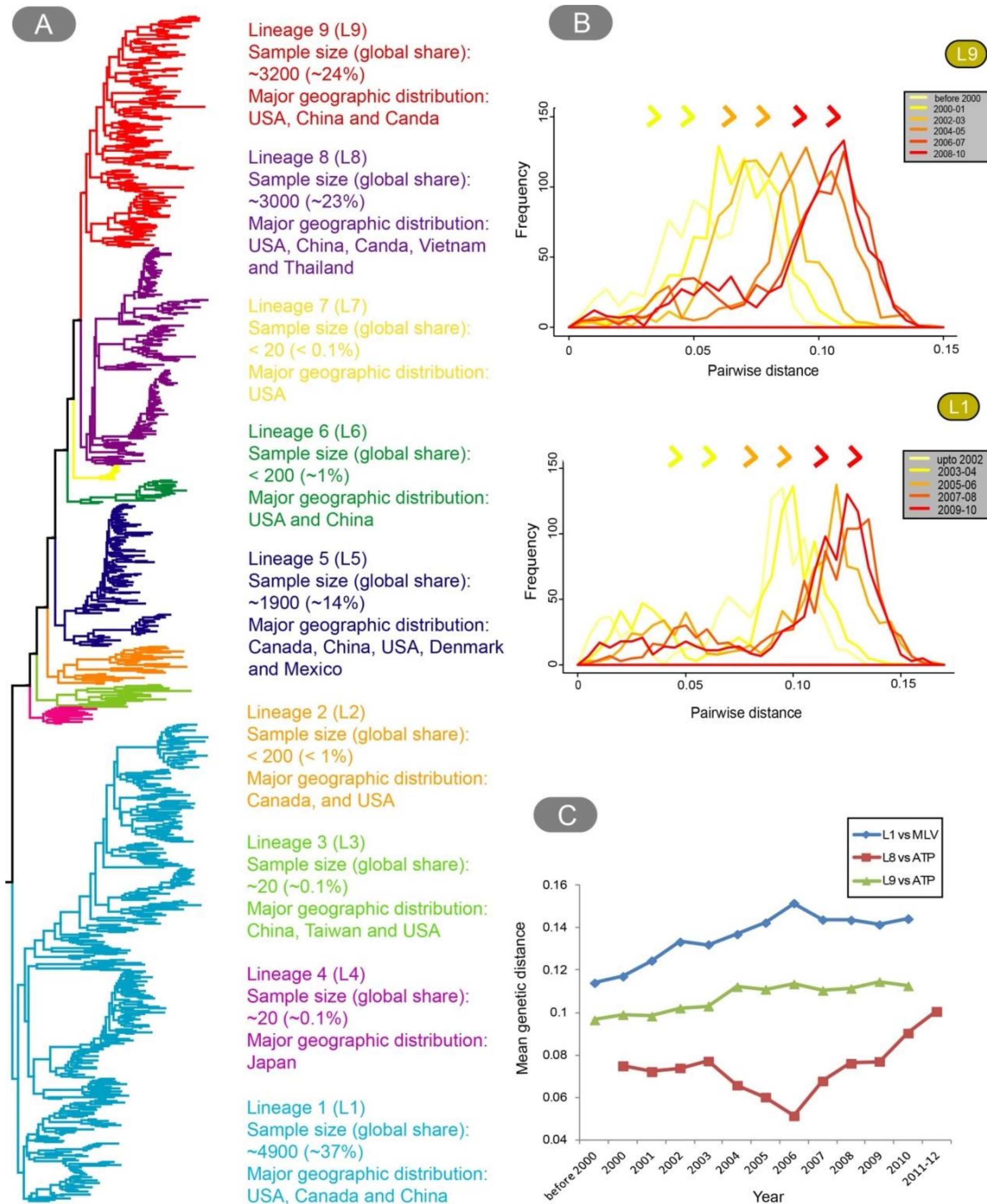

Figure 4. Organization and trends of type 2 PRRSV diversity based on ORF5. (A) Phylogenetic classification of globally sequenced isolates (n > 13,000) into nine lineages established and described earlier (Shi et al., 2010b). Annotations on sample number, share of overall dataset, and key geographic areas impacted are given to the right of each lineage. (B) Increasing diversification of PRRSV over time. Sequences typed to lineages 1 and 9 with sampling dates available were utilized in pairwise comparisons for p-distance determination. A thousand randomly sampled genetic distances for each time frame are summarized for the two lineages. (C) Widening genetic disparity over time between prominent lineages and commercially available vaccines. Sequences were grouped by collection dates separately for lineages 1, 8, and 9. Pairwise p-distances between field isolates and vaccines (RespPRRS MLV and PRRS ATP) were determined and summarized.

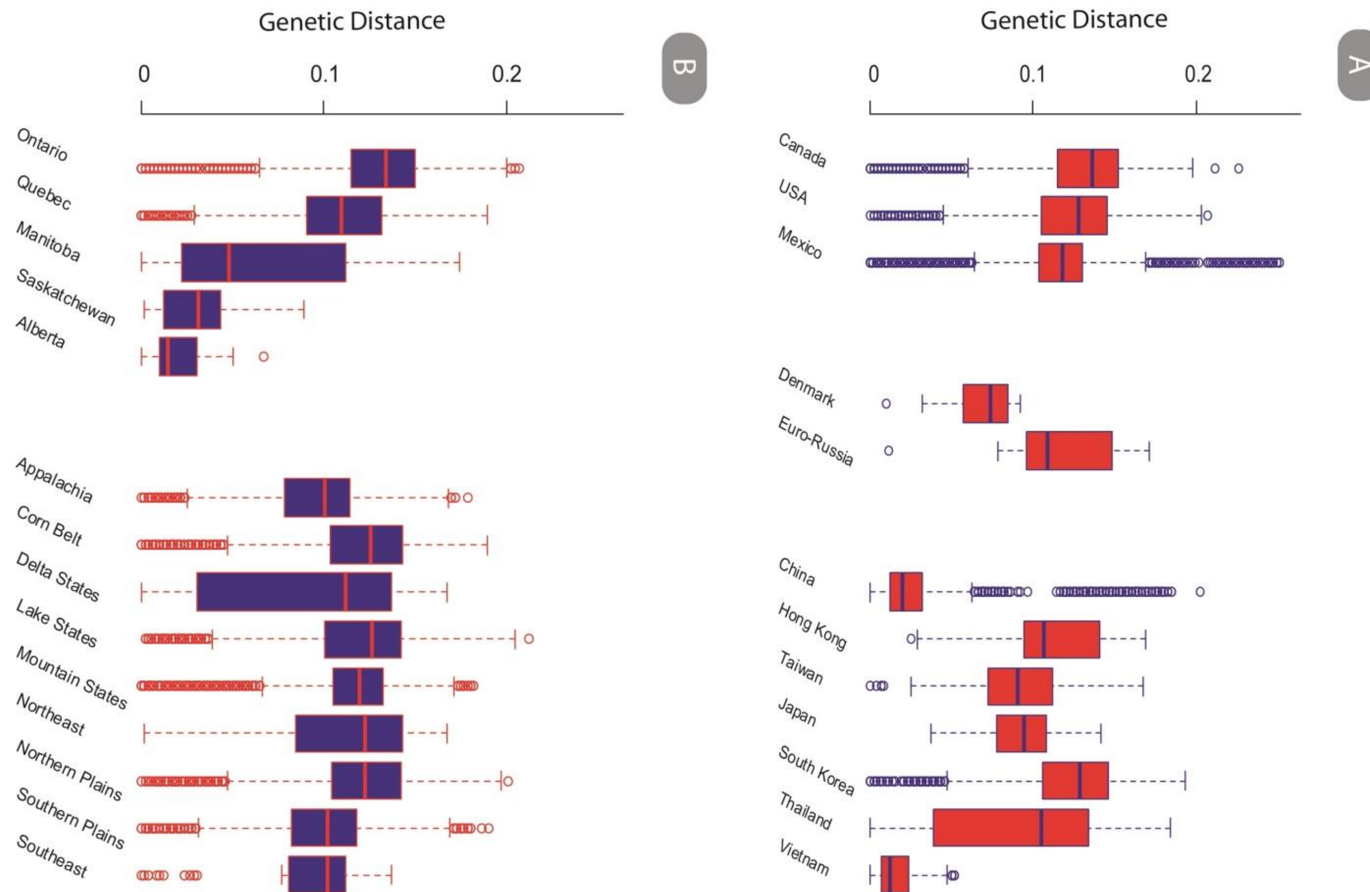

Figure 5. Genetic variability of isolates within major countries and regions impacted by type 2 PRRSV. ORF5 sequences were partitioned based on place of collection. Pairwise p-distances for each partition were determined and a maximum of 10,000 randomly sampled values were summarized as boxplots. Box boundaries represent the first and third quartiles and the division inside the box the median. Whiskers represent at maximum 1.5 x interquartile range. Open circles outside upper and lower whiskers represent outliers. (A) Summary plot based on country-based grouping spanning North America, Euro-Russia, and Asia. (B) Summary plot based on regions within Canada and USA.