Chapter 2

Review literatures

The syndrome of reproductive and respiratory diseases of the unknown etiology was first recognized in 1987 in the swine herds in North Carolina, Iowa and Minnesota (Keffaber, 1989). In the Netherlands, a few years later, the similar syndrome was refered to various names such as mystery swine disease, porcine epidemic abortion and respiratory syndrome (PEARS), Seuchenhafter Spatabort der Schweine, blue ear disease, porcine reproductive and respiratory syndrome (PRRS) and swine infertility and respiratory syndrome (SIRS) (Collins et al., 1992). Finally, PRRS has been used to described the disease characterized by reproductive failure in gilts, sows and respiratory diseases in young pigs. The etiologic agent of the disease was first isolated in the Netherlands in 1991 and was named the Lelystad virus, identified as the European genotype. Later in the United States, the prototype of the American strain (US), VR-2332 was isolated in 1992 (Collins et al., 1992). In Thailand, porcine reproductive and respiratory syndrome virus (PRRSV) might spread via imported breeding pigs from both continents. The serological survey of the swine sera during 1988-1999 demonstrated the earliest detection of seropositive animals in 1989 and the percentage of seropositive animals increased annually from 8.6 in 1996 to over 79 in 1999. PRRSV was first isolated in Thailand from suckling and nursery pigs with severe chronic respiratory distress and the virus was identified as the US genotype (Damrongwatanapokin et al.,

1996). A few years later, both EU and US genotypes have been reported in Thailand by nested multiplex PCR (Thanawongnuwech et al., 2002). However, antigenic variation exists within the US genotype based on the nucleotide sequences of open reading frame 5 (ORF5) (Meng et al., 1995a).

PRRSV is classified within the genus *Arterivirus* in the family *Arteriviridae* placed in the order Nidovirales (Cavanagh, 1997). The Arteriviruses include lactate dehydrogenase - elevating virus (LDV) of mice, equine arteritis virus (EAV) and simian hemorrhagic fever virus (Rowland et al., 1999). The PRRSV has a positive single-stranded polyadenylated RNA molecule of approximately 15 kb in length containing eight open reading frames (ORFs). The genome contain two large open reading frames (ORF 1a and 1b) encoding the non-structural polyproteins (viral RNA polymerase and associated protease) and other six smaller ORFs (ORF2-ORF7). ORFs 2, 3, 4 and 5 encode structural proteins, ORF6 encodes a matrix (M) protein of 18-19 kD and ORF 7 encodes a nucleocapsid (N) protein of 14 to 15 kD (Meulenberg et al., 1993; Mardassi et al., 1995; Meulenberg et al., 1995; Cavanagh., 1997; Meulenberg et al., 1997). Envelope glycoprotein E, encoded by ORF5, is a major viral glycoproteins consisting of a 25 kDa. Both glycoprotein E and the glycoprotein encoded by ORF4 induce neutralizing antibodies (Andreyev et al., 1997). These principal envelope glycoproteins, containing a hypervariable region are responsible for generating the diversity of the PRRSV. The variability in the ectodomain of ORF5 associated with antigenic variability of the GP5 of PRRSV is the result of positive or

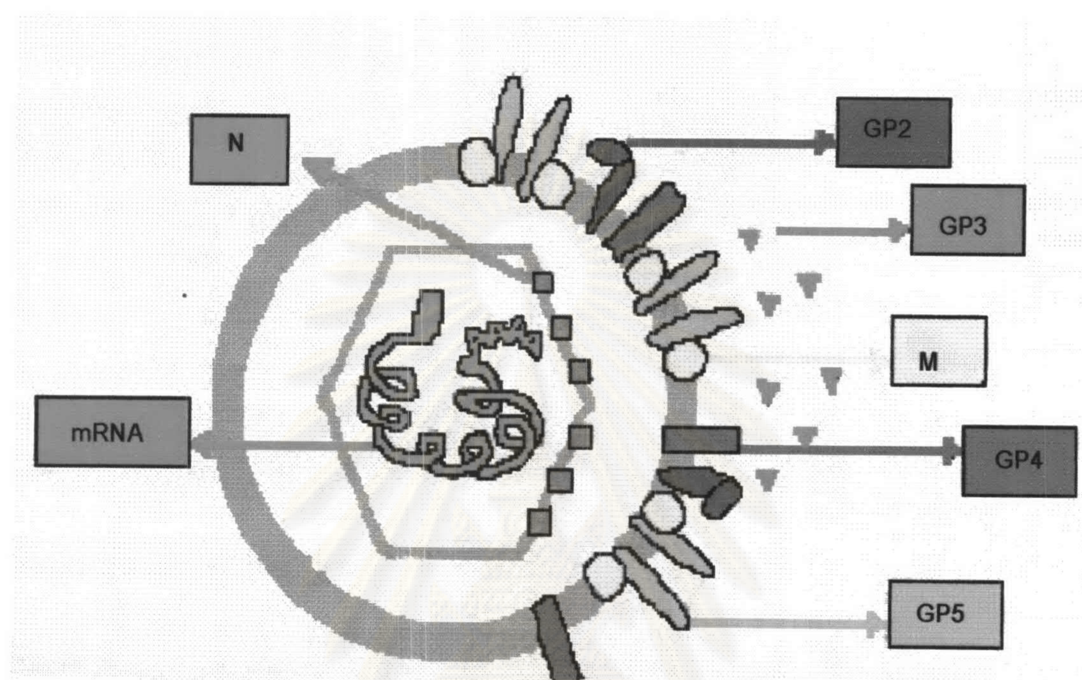

Fig. 1 Schematic representation of the PRRSV. The virion is spherical in shape, enveloped and possesses a non-segmented single strand RNA genome that is encapsidated by the nucleocapsid protein (N), yielding an icosahedral core structure. At least four protein components were envelope-associated: the nonglycosylated matrix protein (M) and the GP5 glycoproteins representing major components, whereas GP2 and GP4 are two minor components (Dea et al., 2000).

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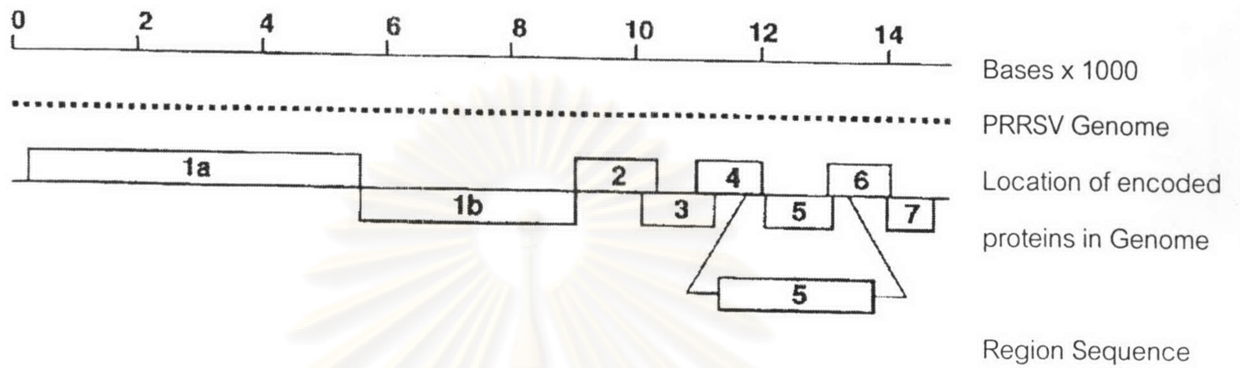

Fig 2 Schematic diagram of the PRRSV genome and gene organization. The nucleotide sequence is represented as a dotted line and the genes, each one of which encodes a viral protein, are shown as numbered boxes (Michael et al., 2001).

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negative neutral selection by antibodies or other host defenses (Pirzadeh et al., 1998; Rowland et al., 1999). Mutations within the antigenic domains of GP5 may contribute to the establishment of a chronic form of the disease and eventually a persistent infection (Pirzadeh et al., 1998). The antigenic drifts of the hydrophilic domains of GP5 could be the result of the host's selective humoral immune response directing against the exposed domains of this envelope glycoprotein. These genome variants may be an effective mechanism for evading the host's immune surveillance. The one point mutation in the epitope recognized by a monoclonal antibody (Mab) may change the reactivity of the strain with Mab (Pirzadeh et al., 1998). For example, the monoclonal antibody, SDOW17 (South Dakota State, USA) reacts with all EU and US isolates but VO17 (South Dakota State, USA) reacts with only the US isolates (Nelson et al., 1993). Similarly, WBE4 reacts with some EU isolates (Drew et al., 1995).

The antibody to nucleocapsid proteins may be detected as early as 14 days post infection (Xiao et al., 2003). Anti-GP5 titers are not present until day 21 and peaked at day 28. Disappearance of virus from the circulation is most closely associated with an increase in antibodies to GP5 (Xiao et al., 2003). The decrease of peripheral $CD4^+$ and $CD8^+$ T subset lymphocytes suggest an early and transient immunosuppression in circulating lymphocytes. Lymphoid tissues, including spleen, inguinal lymph nodes, sternal lymph nodes, mesenteric lymph nodes and bone marrow, harbour PRRSV-specific lymphocytes during acute and persistent infection. Tonsils, the primarily lymphoid tissue sampled in the persistence studies, had high level of PRRSV but no

responding of lymphocytes. The IFNγ-secreting lymphocytes are $CD8^+$ suggesting that PRRSV-specific lymphocytes are $CD8^+$ cytotoxic T lymphocytes. The response of PRRSV-specific IFNγ-secreting lymphocytes was transient, and showed little correlation with clearance of virus from the circulation. However, PRRSV-specific IFNγ-secreting cells were detected in PBMC at day 14 and peaked at 28 days post infection (Xiao et al., 2003).

The envelope glycoprotein E is at least partially responsible for the serological differences between the EU and the US strains of PRRSV. This suggests that PRRSV glycoprotein E in part might account for some antigenic differences that occur among the majority of the US genotype (Andreyev et al., 1997). Interestingly, in one study the low virulence strain had more variation of amino acid sequences than that of the severe virulence isolates and formed a branch distinct from other US isolates (Meng et al., 1995a). In additions, the marked differences in pathogenicity between the US and the EU isolates and among the US isolates have been demonstrated. Experimentally, the Lelystad virus (EU prototype) and the low virulence US isolate (VR 2431) induced mild transient pyrexia, dyspnea and tachypnea while the high virulence US isolates (VR 2385) induced labored and rapid abdominal respiration, pyrexia, lethargy, anorexia and patchy dermal cyanosis. The VR 2385 inoculated pigs had earlier onset of lung lesions, significantly more severe lesions and more persistent lung lesions (Halbur et al., 1995b). PRRSV antigen was detected more in the lung, lymph nodes and tonsils of VR2385 infected pigs than those of the VR 2431 infected and Lelystad infected pigs by

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immunohistochemical evaluation. On the other hand, the cell type and the distribution of PRRSV - positive cells in most tissues were similar for all viruses tested (Halbur et al., 1996a).

The PRRSV is considered to be very infectious since pigs can be infected with relatively small amounts of virus (20-40 virions) by intramuscular (Nelson et al., 1994), intrauterine (Yaeger et al., 1993; Lager et al., 1996; Prieto et al., 1997), oronasal (Terpstra et al., 1991), intraperitoneal (Swenson et al., 1994) or intravenous (Mengeling et al., 1994) infection. The replication cycle is quick with infectious virus particles produced in vitro within 10 hours (Pol et al., 1997) and in vivo within 12 hours after inoculation (Rossow et al., 1995). PRRSV induces cellular lysis of the host cell and also induce apoptosis of the adjacent cells (Suarez et al., 1996; Sur et al., 1997; Sirinarumitr et al., 1998; Sur et al., 1998).

Virus may get into the susceptible cells by several possible ways either adhering at the primary site of exposure or being engulfed by a phagocytic cell. PRRSV may enter the phagocytic cells through an antibody - dependent mechanism referred to as antibody dependent enhancement (ADE) entering a cell through the Fc receptors mediated event or enter a cell via a pH-dependent endocytotic pathway (Kreutz and Ackermann, 1996). A putative PRRSV receptor (210 kd) has been identified on macrophages (Duan et al., 1998). Two receptors are identified for PRRSV on macrophages: heparan sulphate glycosaminoglycans (bind with matrix-protein or GP5) and sialoadherin (both attachment and internalization of PRRSV) (Nauwynck et al.,

2003). However, the macrophage tropism of PRRSV is restricted within only some subpopulations representing some specific states of differentiation and activation (Choi et al., 1994). The restricted tropism for some subpopulations of macrophages by PRRSV is suggestive of a quantitatively heterogenous distribution of the virus in different organs or tissues. The tonsil, lung and tracheobronchial lymph nodes appear to be the replication sites (Shin and Molitor, 2002). PRRSV also induces lysis and decrease bactericidal functions of pulmonary alveolar macrophages (PAMs) (Thanawongnuwech et al., 2000). PAMs are the preferable site of PRRSV replication which may result in replacement of those macrophages by immature cells. The young macrophages have limited ability to cope with bacterial killing resulting in pneumonia and septicemia (Pijoan et al., 1994). Pulmonary intravascular macrophage (PIMs), another population of pulmonary macrophages are very important in clearance of circulating bacteria in pigs. PRRSV induced damages to PIMs have a detrimental effect on removal of bacteria from the bloodstream, which results in increased incidence and severity of secondary bacterial infection, especially *Streptococcus suis* associated disease (Thanawongnuwech et al., 2000). PRRSV can also sensitize the lungs upon exposure to endotoxins of Gram-negative bacteria (lipopolysaccharide; LPS). LPS exert their biological effects after binding to CD14, a specific LPS receptor which is expressed on the membrane of monocytes, macrophages and neutrophils. Binding of LPS to CD14 can be enhanced by LPS-binding protein (LBP), a soluble acute phase protein. LBP increases the effect of LPS about 100 – fold. The PRRSV infection increases CD14

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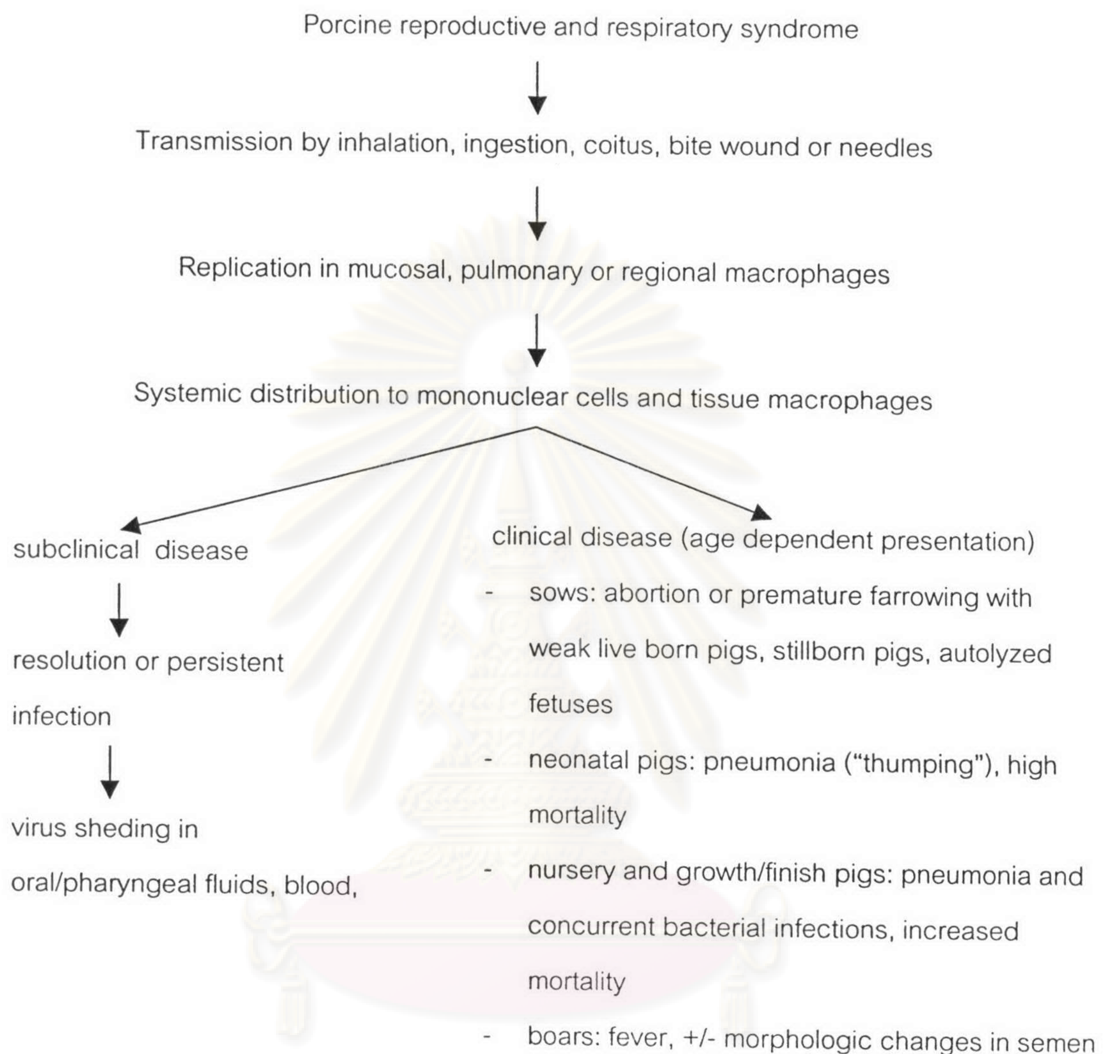

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Fig 3 Pathogenesis of PRRSV infection (Rossow, 1998)

expression and LBP in the lungs, thereby sensitizing the lungs for LPS (Van Gucht et al., 2003).

After PRRSV infection, the virus initiated clearance from systemic circulation at day 5 post infection and slower rate of clearance from 5 to 14 dpi relate with the higher viral load in tissue (Shin and Molitor, 2002). PRRSV antigen has been demonstrated in monocytes and macrophages of multiple tissues eg. skin, kidney and spermatogenic cells by immunohistochemistry (Voicu et al., 1994; Sur et al., 1997; Rossow, 1998; Thibault et al., 1998). PRRSV antigen were also positive in the resident macrophages of salivary gland and skin by fluorescence in situ hybridization (Chuch et al., 1999). The PRRSV induces lesions in multiple tissues, but the most discriminating lesion is in the lung. The pulmonary lesions are observed within 48 hours of infections, peaked by 10 days and resolved by 21-28 days post infection (Halbur et al., 1995b; Rossow et al., 1995; Halbur et al., 1996). Microscopically, multifocal interstitial pneumonia is characterized by three main changes: 1) septal thickening with mononuclear cells, 2) type 2 pneumocyte hypertrophy and hyperplasia and 3) accumulation of normal and necrotic macrophages in alveolar spaces with peribronchiolar and perivascular cuffing (Halbur et al., 1995b). Lymphoid tissues are affected in multiple sites concurrently and characterized by germinal center hypertrophy and hyperplasia with occasionally necrosis. The generalized lymphadenopathy characterized by a follicular hypertrophy and hyperplasia begins about 10-14 days after infection and persists for at least several weeks (Halbur et al., 1995b; Halbur et al., 1996). Vascular lesions can occur in blood

and lymphatic vessels of any size in any organ. Lymphocytes and plasma cells infiltrate beneath the endothelium with occasional transmural infiltration of those affected vessels. Heart lesions contain subendocardial, subepicardial and myocardial infiltration of lymphocytes. Brain lesions compose of infrequently histiocytic choroid plexitis, lymphocytic perivascular cuffing and gliosis. Meningeal lesions are rarely found. Nasal turbinate lesions demonstrate loss of cilia, cystic intraepidermal spaces and submucosal inflammation. Interestingly, fetal infection is only detected in a small percentage of aborted litters and most of these usually are litters aborted within the last 2 weeks of the gestation. Aborted fetuses rarely have lesions except vasculitis involving major organs, myocarditis, encephalitis or just a segmental necrotizing arteritis of the umbilical cord (Swenson et al., 1995; Lager and Halbur, 1996; Rossow et al., 1996). Infected - boars may have decreased semen quality. Infected - sows have mild lymphoplasmacytic inflammation in the reproductive tract. Clinical manifestations of PRRS are diverse and complex. Depending in part on the immune status of the host. The severity and duration of a PRRS outbreak is variable. Some herds may be devastated by high production losses, whereas other herds have no effect. Differences in mortality are possibly due to the dose of virus at exposure, virus strain, pig genetic susceptibility or the differences in management system (Halbur et al., 1995b).

The commercial modified live virus (MLV) vaccine have been used in the United states since late 1994 and then used in some countries but not in Thailand. This particular vaccine is based on a cell culture adaptation of the pathogenic North

American VR 2332 isolate (Collin et al., 1992). A few years later, there was an evidence that vaccine virus could persist, mutate and eventually cause clinically relevant responses in pigs (Botner et al., 1997). In addition, the vaccine-derived field isolate is able to cause reproductive disorders by inoculation into the pregnant sows (Nielsen et al., 1998). Coexisting between the vaccine virus and the field strain is possible and may induce clinical disease (Rossow et al., 1999). The natural recombinant of ORF1 has been reported (Yuan et al., 1999) and the introduction of the American PRRSV to Denmark was determined to be the modified live virus vaccine (Madsen et al., 1998). In addition, 33% of PRRSV field strains, isolated in Korea had the same RFLP pattern for the MLV vaccine (Cheon and Chae, 2000). Accordingly in Japan, some field isolates also have the same RFLP pattern for the vaccine virus, despite that the isolates before using the live vaccine had no RFLP pattern identical to the vaccine virus (Itou et al., 2001). Recently, 86 of 254 of the Canadian isolates demonstrated the RFLP pattern similar to the MLV and the sequence analysis suggested that those isolates might have originated from the spread of the vaccine virus (Cai et al., 2002). These facts may imply that the attenuated vaccine virus has spread to the non vaccinated sows, changed genetically and reverted to virulence (Nielsen et al., 2001). It should be noted that the vaccine strain does not cause detectable pathologic changes other than mild lymph node enlargement while the filed strains having the RFLP pattern for ORF5 identical to the vaccine strains are able to induce pulmonary lesions (Mengeling et al., 1999)

Current methodologies used to detect PRRSV infection include 1) virus isolation, 2) antibody detection, 3) antigen detection and 4) viral nucleic acid detection. Virus isolation is a test of choice used for confirming the presence of the virus and for differentiating the strain by Mab. The serum and lung are excellent samples for virus isolation in acute phase. The lymphoid tissues especially tonsils are excellent for persistent infection. However, boar semen has some toxicity to the cell cultures. RT-PCR, therefore, is a suitable test for detecting PRRSV in the semen. Antibody detection using indirect immunofluorescence (IFA), indirect immunoperoxidase monolayer assay (IPMA) and Enzyme-Linked Immunosorbent Assay (ELISA) provides a sensitive measurement to quantitate as well as to detect antibodies ELISA seems to be the most sensitive method. The ELISA could detect antibodies raised to either the EU or the US strains of PRRSV. The neutralizing antibodies are typically slow to appear following exposure to the PRRSV but are the best indication of protective immunity. Immunohistochemistry and immunofluorescence tests are very sensitive for antigen detection in tissue samples especially in lung and lymphoid tissues (Mengeling and Lager, 2000). RT-PCR is a test used almost exclusively for confirming suspected cases of PRRS. It is fast and very sensitive. The multiplex-PCR has been developed for strain typing (Gilbert et al., 1997) while PCR based-RFLP is for differrentiating DNA variation (Wesley et al., 1998a). The nested set RT PCR was generally as sensitive as the combination of methods used for PRRSV amplification prior to RFLP analysis, and it can markedly reduce the time required for testing (Umthun and Mengeling, 1999). The in

situ hybridization (ISH), the greatest application in experimental studies has been designed to identify the cell and tissue tropism containing PRRSV during persistent infection (Mengeling and Lager, 2000). The fluorimeter-based PCR has the advantage of less time consuming (40 mins) and high specificity (melting point and sequence specific probes) (Spagnudo-Weaver et al., 2000).

The standardized method for RFLP analysis of PRRSV isolates includes digestion of PCR-amplified DNA from ORF5 with selected restriction enzymes (Mengeling and Larger, 2000). There are 3 basic steps including virus amplification by means of isolation and propagation in the cell culture, additional amplification of a portion of the viral genome (mostly ORF 5) by means of a nonnested-set reverse transcriptase polymerase chain reaction (RT-PCR) and then restiction fragment length polymorphism (RFLP) analysis (Umthun and Mengeling, 1999). Regarding to PRRSV replication, genetic variation was able to demonstrate in vitro propagation using MARC-145, MA-104 or pulmonary alveolar macrophage (PAMs) (Kim et al., 1995). The RT-PCR is used for viral RNA amplification containing 2 major steps. Firstly, the viral RNA is converted by the process of reverse transcription into a complementary DNA (cDNA) with reverse transcriptase. The second step is polymerase chain reaction. The number of copies of the target sequence in principle grows exponentially. PCR - based RFLP analysis directly from lung specimens has been demonstrated to be more sensitive, accurate and rapid than from the viral isolation (Cheon and Chae, 2001).

The restriction enzymes give a numerical code to each isolates. The *Mlu*I cutting site is able to distinguish the US vaccine from the field isolates. The MLV vaccine virus could be cut by this enzyme because only this vaccine virus has an alanine (A; GCG) at the residue 137 in a moderately conserved region of ORF5. The consensus sequence has a serine (S; TCA, TCT) at the residue 137 for all the PRRSV strains. A G to T transversion made the 6 nucleotide recognition sequence resulting in *Mlu*I could not cut all the PRRSV isolates. By analyzing the combined *Hinc*II and *Sac*II RFLP patterns, the field isolates could be differentiated from the vaccine (Wesley et al., 1998a). Based on the previous investigation, the RFLP pattern of ORF5 can be differentiated the PRRSV field isolates from the US vaccine strain. However, there were some field isolates having similar pattern with the vaccine strain (Wesley et al., 1998a). Other additional restiction enzymes, *Hae*III was used for investigation in Denmark (Madsen et al., 1998), in Korea (Cheon and Chae, 2000) and Canada (Cai et al., 2002). The *Msp*I was added in the analysis in Japan (Itou et al., 2001). RFLP patterns are able to use investigation of the divergenicity of PRRSV. No evidence for the divergence of RFLP patterns after replication of PRRSV in pigs up to 13 weeks has been found (Wesley et al., 1998b). In contrast to the PRRSV isolates from the Ontario farms, Canada, those viruses have the great variety of RFLP patterns implying that the virus undergoes frequent mutation under field conditions (Cai et al., 2002). The objectives of this study were to study the RFLP patterns of the Thai isolates and to study the pathogenesis of the Thai isolates of each genotype.