CHAPTER 2

LITERATURE REVIEW

2.1 BIOLOGICAL FEATURES

Streptococcus suis is classified in Family Lactobaciles and Genus Streptococcus. It is gram-positive facultative anaerobe that grows as a small, grayish and slightly mucoid colony and exhibits non-complete hemolytic activity (α-hemolysis) on blood agar (Staats *et al*., 1997; Hill *et al*., 2005). The morphology of this organism is coccoid or ovoid bacterium and appears as single cell, in pairs or in short chains. Based on specific polysaccharide capsular antigens, *S. suis* has been identified to 33 serotypes (types 1-31, 33 and 1/2) (serotype 32 and 34 has been classified as *Streptococcus orisratti*); however; not all serotypes can cause diseases (Silva *et al*., 200; Takamatsu *et al*., 2008). *S. suis* serotype 2 is the most prevalence to cause diseases in swine and human in many countries (Wisselink *et al*., 2000; Hill *et al*., 20056).

S. suis is a zoonotic pathogen that mainly transmitted via oral and wounds. Natural habitat of *S. suis* is nasal cavities, tonsils and upper respiratory, but colonization in genital and alimentary tracts of pigs slaughtered have been reported (Robertson and Blackmore, 1989). They colonize not only in disease pigs but also in healthy pigs with asymptomatic sign (Staats *et al*., 1997; Francois *et al*., 1998; Marois *et al*., 2007). Survival of *S. suis* serotype 2 has been demonstrated. This organism is resistant to various environmental conditions. They can survive for 10 min at 60°C, 2 h at 50°C, and 6 weeks in carcasses at 10°C. At 0°C, they can survive for 1 month in

dust and for over 3 months in faeces, whereas at 25°C, they can survive for 24 h in dust and for 8 days in faeces. However, *S. suis* serotype 2 can be killed easily with 5% bleach at 1:799 dilution (Clifton-Hadley and Enright, 1984).

Whole genome of *S. suis*, which has been completely sequenced, contains 20 074 917 bp with a G+C content of 41.3%. Although many genes that plays an important roles in the pathogenesis of *S. suis* including polysaccharide production, capsular transport, iron-restriction factors, suilysin, virulence-associated proteins, various enzymes, arginine deiminase system, and IgG binding proteins have been studied, but the functions of 20–30% of those genes are unknown (Lun *et al*., 2007). Eighty genes of *S. suis* serotype 2 were identified and classified into 5 functional categories include metabolism, cell wall associated proteins, transporters, cell replication, and function unknown. Some of these genes may contribute to the survival and pathogenesis of *S. suis* serotype 2 in the host (Li *et al*., 2010).

2.2 EPIDEMIOLOGY

Streptococcus suis infection in pigs were reported in America (United States, Canada, and Brazil), Europe (United Kingdom, The Netherlands, France, Denmark, Norway, Spain, and Germany), Asia (China, Thailand, Vietnam, and Japan), Australia, and New Zealand (Staats *et al*., 1997). They were also occasionally recovered from other animals such as wild boars, horses, dogs, and cats (Staats *et al*., 1997).

For *S. suis* infection in humans, first case of human-infected *S. suis* was reported from Denmark in 1986, which 2 meningitis and 1 sepsis symptoms (Arends and Zanen, 1988). After that, *S. suis*-infected have been reported in several countries

worldwide. In a period of 1984-1993, 25 *S. suis*-infected patients were reported from 2 hospitals in Hong Kong. Fifteen patients were obvious contact history with pigs or pork, 21 patients have meningitis symptom, and 1 patient dead with toxic shock syndrome (Kay *et al*., 1995). Moreover, 2 cases of *S. suis* infection with meningitis were reported from north Taiwan in 2000 and 2002 (Huang *et al.,* 2005). Subsequently, a significant number of cases had also been reported from China. Two larges human *S. suis* outbreak, which 14 deaths out of 25 reported human cases occurred in 1998 in Jiangsu Province and 38 deaths out of 204 human cases in 2005 in Sichuan Province. The most of the dead cases were characterized by STSS (Tang *et al*., 2006; Ye *et al.* 2006). In a previous review article published, 409 human *S. suis* cases worldwide, mostly occurring in China, Thailand and The Netherlands were reported in 2007 (Lun *et al*., 2007) and human *S. suis* cases has increased to 1,700 cases in 2009 (Wertheim *et al*., 2009a). The most cases reported in 2009 have occurred in Southeast Asia as shown in figure 1.

First two cases of *S. suis* infection were initially reported in Thailand since 1987 (Phuapradit *et al*., 1987). Later, several infected cases were increasingly reported. Six cases of *S. suis* infection were reported from Ramathibodi Hospital in 1993 (Pootong *et al,* 1993) and a further three cases with bacteremia and meningitis caused by *S. suis* were reported in 1997. They were isolated from blood and CSF cultures in all three cases though initially reported to be viridans group of streptococci (Leelarasamee *et al,* 1997). Between 1999 and 2000, 10 and 19 cases of *S. suis* infection were reported from Lamphun province in northern Thailand. Of these, 10 fatal cases have been detected and all cases had a history of raw pork or uncooked

pig's blood consumption prior to their sickness. Additionally, most cases had a history of chronic alcoholic (Fongcom *et al*., 2001 and 2002).

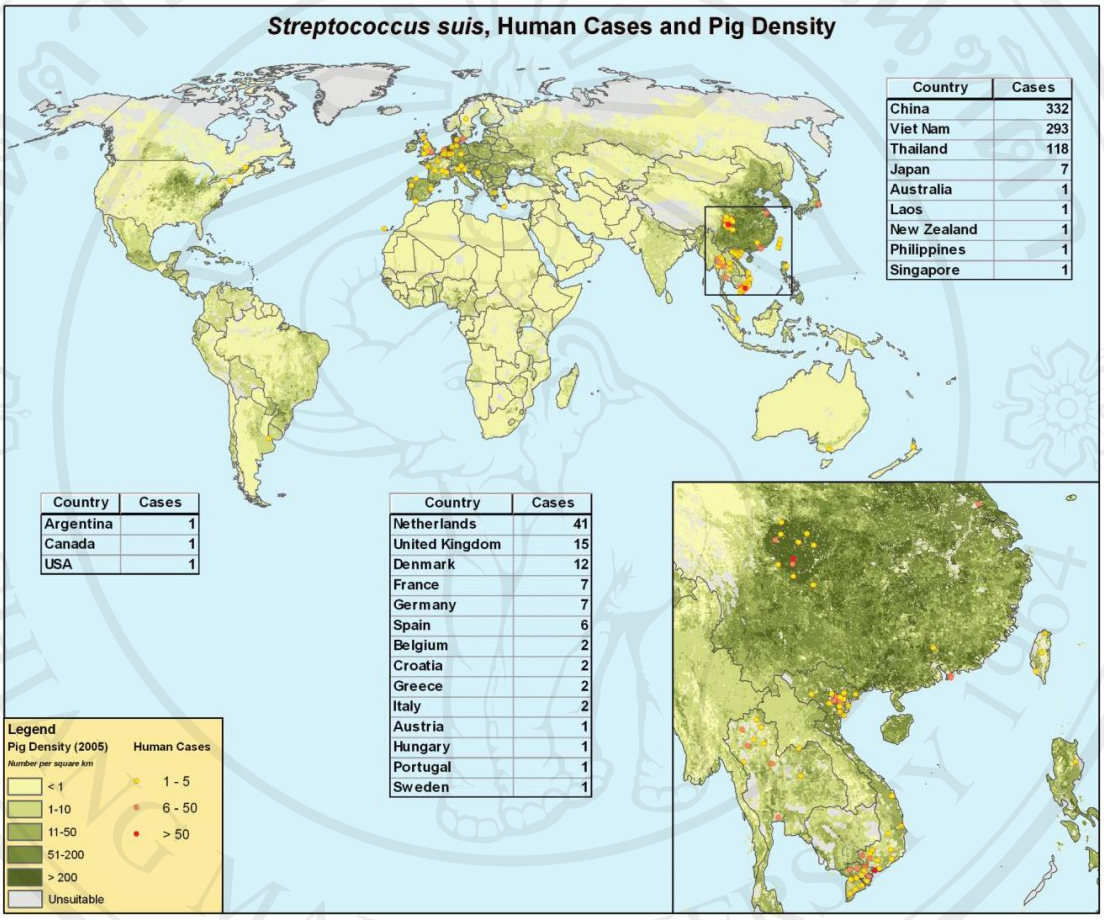

Figure 1 *Streptococcus suis* cases in several countries with swine density data (Wertheim *et al*., 2009a: refer to GeoNetwork. Food and Agricultural Organization, 2005).

In 2003, Donsakul et al. report 8 cases of *S. suis* infection between 1993-1999. Six cases (75%) reported as *S. suis* meningitis and 2 cases (25%) reported as *S. suis* endocarditis. Hearing loss was occurred in all cases of meningitis (Donsakul *et al*., 2003). Between 1997-2002, 10 cases of meningitis and 2 cases of sepsis with *S. suis*

infection have been reported (Suankratay *et al*., 2004). During 2000-2002 in Chiang Mai, Thailand, a retrospective study was conducted. Fourty-one cases of *S. suis* infection were isolated from Chiang Mai, Lamphun, Chaing Rai, Lampang, Phrae, Phayoa and Tak province. The clinical manifestations of the patients included infective endocarditis (39.02%), meningitis (31.71%), sepsis (24.39%), spondylodiscitis (2.44%), and endophthalmitis (2.44%) (Wangkaew *et al*., 2006).

After that, Wongsawan et al. reported that 6 isolates of *S. suis* had been detected from healthy pigs and 67 isolates from patients in Chiang Mai and Lamphun provinces (Wongsawan *et al*., 2006). In addition, risk factors and mortality rates of 66 *S. suis* cases had been demonstrated. Most of cases are men (68%) and 59% of raw pork consumptions cases had been detected. The clinical features were 52% of meningitis, 27% of septicemia, 12% of septic shock, 8% of endocarditis and 1% of artitis. Of these, 35% of cases occurred as hearing loss that correlate to meningitis feature (Wittaya *et al*., 2008). A retrospective study between 2003-2007, 40 *S. suis* infection cases included 30 cases of sepsis, 19 cases of meningitis and 10 cases of endocarditis had been detected from Maharaj Nakorn Chiang Mai Hospital. A past history of the consumption of raw pork and/or pig's blood was found in 62.5% of cases and 25% had contact with pork. Additionally, 73% of meningitis cases had a hearing loss (Navacharoen *et al*., 2009). In 2009, Fongcom et al. reported that 70% of *S. suis* isolated from blood and CSF of patients in Lamphun provincial hospital had misclassified as viridans streptococci (Fongcom *et al*., 2009). To date, the number of *S. suis* infections in humans reported in previous studies from Thailand and on the website of the Bureau of Epidemiology, Ministry of Public Health, Thailand, exceeded 300 cases (Kerdsin *et al*., 2009; Wertheim *et al*., 2009a).

2.3 PREVALENCE OF SEROTYPES

Among serotypes of *S. suis*, serotype 2 is the most common and major cause of severe symptom in swine and human worldwide (Vela *et al*., 2003). However, the serotypes distribution can vary in each area and can change over time. For example, swine infection in European countries such as The Netherlands and Germany, serotype 9 occurred in pig industries as the most common serotype (Wisselink *et al*., 2000). Serotypes 1-9, 1/2, and 14 being among the most prevalent serotypes recovered from infected animals (Wisselink *et al*., 2002; Hill *et al*., 2005). In the United Kingdom, serotypes 1 and 14 were reported frequently in invasive *S. suis* diseases, especially in suckling piglets. Serotype 7 strains were often associated with bronchopneumonia in Scandinavia and less in Germany (Perch *et al*., 1983; Tian *et al*., 2004). Moreover, serotype 2, 1/2, and 3 were mostly prevalent in pigs in Canada and the United States. Most of the *S. suis* isolates in Canada were recovered from the lungs of disease piglets (Galina *et al*., 1996, Messier *et al*., 2008).

In human cases, *S. suis* infection is the most frequently associated with serotype 2 strains as well as swine infection (Gottschalk *et al*., 2007). In North America, 4 human of *S. suis* serotype 2 cases had been reported. All 4 cases included 2 cases of endocarditis and meningitis in Canada and 2 cases of meningitis in the United States (Haleis *et al*., 2009). Two large outbreaks of *S. suis* serotype 2 occurred in China among 14 death cases from Jiangsu Province in 1998 and among 38 death cases from Sichuan Province in 2005 (Tang *et al*., 2006). The distribution of the 385 *S. suis* isolates were from the 16 provinces of China. The prevalence rates of serotype 2 varied between provinces at 16.7% in Fujian to 71.4% in Sichuan (Wei *et al*., 2009).

First case of human meningitis caused by *S. suis* serotype 14 had been occurred in North America in 2007 (Haleis *et al*., 2009). After then, serotype 14 had been reported as a human pathogen in The Netherlands, Thailand, United Kingdom, and Denmark (Messier *et al*., 2008). According to the studies in Thailand, serotype 2 was found to be a common serotype in most published reports, and only a few cases caused by other serotypes (Kerdsin *et al*., 2009). *S. suis* serotype 1 and serotype 1/2 had been reported in Thailand in 2002 and 2006 (Vilaichone *et al*., 2002; Wongsawan *et al*., 2006). Among 177 human isolates of *S. suis* in Thailand, most of all isolates were identified as serotype 2. In addition, 12 isolates of serotype 14 had been demonstrated (Kerdsin *et al*., 2009).

2.4 PATHOGENESIS AND CLINICAL FEATURES

2.4.1 Pathogenesis

Streptococcus suis is localize normally in nasal cavities or tonsils of swine without clinical signs. When *S. suis* expose to stress environment, they can grow in upper respiratory, distribute to the serological system following by septicemia. The most common lesions in pigs infected with *S. suis* are meninges, lymph nodes, and lungs, and the most common histopathological finding is within the choroidal plexus Lun *et al*., 2007). *S. suis* can transmit to human as zoonotic pathogen with high mortality at 13% (Higgins *et al*., 1995; Yu *et al*., 2006). The pathological characteristics of the organs of patients and sick pigs were similar (Zhu *et al*., 2000). In the case of numerous *S. suis* infected, meningitis can occur (Gottschalk and Segura, 2000). They can infect to human via wound or mouth and nasal mucous and colonize in the upper respiratory and tonsils. After that, *S. suis* release suilysin to damage

respiratory and vascular cells. However, how circulating cross the blood-cerebrospinal fluid (CSF) barrier and cause meningitis is not clear (Gottschalk and Segura, 2000). It is generally focused on the mechanism of meningitis caused by *S. suis* serotype 2 (Segura and Gottschalk, 2002). Then they breach the mucosal epithelia for dissemination. Several investigations confirmed that *S. suis* serotype 2 could adhere to and invade epithelial cells such as Hep-2 cells (Segura and Gottschalk, 2002). *S. suis* serotype 2 go into the blood stream as free cells or phagocytose by monocyte or macrophage to reach the central nervous system (CNS) (Segura and Gottschalk, 2002; Baums and Valentin-Weigand, 2009). At least 3 ways could be used by *S. suis* serotype 2 coming across the blood–CNS barriers include the bacteria invaded the porcine brain microvascular endothelial cells (PBMECs) which constructed the blood–brain barrier and the porcine choroid plexus epithelial cells (PCPECs) responsible for the blood–cerebrospinal fluid barrier (Vanier *et al*., 2004; Tenenbaum *et al*., 2009), the bacteria caused apoptosis and necrosis of PCPECs which compromised the integrity of the barrier (Tenenbaum *et al*., 2006), and the proin- flammatory cytokines stimulated by *S. suis* serotype 2 regulate the migration of leukocytes which in turn opened the door for the bacteria trafficking to the CNS (Wei *et al*., 2010). Recently, Feng et al. proposed the two-stage hypothesis for Streptococcal toxic shock syndrome (STSS), which a new severe form observed in all patients during the 2 large outbreaks in China caused by *S. suis* serotype 2 as shown in Figure 2 (Feng *et al*., 2010). In the stage 1, *S. suis* serotype 2 entered blood vessels via an unknown mechanism, and leaded to an early burst of pro-inflammatory cytokines, including Th1 cytokines, interleukin (IL-1b) and tumor-necrosis factor (TNF-a). These inflammatory super-responses could result in STSS with death as

early as 13 hours after *S. suis* serotype 2 infection. In the stage 2, which developed over several days, *S. suis* serotype 2 used virulence factors such as suilysin to cause disease, particularly meningitis (Feng *et al*., 2010).

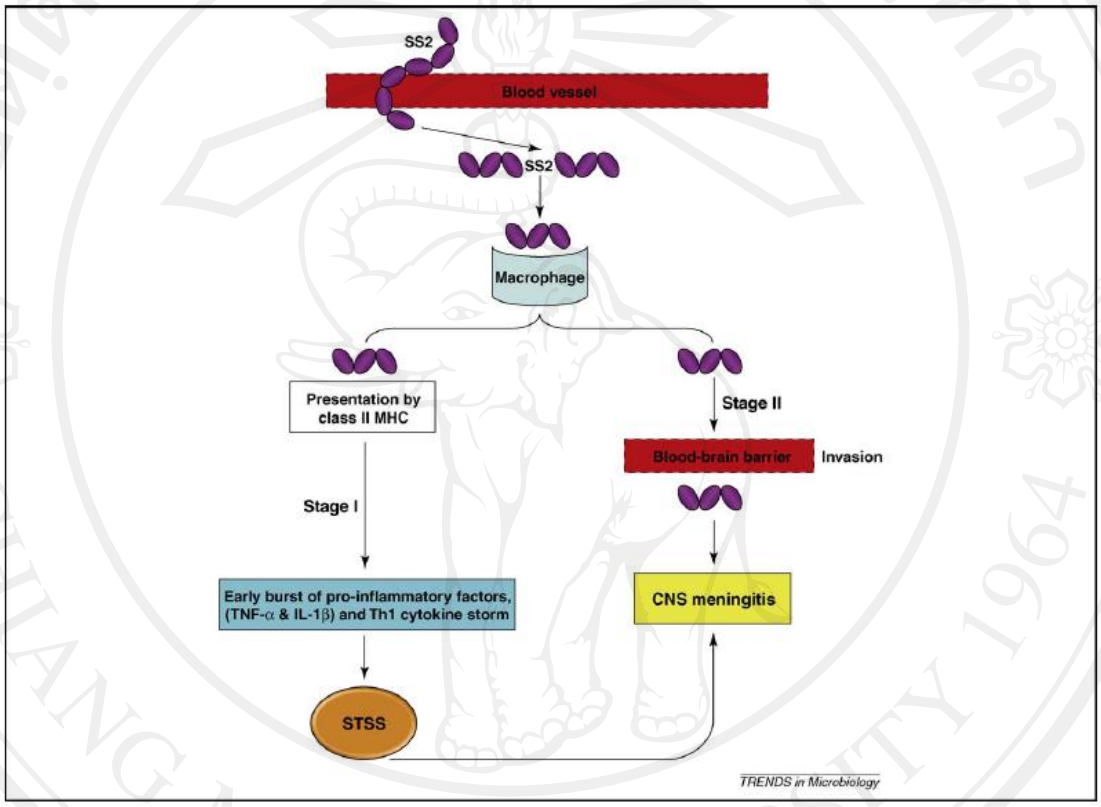

Figure 2 Two-stage hypothesis for Streptococcal toxic shock syndrome caused by *S. suis* serotype 2 (Feng *et al*., 2010).

2.4.2 Clinical features in swine infection

Clinical signs can vary between herds, depending on the pathogenesis of the disease. Pigs with peracute *S. suis* infection may die within hours of the onset of clinical signs. However, it is not unusual for death to occur without apparent clinical signs (Staats *et al*., 1997). Clinical signs may include fever (up to 42 °C), depression, anorexia and lassitude in acute form of the disease, followed by one or more of the following: ataxia, incoordination, tremors, opisthotonus, blindness, loss of hearing, paddling, paralysis, dyspnea, convulsions, nystagmus, arthritis, lameness, erythema, and/or abortion (Staats *et al*., 1997). In 10 to 14 day-old piglets, *S. suis* serotype 1 is usually seen (Taylor, 1986). Joint swelling often occurs, and fever may present in infected animals. Additionally, acute disease can become chronic and cause death. Although *S. suis* type 2 primarily affects weanlings, it has been associated with the fading piglets syndrome in neonates (Sanford and Rosendal, 1984) and has been more recently isolated from two separate herds with meningitis outbreaks in finishing pigs weighing 330-550 kg (Tokach, 1993). In chronic disease, lameness and/or residual central nervous system signs may be apparent. Multiple strains or serotypes are often found within a herd during an *S. suis* outbreak, although no significant differences in clinical signs or lesions occur in pigs infected with multiple or single serotype (Staats *et al*., 1997).

2.4.3 Clinical features in human infection

Clinical signs in human infection can vary depending on the pathogenesis of the diseases. The clinical signs of *S. suis* meningitis are generally similar to those of other bacterial meningitis and include high fever, headache, chills, nausea, vomiting, and vertigo, followed by one or more of the hearing loss, walking ataxia, coma, neck

stiffness, petechia, articular pain, peripheral and facial paralysis, severe myalgia, ecchymosis, rashes, and rhabdomyolysis (Tambyah *et al*., 1997; Fongcom *et al*., 2001; Zhang *et al*., 2002; Matsuo and Sakamoto, 2003). The duration of sickness before hospital admission was 2–5 days (Kopic *et al*., 2002). Hearing loss is the most common feature after cure from meningitis, whereas death often follows septic shock or STSS (Lun *et al*., 2007). In general, STSS is defined based on the following clinical criteria as clear erythematous blanching rash, blood spots and petechia, sudden onset of high fever, hypotension, diarrhea, and dysfunction of multiple organs (e.g. acute respiratory distress syndrome, liver and heart failure, disseminated intravascular coagulation and acute renal failure) (Feng *et al*., 2010). Those skin findings had been reported between 6 to 31% of patients (Mai *et al*., 2008). Less common manifestations of *S. suis* infection such as endocarditis, arthritis, endophthalmitis and uveitis, spondylodiscitis, brain stem ophthalmoplegia, and epidural abscess (McLendon *et al*., 1978; Doube and Calin, 1988; Meecham *et al*., 1992; Ibaraki *et al*., 2003; Huang *et al*. 2005; Tsai *et al*., 2005). Of importance, in Chiang Mai, Thailand, infective endocarditis was reported to be more common than meningitis (Wangkaew *et al*., 2006). Outbreaks in China reported high relative of a severe toxic shock syndrome associated with a high mortality (Tang *et al*., 2006; Yu *et al*., 2006). A review by Wertheim et al. (2009) identified a features of reported cases that mostly occurring in Vietnam, China, Thailand and The Netherland as shown in Table 1 (Wertheim *et al*., 2009a).

Table 1 Clinical characteristics of *Streptococcus suis* infection reported from Vietnam, China, Thailand and The Netherland (adapted from Wertheim *et al*., 2009a).

Variable	**China (*n* = 204)** (Tang *et al*., 2006)	**Vietnam (*n* = 151)** (Mai *et al*., 2008)	**Thailand (*n* = 32)** (Suankratay *et al*., 2004)	**The Netherland (*n* = 30)** (Arends and Zanen, 1988),
Demographic characteristic				
Male sex	171 (83.8)	117 (77.5)	23 (71.9)	26 (86.7)
Age	54 years (NA)	46.5 years (19-84 years)	49 years (1 month to 75 years)	49 years (26-76 years)
Clinical signs				
Duration of illness, days	NA	4 (1-21)	4.5 (1-14)	2 (1-5)
Fever	204 (100)	151 (100)	NA	NA
Headache	164 (80.4)	142 (94.0)	NA	NA
Vomiting	117 (57.4)	NA	NA	NA
Glasgow Coma Scale	NA	12 (5-15)	NA	NA
Stiff neck	NA	142 (94.0)	NA	NA
Skin finding	56 (27.5)	9 (6.0)	8 (25.0)	5 (16.7)
Outcome				
Duration of hospital admission, days	15.1	14 (1-43)	NA	NA
Died in hospital	38 (18.6)	4 (2.6)	2 (6.3)	2 (6.7)
Hearing loss at hospital discharge	NA	93/140 (66.4)	22/32 (68.8)	15/28 (54.0)

NOTE. Data are no.(%) of patients or median value (range).

2.5 VIRULENCE FACTORS

A lot of virulence factors of *S. suis* are different among serotypes and between different strains of the same serotype. Several studies have been done with the *S. suis* serotype 2 that the factors involved in the survival, spread and persistence of the bacterium within the host. The virulence-associated factors known such as muramidase-release proteins (MRP) and extracellular factor (EF), which are required for the phagocytosis resistance; suilysin (SLY) which can damage host cells and increase cell permeability; capsular polysaccharide (CPS) and fibrinogen binding protein (FBPS), other bacterial components have been shown to be related to *S. suis* serotype 2 virulence factors as in Table 2 (Feng *et al*., 2010; Wu *et al*., 2011). The expression of these genes contributes in *S. suis* serotype 2, but production of the disease requires temporal and coordinated expression of a series of genes that allow the prospective pathogen to shift to its pathogenic state and adapt to a hostile environment in the host (Wu *et al*., 2011).

2.5.1 Capsular polysaccharide (CPS)

Serotyping of *S. suis* is based on differences of composition and structure of the polysaccharide capsule. The capsule of *S. suis* serotype 2 has been demonstrated as an important virulence factor (Charland *et al*., 1998; Smith *et al*., 1999b). The capsule of *S. suis* serotype 1 and serotype 2 strains contains N-acetyl neuraminic acid (sialic acid) and four additional sugars included glucose, galactose, N-acetyl glucosamine and N-acetyl galactosamine (serotype 1) or rhamnose (serotype 2) (Elliott and Tai, 1978). Furthermore, *S. suis* serotypes 1, 2, 14 and 1/2 carry the genes involved in sialic acid synthesis (Smith *et al*., 2000). In many bacteria, sialic acid is well known as an anti-phagocytic factor through inhibition of the activation of the

alternative complement pathway by increasing the affinity of C3b to factor H (relative to factor B). This prevents formation of the C3 convertase C3bBb thereby limiting C3b deposition on the surface of the pathogen (Buam and Valentin-Weigand, 2009; Tanabe *et al*., 2009). However, unencapsulated *S. suis* strains might also invade host tissue, though to a lower degree. This indicated the capsule of *S. suis* is not sufficient for full virulence and that other factors have important functions in the pathogenesis of *S. suis* (Buam and Valentin-Weigand, 2009).

2.5.2 Muramidase-release proteins (MRP) and Extracellular factor (EF)

The presence of MRP and EF, which required for the phagocytosis resistance varied among human isolates (Vecht *et al*., 1991; Wertheim *et al*., 2009a). Based on the production of two proteins, a 136-kD MRP and a 110-kD EF protein, *S. suis* serotype 2 could differ in virulence for pigs (Luque *et al*., 1998). Accordingly, MRP had been demonstrated to be a substrate of sortase A (Osaki *et al*., 2002). The mature MRP contained a proline rich region followed by three repeats at the C-terminus (Smith *et al*., 1992). The protein was detected in stationary-growth phase culture supernatants of MRP expressing serotype 2 strains (Vecht *et al*., 1991). Determination of the *mrp* and *epf* genotypes (or the respective phenotypes) in addition to serotyping had been performed in numerous epidemiological studies (Buam and Valentin-Weigand, 2009). Expression of the MRP was found among strains of serotypes 1, 2, 1/2, 14 and 15. Wisselink et al. described that Large (MRP*) and small (MRPs) size variants were detectable in *S. suis* strains of nearly all serotypes investigated (Wisselink *et al*., 2000).

For studies on the 110-kDa EF, it encoded by the gene *epf* that was identified as a protein associated with virulence in serotype 1 and 2 strains (Vecht *et al*., 1991).

So far, EF producing strains had been determined only in serotypes 1, 2, 1/2, 14 and 15 (Wisselink *et al*., 2000). Of these two proteins, various phenotypes were distinguished as phenotype MRP+EF+ (strains which synthesize both MRP and EF proteins), corresponding to strains frequently isolated from diseased pigs and associated with severe clinical signs of disease after experimental infection and another phenotype MRP–EF– (strains which do not produce MRP and EF), corresponding to strains frequently isolated from healthy pigs that were non-pathogenic after experimental inoculation (Vecht *et al.*, 1991). Variants of these proteins, immunologically related to MRP and EF, called MRP* (molecular weight, MW<136 kDa), MRPs (MW>136 kDa), EF* (MW>110 kDa) had also been described. The relationship with virulence of these phenotypes was unclear (Luque *et al*., 1998). Large variants (>110 kDa) of EF (EF*) were although characterized by long C-terminal tandem repeats (each 76 amino acids long). They were not expressed in all *S. suis* serotype 2. These repetitive sequences were missing in the 110 kDa EF. In contrast, the N-terminus, namely the first 811 amino acids, of EF and EF* are nearly identical. Based on the repeats, five different classes of EF* proteins have been differentiated (Buam and Valentin-Weigand, 2009). During the studies of Luque et al. (1998), the MRP and EF proteins were not detected in other streptococcal isolates examined include *S. bovis, S. dysgalactiae, S. equi, E. faecium, E. faecalis* and group L streptococci. This indicated that the MRP and EF proteins were probably exclusively associated with the *S. suis* species (Luque *et al*., 1998). However, MRP was not reported as an essential virulence factor for *S. suis* serotype 2 (Smith *et al*., 1996; Buam and Valentin-Weigand, 2009).

2.5.3 Suilysin (SLY)

Suilysin, encoded by gene *sly* had a cytotoxic effect, might affect complement deposition and might allow invasion of *S. suis* into deeper tissues (Fittipaldi *et al*., 2009). An in vitro study also showed that the presence of SLY enhanced the epithelial invasion and cell lysis by virulent strains of *S. suis* (Lun *et al*., 2003). Suilysin can damage host cells and increase cell permeability by pore forming cholesterol-dependent cytotoxin (CDC) expressed by some strains of *S. suis*. It shares 52% amino acid identity with pneumolysin, its closest known relative within the CDC protein family. The mature protein has an estimated molecular weight of 54 kDa. This protein is part of a family of cytolysins that play a role in disease caused by several bacterial pathogens. Immunization of pigs with suilysin provided partial protection from *S. suis* infection. However, while a suilysin mutant was avirulent in a mouse infection model, it was only slightly attenuated in a model of infection in pigs (Holden *et al*., 2009).

Together with suilysin, the two protein known as MRP and EF had been used as phenotypic markers of virulence. *S. suis* serotype 2 MRP+EF+SLY+ strains were mainly isolated from diseased pigs that showed severe clinical signs of disease while MRP-EF-SLY- strains had been frequently isolated from healthy pigs (Fittipaldi *et al*., 2009). In addition, avirulent strains producing MRP, EF and suilysin have not been reported. However, some European and most Canadian virulent isolates do not produce any of these 3 factors (Fittipaldi *et al*., 2009).

2.5.4 Glutamate dehydrogenase (GDH)

Glutamate dehydrogenase (GDH) is an enzyme that present in most microbes and the mitochondria of eukaryotes. In general, bacterial GDH enzymes are located

in the cytoplasm or the cytoplasmic membrane of the cell. However, the GDH of *Porphyromonas gingivalis* has been shown to be surface associated. For *S. suis*, GDH protein is exposed at the surface of intact cells. The GDH enzyme required for urea synthesis to converts glutamate to α-ketoglutarate and also has a very high affinity for ammonia (1 mM), and therefore toxic levels of ammonia would be present in the body for the reverse reaction to proceed (that is, α-ketoglutarate and ammonia to glutamate and NAD(P)+). In bacteria, the ammonia is assimilated to amino acids via glutamate and amidotransferases. The activity of this enzyme is controlled by the concentration of ammonium and or the like-sized rubidium ion, which binds to an allosteric site on GDH and changes the K_m (Michaelis constant) of the enzyme.

The gene encoding the glutamate dehydrogenase (gdh) was described by Okwumabua et al. (2001 and 2003). It was cloned, sequenced and characterized from a virulent strain of *S. suis* serotype 2. They found that it located within a 1.6-kb *Dra*I DNA fragment and appears to be transcribed from a promoter located within the cloned DNA fragment, since expression is not dependent on insertional orientation. The *gdh* gene encoded a 45-kDa antigen and appeared to be conserved across serotype like other GDHs. The *S. suis* GDH activity is NAD(P)H dependent but, unlike the NAD(P)H-dependent GDH from various other sources, that of *S. suis* utilizes L-glutamate rather than a-ketoglutarate as the substrate. Highly virulent strains of *S. suis* serotype 2 could be distinguished from moderately virulent and avirulent strains on the basis of their GDH protein profile following activity staining on a nondenaturing gel (Okwumabua *et al*., 2001; Okwumabua *et al*., 2003)

2.5.5 Fibrinogen binding protein (FBPS)

FBPS was an adherence protein that binds to fibronectin in host cells (de Greeff *et al*., 2002). Fibronectin was a large glycoprotein composed of two polypeptide chains with a combined molecular weight of 450 kDa. It was found in a soluble form in the body fluids and in an immobilized form in both the extracellular matrix and on the surface of host cells. Some bacteria bound fibronectin in order to colonize or invade host cells. The FBPS encoded by gene *fbps*, which exists in all 33 *S. suis* serotype. It was detectable in virulent, weakly virulent and avirulent serotype 2 strains (de Greeff *et al*., 2002). FBPS was immunogenic and it was found to be expressed in young piglets. In *S. suis* infection, mutant strains deficient FBPS had low efficiency in colonizing the specific organs involved (Wang and Lun, 2008). Accordingly, de Greeff et al. and Esgleas et al. suggested that the recombinant FBPS (rFBPS) bound to human fibronectin and fibrinogen and *S. suis* serotype 2 bound to both plasma and cellular fibronectin (de Greeff *et al*., 2002; Esgleas *et al*., 2005). However, it had not been demonstrated that FBPS conferred binding of *S. suis* to fibronectin (or fibrinogen) (Buam and Valentin-Weigand, 2009).

2.5.6 Other virulence factors

Currently, newly identified elements related to virulence of *S. suis* serotype 2 had been discripted. The 89KPAI encoded a two-component signal transduction system, SalK–SalR, which required for full bacterial virulence of a STSS-causing strain (O5ZYH33) (Li *et al*., 2008). Disruption of salK–salR attenuated greatly the virulence of *S. suis*, whereas functional complementation restored it in experimental infection of piglets. The attenuated virulence of this mutant could, in part, be attributed to decrease colonization capability in susceptible tissues of piglets and

lower resistance to polymorphonuclear leukocyte-mediated killing (Li *et al*., 2008). Although the molecular mechanism or the regulatory network associated with SalK–SalR was not clear yet, these studies provided direct evidence for 89K being a functional PAI (Li *et al*., 2008).

The hyaluronate lyase (HYLA) of *S. suis* serotype 2 was a secreted protein that degraded hyaluronic acid (HA) into an unsaturated disaccharide (Tan *et al*., 2008). HA was a glycosaminoglycan that had high-molecular-weight polysaccharide consisting of repeating disaccharide units with a major part of the extracellular matrix and capsular meterial (Tan *et al*., 2008). In gram-positive bacteria, HA was able to cause infection at the skin and mucosal surfaces of humans and animals. It increased the ability of bacteria to spread and contributed to subsequent virulence (Tan *et al*., 2008). Human tissues known to contain HA include the blood, brain, liver, umbilical tissue, and skin. Members of several streptococcal species were known to produce a cell surface-associated hyaluronate lyase, and research indicates that this lyase may be important in pathogenesis (Tan *et al*., 2008).

Sortase A, a transpeptidase originally found in *S. aureus*, specifically cleaves the LPXTG amino acid sequence between the T and G residues and mediates the covalent linkage of bacterial surface proteins to the peptidoglycan. Genetic evidence of *S. suis* serotype 2 srtA homolog associated with full virulence of this pathogen had been demonstrated (Wang *et al*., 2009; Feng *et al*., 2010).

Surface antigen one (SAO) protein was first reported as an immunogenic surface protein reacting with convalescent sera from pigs infected with *S. suis* serotype 2. Later, three allelic variants of the *sao* gene were reported, namely *sao*-S, *sao*-M and *sao*-L, based on different lengths among *S. suis* isolates (Feng *et al*.,

2010). The function of SAO was still unknown. Immunization of mice and piglets with recombinant SAO (rSAO) elicited protective immune response against serotype 2 strains expressing different variants of SAO (Li *et al*., 2007; Buam and Valentin-Weigand, 2009). Furthermore, Zhang et al. described protection against challenges with serotypes 2 and 7 strains in mice after immunization with rSAO (Zhang *et al*., 2009).

Serum opacity factor (OFS) is a surface-associated protein which opacified serum of various species expresses in *S. suis* serotype 2 (Baums *et al*., 2006). The protein had structural features of an MSCRAMM, namely a signal sequence, a large N-terminal domain, C-terminal repeats and a CWS. OFS had been demonstrated as an important virulence factor of *S. suis* serotype 2. However, the function of OFS in pathogenesis might be restricted to the highly virulent multilocus sequence type (ST) complex 1 as serum opacification activity was not detectable in virulent strains of ST complex 27 (Takamatsu *et al*., 2008; Buam and Valentin-Weigand, 2009).

Di-peptidyl peptidase IV (DPP IV), originally recognized as an antigenic enzyme (CD26) on the surface of eukaryotic cells, was widely distributed in microbial pathogens. Recently, a dppIV homolog in a highly invasive isolate of Chinese STSS-causing *S. suis* serotype 2 was identified, and it was shown that inactivation of dppIV attenuated greatly the virulence of this strain (Feng *et al*., 2010).

Interestingly, Pan et al. recently reported that CovR, an orphan response regulator, negatively regulated virulence of *S. suis* 05ZYH33, a STSS-causing strain. Disruption of covR resulted in a mutant strain with increased hemolytic activity, enhanced adherence to epithelial cells, higher virulence in piglets and increased ability to colonize susceptible tissues of piglets. Microarray analyses suggest that

CovR repressed the expression of hundreds of genes, some of them encoding known or putative virulence factors (Pan *et al*., 2009).

Finally, enolase was generally considered to be a glycolytic enzyme, catalyzing the dehydration of 2-phosphoglycerate to phosphoenolpyruvate. However, it had recently been shown that *S. suis* enolase could be exported to the bacterial surface. Furthermore, it appeared to function as a novel protective antigen conferring full protection upon mice against *S. suis* serotype 2 attack, although some controversy on the protective efficiency existed. Surprisingly, genetic studies showed that two other enzymes associated with central metabolism also contribute to *S. suis* serotype 2 pathogenicity: glutamine synthetase and inosine 5-monophosphate dehydratase (Feng *et al*., 2010).

Table 2 Factors associated with virulence of *Streptococcus suis* serotype 2 (Feng *et al*., 2010).

Gene	Function or gene product	Origin
Known virulence factors		
epf	Extracellular protein factor	Pig isolate (Netherlands)
gdh	Glutamate dehydrogenase	Strain 1933 (USA)
cps	Capsular polysaccharide	Strain S735 (Netherlands)
sly	Suilysin, thio-activated hemolysin	Strain P1/7 (Netherlands)
fbps	Fibronectin binding protein	Pig isolate (Netherlands)
mrp	Muramidase-released protein	Pig isolate (Netherlands)
Newly identified components		
sao	Surface antigen protein	Strains 89/1591 (Canada) and 05ZYH33 (China)
salk-salR	Two-component signal transduction system in the 89K PAI	Strain 05ZYH33 (China)
dltA	Enzyme catalyzing lipoteichoic acid D-alanylation	Strain 31533 (France)
pgdA	Peptidoglycan N-acetylglucosamine deacetylase	Strain 31533 (France)
srtA	Transpeptidase mediating covalent linkage of surface proteins to peptidoglycan	Strains NCTC10234 (Canada) and 05ZYH33 (China)
dppIV	Di-peptidyl peptidase IV	Strain 05ZYH33 (China)

Table 2 Continued

Gene	Function or gene product	Origin
covR	Orphan response regulator	Strain 05ZYH33 (China)
eno	Enolase for dehydration of 2-phosphoglycerate to phosphoenolpyruvate	Strains 166 (France) and 05ZYH33 (China)
impdh	Inosine 5-monophosphate dehydrogenase	Strain SS2-Ha (China)
glnA	Glutamine synthetase	Strain SC19a (China)

2.6 DIAGNOSIS AND TYPING

2.6.1 Biochemical identification

S. suis colonies are small (0.5-1.0 mm diameter), grayish or transpalent, and slightly mucoid (Staats *et al*., 1997). It is able to grow in anaerobic or aerobic conditions (Ma *et al*., 2003). Variable types of hemolysis on sheep blood agar plates caused by strains of *S. suis* can be produced, but the main produce narrow zones of α-hemolysis (Staats *et al*., 1997). *S. suis* is a gram-positive cocci that is seen in pairs, single or in short chains. Based on biochemical tests, optochin, Voges-Proskauer, salicin, trehalose, and 6.5% sodium chloride can be used to determine *S. suis* to the species level for almost all capsular types (Higgins and Gottschalk, 1990). The strains of *S. suis* are positive for hydrolysis of esculin and positive for fermentation of trehalose and amylase synthesis and negative for oxidase, catalase and VP tests. Moreover, *S. suis* is non-motility, optochin-resistant, and unable to grow in 6.5% NaCl solution (Tarradas *et al*., 2001). Currently, commercial biochemical tests (e.g., API Strep; Biomerieux) are available and effective for identification. The commercial kits can also be used to identify (most 90%), but not all *S. suis* strains (Hommez *et al*., 1986). However, although *S. suis* can be cultured from blood or CSF samples by standard microbiological techniques but biochemical characteristics are so variable. It is often misidentified, and commonly reported as *Streptococcus* species, α-hemolytic or viridans streptococci, *E. faecalis, A. viridans,* or even *S. pneumoniae* or infection goes undiagnosed, so that identification is often difficult and may require a combination of biochemical reactions, followed by confirmative serotyping (Lutticken *et al*., 1986; Donsakul *et al*., 2003; Gottschalk M., 2004; Lun *et al*., 2007; Wertheim *et al*., 2009a).

2.6.2 Serological typing

The 35 *S. suis* serotypes could be classified based on antigenic specificities. The three techniques used to identify *S. suis* based on capsular polysaccharide antigens were following capsular reaction test, capillary precipitation test and coagglutination test (Gottschalk *et al*., 1989). Of these, the coagglutination test was the most frequently used in North America (Breton *et al*., 1986; Erickson *et al*., 1984; Sanford, 1987). Characterization of *S. suis* isolates from the diseased pigs in China between 2003 and 2007 had been demonstrated. By using anti-sera against serotype 1–28, 407 *S. suis* strains, serotype 2 was most frequent (43.2%), followed by other serotypes occurred rarely. Takamatsu *et al*. used coagglutination tests to typed 20 isolates of *S. suis*. The result showed that 19 of *S. suis* belonged to serotype 2, while the remaining 1 (MNCM07) was serotype 14 (Takamatsu *et al*., 2008). Recently, Kerdsin et al. reported two isolates of serotypes 5 and 24 as new serotypes in Thailand (Kerdsin *et al*., 2011). However, cross-reactions detected with the coagglutination technique among 14 serotypes had been reported (Gottschalk *et al*., 1989). This cross-reaction was probably due to similar composition of capsular polysaccharides. In coagglutination test, *S. suis* serotype 1 react with the anti-serotype 1 as well as anti-serotype 14 antisera and *S. suis* serotype 1/2 react with the anti-serotype 1 as well as anti-serotype 2 antisera (Gottschalk *et al*., 1989). This suggests that they shared a common epitopes between these serotypes. To date, *S. suis* serotype 1/2 which shared antigens with both types 1 and 2 cross-reacting capsular material in the *S. suis* species had been demonstrated (Perch *et al*., 1981). Moreover, non-specific cross-reactions occurred among different serotypes. Cross-reaction of serotype 6 with type 16 antiserum, serotype 16 with type 6 antiserum, and serotype 22 with type 2 antiserum

had been reported (Higgins and Gottschalk, 1990). These results indicated that the coagglutination test for identification of *S. suis* should not be used alone. Weak or multiple positive reactions must be confirmed by the capsular reaction test or the capillary precipitation test (Gottschalk *et al*., 1989).

2.6.3 Molecular typing

Culture results might be negative as a result of, for example, antibiotic use prior to the obtainment of specimens or misidentification. Currently, PCR is a rapid molecular technique used to detect specific serotypes or strains of *S. suis* in infected or healthy pigs and it also used for clinical diagnosis in sick human. Based on *S. suis*-specific gene, 16S rRNA, glutamate dehydrogenase (*gdh*) and capsule polysaccharide (*cps*) genes were used to identify this microbe as described by Marois et al. (2004), Silva et al. (2006) and Kerdsin et al. (2009).

16S ribosomal RNA (16S rRNA) is a component of the 30S small subunit of prokaryotic ribosomes. It is approximately 1.5 kb (or 1500 nucleotides) in length. Bacterial 16S rRNA genes contain 9 hypervariable regions (V1–V9) that demonstrate considerable sequence diversity among different bacteria. The PCR technique have been used to identify species-specific sequences within a given hypervariable region of the 16S rRNA genes (rDNAs) for species identification (Chakravorty *et al*., 2007). For *S. suis* identification, Rasmussen et al. (1998) and Boye et al. (2000) reported 16S rRNA region and a species-specific probe (serotypes 1–31) targeting 16S rRNA gene could be used to identify *S. suis* strains. Based on 16S rRNA and *cps* genes, multiplex PCR test had been developed to detect *S. suis* species and serotypes from swine tonsils (Marois *et al*., 2004). In 2006, eight swine strains and thirty-seven human strains in China could detect based on 16S rDNA and *cps* genes by multiplex

PCR (Tang *et al.*, 2006). In Thailand, Miscellaneous Bacteriology Laboratory, National Institute of Health (NIH) used PCR technique based on 16S rRNA gene to detect *S. suis* isolated from blood and CSF of patients. Of these, 177 isolates of *S. suis* were detected (Kerdsin *et al*. 2009).

Moreover, PCR targeted to *gdh* house keeping gene encoding glutamate dehydrogenase enzyme was reported to detect *S. suis* (Okwumabua *et al*., 2003). Previous study by Lyerly et al. (1991) has been successfully used the *gdh* gene in the diagnosis of the Clostridium difficile infections. The *gdh* gene of *S. suis* serotype 2 that was reported to be conserve in *S. suis* species was efficiently amplify a 688 bp specific fragment from all *S. suis* strains (Okwumabua *et al*., 2003). After that, Silva et al. (2006) designed primers specific to the *gdh* gene as described by Okwumabua et al. (2003). They found that primers could amplify *S. suis* based on *gdh* specific gene. Additionally, Wei et al. (2009) reported 407 *S. suis* isolates from 719 streptococcal isolates from diseased pigs in China between 2003 and 2007 were detected by PCR based on the *gdh* gene.

Characterization of the *cps* genes specific for *S. suis* serotype 1 and serotype 2 was previously demonstrated by Smith et al. (1999). Cross-hybridization with the individual *cps2* and *cps1* genes as probes showed that the *cps2F*, *cps2H*, *cps2I*, and *cps2J* genes specifically hybridized with serotype 2 and 1/2 strains, whereas the *cps1F*, *cps1G*, *cps1I*, and *cps1J* genes specifically hybridized with serotype 1 and 14 strains. The *cps2* locus of *S. suis* serotype 2 comprised 14 open reading frames (ORFs) and the *cps1* locus of *S. suis* serotype 1 comprised 7 open reading frames (ORFs) as shown in figure 3 (Smith *et al*., 1999). Among the genes in the *cps2* locus,

cps2J exists only in the serotype 2 and 1/2 strains. Of these, *cps2J*-positive strains are suspected to have capsules of serotype 2 or 1/2 (Lakkitjaroen *et al*., 2011).

In many gram-positive and gram-negative bacteria, these *cps* loci show a common genetic organization. A region containing the serotype-specific glycosyltransferases, required for the successive linkage of the monosacchrides to the oligosaccharides, and the putative polysaccharide polymerase, required for the polymerization of the oligosaccharide subunits, is preceded by a region presumed to encode proteins with common functions, such as regulation and transport of polysaccharide across the membrane (Smith *et al*., 1999). As in a proposed function of gene product from *cps1* ORFs, most of all function as glucosyltransferase, following CP polymerase and unknown functions. In *cps2* ORFs, most of proposed function is glucosyltransferase, following chain length determination, transcription regulation, regulation, capsular polysaccharide polymerase and unknown functions (Smith *et al*., 1999).

Previously, PCR method which can be used to detect pathogenic strains of *S. suis* serotype 2 as well as highly pathogenic strains of *S. suis* serotype 1 was developed (Wisselink *et al*., 1999). Type 2-specific PCR had been established for detection of *S. suis* serotype 2 in human infection (Matsuo and Sakamoto, 2003). In a case series of 151 patients in Ho Chi Minh City, Vietnam, 117 *S. suis* isolates were detected by CSF culture, but 149 *S. suis* isolates were detected by monoplex real-time PCR based on *cps*2J gene (Mai *et al*., 2008). In 2007, diagnostics for *S. suis* by PCR were set up at a national hospital in Hanoi. The cerebrospinal fluid samples of *S. suis* positive were 43 cases, of which *S. suis* could be cultured in 32 cases and 11 cases were only positive by PCR (Wertheim *et al*., 2009b).

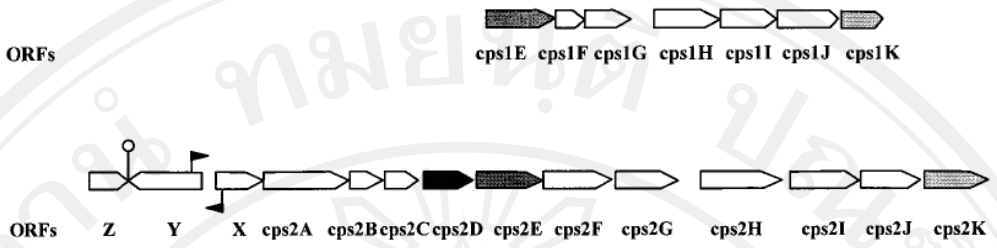

Figure 3 The *cps1* and *cps2* gene clusters of *S. suis* serotype 1 and serotype 2. (A) Genetic organization of the *cps2* gene cluster. The large arrows represent potential ORFs. Gene designations are indicated below the ORFs. Identically filled arrows represent ORFs which showed homology. The small closed arrows indicate the position of the potential promoter sequences. o, position of the potential transcription regulator sequence. (B) Genetic organization of the *cps1* gene cluster. The open arrows represent potential ORFs. As a result of the construction of the plasmids, *cps1E* is incomplete at its 5' end and *cps1K* is incomplete at its 3' end.

Molecular methods such as RFLP, genome fingerprinting, pulsed-field gel eletrophoresis, and multilocus sequence typing had been used to study the genetic diversity of *S. suis* strains, the colonial relations between the strains, and pathogenicity of particular clones (Mogollon *et al*., 1990; Okwumabua *et al*., 1995; Berthelot-Herault *et al*., 2002; King *et al*., 2002; Vela *et al*., 2003). Additionally, immunocapture method, fluorescent antibody techniques, whole-cell antigen-based indirect ELISA, and purified capsular polysaccharide antigen-based indirect ELISA had been developed (Robertson and Blackmore, 1989; Davies and Ossowicz, 1991; Paterson *et al*., 1993; Gottschalk *et al*., 1999).

2.7 TREATMENT AND OUTCOME

The variation of *S. suis* antigenic has limited the effectiveness of vaccines in the prevention of the *S. suis* infection. Thus, antimicrobial agents have become important choice to treat and control the infection by *S. suis*. Treatment of *S. suis* infection depended on the clinical signs, the type of infection or the susceptibility of them. *S. suis* was sensitive to antibiotics, including penicillin, ceftriaxone, cephalosporin, ampicillin, and amoxicillin. Penicillin G is commonly used to treat or control *S. suis* infection (Gottschalk *et al*., 1991; Prieto *et al*., 1994). By Kirby-Bauer method, *S. suis* isolates were susceptible to penicillin between 80% and 95% (Dee *et al*., 1993; Tarradas *et al*., 1994). Penicillin G (24 million U over 24 h for at least 10 days) could used to treat *S. suis* meningitis (Halaby *et al*., 2000). However, Penicillin resistance was reported in a single human case and in some pig isolates (Shneerson *et al*., 1980; Marie *et al*., 2002).

Various resistance patterns among *S. suis* isolated in several countries had been shown as in previous studies (Kataoka *et al*., 2000; Marie *et al*., 2002; Vela *et al*., 2005; Wisselink *et al*., 2006; Zhang *et al*., 2008). In seven European countries, the drug susceptibility of 384 *S. suis* strains from diseased pigs was assessed. The strains were susceptible to penicillin (MIC90, 0.13 mg/ml). Low rates of gentamicin (1.3% of isolates; MIC90, 8 mg/ml) and trimethoprim-sulfamethoxazole (6.0%; MIC90, 2 mg/ml) resistance were observed and a high rate of tetracycline (75.1%; MIC90, 64 mg/ml) resistance was observed (Wisselink *et al*., 2006). In 2008, data from Vietnam showed that *S. suis* was resistant to tetracyclin (83.2% of isolates; MIC50, 16 mg/mL; MIC90, 32 mg/mL), erythromycin (20%; MIC50, 0.064 mg/mL; MIC90, 1256 mg/mL), and cloramphenicol (3.3%) (Mai *et al*., 2008). The principles

of bacterial meningitis treatment were mainly for other clinical features causes by *S. suis* infection. For empirical treatment, ceftriaxone with or without vancomycin was a good choice until the diagnosis was confirmed by laboratory. The same treatment dose and duration that was used for pneumococcal meningitis was also recommended for *S. suis* meningitis (Nguyen *et al*., 2007; Mai *et al*., 2008). In the case of *S. suis* infection in other sites (not associated to CNS), such as endocarditis, endophthalmitis, or arthritis, the recommended guidelines should be observed in terms of duration of treatment, monitoring, and surgical intervention. There were no clinical data on the treatment of penicillin-or ceftriaxone-resistant *S. suis* infections (Wertheim *et al*., 2009a). In a multivariate analysis, severe hearing loss was associated with older age (>50 years) and not receiving corticosteroid treatment (Mai *et al*., 2008). After that, dexamethasone (0.4 mg/kg twice daily for 4 days) was given to adult patients in southern Vietnam who had a short disease onset (<7 days), cloudy CSF, WBC count >1000 cells/mL (with >60% neutrophils), high CSF lactate level (>4 mmol/L), and low CSF glucose level (<50% of blood glucose) or to patients who had a positive result of at least 1 of the following tests: Gram stain, bacterial culture, bacterial antigen test, or PCR. However, an adjunctive treatment by using the dexamethasone to reduce mortality and improve the outcome of bacterial meningitis remained controversial (Greenwood, 2007). The reported case-fatality rates associated with *S. suis* meningitis were generally low in several meningitis series, compared with rates among patients in the same age group with meningitis due to *S. pneumoniae* and other bacterial agents (e.g., 2.6% in southern Vietnam; Mai *et al*., 2008 and 7% in The Netherlands; Perch *et al*., 1968). An outbreak in China was associated with an overall

case-fatality rate of 18%, but this reached 63% among patients with septicemia and septic shock (Tang *et al*., 2006).