Development of a Chimeric Strain of Porcine Reproductive and Respiratory Syndrome Virus with an Infectious Clone and a Korean Dominant Field Strain

Jung-Ah Lee, Nak-Hyung Lee, Sang-Won Lee, Seung-Yong Park, Chang-Seon Song, In-Soo Choi, and Joong-Bok Lee*

Laboratory of Infectious Diseases, College of Veterinary Medicine, Konkuk University, Seoul 143-701, Republic of Korea

(Received Feb 5, 2014 / Revised Mar 11, 2014 / Accepted Mar 13, 2014)

The K418 chimeric virus of porcine reproductive and respiratory syndrome virus (PRRSV) was engineered by replacing the genomic region containing structure protein genes of an infectious clone of PRRSV, FL12, with the same region obtained from a Korean dominant field strain, LMY. The K418 reached 10^6 TCID50/ml of viral titer with similar growth kinetics from a Korean dominant field strain, LMY. The K418 infectious clone of PRRSV, FL12, with the same region of the genomic region containing structure protein genes of an infectious clone of PRRSV, FL12, with the same region obtained from a Korean dominant field strain, LMY. The K418 reached 10^6 TCID50/ml of viral titer with similar growth kinetics to those of parental strains and had a cross-reactive neutralizing antibody response to field serum from the entire country. The chimeric clone pK418 can be used as a practical tool for further studying the molecular characteristics of PRRSV proteins through genetic manipulation. Furthermore, successful construction of the K418 will allow for the development of customized vaccine candidates against PRRSV, which has evolved rapidly in Korea.

Keywords: PRRSV, reverse genetics, infectious clone, chimeric, customized vaccine

Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV), which causes a reproductive and respiratory disease in pigs, contains a positive-sense single-strand RNA genome measuring approximately 15.4 kb. The virus is classified into two genotypes, a North American (NA) type and a European (EU) type. The genome of PRRSV contains nine open reading frames (ORFs). ORF1a and ORF1b occupy approximately 80% of the viral genome and encode nonstructural proteins (NSPs) (Meulenberg et al., 1993). The remaining ORFs encode glycoprotected structure proteins, including the glycoprotein (GP)2, GP3, GP4, and GP5, unglycosylated membrane (M) protein and nucleocapsid (N) protein (Meulenberg, 2000). Previous studies indicated that B-cell epitopes for viral neutralization appeared to reside on structure proteins, including GP3, GP4, GP5, and M (van Nieuwstadt et al., 1996; Yang et al., 2000; Cancel-Tirado et al., 2004; Ansari et al., 2006; Plagemann, 2006).

PRRS is characterized by reproductive failure, including aborted, stillborn, mummified and weak-born piglets, and respiratory disorder, which can result in high death rates in suckling and weaned pigs. PRRS is responsible for significant economic losses for the swine industry worldwide (Neumann et al., 2005). Control methods including management of incoming replacement gilts, implementation of biosecurity protocols and vaccination have been applied to reduce the risk of PRRSV outbreak. Among them, vaccination has been considered as the most effective tool for preventing the disease (Thawongnuwech and Suradhat, 2010). However, current commercial PRRSV vaccines have the drawback of featuring a high level of antigenic variation between the vaccine and field strains of PRRSV (Meng, 2000). Heterologous strains of PRRSV compared to a vaccine strain possessing antigenic diversity resulting from amino acid sequence variation in GP5 have been constantly isolated and reported from Korea (Cha et al., 2006; Yoon et al., 2008; Kim et al., 2012; Choi et al., 2013). Generally, field strains, which are the most frequently isolated from regional areas, have been used to develop vaccines in each country that has had an endemic PRRSV outbreak.

Reverse genetics (RG) technology is a practical system to study virus characteristics, in vivo pathogenesis and viral protein function. The manipulation of viral RNA genomes is generally difficult due to the instability of RNA genomes and the lack of research tools for direct RNA editing. However, with RG technology, the modification of infectious RNA viruses can be easily achieved. In previous studies, RG technology has been applied to the genetic modification of genomes of positive-sense and negative-sense RNA viruses and to rescue mutant viruses from cDNA infectious clones (Taniguchi et al., 1978; Racaniello and Baltimore, 1981; Castrucci and Kawaoka, 1995).

RG technology has widespread implications in the fields of virology and vaccinology (Ito et al., 2001; Collins and Murphy, 2005; Almazan et al., 2013). Using RG technology, a highly pathogenic strain of a virus can be attenuated by the construction of a chimeric virus with highly and weakly pathogenic viruses. In addition, a chimera virus can serve as a viral vector expressing heterologous antigen. The infectious clone of PRRSV has been developed and used in previous studies (Nielsen et al., 2003; Truong et al., 2004; Yoo et al., 2004; Lee et al., 2005; Fang et al., 2006). In previous studies, diverse chimera viruses of PRRSV have been generated using RG technology to manipulate viral genomes.
to determine the viral protein involved in the pathogenicity of field strains in pigs (Kwon et al., 2006; Zhou et al., 2012; Ni et al., 2013). However, RG technology has been used mainly to characterize the function of viral proteins but has not been used to develop recombinant vaccine candidates against PRRSV. In this study, a chimeric virus was constructed using an infectious clone of PRRSV containing genomes of the FL12 strain and a Korean dominant field strain, LMY. The chimeric virus, named K418, contains NSPs from the FL12 strain and structure proteins from the LMY strain.

Materials and Methods

Cells and virus

MARC-145 cells were used to rescue virus and to determine viral growth kinetics. The PRRSV field isolate, LMY, was isolated at the Animal, Plant and Fisheries Quarantine and Inspection Agency (Korea) from a case of PRRSV infection associated with clinical disease. Virus titers were calculated and expressed as tissue culture infectious doses 50 (TCID50)/ml.

Construction of a chimeric clone and recovery of chimeric virus

The PRRSV infectious cDNA clone, pFL12, was generously provided by Dr. Fernando Osorio and Dr. Asit Pattnaik of the University of Nebraska-Lincoln (Truong et al., 2004). A genomic region including whole-structure genes from the LMY strain of PRRSV was amplified using reverse transcription-polymerase chain reaction (RT-PCR) with a primer pair of 5'-GTGGATGCTTTCACGGAGTTC-3' and 5'-CACAATTAAACGTGTTTTTTTTTTTTTTTTTTTTT TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT
RNA for the recovery of infectious PRRSV as efficiently as baby hamster kidney (BHK)-21 cells (Truong et al., 2004; Ansari et al., 2006). These results showed that the chimeric virus, K418, recovered and replicated well in MARC-145 cells.

**Growth properties of the chimeric virus**

Upon the initial development of CPE, the supernatant from the electroporated cells was collected and infected into MARC-145 cells to propagate recovered viruses. The once-passaged culture supernatant produced 80% CPE at 5 days post-infection and yielded virus titers of 10^6 TCID_{50}/ml. The multi-step growth kinetics of the chimera virus was compared to those of parental strains in MARC-145 cells (Fig. 2). Genetic engineering did not greatly affect viral replication. The result of the growth kinetics study demonstrated that the final titers of the chimera and parental viruses were similar, whereas the chimeric virus showed slightly delayed viral replication.

**Cross neutralization of field samples by K418 chimeric virus**

The serum samples were divided into three groups depending on the K418-specific neutralizing antibody titers, cross-reactive, partially cross-reactive and resistant. The neutralizing antibody titers of cross-reactive group were greater than 1:8, the titers of partially cross-reactive group were 1:8 and the titers of resistant group were less than 1:8. The cross-reactive samples against K418 virus, including partially cross-reactive ones, accounted for 27.06% whereas cross-reactive samples against MLV which widely used commercial live vaccine accounted for 5.88% of the total field samples (Table 1).

**Discussion**

RG technology has been used to produce numerous vaccine candidates against other viruses, including the influenza, human respiratory syncytial, Newcastle disease and foot-and-mouth disease viruses (Castrucci and Kawaoa, 1995; Collins and Murphy, 2005; Hu et al., 2009; Blignaut et al., 2011). The chimeras developed using RG technology could be used as a multivalent vaccine to control diseases caused by rapidly changing RNA viruses. In this study, a chimeric virus was constructed using an infectious clone of PRRSV, FL12, and a Korean dominant field strain, LMY, using RG technology. Many chimeric or mutant viruses of PRRSV have shown reduced viral titers relative to those of parental viruses (Bentley et al., 2013; Ni et al., 2013). However, K418 produced a viral yield similar to the yields of parental viruses. These results might be caused by the different characteristics of the strains used in this study, FL12 and LMY. The two strains have previously been incorporated into MARC-145 cells through multiple passages. Therefore, the recovered chimeric virus, K418, may have inherited its susceptibility to growth conditions in MARC-145 cells from parental viruses.

K418 showed a cross-reactive neutralizing antibody response against a considerable proportion of field serum samples collected from the entire country. Generally, protection against PRRSV in pigs can be established starting from the serum neutralization titers of 1:8 (Osorio et al., 2002). Korean field strains of PRRSV were classified into NA or EU genotype with similar rate. There is a considerable antigenic variability between the NA and EU strains of PRRSV (Cha et al., 2006; Yoon et al., 2008; Kim et al., 2012; Choi et al., 2013). Furthermore, sequence diversity on the ORF5 decreased serological cross-reactivity between PRRSV strains belonging to the same genotype (Kim et al., 2013). The MLV containing a representative attenuated strain of NA type virus is the most commonly used PRRSV vaccine worldwide in field. The MLV virus was frequently isolated from vaccinated pigs in fields. However, only 5.88% of the total PRRSV ELISA positive serum samples neutralized MLV, while 27.06% of PRRSV ELISA positive serum samples neutralized the chimeric virus, K418. The serological cross-reactivity against the K418 virus suggests that the humoral immune response induced by the chimeric virus would cross-react with approximately half percentage of the NA field strain of PRRSV in Korea. The polyclonal antiserum against the K418 neutralized the LMY strain, but not the FL12 and MLV strain of PRRSV (Lee et al., manuscript in preparation). These results supported that the chimeric virus would be more effective as a potential regional vaccine candidate to protect pigs than the MLV vaccine.

To generate customized vaccine candidate, the genomic region of established PRRSV infectious clone encoding structure proteins that play a critical role in the virus neutralizing response were replaced with the same genomic region from a Korean dominant field strain of PRRSV. It is highly possible that K418 would induce a host immune response, which can broadly cross-protect most of the NA genotype of Korean field strains of PRRSV, because the virus expresses all structure proteins from one of the current dominant field.
strains of PRRSV. Further studies are in progress to determine a possible application of K418 as a vaccine candidate in Korea and to evaluate the efficacy of other genetically modified K418 viruses.

Acknowledgements

We thank Dr. Fernando Osorio, Dr. Asit Pattnaik and Dr. Byungjoon Kwon of the University of Nebraska-Lincoln for providing the PRRSV infectious clone, FL12. This study was supported by a grant of Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries.

References

Almazan, F., DeDiego, M.L., Sola, I., Zuniga, S., Nieto-Torres, J.L., Marquez-Jurado, S., Andres, G., and Enjuanes, L. 2013. Engineering a replication-competent, propagation-defective Middle East respiratory syndrome coronavirus as a vaccine candidate. MBio 4, e00650–00613.

Ansari, I.H., Kwon, B., Osorio, F.A., and 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.

Bentley, K., Armesto, M., and Britton, P. 2013. Infectious bronchitis virus as a vector for the expression of heterologous genes. PLoS One 8, e67875.

Blignaut, B., Visser, N., Theron, J., Rieder, E., and Maree, F.F. 2011. Custom-engineered chimeric foot-and-mouth disease vaccine elicits protective immune responses in pigs. J. Gen. Virol. 92, 849–859.

Cancel-Tirado, S.M., Evans, R.B., and Yoon, K.J. 2004. Monoclonal antibody analysis of porcine reproductive and respiratory syndrome virus epitopes associated with antibody-dependent enhancement and neutralization of virus infection. Vet. Immunol. Immunopathol. 102, 249–262.

Castrucci, M.R. and Kavaoka, Y. 1995. Reverse genetics system for generation of an influenza A virus mutant containing a deletion of the carboxyl-terminal residue of M2 protein. J. Virol. 69, 2725–2728.

Cha, S.H., Choi, E.J., Park, J.H., Yoon, S.R., Song, J.Y., Kwon, J.H., Song, H.J., and Yoon, K.J. 2006. Molecular characterization of recent Korean porcine reproductive and respiratory syndrome (PRRS) viruses and comparison to other Asian PRRS viruses. Vet. Microbiol. 117, 248–257.

Choi, E.J., Lee, C.H., Song, J.Y., Song, H.J., Park, C.K., Kim, B., and Shin, Y.K. 2013. Genetic diversity of porcine reproductive and respiratory syndrome virus in Korea. J. Vet. Sci. 14, 115–124.

Collins, P.L. and Murphy, B.R. 2005. New generation live vaccines against human respiratory syncytial virus designed by reverse genetics. Proc. Am. Thorac. Soc. 2, 166–173.

Fang, Y., Rowland, R.R., Roof, M., Lunney, J.K., Christopher-Hennings, J., and Nelson, E.A. 2006. 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.

Hu, S., Ma, H., Wu, Y., Liu, W., Wang, X., Liu, Y., and Liu, X. 2009. A vaccine candidate of attenuated genotype VII Newcastle disease virus generated by reverse genetics. Vaccine 27, 904–910.

Ito, N., Takayama, M., Yamada, K., Sugiyama, M., and Minamoto, N. 2001. Rescue of rabies virus from cloned cDNA and identification of the pathogenicity-related gene: glycoprotein gene is associated with virulence for adult mice. J. Virol. 75, 9121–9128.

Kim, W.L., Kim, J.J., Cha, S.H., Wu, W.H., Cooper, V., Evans, R., Choi, E.J., and Yoon, K.J. 2013. Significance of genetic variation of PRRSV ORF5 in virus neutralization and molecular determinants corresponding to cross neutralization among PRRS viruses. Vet. Microbiol. 162, 10–22.

Kim, H.K., Nguyen, V.G., Kim, I.O., Park, J.H., Park, S.J., Rho, S.M., Han, J.Y., and Park, B.K. 2012. Epidemiologic and phylogenetic characteristics of porcine reproductive and respiratory syndrome viruses in conventional swine farms of Jeju Island as a candidate region for PRRSV eradication. Transbound Emerg. Dis. 59, 62–71.

Kwon, B., Ansari, I.H., Osorio, F.A., and Pattnaik, A.K. 2006. Infectious clone-derived viruses from virulent and vaccine strains of porcine reproductive and respiratory syndrome virus mimics biological properties of their parental viruses in a pregnant sow model. Vaccine 24, 7071–7080.

Lee, C., Calvert, J.G., Welch, S.K., and 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.

Meng, X.J. 2000. Heterogeneity of porcine reproductive and respiratory syndrome virus: implications for current vaccine efficacy and future vaccine development. Vet. Microbiol. 74, 309–329.

Meulenbergh, J.J. 2000. PRRSV, the virus. Vet. Res. 31, 11–21.

Meulenbergh, J.J., Hulst, M.M., de Meijer, E.J., Moomen, P.L., den Besten, A., de Kuyper, E.P., Wensvoort, G., and Moormann, R.J. 1993. Lelystad virus, the causative agent of porcine epidemic abortion and respiratory syndrome (PEARIS), is related to LDV and EAV. Virology 192, 62–72.

Neumann, E.J., Klieberstein, J.B., Johnson, C.D., Mabry, J.W., Bush, E.J., Seitzinger, A.H., Green, A.L., and Zimmerman, J.J. 2005. Assessment of the economic impact of porcine reproductive and respiratory syndrome on swine production in the United States. J. Am. Vet. Med. Assoc. 227, 385–392.

Ni, Y.Y., Opriessnig, T., Zhou, L., Cao, D., Huang, Y.W., Halbur, P.G., and 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.

Nielsen, H.S., Liu, G., Nielsen, J., Oleksiewicz, M.B., Botner, A., Storgaard, T., and 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.

Osorio, F.A., Galeota, J.A., Nelson, E., Brodersen, B., Doster, A., Wills, R., Zuckermann, F., and Laegreid, W.W. 2002. Passive transfer of virus-specific antibodies confers protection against reproductive failure induced by a virulent strain of porcine reproductive and respiratory syndrome virus and establishes sterilizing immunity. Virology 302, 9–20.

Plagemann, P.G. 2006. Neutralizing antibody formation in swine infected with seven strains of porcine reproductive and respiratory syndrome virus as measured by indirect ELISA with peptides containing the GP5 neutralization epitope. Virology Immunol. 19, 285–293.

Racaniello, V.R. and Baltimore, D. 1981. Molecular cloning of poliovirus cDNA and determination of the complete nucleotide sequence of the viral genome. Proc. Natl. Acad. Sci. USA 78, 4887–4891.

Taniguchi, T., Palmieri, M., and Weissmann, C. 1978. QB DNA-containing hybrid plasmids giving rise to QB phage formation in the bacterial host. Nature 274, 223–228.

Thanawongnuwech, R. and Suradhat, S. 2010. Taming PRRSV: re-
visiting the control strategies and vaccine design. *Virus Res.* 154, 133–140.

Truong, H.M., Lu, Z., Kutish, G.F., Galeota, J., Osorio, F.A., and 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.

van Nieuwstadt, A.P., Meulenberg, J.J., van Essen-Zanbergen, A., Petersen-den Besten, A., Bende, R.J., Moormann, R.J., and Wensvoort, G. 1996. Proteins encoded by open reading frames 3 and 4 of the genome of Lelystad virus (Arteriviridae) are structural proteins of the virion. *J. Virol.* 70, 4767–4772.

Yang, L., Frey, M.L., Yoon, K.J., Zimmerman, J.J., and Platt, K.B. 2000. Categorization of North American porcine reproductive and respiratory syndrome viruses: epitopic profiles of the N, M, GP5, and GP3 proteins and susceptibility to neutralization. *Arch. Virol.* 145, 1599–1619.

Yoo, D., Welch, S.K., Lee, C., and Calvert, J.G. 2004. Infectious cDNA clones of porcine reproductive and respiratory syndrome virus and their potential as vaccine vectors. *Vet. Immunol. Immunopathol.* 102, 143–154.

Yoon, S.H., Song, J.Y., Lee, C.H., Choi, E.J., Cho, I.S., and Kim, B. 2008. Genetic characterization of the Korean porcine reproductive and respiratory syndrome viruses based on the nucleocapsid protein gene (ORF7) sequences. *Arch. Virol.* 153, 627–635.

Zhou, L., Ni, Y.Y., Pineyro, P., Sanford, B.J., Cossaboom, C.M., Dryman, B.A., Huang, Y.W., Cao, D.J., and 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.