CHAPTER 2
Literature review

2.1 Porcine reproductive and respiratory syndrome

Porcine reproductive and respiratory syndrome (PRRS) was first known as blue-eared pig disease in the United Kingdom and the causative agent was called Lelystad virus. The virus that causes PRRS infection in the USA (VR-2332) and Europe (Lelystad virus) had genomic and serologic differences. The virus is a small, enveloped single-stranded RNA virus, which tentatively can be included in a new virus family, Arteriviridae. It infects almost exclusively pig monocytes or macrophages. The virus has varied selective tropism for macrophages of the alveolar spaces and alveolar septa. The tropism of PRRS virus for porcine alveolar macrophage (PAMs) has led to the central hypothesis that lung defense mechanisms are suppressed following PRRS virus infection. For unexplained reasons, the virus may persist in the body for extended periods (several weeks) after an antibody immune response has been mounted. Within herds, the virus may persist for several years[10].

The disease is characterized by very variable clinical signs, including reproductive failure and respiratory diseases. Clinical signs depend on the age of the infected pig and on the pregnancy status and gestation period (Figure 2.1) of the infected sow[11]. Clinical signs of PRRS virus are characterized by late term abortions and stillbirth in sows, and respiratory disease in piglets[12]. However, some seropositive herds show no clinical signs of disease[11]. The respiratory syndrome is often associated with severe infection with secondary bacterial agents including *Pasteurella multocida*, *Haemophilus parasuis* and *Streptococcus suis*. The insemination of either seronegative or preimmunized gilts with boar semen containing PRRS virus may have an insignificant effect or no effect on conception and fertilization rates, although it can result in transplacental infection and embryonic infection and death[13]. Herd performance was severely affected by PRRS over a period of 4-6 months owing to prolonged farrowing interval and a higher replacement rate of sows[2] (Figure 2.1).

PRRS virus is transmitted by contact with blood, oropharyngeal fluids, semen, feces, or urine of an infected animal[11]. Transmission of PRRS virus by aerosolization is possible over short distances. After infection and replication in resident mucosal macrophages, PRRS virus is transported either intracellularly or in free lymph to regional lymph nodes[11].

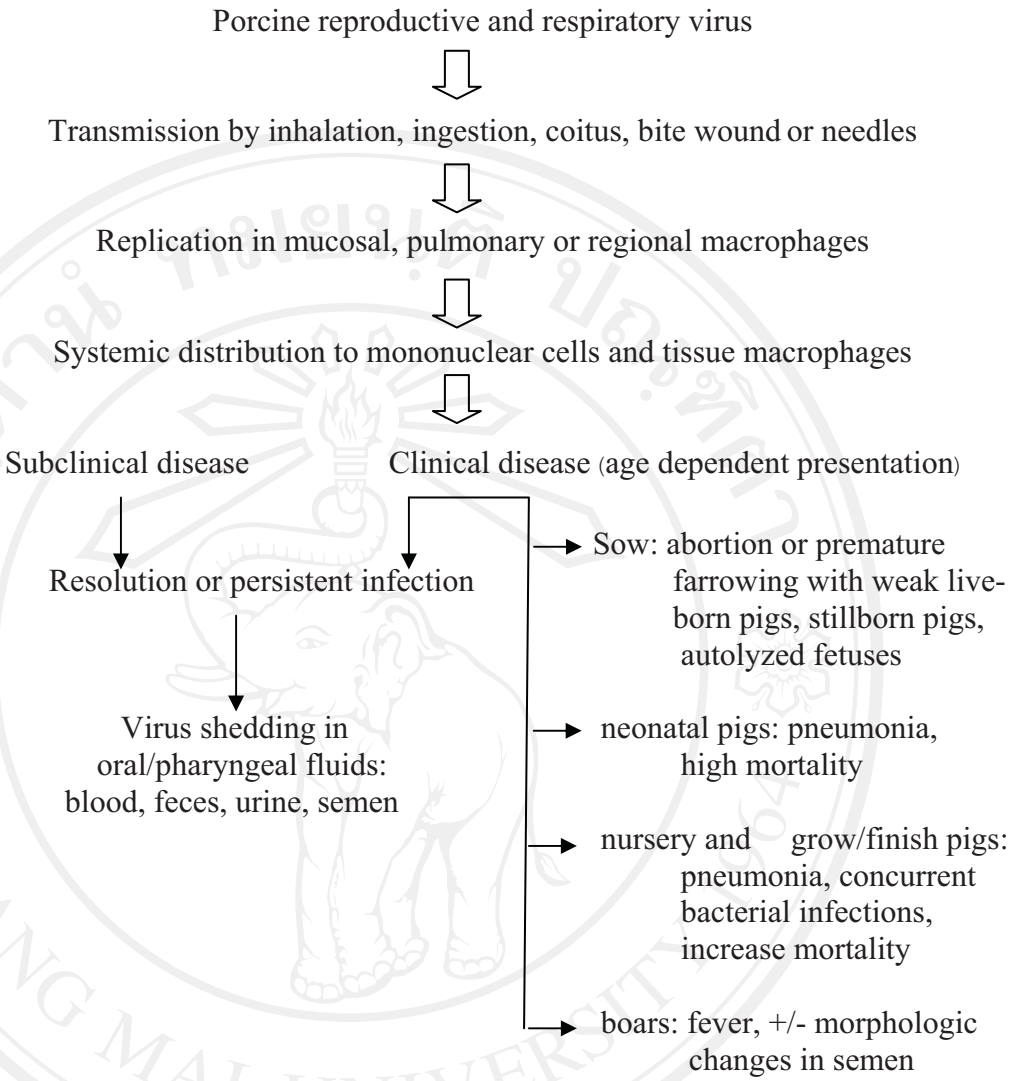

Figure 2.1: Pathogenesis of PRRS virus infection [11]

The diagnostic procedures currently used for investigating PRRS are pathologic examination, serologic testing, and virus detection. The diagnosis of PRRS virus-induced reproductive failure with gross and microscopic lesions occurred in fetuses transplacentally infected with PRRS virus during late gestation. The gross lesions in the umbilical cords ranged from segmental hemorrhagic areas of 1-2 cm in length to a full length involvement of the cord and histopathologic examination revealed a necrotizing umbilical arteritis with periarterial hemorrhage[14].

The development of the humeral immune response against PRRS virus can be monitored by an indirect fluorescent antibody (IFA) test, an immunoperoxidase monolayer assay (IPMA), an enzyme-linked immunosorbent assay (ELISA), or a serum virus neutralization (SVN) test[15]. The direct FA test on fresh tissue utilizing monoclonal antibodies is useful for investigating PRRS virus-associated pneumonia. Virus isolation utilizing swine alveolar macrophages has also been a useful diagnostic procedure[16]. The indirect FA test detects antibodies sooner than the serum neutralization test and will likely become the serologic test of choice. ELISA is technically superior to IFA and IPMA in several aspects: it is less time consuming, is low cost, and is suitable for testing a large number of samples over a short period of time. Thus, ELISA may be a better method for routine detection of PRRS viral antibodies in swine sera[17]. Serological diagnosis provides a high specificity and sensitivity and is easy to perform on a herd level. However, no serological test has proven to be suitable for individual animal certification[18].

A substantial effort toward successfully controlling and eradicating PRRS has emphasized reducing the negative production and economic effects of the disease in swine production. Emphasis is placed on controlling PRRS virus circulation in the breeding herd in an attempt to prevent vertical and horizontal transmission, particularly before weaning[19].

Several methods of eradication have proven to be effective in eliminating PRRSV from positive herds, including whole herd depopulation/repopulation, test and removal, herd closure, and mass vaccination.

Whole herd depopulation/repopulation has been used to eliminate multiple swine pathogens including the PRRS virus. Key elements in maintaining this strategy include purchasing PRRS virus-negative animals and consistent diagnostic testing of each incoming group of animals. However, disadvantages include inability to preserve the genetic material and an increase in production down-time, thus resulting in high implementation cost.

Test and removal methods have also resulted in the successful elimination of PRRS virus from positive populations[20]. This procedure emphasizes testing the entire breeding herd population in order to detect carriers and remove them from the herd. Potential carrier animals are detected through the testing of sera from all animals by ELISA and PCR. Although highly successful in eliminating PRRS virus from endemically infected populations, disadvantages include the high cost of diagnostic procedures and the potential removal of previously exposed animals that no longer have the virus.

Herd closure has also been shown to be a highly efficacious method for eliminating PRRS virus. The basis of herd closure is the cessation of replacement gilt introduction for an extended period (4-8 months), resulting in reduced viral shedding

and the elimination of carrier animals. However, it can be costly and can result in improper parity distribution within the breeding herd[19].

Currently, a PRRS virus vaccine, of either modified live or killed vaccines, is available on the market. Protective immunity develops 7 days after vaccination and persists for about 16 weeks[21]. Using live vaccine in seropositive sows would interrupted shedding and spread of the virus within the herd[22]. Killed vaccines has no effect on virus shedding[23]. There are mixed results regarding the efficacy of these vaccines against the genetically diverse field strains of PRRS virus.

However, the ability to successfully control PRRS depends on identifying the source of virus for the farm, where the virus re-circulates, how the gilt pool is managed, and whether there are available negative sources for replacements and semen.

2.2 Porcine pleuropneumonia

The etiological agent of porcine pleuropneumoniae is *Actinobacillus pleuropneumoniae* (*A. pleuropneumoniae*; APP). This gram-negative capsulated rod bacterium can be divided into two biotypes based on nicotinamide adenine dinucleotide (NAD) requirements. Biovar 1 is dependent upon nicotinamide adenine dinucleotide (NAD), *V* factor, for growth in vitro while biovar 2 is NAD-independent. To date, 15 serotypes have been described on the basis of the antigenic diversity of the capsular polysaccharides and lipopolysaccharides.

Direct transmission of *A. pleuropneumoniae* from nose to nose contact is the most predominant transmission route[24], because the bacterium does not survive long in the environment. Furthermore, it is believed that carriers, pigs that are infectious but do not show clinical disease, are important in the transmission of *A. pleuropneumonia*[25]. Therefore, prevention or reduction of direct transmission from carriers to susceptible pigs by an intervention, such as vaccination, may lead to eradication of *A. pleuropneumoniae* from a population. However, it is not known what magnitude of this intervention can reduce the transmission of *A. pleuropneumoniae* among pigs[24].

The pathogenesis of porcine pleuropneumonia is complex and a large number of virulence factors have been described. *A. pleuropneumoniae* enters the lungs after inhalation. The bacteria bind preferentially to cells of the lower respiratory tract, such as ciliated cells of the terminal bronchiolar and alveolar epithelial cells. In the lower respiratory tract, essential nutrients for the growth of bacteria are restricted. Therefore, *A. pleuropneumoniae* has developed several ways for the uptake of nutrients such as iron, nickel and sugars. Iron can be acquired by means of transferring binding proteins, siderophore receptors, and binding of porcine hemoglobin by both lipopolysaccharides and outer membrane proteins. The mechanism for the uptake of nickel is not yet known, although an operon for transportation of nickel has been demonstrated. An outer membrane protein of 42 kDa could be involved in the uptake of maltose.

Most of the pathological consequences of pleuropneumonia can be attributed to the production of pore-forming exotoxins (Apx toxins). Four different Apx toxins have been found to be produced by the 15 serotypes: Apx I, Apx II, Apx III and Apx IV (Table 2.1). All virulent *A. pleuropneumoniae* strains express one or two of these

toxins. Secretion of Apx toxins may result in lysis of alveolar epithelial cells, endothelial cells, red blood cells, neutrophils, and macrophages. Moreover, purified recombinant Apx toxins are able to cause lesions upon endobronchial instillation, and mutant strains which are unable to produce Apx toxins do not induce lesions. Use of transposition mutagenesis and complementation experiments also proves that Apx toxins are essential in the pathogenesis of porcine pleuropneumonia. A fourth toxin (Apx IV) has been demonstrated in all *A. pleuropneumoniae* strains but its role in pathogenesis is unclear[26]. As Apx toxins are immunogenic, they form the essential part of efficient vaccines.

Table 2.1: Apx toxins produced by different serotypes of *Actinobacillus pleuropneumonia[27]*

	Operon			**Activity**		**MW (kDa)**	**Serotype**
	Activator	**Structural**	**Export**	**Hemolysis**	**Cytotoxic**		
Apx I	Apx IC	Apx IA	Apx I BD[a]	strong	strong	105-110	1, 5a 5b, 9, 10, 11
Apx II	Apx IIC	Apx IIA	none[a]	weak	moderate	103-105	all but 10
Apx III	Apx IIIC	Apx IIIA	Apx III BD	none	strong	120	2, 3, 4, 6, 8
Apx IV[b]	ORF1[c]	Apx IVA	none[a]	weak	ND[d]	200[e]	all

[a] Apx IBD genes are found in all serotypes except serotype 3.
[b] Unlike the other Apx toxins, Apx IV is only produced in vivo.
[c] ORF1 seem to be required for activity of Apx IV, although it shares no homology with Apx IC, Apx IIC or Apx IIIC.
[d] ND: not determined.
[e] The molecular mass of Apx IV is predicted from the protein sequence.

The pace of the disease can range from per-acute to chronic depending on the serotype of the bacteria reaching the lung [28]. Per-acute, pigs die within hours. Acute, they symptoms from respiratory disease, high fever, cyanosis, vomiting, and coughing to non-symptomatic carriers. In acute cases, pigs may die within one or two days or survive after optimal antibiotic therapy. Surviving pigs often become chronically infected, showing few clinical symptoms like sporadic coughing and a reduced growth rate. Survivors frequently remain silent carriers of *A. pleuropneumoniae*, which resides in their tonsils, nasal cavities and focal pulmonary lesions. They are a source of infection for other pigs[26].

Infection of the upper respiratory tract without lung involvement does not result in development of Apx toxin neutralizing antibodies, so serological assays cannot be used for the detection of subclinically infected animals[29]. Therefore, PCR from tonsillar swabs is a valuable tool for the detection of infected animals. However, the Apx IV ELISA is a valuable tool for the detection of latently infected herds[30]. The

advantages and limitations of the tools currently available for diagnosis of APP are summarized in Table 2.2.

Success in controlling pleuropneumonia depends on the possibility of weaning piglets free from *A. pleuropneumoniae* infection[24]. In previous studies, control of APP was mainly accomplished by medication, vaccination and management[32]. However, resistance to sulfamethoxazole, the combination sulfamethoxazole-trimethoprim, tiamulin, tilmicosin, tetracycline, penicillin, and ampicillin was found in slaughtered pigs in Switzerland[33]. Several vaccines against pleuropneumonia have been developed. Vaccination with a whole-cell bacterin, capsular extract, lipopolysaccharide, and outer membrane protein reduces mortality clinical symptoms and improves performance but fails to eliminate subclinical tonsillar carriers and does not confer cross-protection against heterologous serotypes.

Table 2.2: Comparative merits of diagnostic tools for *Actinobacillus pleuropneumoniae*[31]

Diagnostic tools	Advantages	Disadvantages
PCR on clinical samples or primary mixed cultures	High sensitivity	Limited availability Specificity varies with technique Usually species-specific
Bacterial isolation on selective medium	Low cost	Low sensitivity Limited availability Skilled technicians needed
Selective bacterial isolation after IMS	High sensitivity	Costly Limited availability
Serotyping	Identifies the serotype of an isolate	Limited availability Cross-reactions reported
LC-LPS ELISA	Serotype-specific High sensitivity and specific Validated with large numbers of field sera Reference test Commercially available	Serotype-specific Costly for multiple serotypes
Apx IV ELISA	Low cost as a screening test	Only partially validated in the field