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จากการบริโภคเนื้อสุกร

นายพฤษมน วงศ์นาค

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
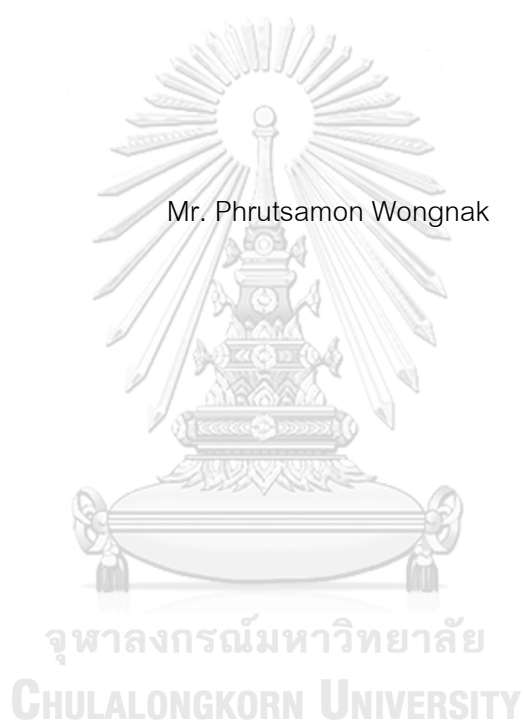สาขาวิชาสัตวแพทยสาธารณสุข ภาควิชาสัตวแพทยสาธารณสุข
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QUANTITATIVE MICROBIAL RISK ASSESSMENT OF *STREPTOCOCCUS SUIS*

SEROTYPE 2 FROM PORK CONSUMPTION

Mr. Phrutsamon Wongnak

A Thesis Submitted in Partial Fulfillment of the Requirements

for the Degree of Master of Science Program in Veterinary Public Health

Department of Veterinary Public Health

Faculty of Veterinary Science

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Thesis Title	QUANTITATIVE MICROBIAL RISK ASSESSMENT OF *STREPTOCOCCUS SUIS* SEROTYPE 2 FROM PORK CONSUMPTION
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พฤษมน วงศ์นาค : การประเมินความเสี่ยงจุลินทรีย์เชิงปริมาณของสเตร็ปโตค็อกคัส ซู อิส ซีโรทัยป์ 2 จากการบริโภคเนื้อสุกร (QUANTITATIVE MICROBIAL RISK ASSESSMENT OF *STREPTOCOCCUS SUIS* SEROTYPE 2 FROM PORK CONSUMPTION) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ. น.สพ. ดร. ศุภชัย เนื้อนวลสุวรรณ, 98 หน้า.

การศึกษานี้มีวัตถุประสงค์เพื่อประเมินหาความชุกและความเข้มข้นของเชื้อสเตร็ปโต ค็อกคัส ซูอิสที่ปนเปื้อนภายในสายโซ่การผลิตเนื้อสุกร อีกทั้งเพื่อประเมินความเสี่ยงสุขภาพที่เกิด จากการติดเชื้อสเตร็ปโตค็อกคัส ซูอิส ซีโรทัยป์ 2 ผ่านการบริโภคเนื้อสุกรในประเทศไทย ตัวอย่าง จากกรุงเทพมหานคร ถูกเก็บมาจาก 4 สายโซ่การผลิตเนื้อสุกร ซึ่งประกอบด้วยตัวอย่างสุกรและ ผลิตภัณฑ์สุกร 492 ตัวอย่าง และตัวอย่างจากสิ่งแวดล้อม 480 ตัวอย่าง อีกทั้งตัวอย่างจากพื้นที่ ต่างจังหวัด ซึ่งได้แก่จังหวัดเชียงใหม่ พะเยา น่าน ขอนแก่น มุกดาหาร สระบุรี นครปฐม และ พังงา ประกอบด้วยเนื้อสุกรที่ถูกจำหน่ายในตลาดแบบดั้งเดิม และตลาดทันสมัยทั้งสิ้น 1,036 ตัวอย่าง ถูกเก็บเพื่อตรวจหาเชื้อสเตร็ปโตค็อกคัส ซูอิสเชิงปริมาณ โดยวิธีตรวจนับจุลินทรีย์บน อาหารเลี้ยงเชื้อ การยืนยันซีโรทัยป์ 2 ใช้วิธีการตรวจหายีน *cps2-J* ร่วมกับปฏิกิริยา Quellung การศึกษานี้พัฒนาแบบจำลองการประเมินความเสี่ยงชนิดสโตแคสติกโดยอาศัยข้อมูลที่ถูก เผยแพร่สาธารณะ ร่วมกับผลการศึกษาจากการศึกษาครั้งนี้ ผลการศึกษาพบว่า ความชุกของ เชื้อสเตร็ปโตค็อกคัส ซูอิสในตัวอย่างสุกรและผลิตภัณฑ์สุกรและตัวอย่างจากสิ่งแวดล้อมใน กรุงเทพมหานครคิดเป็น 11.4% (56/492) และ 5.2% (25/480) ตามลำดับ มีการตรวจพบเชื้อส เตร็ปโตค็อกคัส ซูอิสจากตัวอย่างเนื้อสุกรจากทั้งตลาดแบบดั้งเดิม และตลาดทันสมัยทั้งสิ้น 1% (10/1,036) และมีความเข้มข้นเฉลี่ย 4.22 log cfu ต่อกรัม ไม่มีการตรวจพบสเตร็ปโตค็อกคัส ซูอิส ซีโรทัยป์ 2 ในตัวอย่างเนื้อสุกรจากการศึกษาครั้งนี้ ค่าประมาณความเสี่ยงต่อวันจากความ เจ็บป่วยโดยเชื้อสเตร็ปโตค็อกคัส ซูอิส ซีโรทัยป์ 2 อยู่ที่ 1.3×10^{-7} หรือเทียบเท่าผู้ป่วย 4.6 รายต่อ ประชากร 100,000 คนต่อปี ผลจากการวิเคราะห์ความอ่อนไหวพบว่า ปริมาณสัมผัสต่อวัน ความ เข้มข้นของเชื้อขณะบริโภค และเวลาในการเก็บรักษาเนื้อสุกรในครัวเรือน มีอิทธิพลมากต่อ ค่าประมาณความเสี่ยง

ภาควิชา	สัตวแพทยสาธารณสุข	ลายมือชื่อนิสิต	....
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PHRUTSAMON WONGNAK: QUANTITATIVE MICROBIAL RISK ASSESSMENT OF *STREPTOCOCCUS SUIS* SEROTYPE 2 FROM PORK CONSUMPTION. ADVISOR: ASSOC. PROF. SUPHACHAI NUANUALSUWAN, Ph.D., 98 pp.

This study aimed at determine prevalence and concentration of *Streptococcus suis* contamination in pork production chains as well as evaluate the health risk attributable to *S. suis* serotype 2 from pork consumption in Thailand. In total, 492 pig-to-pork and 480 environmental samples were collected from 4 pork production chains in Bangkok. Besides, total 1,036 pork samples were also collected from traditional and modern trades in Chiangmai, Phayao, Nan, Khonkaen, Mukdahan, Saraburi, Nakhonpathom, and Phang-nga provinces. All samples were enumerated using plate count method. *S. suis* isolates were confirmed by detecting *cps2-j* gene, followed by Quellung reaction. A stochastic risk assessment model was constructed using the available information together with the results from this study. Total 11.4% (56/492) of pig-to-pork samples and 5.2% (25/480) of environmental samples from Bangkok were positive to *S. suis*. Total 1% (10/1,036) of pork samples from both traditional and modern trades were positive to *S. suis*, with an average concentration of 4.22 log cfu/g. There was no *S. suis* serotype 2 detected from all pork samples. The estimated daily risk of *S. suis* serotype 2 illness from pork consumption was 1.3×10^{-7} or equilvalent to 4.6 cases per 100,000 persons, annually. A sensitivity analysis revealed that exposure dose, bacterial concentration at consumption and storage time at home greatly impacted the risk estimate.

Department: Veterinary Public Health   Student's Signature ....

Field of Study: Veterinary Public He   lth   Advisor's Signature ....

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LIST OF ABBREVIATIONS

µl	Microliter
µm	Micrometer
ml	Milliliter
mm	Millimeter
cm^2	Square centimeter
g	Gram
h	Hour
min	Minute
s	Second
%w/v	Weight/Volume percentage
cfu	Colony forming unit
UV light	Ultraviolet light
PCR	Polymerase chain reaction
NaCl	Sodium chloride
CO_2	Carbon dioxide
TBE buffer	Tris/Borate/EDTA buffer
BPW	Buffered peptone water
BHI	Brain heart infusion
CPS	Capsular polysaccharide
EF	Extracellular factor
SLY	Suilysin
MRP	Muramidase-released protein
ADS	Arginine deaminase system
BOE	Bureau of Epidemiology
FAO	Food and Agriculture Organization of the United Nations
WHO	World Health Organization
CAC	Codex Alimentarius Commission

EFSA	European Food Safety Authority
NIH	National Institute of Health
MRA	Microbial risk assessment
QMRA	Quantitative microbial risk assessment
FSO	Food safety objective
ALOP	Appropriate Level of Protection

จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY

CHAPTER 1

INTRODUCTION

1.1 Importance and Rationale

Streptococcus suis (*S. suis*) is an important pathogenic bacterium circulating in pork production industry worldwide. Recently, *S. suis* has been recognized as an emerging zoonotic pathogen for pigs and humans(Wertheim et al., 2009). Among a wide variety of serotypes, which is based on capsular polysaccharide (CPS) antigens, *S. suis* serotype 2 has been the most prevalent and the most virulent for pigs and humans. Primarily, *S. suis* colonizes at the upper respiratory tract and palatine tonsils of pigs. However, *S. suis* have also been isolated from the alimentary and reproductive tracts (Staats et al., 1997; Huang et al., 2005). .Although *S. suis* generally causes low disease incidence in swine populations, the carrier rate of clinically healthy pigs could have reached 80% at the herd level (Wertheim et al., 2009). These healthy pigs at the slaughterhouses might be the important sources of human exposure to pathogenic *S. suis* (Ngo et al., 2011). Eventually, *S. suis* could threaten the susceptible population from swine farms, abattoirs, retail markets and also the tables (Fauveau et al., 2007; Gustavsson and Rasmussen, 2014; Wongjittraporn et al., 2014).

S. suis infections are typically sporadic in humans, however, outbreaks involving a large number of patients have been frequently reported (Yu et al., 2006; Wang et al., 2007). Most patients suffer from meningitis, followed by sepsis, arthritis and endocarditis, respectively. Deafness and vestibular dysfunction might occur as the adverse consequences in some recovered patients.

Historically, *S. suis* infection has been an occupational disease particularly from direct invasion of pathogen through the cutaneous lesions. The risk occupational groups are those who are frequently exposed to infected pigs or pork products e.g. swine farmers, veterinarians, slaughter workers or even butchers. Recently, foodborne infection cases attributed to this pathogen have been increasingly reported. In Asian,

especially Thailand and Vietnam, consumption of raw or undercooked pork and pork products has been associated with several cases (Navacharoen et al., 2009; Nghia et al., 2011). Ferrando et al. (2015) concluded that *S. suis* can infect not only through skin lesions, but also translocate across human intestinal epithelial cells. It should be considered as an emerging foodborne pathogen.

In Thailand, annual morbidity and mortality rates of *S. suis* infection between 2013 and 2016 ranged between 0.29-0.55 and 0.02-0.03 per 100,000 people, respectively.[1] However, the actual number of *S. suis* infection could have been even higher due to the underreport and unawareness of laboratories or health officers (Navacharoen et al., 2009). The northern region of Thailand has been reported with the highest morbidity rate annually. High incidence in this region has long been believed to be mainly attributed to the backyard slaughter for religious ceremonies and traditional food preparations. However, the epidemiological studies of *S. suis* as a foodborne pathogen are still rarely reported. The true sources of *S. suis* contaminating pork and pork products in retail markets, especially in Thailand, are still needed.

Microbial risk assessment (MRA) is a scientific evaluation of adverse health effects from the exposure of pathogens from food consumption. MRA could be conducted in either qualitative or quantitative approaches. Qualitative microbial risk assessment describes the degree of risk by using rating system. The risk is rated using texts such as "low", "medium" or "high". While, quantitative microbial risk assessment (QMRA) evaluates the risk as the number by using statistical models and probability distributions. QMRA provides scientific-based information for risk managers in order to reduce the infection risks and improve the public health (Haas et al., 2014). QMRA of the pathogenic *S. suis,* an emerging foodborne pathogen, has never been reported.

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[1]Bureau of Epidemiology 2017. "Subject: National Disease Surveillance: *Streptococcus suis*" (online). Available: http://www.boe.moph.go.th/boedb/surdata/disease.php?ds=82. Accessed April 12, 2017.

To reduce the risk of *S. suis* serotype 2 infection from consuming pork, this study evaluated the prevalence and concentration of *S. suis* contamination along the pork supply chains and developed a QMRA framework for describing the health risk from *S. suis* serotype 2 using currently available information. Our findings revealed the important risk factors, from farm to fork, that influence the risk estimates and applicable to proper risk management options.

1.2 Objectives of this study

1.2.1 To quantify the prevalence and concentration of *S. suis* contamination along the pork production chain.

1.2.2 To evaluate the health risk attributable to *S. suis* serotype 2 from pork consumption in Thailand.

CHAPTER 2

LITERATURE REVIEW

2.1 Microbiology of *Streptococcus suis*

Streptococcus suis, a member of the family *Streptococcaceae*, is a spherical-shaped, non-motile, non-sporeforming, encapsulated, facultative anaerobic, Gram-positive bacterium (Gottschalk, 2012). Biochemical properties are used to differentiate between *S. suis* and other group D *streptococci* (Table 2.1). The bacterial cells, approximately 0.1 µm in diameter, were divided by one plane to form a pair or chain arrangement. This fastidious bacterium demanded a culture medium supplemented with blood or serum to support growth. Additionally, the optimal growth condition for *S. suis* is at 37 °C with elevated concentration of CO_2 levels (Stewart, 2013).

On blood agar, *S. suis* produces small, approximately < 1 mm in diameter, round, white-grayish colonies with a green discoloration zone around the colonies, called incomplete or alpha hemolysis. According to cell wall polysaccharide antigens, *S. suis* has been serologically classified as a Lancefield group D streptococcus (Okura et al., 2016).

Table 2.1 Biochemical properties of *S. suis* and other Lancefield group D *streptococci*[2]

Species	Biochemical test [a]								
	Opt	BE	Esc	Na	Vp	Man	Sbl	Tre	St
S. suis	-	-	+	-	-	-	-	+	+
S. equinus	-	+	+	-	+	-	-	+/-	-
S. gallolyticus	-	+	+	-	+	+/-	-	+	+/-
S. infantarius	-	+/-	+/-	-	+	-	-	-	+/-

[a]Abbreviations: Otp, optochin; BE, bile-esculin reaction; Esc, esculin hydrolysis; Na, growth in 6.5% NaCl; VP, Voges-Proskauer reaction; Man, manitol; Sbl, sorbitol; Tre, trehalose; and St, hydrolysis of starch.

S. suis naturally inhabits the mucous membrane of swine upper respiratory tract, predominantly at palatine tonsil and nasal cavities, gastrointestinal and genital tracts. However, it has been increasingly isolated from a wide range of mammalian (Table 2.2) and avian species (Devriese et al., 1994). Moreover, *S. suis* has also been detected from the environment of pork production systems e.g. equipment surfaces (Soares et al., 2015) and air samples (Bonifait et al., 2014). Even *S. suis* is capable of surviving and circulating around the environment of pork production systems, the bacterium is effectively inactivated by conventional disinfectants and heating (Dee and Corey, 1993). Additionally, *S. suis* remains viable in pig carcasses and secretions for an extended period of time (Tables 2.3-2.4) (Clifton-Hadley and Enright, 1984; Clifton-Hadley et al., 1986).

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[2]CDC 2006. "Subject: Identification of Other Streptococcus Species: Streptococcus General Methods" (online). Available: https://www.cdc.gov/streplab/strep-doc/general-methods-section2.html. Accessed August 10, 2016.

Table 2.2 *S. suis* isolated from mammals other than swine host

Host	Isolation site	Serotype	Reference
Cat	Brain, Liver, Kidney, and Spleen	Non-typable	Roels et al. (2009)
Dog	Urine	8	Muckle et al. (2010)
Wild rabbit	Nasal and Tonsil swab	9, 31	Sanchez del Rey et al. (2013)
Wild cat	Tonsil swab	9	Tang et al. (2016)
Cattle	Lung, Kidney, Liver, Spleen, Intestine, Heart, Milk, Conjunctiva, and Fetus	Non-typable	Okwumabua et al. (2017)

Table 2.3 Survival of *S. suis* in different media and temperatures

Medium	Survival period [a]					
	0 °C	4 °C	9 °C	20-25 °C	50 °C	60 °C
Feces	104 days	N/A	10 days	8 days	N/A	N/A
Dust	54 days	N/A	25 days	< 1 day	N/A	N/A
Broth	N/A	9 months	N/A	N/A	120 min	10 min
Water	N/A	7-14 days	N/A	N/A	120 min	10 min
Carcass	N/A	42 days	N/A	12 days	N/A	N/A
Urine	N/A	N/A	N/A	10 days	N/A	N/A
Whole blood	N/A	N/A	N/A	10 days	N/A	N/A
Brain	N/A	N/A	N/A	10 days	N/A	N/A
Semen	N/A	N/A	N/A	10 days	N/A	N/A

[a] Note: N/A, not available　　　　(Clifton-Hadley and Enright, 1984; Dee and Corey, 1993)

Encapsulated *S. suis* strains, which produce the antigenic capsular polysaccharide (CPS), have been categorized up to 35 serotypes, namely serotypes 1 to 34 and serotype 1/2. However, later on *S. suis* serotypes 20, 22, 26, 32, 33 and 34 were later taxonomically excluded from *S. suis* (Okura et al., 2016). Serotypes 32 and 34 were considered as *S. orisratti* (Hill et al., 2005), serotypes 20, 22 and 26 were recently proposed as *S. parasuis* (Nomoto et al., 2015), and serotype 33 was classified as *S. ruminantum* (Tohya et al., 2017). *S. suis* serotype 2 is the most prevalent and the most virulent serotype in humans and pigs (Goyette-Desjardins et al., 2014).

Table 2.4 Survival of *S. suis* on different surfaces (Dee and Corey, 1993)

Surface	Manure-coated	Survival period [a]	
		20 °C	49 °C
Plywood	+	< 4 h	< 4 h
	-	12 h	N/A
Plastic	+	20 h	< 4 h
	-	24 h	4 h
Concrete	+	< 4 h	< 4 h
	-	24 h	4 h
Metal	+	< 4 h	< 4 h
	-	8 h	N/A
Rubber (Boots)	+	72 h	N/A

[a] Note: N/A, not available

2.2 *Streptococcus suis* infection in pigs

      S. suis, an opportunistic pathogen, is commensal in several organs of pigs e.g. oral and nasal cavities, palatine tonsils, alimentary and genital tracts (Gottschalk, 2012). This bacterium is globally distributed among swine populations in both backyard and industrial farms. Most clinically healthy pigs are colonized by virulent or non-virulent strains of *S. suis* and served as the infectious reservoirs for their herds, and also humans. While the carrier rates of healthy pigs could be as high as 80%, the incidence rates of clinical infection are usually less than 5%. In the absence of appropriate antimicrobial treatment, the case-fatality rates could have reached to 20% (Staats et al., 1997).

      S. suis serotypes 1/2, 2, 3, 7 and 9 have been frequently isolated from diseased pigs. Of these serotypes, *S. suis* serotype 2 has been the most prevalent (27.9%) worldwide (Goyette-Desjardins et al., 2014). In Thailand, however, the most frequently reported serotype associated with infected pigs is serotype 9, followed by serotypes 2 and 1, respectively (Padungtod et al., 2010; Nutravong et al., 2014). Additionally, the occurrence of *S. suis* and its serotypes in healthy carrier pigs in Thailand are shown in Table 2.5.

      Primary pathway of animal-to-animal transmission of *S. suis* is through the oronasal route. Piglets may receive the bacteria from vaginal secretion of their sows (a vertical transmission) during parturition (Amass et al., 1996). The infected pigs may spread the bacteria to their pen mates by nose-to-nose contact (a horizontal transmission). Additionally, recent studies have increasingly suggested the possibility of aerosol transmission route of *S. suis* in pigs (Bonifait et al., 2014; Gauthier-Levesque et al., 2016).

      S. suis predominantly causes the clinical infections in weaned and growing pigs, where they are no longer protected by the maternal antibodies, but less frequent in suckling and adult pigs. Fever is usually developed in the infected pigs as the earliest clinical sign (Gottschalk, 2012). This may occur with or without any other consequential clinical signs. However, some affected pigs typically suffer from the neurological signs

e.g. ataxia, paddling, opisthotonus, nystagmus or seizure as a result of meningitis. Endocarditis, which is common in older pigs, causes cyanosis, dyspnea, cachexia or even sudden death. Septicemia, pneumonia, arthritis, rhinitis and vaginitis are less common and produce less remarkable clinical manifestations (Goyette-Desjardins et al., 2014). In peracute infections, pigs usually suddenly die even without developing any precautionary signs (Gottschalk, 2012).

Generally, a tentative diagnosis of *S. suis* infection in pigs is based on clinical manifestations, age of the affected pigs, and macroscopic pathological findings. Bacterial isolation and histological lesions in tissues could confirm the *S. suis* infection (Goyette-Desjardins et al., 2014). Additionally, serotype identification is advised as a part of a routine diagnostic procedure (Gottschalk, 2012).

To minimize production loss from clinical *S. suis* infections, early recognition of the clinical signs, followed by instantaneous empirical antimicrobial treatment is suggested. The choice of antimicrobial agent should be based on the results of susceptibility test (Gottschalk, 2012). Sensitivity rates to extended spectrum beta-lactams, e.g. ampicillin, of *S. suis* isolates were approximately 90% (Noppon et al., 2014; Athey et al., 2016; Oh et al., 2017).

Stress is a crucial predisposing factor involved in development of clinical *S. suis* infections. Management of stress e.g. herd intensity, immune status, concurrent health problems, weaning, pig movement, air ventilation, temperature and other environment quality could control the disease occurrence. Additionally, all-in/all-out pig flow practice could also reduce the risk of infection (Goyette-Desjardins et al., 2014). Immunization against *S. suis* is serotype-specific and relatively ineffective to prevent the outbreaks (Gottschalk, 2012).

Eradication of *S. suis* from pig herds is economically inefficient and limit only serotype 2. Since *S. suis* colonizes the piglets immediately after birth, eliminating this bacterium by medicated early weaning tends to be unsuccessful (Gottschalk, 2012).

Table 2.5 Occurrence of *S. suis* and serotypes in healthy pigs in Thailand

Sampling site (Region/Province)	No. (%) of *S. suis*		Serotype	No. (%) of serotype		Reference
Chiang Mai	19/212	(8.9)	2	12/28	(42.9)	Padungtod et al.
			7	4/28	(14.3)	(2010)
			9	1/28	(3.9)	
			1	1/28	(3.9)	
Phayao	151/180	(83.8)	23	20/196	(10.2)	Thongkamkoon et al.
			9	16/196	(8.2)	(2017)
			7	16/196	(8.2)	
			2	11/196	(5.6)	
Central	125/350	(35.7)	16	15/135	(11.0)	Meekhanon et al.
			2	3/135	(2.0)	(2017)
North-eastern	42/741	(5.7)	7	5/42	(11.9)	Nutravong et al.
			8	5/42	(11.9)	(2014)
			2	1/42	(2.4)	

2.3 *Streptococcus suis* infection in humans

S. suis has been recognized as an emerging zoonotic pathogen circulating along the swine production industry (Sriskandan and Slater, 2006). *S. suis* infection in humans has been increasingly reported since it was first described in Denmark by Perch et al. (1968). Although, *S. suis* typically causes sporadic infections, outbreaks involving hundreds of patients with serious illness have repeatedly occurred in China in 1998, 1999 and 2005 (Lun et al., 2007).

Human infection has been frequently associated with *S. suis* serotype 2 (74.7%), followed by serotype 14 (2.0%) worldwide (Goyette-Desjardins et al., 2014). However, 23.0% of overall reported cases did not properly identify the corresponding serotype. In addition, human infections caused by serotypes 1, 4, 5, 9, 16, 21, 24 and 31 have also

been documented (Nghia et al., 2008; Callejo et al., 2014; Gustavsson and Rasmussen, 2014; Hatrongjit et al., 2015; Kerdsin et al., 2015; Kerdsin et al., 2016).

Typically, *S. suis* causes an occupational zoonotic infection via direct invasion through skin lesions. People who are continually exposed to pigs, carcasses or pork products are at risk of infection e.g. pig handlers, farmers, veterinarians, slaughterhouse workers and butchers (Soares et al., 2015). However, the major risk factor of infection in some Asian countries, especially Thailand and Vietnam, is the consumption of undercooked pork or pork products (Navacharoen et al., 2009; Huong et al., 2014). Since a recent study has demonstrated that *S. suis* has an ability to translocate across human intestinal epithelial cells, it now becomes an emerging foodborne pathogen (Ferrando et al., 2015).

After exposure to *S. suis*, the patient could develop the disease within a wide range of incubation period of 2 hours to 2 weeks (Navacharoen et al., 2009). The onset-to-hospitalization interval ranged between 2.0 to 11.4 days (median 3.5 days), while the duration of hospitalization are varied from 13.0 to 19.2 days (median 17.4 days) (Huong et al., 2014).

Most of the patients suffered from meningitis, followed by sepsis, arthritis, endocarditis and endopthalmitis, respectively. Streptococcal toxic shock-like syndrome (STSS), a severe clinical feature, was also reported at a rate of 2.9% (Huong et al., 2014). Case-fatality rate for *S. suis* infection was 12.8% in adults. Additionally, a number of survived patients developed deafness (39.1%) and vestibular dysfunction (22.7%) as the consequences (Huong et al., 2014), presumably, of exotoxin-damaged cochlear (Tan et al., 2010).

2.4 *Streptococcus suis* situation in Thailand

S. suis has been a serious public health burden in Thailand, since the human infection was first described in 1987 (Teekakirikul and Wiwanitkit, 2003). At the end of 2012, global reported *S. suis* infection cases were primarily from Thailand (36% of total cases worldwide) (Huong et al., 2014). During 1999 and 2000, aggregated mortality

cases were reported in Lumphun province (Fongcom et al., 2001). Annual morbidity and mortality rates in Thailand during 2013 to 2016 ranged between 0.29-0.55 and 0.02-0.03 per 100,000 people, respectively. In addition, the case-fatality rate was 6.21% during the same period[3]. In Thailand, seasonal effect appeared to influence the number of *S. suis* infections, where higher prevalence observed during rainy season than cold season (Figure 2.1).

Geographically, the annual morbidity rate of *S. suis* infection was highest in the Northern region of Thailand, followed by North-eastern and Central regions, respectively, while the incidence of human infections rarely happen in the Southern region. The cumulative numbers of S. suis reported cases in Thailand were illustrated in Figure 2.2. The actual number of *S. suis* infection, however, could have been underestimated as a result of the unawareness of either physicians or laboratories since *S. suis* infection has been commonly misdiagnosed as other type of streptococcal infections (Navacharoen et al., 2009).
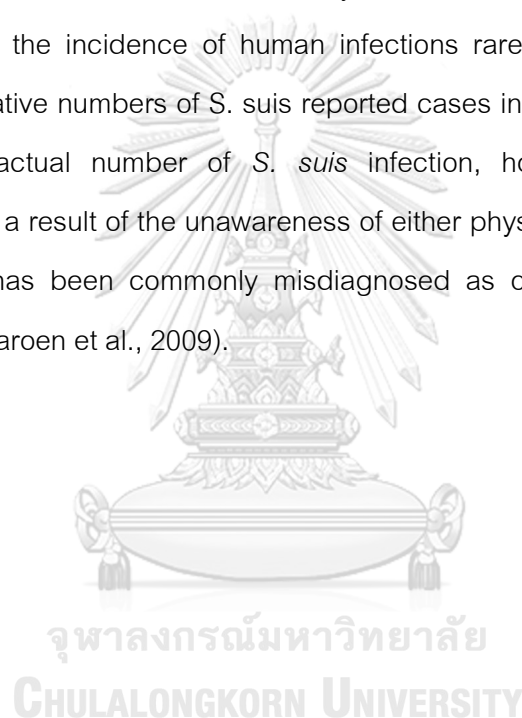
[3]Bureau of Epidemiology 2017. "Subject: National Disease Surveillance: *Streptococcus suis*" (online). Available: http://www.boe.moph.go.th/boedb/surdata/disease.php?ds=82. Accessed April 10, 2017.
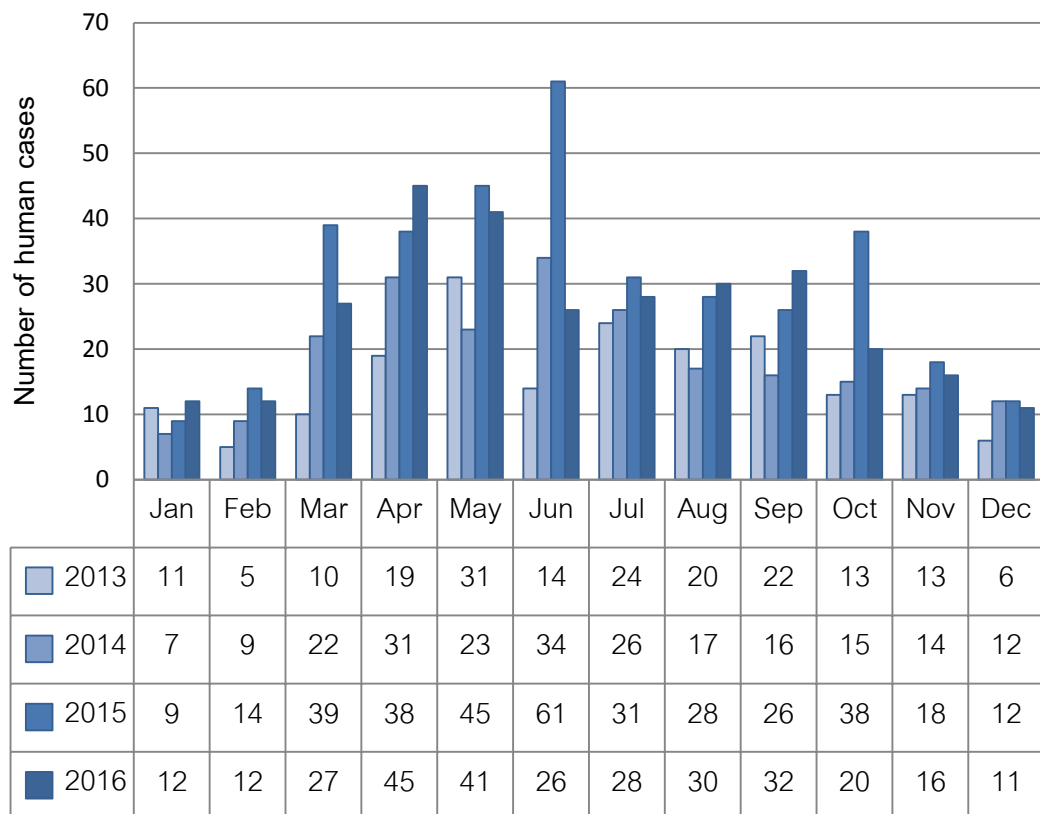
Figure 2.1 Number of reported *S. suis* infections in Thailand by months during 2013 to 2016[3]

As a majority of Thai patients was from the Northern region, consumption of traditional food preparations in this area, served as raw pork and pig blood, has been a risky eating culture. These undercooked foods are conventionally available at the fresh markets in this region. Moreover, people may even share their raw dishes in some rituals, such as wedding or religious ceremonies, and spread the infections. The residents of this area posed low to moderate levels of perception and knowledge about risk and prevention of *S. suis* infection (Unnankrad, 2008; Kaewmoon, 2009; Yana, 2009).
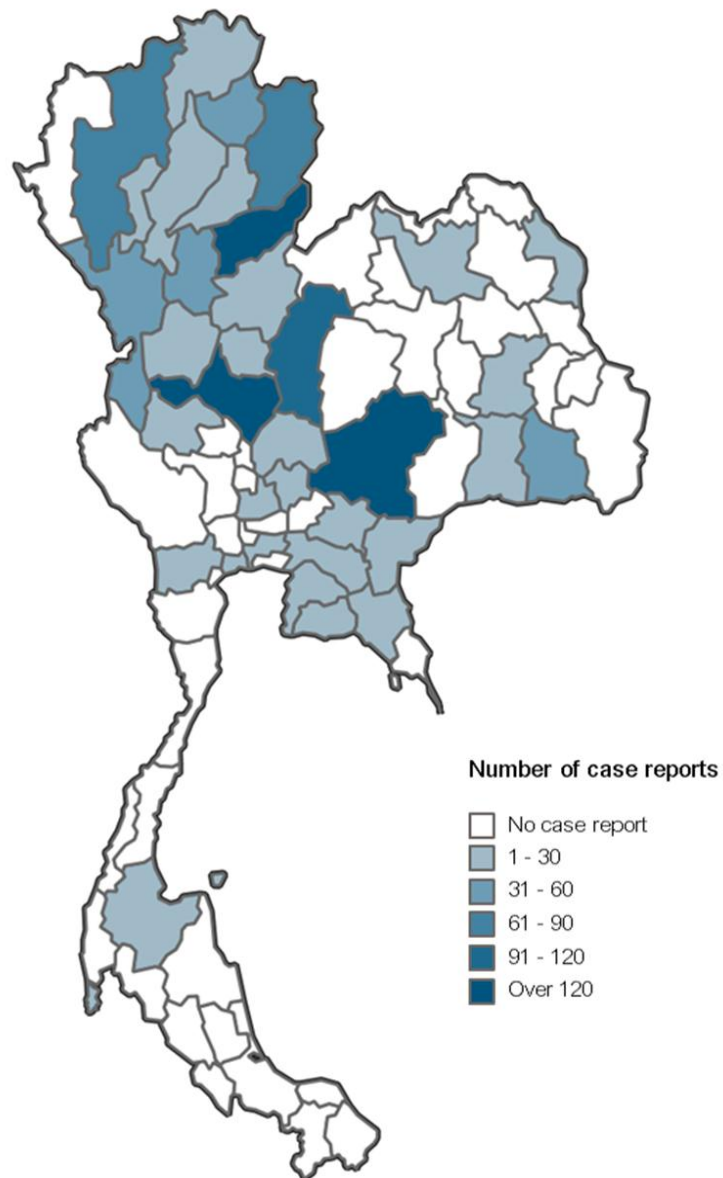
Figure 2.2 Geographical distribution of cumulative *S. suis* cases in Thailand during 2013 to 2016[3]

Takeuchi et al. (2017) has demonstrated that a food safety campaign aiming at strengthening the levels of risk perception and knowledge on *S. suis* infection to the risk population has effectively reduced the incidence rates in Phayao province for few years (from 6.4/100,000 persons in the year before the campaign to 2.7/100,000 persons and to 2.0/100,000 persons in two years after the campaign). However, a continuous campaign and additional interventions are strongly recommended to sustain the effectiveness of disease control program (Takeuchi et al., 2017). In addition, proper pork cooking temperature and time recommended by National Institute of Health is 70 ºC for at least 10 minutes[4].

2.5 *S. suis* identification and serotyping

Identification of *S. suis* that is based solely on colony morphology, Gram-stain and biochemical tests may cause a false negative result (Gottschalk et al., 2010). Many human diagnostic laboratories, which are less familiar with *S. suis* compared to veterinary laboratories, frequently misdiagnosed *S. suis* as viridans streptococci, *Streptococcus bovis*, *Streptococcus pneumoniae* or other enterococci (Goyette-Desjardins et al., 2014).

Polymerase chain reaction (PCR) assay targeting at glutamate dehydrogenase gene (*gdh*), a house-keeping gene of *S. suis*, has been widely used to avoid such misdiagnoses (Okwumabua et al., 2003). However, after serotypes 20, 22, 26, 32, 33, and 34 have been taxonomically excluded from taxon *S. suis*, *recN* gene was recently proposed as a novel PCR identification target (Ishida et al., 2014).

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[4]National Institute of Health2012. "Subject: *Streptococcus suis* infection Fact sheet" (online). Available: http://nih.dmsc.moph.go.th/login/showimgpic.php?id=23. Accessed August 2, 2017

Serotyping is an important means for the diagnosis of *S. suis* infection, since the virulence is rather relevant to serotypes than several virulence factors. Instead, some of these virulence factors e.g. extracellular factor (EF), suilysin (SLY) and muramidase-released protein (MRP) and their encoding genes, have been widely used as the tools for determining genetic diversity of *S. suis* (Martinez et al., 2003; Tharavichitkul et al., 2014).

Several multiplex PCR assays have recently been developed to differentiate several serotypes of *S. suis* (Kerdsin et al., 2014a; Okura et al., 2014; Hatrongjit et al., 2016). However, a major limitation of PCR assay, targeting serotype-specific capsular polysaccharide (*cps*) genes, is that some serotypes share the same *cps* genes. Detection of serotype-specific *cps* genes could not distinguish between *S. suis* serotype 2 and 1/2, and between serotype 1 and 14 (Okura et al., 2013). Additional serological techniques are required for these serotype identifications.

The biochemical properties of *S. suis* posed no correlation with any specific serotype. The PCR techniques could not distinguish some *S. suis* serotypes. The appropriate serotyping should be performed by using serological techniques e.g. capillary precipitation, agglutination or Quellung (or Neufeld's) reaction (Goyette-Desjardins et al., 2014). Even though, these serological tests are simple and rapid, the production of antisera used in these techniques is time-consuming and costly. Cross-reactions between some serotypes have been demonstrated: serotypes 1 and 14, serotypes 2 and 22, serotypes 6 and 16 and serotype 1/2 with serotypes 1 and 2. Additionally, some *S. suis* strains do not react to any 35 serotype-specific antisera, described as non-typable strains (Goyette-Desjardins et al., 2014).

However, each of the above-mentioned identification and serotyping techniques were not perfect. Combination of these techniques would complement and form an appropriate solution to truly identify *S. suis* for diagnosis.

2.6 Pathogenesis of *S. suis* infection

The pathological processes of *S. suis* infection in pigs and humans share several similarities. Three significant pathologic phases consist of initial phase, systemic phase and central nervous phase (Segura et al., 2017).

2.6.1 Initial phase

The initial phase is an early step when *S. suis* interacts with host mucosal defense system and initiate the infection. The pathogen may colonize host tissues for long duration without causing disease. In pigs, *S. suis* primarily adheres to mucosal epithelium of respiratory tract, while it prefers epidermal or intestinal epithelial cells in humans. Several virulence factors are associated with adhesion, including extracellular matrix binding-factors, immunoglobulin A protease, adhesins and biofilm (Bonifait et al., 2008; Fittipaldi et al., 2012).

Invasion of *S. suis* through the host epithelial cells may be either direct or indirect, depending on its toxin, invasion. *S. suis* down-regulates its capsular polysaccharide (CPS) expression during colonization allowing better interaction between the bacteria and host cells (Gottschalk and Segura, 2000; Willenborg et al., 2011). Additionally, arginine deaminase system (ADS) promotes the survival rate of *S. suis* in acidic environment (Winterhoff et al., 2002; Gruening et al., 2006).

2.6.2 Systemic phase

In this phase, *S. suis* reaches and survives in blood circulation causing several organ infections. Polysaccharide capsule of *S. suis* plays a crucial role in its survival against phagocytic cells of both innate and adaptive immunities (Fittipaldi et al., 2012). Capsular structure of virulent strains, including serotype 2, contains sialic acid allowing the bacteria to attach to monocytes and to travel along the blood stream without being phagocytized (Gottschalk and Segura, 2000). The examples of the virulence factors that restrict the neutrophil functions are the modified cell wall structure, serine protease and

suilysin. Massive pro-inflammatory cytokine production could develop in some cases resulting in septic shock-like syndrome and sudden death (Fittipaldi et al., 2012).

2.6.3 Central nervous phase

The final phase, *S. suis* causes meningitis via translocation across blood-brain-barrier or blood-cerebrospinal fluid barrier. Similar to the initial phase, invasion of the bacteria through these barriers could be either direct or indirect invasion. Additionally, lytic activity of its exotoxins has been speculated to cause the cochlear damages resulting in loss of auditory and vestibular functions (Tan et al., 2010).

2.7 Food safety risk analysis

In terms of food safety, "risk" refers to a likelihood of adverse health effects consequential to physical, chemical or biological hazards in foods or beverages (FAO/WHO, 2006). Joint collaboration between Food and Agriculture Organization of United Nation (FAO) and World Health Organization (WHO) has played a major role in fostering food safety risk analysis frameworks. Food safety risk analysis provides scientific information and appropriate decisions for the regulators, resulting in improvement of public health and food safety outcomes. According to definition by Codex Alimentarius Commission (CAC), risk analysis comprises three components; risk assessment, risk management and risk communication (FAO/WHO, 2006).

2.7.1 Risk assessment

Risk assessment, a scientific component of risk analysis, provides information on probability or likelihood of illness. Structural framework of risk assessment established by CAC comprises 4 steps, namely, hazard identification, exposure assessment, hazard characterization, and risk characterization (FAO/WHO, 2006). These structural components are applicable to both chemical and microbial risk assessments.

2.7.2 Risk management

Risk management is a process of evaluating policies, selecting, implementing and monitoring the appropriate interventions to control the corresponding risks. Risk management options are decided based on the scientific information acquired from risk assessment with the socio-economic factors e.g. cost effectiveness and cultural influence. Risk managers, also known as regulators, are generally portrayed by government organizations and should be independent of the risk assessors.

2.7.3 Risk communication

Risk communication is a process of interactive information exchange, considering findings from risk assessment, risk perceptions, the principle of risk management interventions, among the risk assessors, risk managers, industries, consumers or other third parties throughout the risk analysis processes.

2.8 Microbial risk assessment

Microbial risk assessment (MRA) scientifically evaluates adverse consequences on human health of contaminated microbial pathogens in foods or drinks (FAO/WHO, 2006). Unlike chemical contaminants, levels of microbial contamination are dynamic throughout the food supply chains. MRA could be performed either qualitatively or quantitatively.

Qualitative microbial risk assessment describes the degree of risk by using rating system. This approach requires less complicated calculation methods and the risk can be easily communicated with the regulators and the public. Levels of exposure (1) and potential of infection (2) are rated using texts such as "low", "medium" and "high", and then the risks are characterized by combining these two elements.

In quantitative microbial risk assessment (QMRA), on the other hand, the risk estimates are numerically calculated and expressed by utilizing complex mathematical equations and/or statistical models (FAO/WHO, 2006). The numeric outputs from QMRA are applicable for establishing the control measures in terms of risk-based

microbiological metric e.g. food safety objectives (FSO) or appropriate level of protection (ALOP).

Risk assessors could conduct QMRA by using either deterministic or stochastic (probabilistic) approach (Wooldridge, 2008). Deterministic approach to QMRA requires only a single value (point estimate) of each variable and establishes only one possible risk estimate. Worst-case and best-case scenarios are frequently examined through this approach. In stochastic QMRA, the probability distributions and mathematical models are employed to characterize variability and uncertainty of variables and model parameters. The stochastic approach is more realistic than the deterministic approach, since it considers wider ranges of possible outcomes of model parameters and different possible scenarios will be assessed. In addition, MRA should be reassessed whenever more novel relevant information is available (FAO/WHO, 2006).
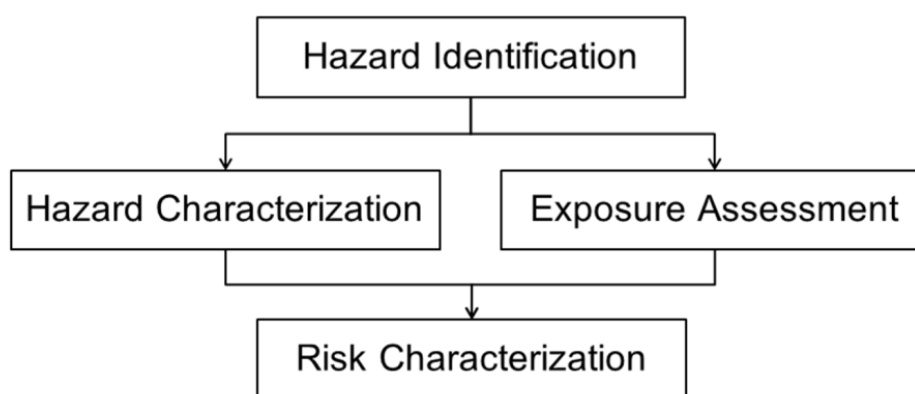
Figure 2.3 A structural framework of MRA established by CAC

2.8.1 Hazard identification

Hazard identification is an initial step of MRA where the biological hazards in foods or commodity is addressed. Microbiological, epidemiological and clinical information of the pathogen of interest and its characteristic along the food supply chain should be acquired from scientific evidences (FAO/WHO, 2009).

2.8.2 Exposure assessment

Exposure assessment is an estimation of total amount and frequency of population exposure to contaminated microbial hazards during a certain period of time. The major influences of exposure estimates are food consumption patterns, prevalence and concentration of bacterial contamination in foods.

A modular process risk model (MPRM) is frequently developed for food chain exposure pathway. MRPM provides a structured approach to exposure assessment by dividing the food production pathway into different modules. Each module evaluates the contamination dynamics of the pathogens in each food production step (Nauta, 2008). In addition, predictive microbiological models are commonly used in QMRA to describe the contamination dynamics from microbial growth and inactivation throughout the food production chains (FAO/WHO, 2009).

2.8.3 Hazard characterization

Hazard characterization, previously known as dose-response assessment, describes the relationship between the level of exposed pathogen and the probability of adverse health consequences. In QMRA, dose-response models are expressed as mathematical functions. The dose-response relationships are established using information of either human subject or laboratory animal experiments (FAO/WHO, 2009).

2.8.4 Risk characterization

Risk characterization expresses the risk estimate as a product of the outcomes from exposure assessment and hazard characterization. Risk estimates could be presented as risk per serving, individual-based risk or population-based risk. In stochastic MRA, Monte Carlo simulation allows risk assessors to evaluate the risk across all different possible scenarios (FAO/WHO, 2009).

CHAPTER 3

MATERIALS AND METHODS

This study was divided into 2 phases (Figure 3.1) according to its objectives as described below:

Phase 1: Evaluation of *S. suis* contamination throughout pork production chains

Phase 2: Quantitative microbial risk assessment of *S. suis* serotype 2 from pork consumption
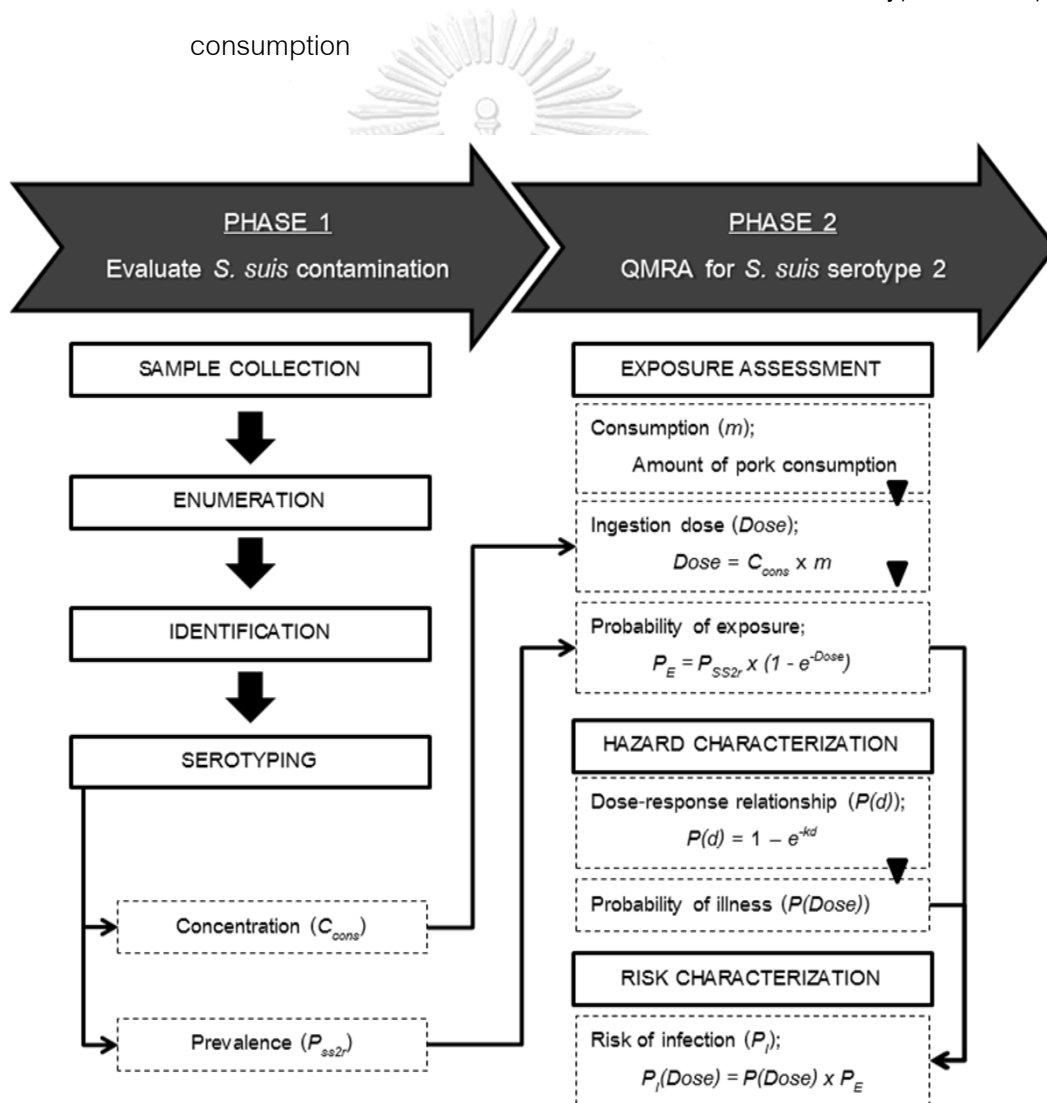
Figure 3.1 Overall work flow according to phases of this study

3.1 Evaluation of *S. suis* contamination throughout pork production chains

3.1.1 Sample collection

Two sets of samples were collected to evaluate the distribution and level of *S. suis* contamination in different aspects. The first sample collection was from Bangkok, which is more focused on *S. suis* contamination in pigs, pork products and the environment along pork production pathway. Another set of sample collection from provincial areas was to assess the contamination of *S. suis* in pork from different retails.

3.1.1.1 Bangkok sample collection

This sample collection was obtained from pork supply chains in Bangkok. Each step through food chain consisted of an abattoir and two corresponding retail sites. Total four pork supply chains, which included four abattoirs and eight retail sites receiving pigs from different sources, were visited during May 2016 to December 2016.

Two sample groups were collected from pork supplying chains, classified as "pig-to-pork" and "environmental" samples. Pig-to-pork samples were directly obtained from pigs, carcasses and pork products along pork production pathway, while environmental samples were surface samples collected from surroundings e.g. wall, floor, equipment, and water. Furthermore, both sample groups were categorized by the sources of contamination throughout the production chain steps including swine farm, transportation, abattoir, and retail market (Table 3.1).

A total of 492 and 480 samples were collected from abattoirs and retail markets, respectively. Details of Bangkok samples are described in Tables 3.2-3.4.

Table 3.1 Bangkok sample collection classified by sampling sites and food chain step

Site	Food chain step	Sample group	
		Pig-to-pork	Environmental
Abattoir	Farm	Pig	N/A
		Tonsil	
		Blood	
	Transportation	N/A	Truck wall
			Truck floor
	Abattoir	Carcass	Walls
		Thoracic organ	Floor
		Abdominal organ	Knife
			Weight tray
			Dehair tray
			Rail
			Hook
			Worker's hands
			Boots
			Hose
			Water
Retail	Retail	Pork	Table
		Intestine	Knife
		Liver	Chopping board
		Lung	Fridge
		Spleen	Weight

Table 3.2 Number of samples in the Bangkok sample collection

Abattoir				Retail			
Supplier	Pork product	Environment	Total	Supplier	Pork product	Environment	Total
A	66	60	126	A1	30	30	60
				A2	30	30	60
B	64	60	124	B1	30	30	60
				B2	30	30	60
C	62	60	122	C1	30	30	60
				C2	30	30	60
D	60	60	120	D1	30	30	60
				D2	30	30	60
Total	252	240	492	Total	240	240	480

Table 3.3 Details of pig-to-pork samples in Bangkok sample collection

Site	Type	Form	No.	Analytical unit
Abattoir	Pig	Swab	48	400 cm^2
	Carcass	Swab	96	400 cm^2
	Thoracic organ	Swab	24	100 cm^2
	Abdominal organ	Swab	24	100 cm^2
	Blood	Product	48	25 g
	Palatine tonsil	Product	12	5 g
Retail	Pork	Product	144	25 g
	Intestine	Product	24	25 g
	Spleen	Product	24	25 g
	Lung	Product	24	25 g
	Liver	Product	24	25 g
Total			492	

Table 3.4 Details of environmental samples in Bangkok sample collection

Site	Type	Form	No.	Analytical unit
Abattoir	Truck floor	Swab	12	100 cm^2
	Truck wall	Swab	12	100 cm^2
	Lairage floor	Swab	12	100 cm^2
	Lairage wall	Swab	12	100 cm^2
	Slaughter floor	Swab	12	100 cm^2
	Slaughter wall	Swab	12	100 cm^2
	Dehair knife	Swab	12	100 cm^2
	Killing knife	Swab	12	100 cm^2
	Hook	Swab	24	10 cm^2
	Dehair tray	Swab	12	100 cm^2
	Weight tray	Swab	24	100 cm^2
	Worker's hands	Swab	24	100 cm^2
	Rail	Swab	12	10 cm^2
	Boots	Swab	12	100 cm^2
	Water hose	Swab	12	100 cm^2
	Water	Water	24	25 ml
Retail	Table	Swab	48	100 cm^2
	Chopping board	Swab	48	100 cm^2
	Knife	Swab	48	100 cm^2
	Weight	Swab	48	100 cm^2
	Fridge	Swab	48	100 cm^2
Total			480	

3.1.1.2 Provincial sample collection

Pork samples in the provinces were collected in two groups of retailing, which were markets and food stalls. "Markets" were defined as retail sites where pork products are sold in a permanent structure either in the indoor or outdoor building, including fresh markets, modern trades and flea markets. Whereas "food stalls" were unofficial and transient retail sites where pork products are retailed in an outdoor or ephemeral ground, some of which are stationary food stall and mobile (truck) food stalls. A total of 1,036 pork samples from all retail types were collected from 8 provinces representing all areas of Thailand, including Chiangmai, Phayao, Nan, Khonkaen, Mukdahan, Saraburi, Nakhonpathom, and Phang-nga (Table 3.5) during September 2016 to June 2017. These provinces were selected on the basis of sampling cooperation.

3.1.1.3 Sample shipping and handling

Sampling procedures of both pork products and surface followed a recommendation by European Food Safety Authority (EFSA). All samples were carefully packaged in sterile double-sealed plastic bags to avoid cross contamination, and then stored in a leak-proof container between 2-8 °C during transportation. The samples arrived at the laboratory within 24 h after collection.

Table 3.5 Provincial samples sorted by retail type and province

| Province | Retail type | | | | | Sum |
| | Market | | | Food stall | | |
	Fresh	Modern	Flea	Stationary	Mobile	
Chiangmai	27	30	30	13	15	115
Phayao	30	30	30	15	15	120
Nan	34	33	25	24	15	131
Khonkaen	30	30	30	15	15	120
Muukdahan	60	30	30	26	5	151
Saraburi	31	30	25	15	15	116
Nakhonpathom	30	28	31	25	12	126
Phang-nga	40	35	32	31	19	157
Total	282	246	233	164	111	1036

3.1.2 Enumeration and isolation of *S. suis*

Since there has been no conventional protocol to quantify *S. suis* concentration for non-clinical samples, this study proposed a direct plate count procedure for enumerating and isolating *S. suis* as described below:

3.1.2.1 An appropriate amount of samples depending on sample types (Tables 3.3-3.4) was aseptically taken for enumeration.

3.1.2.2 The samples were homogenized with 225 ml buffered peptone water (BPW), and then the homogenous mixture was 10-fold serially diluted with BPW for a spread plate procedure.

3.1.2.3 Aseptically distributing 0.1 ml of the diluted inoculum over the surface of Columbia blood agar containing 5% sheep blood with streptococcus selective supplement and then incubated at 37°C for 18-24 h.

3.1.2.4 After overnight incubation, small, round, white-grayish colonies with a surrounded incomplete hemolysis zone were counted as suspected colonies of *S. suis*.

3.1.2.5 Up to 6 suspected colonies per sample were isolated for further identification using biochemical and molecular techniques.

3.1.3 Identification of *S. suis*

S. suis suspected isolates were subjected to presumptive biochemical tests (see Table 3.6) and then confirmed by molecular identification.

3.1.3.1 Presumptive biochemical tests

3.1.3.1.1 <u>Catalase test</u>: One single suspected colony was transferred to 3% hydrogen peroxide solution on a glass slide, and then observed for the gas production in the form of bubbles.

3.1.3.1.2 <u>Growth in a presence of sodium chloride</u>: Inoculating a loopful of the suspected colony to the brain heart infusion (BHI) broth with 6.5% sodium chloride, and then incubated for 18-24h at 37°C.

3.1.3.1.3 <u>Starch hydrolysis test</u>: A suspected colony was streaked on starch agar, and incubated at 37°C for 18-24h. Amylase activity around the colonies was assessed by adding the iodine solution over the surface of incubated agar. Clear zones around the colonies indicate the positive result, which the starch was hydrolyzed.

Table 3.6 Results of biochemical tests for *S. suis*[5]

Biochemical test	Result
Catalase test	No bubble production
Growth in presence of NaCl	No growth
Starch hydrolysis test	Starch was hydrolyzed

3.1.3.2 Molecular identification of S. suis

After biochemical tests, the *S. suis* candidates were, then, confirmed by polymerase chain reaction (PCR) technique targeting at a conserved gene, namely glutamate dehydrogenase (*gdh*) gene.

3.1.3.2.1 <u>DNA extraction</u>: The DNA template for PCR was prepared by boiling lysis of bacterial cells. The bacterial pellets were suspended in 150 µl of nuclease-free water, heated to 100°C for 10 minutes, cooled down on ice, and then centrifuged at 12,000 x g for 2 minutes. The DNA template was stored at -20 °C.

3.1.3.2.2 <u>Detection of *gdh* gene</u>: The primers used to detect *gdh* gene are shown in Table 3.7. Amplification of this gene was performed in a 20 µl reaction volume containing 10 µl of 2x PCR Master mix solution (i-Taq, iNtRON Biotechnology), 0.5 µl of forward primer, 0.5 µl of reverse primer, 8 µl of nuclease-free water and 1 µl of the DNA template. *S. suis* serotype 2 NCTC 10234 strain was used as a positive control.

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[5] CDC 2006. "Subject: Identification of Other Streptococcus Species: Streptococcus General Methods" (online). Available: https://www.cdc.gov/streplab/strep-doc/general-methods-section2.html. Accessed August 10, 2016

The PCR was conducted, using thermal cycler machine (Mastercycler personal, eppendorf, USA), with 5 min at 95 °C for initial denaturation, followed by 35 cycles of 45 s at 95 °C for denaturation, 45 s at 58 °C for annealing, 45 s at 72 °C for extension and finally, 7 min at 72 °C for the final extension. The amplicons were visualized under UV light after completion of electrophoresis at 120 V for 30 min in a 1.5% (w/v) agarose gel in 0.5x TBE buffer containing nucleic acid staining solution (RedSafe, iNtRON biotechnology, South Korea).

3.1.4 Identification of *S. suis* serotype 2

S. suis serotype 2 isolates were identified by PCR assay targeting at *cps2J*-gene, followed by serological serotyping using Quellung method (Segura et al., 2014).

3.1.4.1 Screening for S. suis serotype 2 by PCR assay

The primers used for *cps2J*-gene amplification are shown in Table 3.7. Total reaction volume was 20 µl consisting of 10 µl of 2x PCR Master mix solution (i-Taq, iNtRON Biotechnology, South Korea), 0.5 µl of forward primer, 0.5 µl of reverse primer, 8 µl of nuclease-free water and 1 µl of the DNA template. *S. suis* serotype 2 NCTC 10234 strain was used as a positive control. Detection of *cps2J*-gene used the same conditions as *gdh* gene detection

Table 3.7 Primers for species and serotype identification of *S. suis* serotypes 2 and 1/2

Gene	Primer sequences (5′ to 3′)	Product size (bp)
gdh	F: TTCTGCAGCGTATTCTGTCAAACG	695
	R: TGTTCCATGGACAGATAAAGATGG	
cps2J	F: GATTTGTCGGGAGGGTTACTTG	450
	R: TAAATAATATGCCACTGTAGCGTCTC	

(Kerdsin et al., 2014a)

3.1.4.2 Serological serotyping

S. suis isolates with *cps2J* gene were then confirmed as serotype 2 by using Quellung technique as described below:

3.1.4.2.1 Suspended a freshly grown bacterial colony on sheep blood agar in sterile normal saline solution.

3.1.4.2.2 Transferred small amount of the bacterial suspension to a glass slide using a loop.

3.1.4.2.3 Mixed the bacterial suspension with the same amount of serotype-specific antisera (StatensSerum Institut, Denmark) and then covered with a cover glass.

3.1.4.2.4 Examined the results under a 100X light microscope. Swollen bacterial cells indicated a positive result.

3.2 Quantitative microbial risk assessment of *S. suis* serotype 2 from pork consumption

This study developed a quantitative microbial risk assessment following the guideline established by CAC (FAO/WHO, 2006). Risk of *S. suis* serotype 2 infection from consuming pork was numerically estimated with a stochastic approach. All equations and models were constructed and analyzed by Monte Carlo simulation with "mc2d" and "fitdistrplus" packages in a computing language R version 1.0.143 (R Development Core Team, 2017).
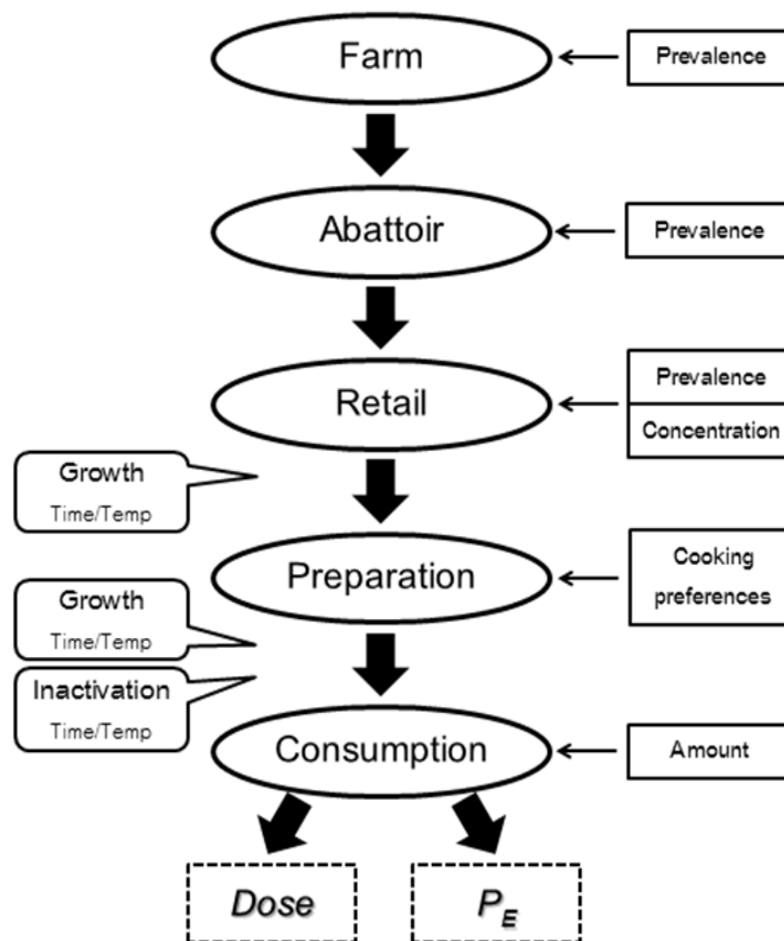
Figure 3.2 A conceptual framework of the modular process risk model for exposure assessment of this study

3.2.1 Hazard identification

The evidences of illness caused by *S. suis* serotype 2 resulting from pork exposure was collected from scientific literatures. The key information was microbiological, clinical, and epidemiological characteristics of the pathogen throughout the pork production chains.

3.2.2 Exposure assessment

In this section, probability of exposure to *S. suis* serotype 2 from daily pork consumption was evaluated using a modular process risk model (MPRM) approach. In the MPRM model, the pork production chain was divided into 5 modules to assess the contamination dynamics: farm, abattoir, retail, preparation and consumption (Figure 3.2). Each module generated output distributions which were used either the input variables of the following modules or the final estimates of exposure assessment. Probability distributions were assigned to the variables to describe variability and uncertainty.

3.2.2.1 Farm module

This module predicted the prevalence of healthy pigs that are infected with *S. suis* serotype 2 at the production farms (P_{ss2f}) and transported to the abattoirs. Beta distribution was assigned to describe the variability of the prevalence in the stochastic model as displayed:

$$p = \text{Beta}(s+1, n-s+1) \qquad (1)$$

Where, p is the probability of success (prevalence), s is the number of success events (positive samples) and n is the number of observed events (sample size). The model parameters were estimated by using the epidemiological data of *S. suis* in pigs in Thailand from previously published literatures together with the results from this study using Bayesian inference. The

model assumes that the proportion of *S. suis* serotype 2 is constant along the pork production chain (Table 3.10).

Table 3.8 Prevalence of *S. suis* in healthy pigs used in farm module

Sampling site (Region/Province)	No. of positive samples (s)	Sample size (n)	Reference
Chiang Mai	19	212	Padungtod et al. (2010)
Phayao	151	180	Thongkamkoon et al. (2017)
Central	125	350	Meekhanon et al. (2017)
North-eastern	42	741	Nutravong et al. (2014)
Bangkok	5	48	This study (Table 4.2)
Total	342	1,531	

Table 3.9 Proportion of *S. suis* serotype 2 in healthy pigs used in farm module

Sampling site (Region/Province)	No. of positive isolates (s)	Sample size (n)	Reference
Chiang Mai	12	28	Padungtod et al. (2010)
Phayao	11	196	Thongkamkoon et al. (2017)
Central	3	135	Meekhanon et al. (2017)
North-eastern	1	42	Nutravong et al. (2014)
Total	27	127	

3.2.2.2 Abattoir module

This module estimated the prevalence of pig carcasses contaminated with *S. suis* serotype 2 (P_{ss2a}) at the end of slaughtering process. The results of *S. suis* contamination along the same production chain from this study (Table 4.2) displayed both increased and decreased in prevalence during the slaughtering processes, and were used to estimate the cross contamination factor. Changes in prevalence are expressed by an equation (Nauta, 2008):

$$P_{out} = \frac{P_{in} \times f}{(1 - P_{in} + (P_{in} \times f))} \qquad (2)$$

Where *f* is the factor for cross contamination, P_{in} and P_{out} is the prevalence before and after cross contaminating (Table 3.8).

3.2.2.3 Retail module

This module evaluated the prevalence (P_{ss2r}) and concentration (C_{pur}) of *S. suis* serotype 2 in pork at the retail displays. Changes in prevalence of the bacterial pathogen were estimated in the same fashion as in an abattoir module (Table 3.10).

The results of bacterial concentration in pork at retail markets (Table 4.5 and Table 4.7) were fitted into a lognormal distribution as initial concentration of *S. suis* (Ini_{ss}) using maximum likelihood estimation. The uncertainty of distribution parameters were defined by bootstrap method. In addition, the initial concentration of *S. suis* serotype 2 (Ini_{ss2}) was assumed to be uniformly distributed from 0 cfu/g to the maximum initial *S. suis* concentration.

Table 3.10 Model input parameters of assessing prevalence in farm, abattoir and retail modules

Module	Variable	Description	Unit	Distribution/Model	Reference
Farm	P_{ssf}	Prevalence of S. *suis* in pigs at farms		Beta(342+1, 1531-342+1)	Literatures and this study (Table 3.8)
	$Prob_{ss2}$	Probability of S. *suis* serotype 2 isolated from pigs		Beta(27+1, 401-27+1)	Literatures and this study (Table 3.9)
	P_{ss2f}	Prevalence of S. *suis* serotype 2 in pigs at farms		P_{ssf} x $Prob_{ss2}$	
Abattoir	P_{pig}	Prevalence of S. *suis* in pigs in production chains		Beta(5+1, 48-5+1)	This study (Table 4.2)
	$P_{carcass}$	Prevalence of S. *suis* in carcasses in production chains		Beta(5+1, 48-5+1)	This study (Table 4.2)
	$Factor_{PC}$	Cross-contamination factor for pigs to carcasses		$P_{carcass}$ / P_{pig}	
	P_{ss2a}	Prevalence of S. *suis* serotype 2 in carcasses at abattoirs		$(P_{ss2f}$ x $Factor_{PC})$ / $(1- P_{ss2f} + (P_{ss2f}$ x $Factor_{PC}))$	

Table 3.10 Model input parameters of assessing prevalence in farm, abattoir and retail modules (continued)

Module	Variable	Description	Unit	Distribution/Model	Reference
Retail	P_{pork}	Prevalence of S. suis in pork in production chains		Beta(5+1, 48-5+1)	This study (Table 4.2)
	$Factor_{CP}$	Cross-contamination factor for carcasses to pork		$P_{pork} / P_{carcass}$	
	P_{ss2r}	Prevalence of S. suis serotype 2 in carcasses at retail markets		$(P_{ss2a} \times Factor_{CP}) / (1 - P_{ss2a} + (P_{ss2a} \times Factor_{CP}))$	

The bacterial growth during retails was estimated by a predictive microbial technique. Since, there is currently no available specific growth model of *S. suis*, this study used a published growth model of *Streptococcus iniae* as a surrogate (Zhou et al., 2008) as demonstrated in the following equations:

$$y(t) = y_0 + \mu_{max} \, F(t) - \ln\left(1 + \frac{e^{\mu_{max} F(t)} - 1}{(y_0 - y_{max})}\right) \tag{3}$$

and

$$F(t) = t + \frac{1}{v}\ln\left(e^{-vt} + e^{-h_0} - e^{(-vt - h_0)}\right) \tag{4}$$

where;

$y(t)$ = bacterial concentration at time t (ln cfu/g)

t = time (h)

y_0 = initial concentration (ln cfu/g)

μ_{max} = maximum specific growth rate (h^{-1})

h_0 = proportionality constant (equal to 4.3)

v = increasing rate of limiting substrate

(Assumed to be equal to μ_{max}).

Additionally, a secondary model (Zhou et al., 2008) was used to calculate μ_{max} at growth temperature T (ºC) as the following equation:

$$\mu_{max} = 0.00140(T - 1.590)^2(1 - e^{(2.10(T - 41.11))}). \qquad (5)$$

Temperature and time for bacterial growth depends on retail storage types (R_{Sto}); refrigerated and ambient storages. The conditions used in the models were observed from retail sites in Bangkok together with the available official databases[6] (see Table 3.11).

3.2.2.4 Preparation module

This model assumes that the purchased pork was kept under refrigeration until cooked. Maximum storage time (Sto_{max}) for home refrigeration was determined by undesirable physical changes of pork, as a result of the growth of food spoilage *Psuedomonas* spp. (Signorini and Tarabla, 2009). Time to consumption was assumed to be uniformly distributed ranging from 0 hour to the maximum storage time. Growth of *S. suis* during home storage was predicted by equations (3) to (5) (see Table 3.12).

Estimation of initial concentration of *Pseudomonas* spp. in pork was derived from the experiment of Department of Veterinary Public Health, Chulalongkorn University (unpublished data).

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[6]Office of Natural Resources and Environmental Policy and Planning 2016."Subject: Annual average temperature of Thailand during 2012-2016" (online). Available: http://www.onep.go.th/env_data/wp-content/uploads/2016/08/70.pdf. Accessed September 22, 2017

Table 3.11 Model input parameters for assessing concentration in retail module

Module	Variable	Description	Unit	Distribution/Model	Reference
Retail	Ini_{ss}	Initial concentration of S. suis in pork	ln cfu/g	Normal distribution	Fitted from this study (Table 4.3 and Table 4.7)
	R_{Sto}	Retail storage type		Discrete({1,2}, {0.25, 0.75}), where 1 = refrigerated; 2 = ambient storage	Observation
	T_{amb}	Average ambient temperature	°C	Pert(uniform(22.8,25.4), uniform(27.5,27.9), uniform(29.4,31.7))	Office of Natural Resources and Environmental Policy and Planning (2016)[6]
	SL_{amb}	Shelf life of ambient storage	h	Uniform(6,8)	Observation
	t_{amb}	Time on retail display of ambient storage	h	Pert(1, uniform(1, SL_{amb}), SL_{amb})	Observation
	T_{ref}	Temperature of refrigerated storage at retail site	°C	Uniform(-1, 6)	Observation

Table 3.11 Model input parameters for assessing concentration in retail module (continued)

Module	Variable	Description	Unit	Distribution/Model	Reference
Retail	SL_{ref}	Shelf life of refrigerated storage	h	Uniform(96, 144)	Observation
	T_{ref}	Time on retail display of refrigerated storage	h	Pert(1, 48, SL_{ref})	Observation
	μ_{max}	Maximum specific growth rate	h^{-1}	$0.0014 \times (T - 1.590)^2 \times (1 - e^{2.1(T-41.11)})$, Where T is either T_{amb} or T_{ref}	Zhou et al. (2008)
	Y_{max}	Maximum cell concentration	ln cfu/g	$\ln(10^{Pert(6,\ Uniform(6,8),\ 8)})$	Zhou et al. (2008)
	C_{pur}	Concentration at purchase	ln cfu/g	$Ini_{ss2} + \mu_{max} \cdot F - \ln(1 + (e^{\mu max \cdot F} - 1/e^{Ymax - Iniss2}))$, Where; $F = t + \ln(e^{-\mu max \cdot t} + e^{4.3} - e^{(-\mu max \cdot t - 4.3)})/\mu_{max}$ And t is either t_{amb} or t_{ref}	Zhou et al. (2008)

Table 3.12 Model input parameters for assessing concentration during home storage in preparation module

Module	Variable	Description	Unit	Distribution/Model	Reference
Preparation	T_{home}	Temperature of refrigerated storage at home	°C	Uniform(0,6)	Observation
	Ini_{Pseud}	Initial concentration of *Pseudomonas* spp.	ln cfu/g	Triangle(3,4,5)	Experiment (Data not shown)
	μ_{Pseud}	*Pseudomonas* spp. specific growth rate	h^{-1}	$e^{Normal(-0.4863+0.1155ln(T\_home),0.12)}$	Signorini and Tarabla (2009)
	Λ	*Pseudomonas* spp. lag time	h	$e^{Normal(-1.568+(-0.33ln(T\_home),0.32)}$	Signorini and Tarabla (2009)
	Sto_{max}	Maximum home storage time	days	$((Uniform(8,9) - Ini_{Pseud})/\mu_{Pseud}) + \Lambda$	
	t_{con}	Time to consumption	h	$24 \times Uniform(0, Sto_{max})$	

Table 3.12 Model input parameters for assessing concentration during home storage in preparation module (continued)

Module	Variable	Description	Unit	Distribution/Model	Reference
	μ_{sto}	Maximum specific growth rate at home storage	h^{-1}	$0.0014 \times (T_{home} - 1.590)^2 \times (1 - e^{2.1(Thome - 41.11)})$	Signorini and Tarabla (2009)
	C_{sto}	Concentration after home storage	ln cfu/g	$C_{pur} + \mu_{sto} \cdot F$ $- \ln(1 + (e^{\mu sto \cdot F} - 1/e^{Ymax - Ini}))$, Where; $F = t$ $+ \ln(e^{-\mu sto \cdot tcon} + e^{4.3} - e^{(-\mu sto \cdot tcon - 4.3)})/ \mu_{sto}$	Signorini and Tarabla (2009)

Cooking preference (fully or lightly cooked) and cooking time were included in the exposure assessment model. Proportions of cooking preference of Thai people in different regions are shown in Table 3.13.

A thermal inactivation model was used to evaluate log reduction of bacterial concentration in pork during cooking. Decimal reduction time (DRT) at 70 °C (D_{70}) and Z-value (Z), derived from *Enterococcus feacalis*, used in this model were 2.79 min and 12.82 °C, respectively (Magnus et al., 1986). DRT on other temperatures T °C (D_T) was calculated from equation (6).

$$\frac{D_T}{D_{70}} = 10^{\frac{70-T}{Z}} \tag{6}$$

Table 3.13 Percentages of undercooked food preparation across regions in Thailand

Region	Undercooked food preference (%)
Bangkok	0.30
Central	0.17
North	0.35
North-east	0.48
South	0.04
Average	0.30

(National Statistical Office, 2010)

Log reduction (*R*) of *S. suis* concentration at the point of consumption (C_{cons}) were estimated by using equations (7) and (8), respectively (Table 3.14).

$$R \quad = \quad \frac{t}{D_T} \tag{7}$$

and $$C_{cons} \quad = C - R \tag{8}$$

Where;   R   = log reduction of *S. suis* (cfu/g)

             t   = cooking time (min)

             C   = bacterial concentration before cooking (cfu/g)

Since there was no currently available quantitative information on the proportion of *S. suis* serotype 2 in pork, the uncertain concentration at consumption of *S. suis* serotype 2 (C_{ss2}) was assumed to be uniformly distributed from 0 cfu/g to the maximum concentration at consumption of total contaminated *S. suis* (C_{cons}) (Table 3.12).

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Table 3.14 Model input parameters in preparation module

Module	Variable	Description	Unit	Distribution/Model	Reference
Preparation	P_{unc}	Proportion of people who prefer undercooked foods		Table 3.13	National Statistical Office (2010)
	CP	Cooking preference		Discrete({1,2}, {P_{unc}, 1-P_{unc}}), Where; 1 = Undercooked and 2 = Propercooked	
	T	Cooking temperature of proper cooked foods	°C	Uniform(60,70)	
	t_{unc}	Cooking time of undercooked foods	min	Triangle(0, 0.5, 1)	
	t_{cook}	Cooking time of proper cooked foods	min	Pert(1,uniform(1,10),10)	

Table 3.14 Model input parameters in preparation module (continued)

Module	Variable	Description	Unit	Distribution/Model	Reference
Preparation	D_{70}	DRT at 70 °C	min	2.79	Magnus et al. (1986)
	Z	Z value	°C	12.82	Magnus et al. (1986)
	D_T	Decimal reduction time at T °C	min	$D_{70} \times 10^{((70-T)/Z)}$	
	T	Cooking temperature	°C	Uniform(70,90)	
	t_{cook}	Time for fully cooked preparation	min	Pert(0,1,3)	
	t_{unc}	Time for undercooked preparation	min	Pert(3,7,10)	
	R	Log reduction from thermal inactivation	log cfu/g	t / D_T, Where T is either t_{unc} or t_{cook}	
	C_{cons}	Concentration of S. suis at consumption	log cfu/g	$\log(e^{C_{sto}}) - R$	
	C_{ss2}	Concentration of S. suis serotype 2 at consumption	log cfu/g	Uniform(0, C_{cons})	

3.2.2.5 Consumption module

This module evaluates the final outcomes of exposure assessment, which are daily ingestion dose of *S. suis* serotype 2 (*Dose*) and probability of exposure to the pathogen (P_E) (Table 3.15). Daily ingestion dose was calculated by the following equation:

$$Dose = C \times m \tag{9}$$

Where;   *Dose* = number of ingested bacterial cells (cfu/day)

C   = concentration of the bacteria in pork (cfu/g)

m   = daily pork consumption per person in (g/day)

Average amount of pork consumption in Thai population in a day[7] was 20.7 g with a 95th percentile at 90 g. The amount of pork consumption was assumed to be log-normally distributed.

Finally, the probability of exposure was evaluated by:

$$P_E = P_{ss2r} \times (1 - e^{-Dose}). \tag{10}$$

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[7]National Bureau of Agricultural Commodity and Food Standards 2010. "Subject: Database of Food Consumption of Thai People" (online). Available: http://consumption.acfs.go.th/index.php. Accessed August 1, 2017

Table 3.15 Model input parameters in consumption module

Module	Variable	Description	Unit	Distribution/Model	Reference
Consumption	m	Amount of pork consumption of Thai people	g/person/day	Lognormal(ln(20.7), (ln(90)-ln(20.7))/1.647)	National Bureau of Agricultural Commodity and Food Standards (2010)
	$Dose$	Number of ingested dose	cfu/person/day	$10^{Css2} \times m$	
	P_E	Probability of exposure to *S. suis* serotype 2		$P_{ss2r} \times (1-e^{-Dose})$	
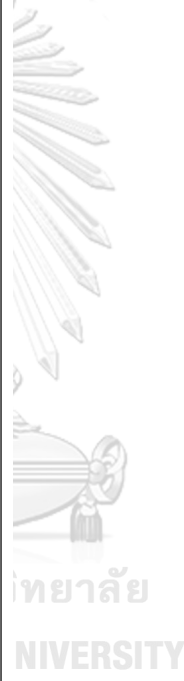
3.2.3 Hazard characterization

The dose-response relationship of *S. suis* infection in human has never been established. This study applied an exponential dose-response model of *Staphylococcus aureus* infection as a surrogate (Rose and Haas, 1999). This model was optimized from experimental infections in humans (Singh et al., 1971) by maximum likelihood estimation. Additionally, the uncertainty on the model parameter was described by using a bootstrap technique. The relationship between exposure doses and infections are shown in equation (11).

$$P(d) = 1 - e^{-kd} \qquad (11)$$

Where; $P(d)$ = probability of infection as a function of d

   k = exponential model parameter

   d = exposure dose (cfu)

3.2.4 Risk characterization

This step evaluates the risk of *S. suis* serotype 2 infection from consuming pork (Table 3.16). Probability of illness from a single exposure ($P_I(d)$) was calculated from the outcomes of exposure assessment and hazard characterization steps as displayed below:

$$P_I(d) = P_E \times P(d) \qquad (12)$$

Additionally, the annual risk estimate was estimated by an expression for multiple exposures as:

$$\text{Risk from } n \text{ exposure} = 1 - (1 - \pi)^n \qquad (13)$$

Where; π = risk estimate from a single exposure.

In addition, a sensitivity analysis, using Spearman's rank correlation, was performed to identify important variables that pose great impacts on the risk estimate. Finally, the risks of different what-if scenarios were evaluated by adjusting model variables.

Table 3.16 Model parameters for hazard characterization and risk characterization

Variable	Description	Distribution/Model
k	Parameter for exponential model	Lognormal(ln(7.64 x 10^{-8}), (ln(1.00x10^{-7})- ln(7.64 x 10^{-8}))/1.647), (Rose and Haas, 1999)
P_D	Probability of illness	$1 - e^{-kDose}$
$Risk$	Daily risk of infection from *S. suis* serotype 2	$P_E \times P_D$
$Risk_{An}$	Annual risk of infection from *S. suis* serotype 2	$1 – (1-Risk)^{365}$

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CHAPTER 4

RESULTS

4.1 Levels of *S. suis* contamination in pig-to-pork products and environment throughout the pork production chains

Prevalence and concentration of *S. suis* in pig-to-pork products and environment along the pork production chains were described by the samples from Bangkok. Prevalence of *S. suis* in pig-to-pork products was 11.4 % (56/492) and in environmental samples was 5.2% (25/480). Additionally, *S. suis* was contaminated in both pig-to-pork product and environment samples from all slaughterhouses that participated in this study, while it was not detected in some retail markets (Table 4.1).

Among pig-to-pork product samples, The highest prevalent of *S. suis* was observed in palatine tonsils (56.3%) with average concentration of 5.58 log cfu/g, followed by thoracic organs swabs (33.3%), and carcass swabs (28.1%), respectively. The prevalence of *S. suis* in pig skins before dehairing, scalding and eviscerating processes was 10.4%. In addition, prevalence of *S. suis* among three abattoirs (A, C and D) increased after a series of carcass handling processes, while observed decrease in abattoir B (Table 4.2). Prevalence of *S. suis* in pork was 0.7% (1/144) with an average concentration of 4.38 log cfu/g (Table 4.3).

Results from the environmental samples were shown in Table 4.4 and 4.5. The surface samples taken from floors and walls of slaughter rooms yielded the highest *S. suis* prevalence (20.8%), followed by worker's hands (16.7%) and boots (16.7%), while the wall and floor surfaces of pig transport trucks showed the highest average *S. suis* density (5.87 log cfu/cm^2).

Interestingly, 3 out of 16 tonsilar samples were positive for *S. suis* serotype 2, while it was not detected in all other pork product and all environmental sample types.

Table 4.1 Prevalence of *S. suis* in pork production chains

Abattoir[a]			Retail[a]		
Supplier	Pork product	Environment	Supplier	Pork product	Environment
A	42% (28/66)	10% (6/60)	A1	3% (1/30)	3% (1/30)
			A2	0% (0/30)	0% (0/30)
B	23% (15/64)	7% (4/60)	B1	0% (0/30)	0% (0/30)
			B2	0% (0/30)	3% (1/30)
C	11% (7/62)	10% (6/60)	C1	7% (2/30)	7% (2/30)
			C2	0% (0/30)	3% (1/30)
D	5% (3/60)	5% (3/60)	D1	0% (0/30)	0% (0/30)
			D2	0% (0/30)	0% (0/30)
Total	21% (53/252)	8% (19/240)	Total	1% (3/240)	3% (6/240)

[a]The results were displayed as prevalence (number of positive samples/sample size)

Table 4.2 Prevalence of *S. suis* from pig-to-pork product samples

Site	Sample	Supply chain[a]			
		A	B	C	D
Abattoir	Pig	0% (0/12)	42% (5/12)	0% (0/12)	0% (0/12)
	Carcass	58% (14/24)	25% (6/24)	17% (4/24)	13% (3/24)
	Thorax	67% (4/6)	17% (1/6)	50% (3/6)	0% (0/6)
	Abdomen	0% (0/6)	0% (0/6)	0% (0/6)	0% (0/6)
	Blood	17% (2/12)	0% (0/12)	0% (0/12)	0% (0/12)
	Palatine tonsil	75% (6/8)[b]	50% (3/6)	0% (0/2)	-
Retail	Pork	0% (0/36)	0% (0/36)	3% (1/36)	0% (0/36)
	Intestine	17% (1/6)	0% (0/6)	0% (0/6)	0% (0/6)
	Spleen	0% (0/6)	0% (0/6)	17% (1/6)	0% (0/6)
	Lung	0% (0/6)	0% (0/6)	0% (0/6)	0% (0/6)
	Liver	0% (0/6)	0% (0/6)	0% (0/6)	0% (0/6)

[a]The results were displayed as prevalence (positive samples/sample size)

[b]*S. suis* serotype 2 was detected

Table 4.3 Concentration of *S. suis* from pig-to-pork product samples

Site	Sample	Unit	Supply chain[a]			
			A	B	C	D
Abattoir	Pig	log cfu/cm^2	-	3.03	-	-
	Carcass	log cfu/cm^2	3.09	3.05	3.30	1.95
	Thorax	log cfu/cm^2	3.26	2.78	3.41	-
	Abdomen	log cfu/cm^2	-	-	-	-
	Blood	log cfu/g	2.69	-	-	-
	Palatine tonsil	log cfu/g	5.46	5.83	-	-
Retail	Pork	log cfu/g	-	-	4.38	-
	Intestine	log cfu/g	4.73	-	-	-
	Spleen	log cfu/g	-	-	5.20	-
	Lung	log cfu/g	-	-	-	-
	Liver	log cfu/g	-	-	-	-

[a]The results were displayed as mean of bacterial concentration

Table 4.4 Prevalence of *S. suis* from environmental samples

Site	Sample	Supply chain[a]							
		A		B		C		D	
Abattoir	Truck floor/wall	0%	(0/6)	0%	(0/6)	17%	(1/6)	17%	(1/6)
	Lairage	17%	(1/6)	0%	(0/6)	0%	(0/6)	0%	(0/6)
	Slaughter site	50%	(3/6)	17%	(1/6)	17%	(1/6)	0%	(0/6)
	Knife	0%	(0/6)	0%	(0/6)	0%	(0/6)	0%	(0/6)
	Hook	0%	(0/6)	17%	(1/6)	0%	(0/6)	0%	(0/6)
	Tray	0%	(0/9)	0%	(0/9)	22%	(2/9)	11%	(1/9)
	Worker's hand	17%	(1/6)	33%	(2/6)	17%	(1/6)	0%	(0/6)
	Rail	0%	(0/3)	0%	(0/3)	0%	(0/3)	0%	(0/3)
	Boots	33%	(1/3)	0%	(0/3)	0%	(0/3)	33%	(1/3)
	Water hose	0%	(0/3)	0%	(0/3)	33%	(1/3)	0%	(0/3)
	Water	0%	(0/6)	0%	(0/6)	0%	(0/6)	0%	(0/6)
Retail	Table	0%	(0/12)	0%	(0/12)	8%	(1/12)	0%	(0/12)
	Cutting board	0%	(0/12)	8%	(1/12)	17%	(2/12)	0%	(0/12)
	Knife	0%	(0/12)	0%	(0/12)	0%	(0/12)	0%	(0/12)
	Weight	0%	(0/12)	8%	(1/12)	0%	(0/12)	0%	(0/12)
	Fridge	8%	(1/12)	0%	(0/12)	0%	(0/12)	0%	(0/12)

[a]The results were displayed as prevalence (positive samples/sample size)

Table 4.5 Concentration of *S. suis* from environmental samples

Site	Sample	Supply chain[a]			
		A	B	C	D
Abattoir	Truck floor/wall	-	-	5.48	6.26
	Lairage	3.04	-	-	-
	Slaughter site	3.51	3.44	3.72	-
	Knife	-	-	-	-
	Hook	-	4.30	-	-
	Tray	-	-	3.42	4.48
	Worker's hand	1.95	2.43	2.48	-
	Rail	-	-	-	-
	Boots	3.00	-	-	2.70
	Water hose	-	-	2.00	-
	Water	-	-	-	-
Retail	Table	-	-	3.44	-
	Cutting board	-	3.99	3.62	-
	Knife	-	-	-	-
	Weight	-	3.74	-	-
	Fridge	2.85	-	-	-

[a]The results were displayed as mean of bacterial concentration in log cfu/cm^2

4.2 Levels of *S. suis* contamination in pork from different retail types

Contamination of *S. suis* in pork in the provinces were demonstrated by provinces and retail types. Prevalence of *S. suis* in pork samples was only 0.97% (10/1,036) with an average concentration of 4.22 log cfu/g. In addition, *S. suis* was detected in 4 out of 5 retails. Prevalence of *S. suis* in pork collected from mobile food stalls, flea markets, modern markets and fresh markets were 2.7% (3/111), 1.3% (2/233), 0.8% (2/246) and 0.7% (3/282), respectively. While it was not detected in pork samples collected from stationary food stalls (0/164). *S. suis* was detected in pork samples from 4 out of 8 provinces, namely Nakhonpathom (3.1%), Mukdahan (2.0%), Saraburi (1.7%) and Khonkaen (0.8%) (Table 4.6). The bacterial concentration ranged between 2.9 and 5.4 log cfu/g (Table 4.7). Interestingly, *S. suis* isolates from pork samples were not positive in serotype 2.

Table 4.6 Prevalenceof *S. suis*in pork from various retail types

Province	Retail type [a]				
	Fresh	Modern	Flea	Stationary	Mobile
Chiangmai	0% (0/27)	0% (0/30)	0% (0/30)	0% (0/13)	0% (0/15)
Phayao	0% (0/30)	0% (0/30)	0% (0/30)	0% (0/15)	0% (0/15)
Nan	0% (0/34)	0% (0/33)	0% (0/25)	0% (0/24)	0% (0/15)
Khonkaen	0% (0/30)	0% (0/30)	0% (0/30)	0% (0/15)	7% (1/15)
Muukdahan	0% (0/60)	3% (1/30)	0% (0/30)	0% (0/26)	40% (2/5)
Saraburi	6% (2/31)	0% (0/30)	0% (0/25)	0% (0/15)	0% (0/15)
Nakhonpathom	3% (1/30)	0% (1/28)	6% (2/31)	0% (0/25)	0% (0/12)
Phang-nga	0% (0/40)	0% (0/35)	0% (0/32)	0% (0/31)	0% (0/19)
Total	1% (3/282)	1% (2/246)	1% (2/233)	0% (0/164)	3% (3/111)

[a]The results were displayed as prevalence (positive samples/sample size)

Table 4.7 Concentration of *S. suis* in pork from various retail types

Province	Retail type [a]				
	Fresh	Modern	Flea	Stationary	Mobile
Chiangmai	-	-	-	-	-
Phayao	-	-	-	-	-
Nan	-	-	-	-	-
Khonkaen	-	-	-	-	5.15
Muukdahan	-	2.90	-	-	4.64
Saraburi	4.09	-	-	-	-
Nakhonpathom	2.90	5.39	4.20	-	-
Phang-nga	-	-	-	-	-
Total	3.69	4.15	4.20	-	4.81

[a]The results were displayed as mean of bacterial concentration in log cfu/g

4.3 Quantitative microbial risk assessment of *S. suis* serotype 2 from pork consumption

    4.3.1 Hazard identification

        The comprehensive review of *S. suis* was described in Chapter 2. Briefly, *S. suis*, an encapsulated, Gram-positive, facultative anaerobic bacterium, causes potential health problems in human and pig populations. The natural habitats of this pathogen are the mucosal membranes of respiratory tract, alimentary tract and genital tract of pigs. Additionally, *S. suis* could survive in pig carcasses, secretions, or even the environment of pork production systems (Soares et al., 2015).

        S. suis shows a low incidence in pig herds, while the carrier rates of this bacteria could have reached 80% among healthy pigs. In humans, *S. suis* typically causes sporadic infections, however, some outbreaks of *S. suis* infection involving hundreds patients have been reported (Lun et al., 2007). *S. suis* infected patients develop meningitis, sepsis, arthritis, endocarditis or toxic shock-like syndrome with 12.8% case-fatality rate. Some survived patients would develop vestibular dysfunction and deafness. *S. suis* serotype 2 has been considered as the most common and the most virulent serotype for both pigs and humans (Goyette-Desjardins et al., 2014). However, some unencapsulated, also called non-typable, *S. suis* strains have been isolated from human and swine cases (Kerdsin et al., 2014b).

        Typically, the risk factors of *S. suis* infection is direct exposure to infected pigs, carcasses, or pork products. However, the potential risk factor in Thailand and some other Southeast Asian countries is consumption of undercooked pork or pork products. In addition, Noppon et al. (2014) and Arai et al. (2015) have demonstrated that *S. suis* and *S. suis* serotype 2 contaminate pork at retail markets.

The annual morbidity and mortality rates of *S. suis* infection in Thailand during 2013 to 2016 ranged between 0.29-0.55 and 0.02-0.03 per 100,000 people, respectively[8].
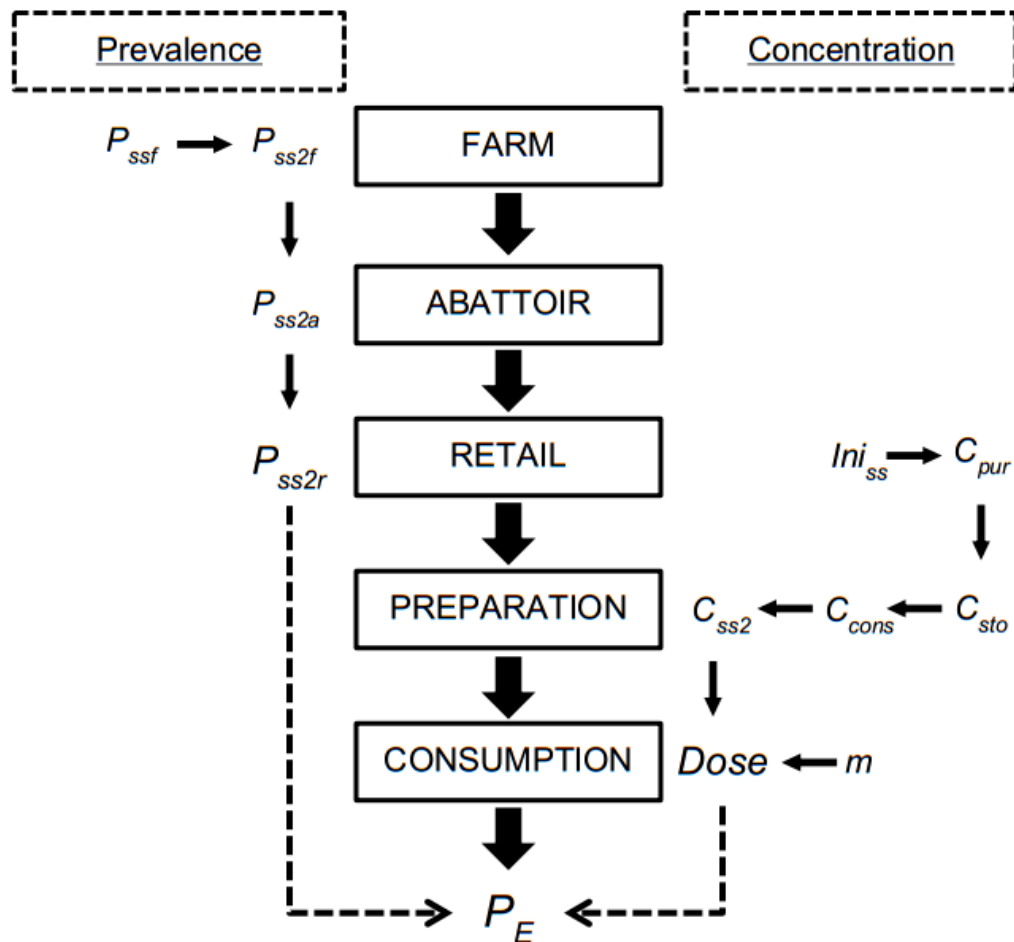
Figure 4.1 A conceptual modular process risk model describing changes in prevalence (*P*) and concentration (*C*) of *S. suis* and the probability of exposure (*P_E*) in exposure assessment. Other variables were shown in Chapter 3.

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[8]Bureau of Epidemiology 2017. "Subject: National Disease Surveillance: *Streptococcus suis*" (online). Available: http://www.boe.moph.go.th/boedb/surdata/disease.php?ds=82. Accessed August 10, 2016

4.3.2 Exposure assessment

Exposure assessment evaluated the probability of exposure (P_E) to *S. suis* serotype 2 from consuming pork by using the modular process risk model (MPRM) provided in Chapter 3 together with the results from sections 4.1 and 4.2 of Chapter 4. The MPRM described changes of *S. suis* prevalence and concentration throughout the modules. Figure 4.1 demonstrated key variables of the MPRM.

4.3.2.1 Estimated prevalence of S.suis in pork production chains

Pig-to-carcass ($Factor_{pc}$) and carcass-to-pork ($Factor_{cp}$) contamination factors were calculated from the results in Table 4.2. These factors were used to estimate the prevalence of *S. suis* serotype 2 at farms (P_{ss2f}), abattoirs (P_{ss2a}) and retail markets (P_{ss2r}). Estimated prevalence of *S. suis* and *S. suis* serotype 2 at each step of pork production chains showed in Table 4.8. The proportion of *S. suis* serotype 2 was assumed to be constant throughout the supply chains. Additionally, the model assumed that the prevalence of the *S. suis* in pork did not change after retail module.

Table 4.8 Estimated median prevalence of *S. suis* and *S. suis* serotype 2 in the pork production chain

Module	Product	*S. suis*[a]	*S. suis* serotype 2[a]
Farm	Pig	22.4% (20.4 - 24.5%)	1.5% (1.0 - 2.2%)
Abattoir	Carcass	42.1% (26.6 - 65.1%)	3.84% (2.0 - 9.3%)
Retail	Pork	2.6% (0.4 - 11.7%)	0.15% (0.02 - 0.7%)

[a]The results were displayed as median prevalence with 95% confidence interval

4.3.2.2 Estimated concentration of S. suis in pork

Unlike estimated prevalence, the concentration of *S. suis* was estimated by the models as a retail module. The initial concentration of *S. suis* (Ini_{ss}) contaminated in pork was estimated by fitting distribution to data (Table 4.3 and Table 4.7). Estimated

initial *S. suis* load in pork was 1.6 x 10^{4}cfu/g (95% confidence interval between 4.6 x 10^{2}cfu/g and 5.8 x 10^{5}cfu/g). Different retail types significantly affect the bacterial concentration at purchase (C_{pur}) ($p<0.05$) and, subsequently, before cooking (C_{sto}) ($p<0.05$) (Table 4.9). Additionally, maximum concentration of *S. suis* in pork at purchase and prior to cooking were predicted from the model were 5.6 x 10^{7}cfu/g and 5.6 x 10^{7}cfu/g, respectively.

Table 4.9 Estimated concentration of *S. suis* in pork at purchase and prior to cooking

Retail type	At purchase[a]	Prior to cooking[a]
Traditional	3.3 x 10^4	3.4 x 10^4
Modern	1.6 x 10^4	1.7 x 10^4
Overall	3.0 x 10^4	3.2 x 10^4

[a] The results are displayed as average bacterial concentration in cfu/g

Thermal inactivation from cooking processes effectively decreased the amount of *S. suis* in pork. The log inactivation of different cooking styles on the concentration of *S. suis* and *S. suis* serotype 2 in pork at consumption (C_{cons}) were shown in Table 4.10.The concentration of *S. suis* serotype 2 in pork at consumption (C_{ss2}) was described by a uniform distribution ranging from 0 cfu/g to the maximum total *S. suis* concentration at consumption (C_{cons}). In addition, concentration of *S. suis* serotype 2 in raw pork preparation utilized the concentration of *S. suis* prior to cooking (C_{sto}) in calculation instead of C_{cons}.

Table 4.10 Predicted concentration of *S. suis* and *S. suis* serotype 2 in pork at consumption from different cooking styles

Cooking style	Total *S. suis*[a]	*S. suis* serotype 2 [a]
Raw	3.2×10^4	1.2×10^4
Undercooked	1.5×10^2	5.1×10^1
NIH[9] recommended	8.2×10^0	3.2×10^0
Proper cooked	3.6×10^{-10}	1.4×10^{-10}

[a] The results are displayed as average bacterial concentration in cfu/g

4.3.2.3 Estimation of exposure dose of S. suis serotype 2 from pork consumption

The estimated daily exposure dose of *S. suis* serotype 2 per person (*Dose*) was calculated by the product of the daily amount of pork consumption (*m*) and corresponding concentration (C_{ss2}). The daily exposure doses in each region of Thailand depending on the preference of cooking were shown in Table 4.11. The average daily exposure dose of *S.suis* in Southern region was significantly lower than those in North-eastern ($p < 0.05$).

4.3.2.4 Estimation of probability of exposure to S. suis serotype 2 from pork consumption

Probability of exposure of *S. suis* serotype 2 (P_E) was the final output of exposure assessment deriving from prevalence of *S. suis* serotype 2 (P_{ss2r}) and exposure dose (*Dose*). Probabilities of exposure, based on proportions of people who prefer undercooked preparation in each region of Thailand, were shown in Table 4.12.There

[9]National Institute of Health2012. "Subject: *Streptococcus suis* infection Fact sheet" (online). Available: http://nih.dmsc.moph.go.th/login/showimgpic.php?id=23. Accessed August 10, 2016

was no significant difference on the average probability of exposure *S. suis* serotype 2 among different regions of Thailand

Table 4.11 Average exposure dose and probability of exposure of *S. suis* serotype 2 from pork consumption

Region	Exposure dose[a] (cfu/person/day)	Probability of exposure
Bangkok	2.7×10^{-9}	3.7×10^{-4}
Central	2.5×10^{-9}	3.6×10^{-4}
North	2.8×10^{-9}	3.7×10^{-4}
North-east	2.8×10^{-9}	3.7×10^{-4}
South	2.5×10^{-9}	3.6×10^{-4}
Overall	2.7×10^{-9}	3.7×10^{-4}

4.3.3 Hazard characterization

Probability of illness as a function of the exposure dose was described by an exponential model. The average model parameter k was 7.77×10^{-8}. The model parameters used in risk characterization were randomly selected from an uncertainty distribution ranged from 5.07×10^{-8} to 1.33×10^{-7}. The relationship between exposure dose and the probability of illness was illustrated in Figure 4.2.
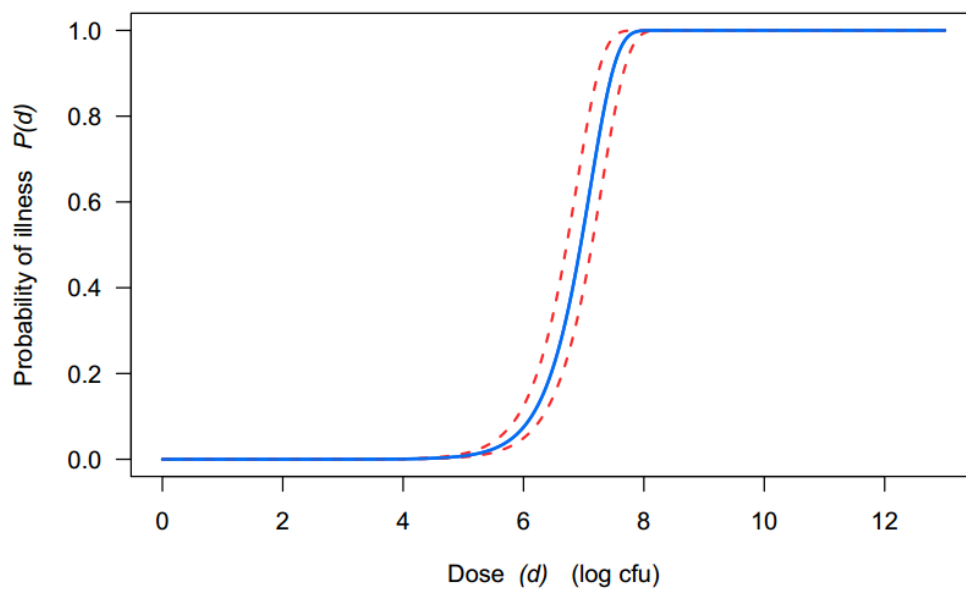
Figure 4.2 An exponential dose-response relationship with uncertainty distributions. Two dot lines represented the upper and lower bounds.

4.3.4 Risk characterization

4.3.4.1 Risk of S. suis serotype 2 infection from pork consumption

Risk estimate of *S. suis* serotype 2 infection (*Risk*) was the product of the probability of exposure (P_E) and the probability of illness (*P(d)*) corresponding to the exposure dose (*Dose*). Estimated daily risks and annual cases of *S. suis* serotype 2 infection from pork consumption in different regions of Thailand were shown in Table 4.12 and Figure 4.3. The risk estimate in Southern region was significantly lower than those in North-eastern (*p*<0.05), Northern (*p*<0.05) regions.

Table 4.12 Daily risk estimates and expected annual case number of *S. suis* serotype 2 infection by regions

Retail type	Daily risk[a]	Annual cases per 100,000 persons[a]
Bangkok	1.3×10^{-7}	4.6
	$(0.00; 4.3 \times 10^{-7})$	$(0.00; 15.7)$
Central	1.1×10^{-7}	3.9
	$(0.00; 3.9 \times 10^{-7})$	$(0.00; 15.0)$
North	1.3×10^{-7}	4.8
	$(0.00; 4.3 \times 10^{-7})$	$(0.00; 15.7)$
North-east	1.4×10^{-7}	5.0
	$(0.00; 4.8 \times 10^{-7})$	$(0.00; 17.5)$
South	9.9×10^{-8}	3.6
	$(0.00; 3.9 \times 10^{-7})$	$(0.00; 14.3)$
Overall	1.3×10^{-7}	4.6
	$(0.00; 4.3 \times 10^{-7})$	$(0.00; 15.7)$

[a] The results were displayed as mean (95% confidence interval)
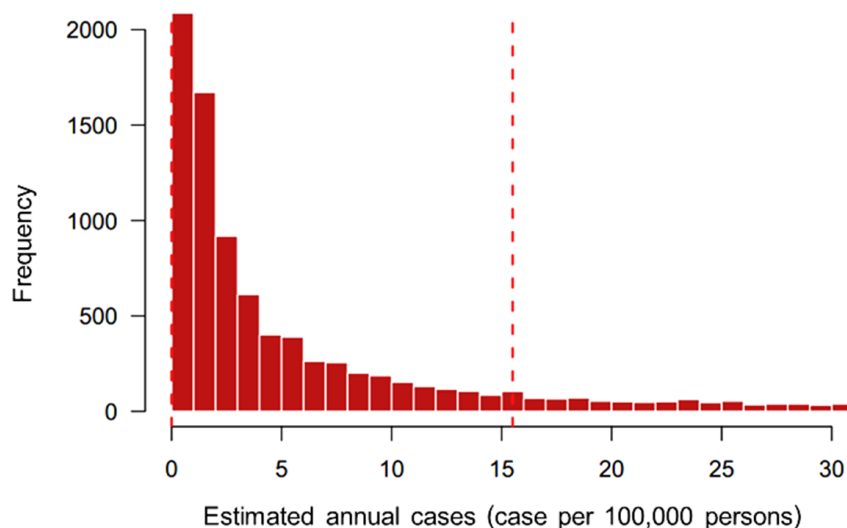

Figure 4.3 Distribution of estimated annual *S. suis* serotype 2 cases. The dash lines indicated the 95% confidence interval.

4.3.4.2 Sensitivity analysis

A sensitivity analysis was performed to assess the key factors that influenced the risk estimate using Spearman's rank correlation. The magnitude of the Spearman's rho (r) indicates strength of impacts over the risk estimate, in that positive r indicates the risk factor and in turn negative r suggests the protective factor.

The results suggested that the risk estimate was greatly influenced by the daily exposure doses ($r = 0.94$), concentration of *S. suis* serotype 2 at consumption ($r = 0.94$), followed by time of refrigerated storage at home ($r = 0.23$), and cooking time ($r = -0.12$) (Figure 4.4). In addition, the risks appeared to be negligibly contributed by amount of pork consumption ($r = 0.09$), initial concentration ($r = 0.08$), and prevalence ($r = 0.01$) of *S. suis* in pork.
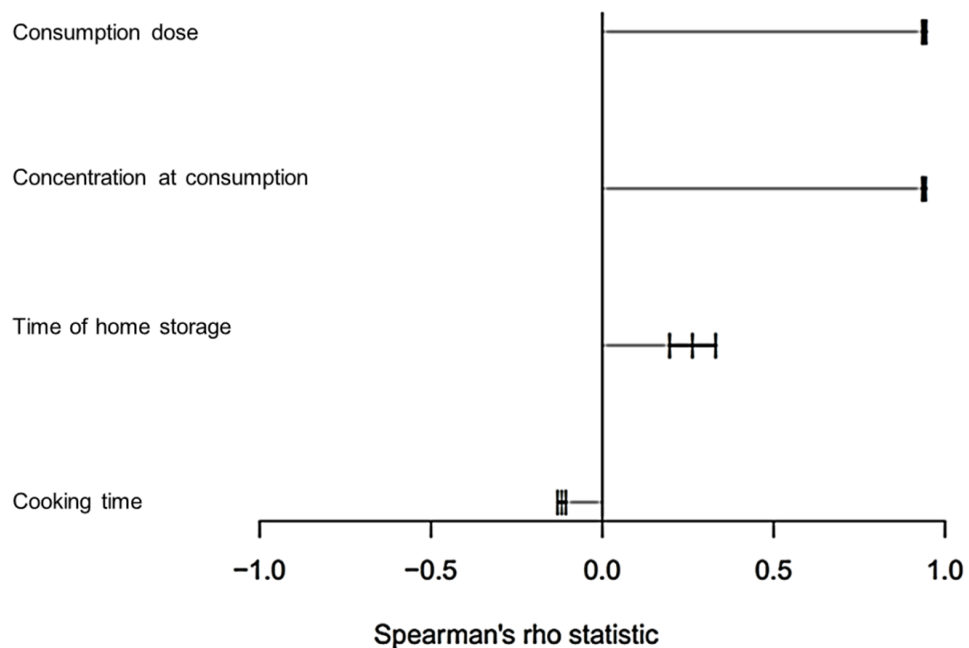

Figure 4 4 Spearman's rho statistic between the risk of *S. suis* serotype 2 infection and important factors

4.3.4.3 Risks of S. suis infection in what-if scenarios

The risks of *S. suis* illness in different scenarios were evaluated by adjusting the model variables in the what-if analysis. The results revealed that the daily risk of *S. suis* serotype 2 illness from consuming raw pork preparations was 2.5×10^{-4}. When assuming that people always prefer raw pork consumption, the expected annual risk of *S. suis* serotype 2 was 4,987 per 100,000 persons. In addition, the risk of illness in the worst scenario, assuming all *S. suis* isolates in raw pork preparations were pathogenic, was as high as 4.3×10^{-3} (expected 46,307 cases per 100,000 persons annually).

Even, the results of sensitivity analysis suggested that longer storage time before cooking greatly impacted the risk estimate, the risk of *S. suis* serotype 2 infection only slightly changed (risk = 1.3×10^{-7}) in the scenario where pork was immediately cooked and consumed right after purchased (assumed t_{con} = 0 h).

The risk of the scenario, where the minimum cooking condition was recommended by National Institute of Health (70ºC for 10 min), was 7.3×10^{-8} or equivalent to 2.7 cases per 100,000 persons. In addition, the expected cases were reduced to 0.2 and 0.04 cases per 100,000 persons when the cooking time at 70 ºC extended to 13 min and 15 min, respectively.

CHAPTER 5

DISCUSSION AND CONCLUSION

5.1 Contamination of *S. suis* in pork production chains

This study was the first evidence to enumerate *S. suis* from non-clinical samples in Thailand using plate count method. This results revealed that among 1,084 retailed pork samples *S. suis* was isolated from 11 samples (1.0%), including the samples from Bangkok and provincial areas. There was no *S. suis* serotype 2 detected in any pork samples. Since objective of this study focused on only pathogenic strain, this study did not attempt to characterize other than serotype than serotype 2. However, several other *S. suis* serotypes have been increasingly reported as causative agents of Thai patients, including non-typable strains (Kerdsin et al., 2014b; Hatrongjit et al., 2015; Kerdsin et al., 2015; Kerdsin et al., 2016). Presence of *S. suis* other than serotype 2 in pork should also be concerned as the potential risk to consumers.

The prevalence of *S. suis* serotype 2 isolated from pork in this study was comparably lower than that from previous study in Khon Khaen province, which reported the prevalence in fresh pork (10.8%) and minced pork (2.7%) based solely on the results of *cps2-J* gene detection (Noppon et al., 2014). While this study identified *S. suis* serotype 2 by detecting *cps2-J* gene together with a serological test, namely Quellung reaction. Okura et al. (2013) has demonstrated that detection of serotype-specific *cps* genes could not distinguish between *S. suis* serotype 2 and 1/2, and between serotype 1 and 14. Thus, aside from the detection of serotype-specific capsular polysaccharide (*cps*) gene, it has been suggested that serotyping technique using specific antisera should be performed to avoid such misidentifications.

In this study, *S. suis* was isolated from pork at retail step in both traditional and modern trades with the concentration ranged from 2.9 to 5.6 log cfu/g. The situation was similar to that in Hong Kong, where *S. suis* was detected, using MPN-PCR technique without bacterial isolation, in pork from both wet markets and supermarkets (Cheung et al., 2008). Their study reported that 60.3% of retailed pork samples contains low concentration of *S. suis* (less than 2 log MPN/g), while the concentration was higher than 3 log MPN/g in only 15.4% of pork samples. From our preliminary experiment, using direct PCR-based identification without isolation similar to their technique, also yielded high prevalence of *S. suis* (57.5%) in retailed pork (data not shown).These evidences all agreed that PCR-based bacterial identification is more sensitive than culture-based techniques. However, conventional isolation of the bacteria is still important screening tool for making final diagnosis or evaluating the epidemiological situations.

This study also observed the contamination patterns of *S. suis* in pork production chains. The results indicated that *S. suis* contaminates pigs, carcasses, pork products, as well as the environment, particularly at slaughterhouses. *S. suis* was detected in all 4 participated slaughterhouses, while it was not detected in some corresponding retail markets. Among pig-to-pork samples, palatine tonsils yielded the highest prevalence (56.3%) and the highest concentration (5.58 log cfu/g) of *S. suis*. In addition, *S. suis* serotype 2 was detected only in tonsilar samples. These suggested that palatine tonsils, a natural habitat of *S. suis*, could be the source that introduces the pathogenic strain of *S. suis* into the environment and pork production chain.

Results from this study also demonstrated that *S. suis* was isolated from blood samples (4.2%), taken from pooled blood of several pigs in the same container. This might suggest that some sepsis pigs were allowed to enter the slaughtering process or the pooled blood got contaminated from the environment. Strengthening antemortem inspection practice could prevent the diseased pigs from entering the slaughter process.

S. suis was also frequently isolated from the skins of pigs (10.4%) and carcasses (28.1%).In abattoir A, C, and D, the prevalence of *S. suis* in carcasses skin increased after they were undergone the carcass handling processes, while that of abattoir B decreased. These results indicated that the prevalence of *S. suis* could either increase or decrease during dehairing, scalding, and eviscerating processes depending on the slaughterhouses, probably form cross-contamination or different hygiene managements.

Aside from the surface samples of walls, floors and equipment, we also isolated *S. suis* from 16.7% of swab samples taken from worker's hands in 3 of 4 participated abattoirs. Similar findings had been reported in Brazil, where *S. suis* was detected from 21.3% of worker's hands and several equipment in all 4 observed slaughterhouses (Soares et al., 2015). In addition, all slaughterhouse workers observed in this study did not wear proper protective equipment while working with pigs and carcasses. These findings exhibited that slaughterhouse workers were at risk of *S. suis* infection from direct exposure to their skin. Besides, they might even distribute the pathogen around the environment and cause cross-contamination to the carcasses. Improving hygiene practices in slaughterhouses could reduce the risk of *S. suis* infection among workers and even to the pork consumers.

5.2 Quantitative microbial risk assessment of *S. suis* serotype 2 form pork consumption

This study was the first approach to develop a food safety risk assessment framework for *S. suis* using stochastic models. These models considered the contamination dynamics of *S. suis* along pork production chains, farm-to-fork. Cooking preference was also taken into account, since consuming raw or undercooked pork has been the crucial risk behavior of *S. suis* infection in Thailand. Currently, there were only few published scientific literatures about *S. suis* in food safety aspect. Thus, this risk assessment models were constructed from imperfect knowledge using readily accessible information on *S. suis,* and those of the surrogate pathogens together with the results from this study. These uncertain variables could be reduced when more precise information upon *S. suis* are available.

The models in this study estimated low risk of illness from *S. suis* serotype 2 with expected annual cases at 4.6 per 100,000 persons, which is higher than those reported by Bureau of Epidemiology (BOE). BOE reported annual morbidity rates of *S. suis* infection during 2013 to 2016[10] ranged from 0.29 to 0.55 cases per 100,000 persons, while the highest morbidity rate was from Northern region (2.03 cases per 100,000 persons). It has been suggested that the actual number of *S. suis* cases were underreported. Since making definite diagnosis and identifying the serotypes of *S. suis* infections are less aware for the physicians and then less likely for further diagnosis in laboratories in some regions (Navacharoen et al., 2009). However, the difference between estimated and reported cases could also arise from the model uncertainty.

The sensitivity analysis revealed that the risk of illness was highly correlated with the daily exposure doses and microbial concentration at consumption, while it was negligibly correlated with the prevalence and initial concentration of *S. suis* in pork. This

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[10]Bureau of Epidemiology 2017. "Subject: National Disease Surveillance: *Streptococcus suis*" (online). Available: http://www.boe.moph.go.th/boedb/surdata/disease.php?ds=82. Accessed August 10, 2016

model demonstrated that the risk of *S. suis* illness in Southern was significantly lower than those of North-eastern and Northern regions. These findings indicated that proportions of cooking preferences greatly affected the exposure doses, and consequently, the risk of *S. suis* illness. It has been demonstrated that people in Northern region preferring undercooked pork, particularly in rural areas, pose low perception and knowledge about risk and prevention of *S. suis* infection (Unnankrad, 2008; Kaewmoon, 2009; Yana, 2009). Thus, we suggested that the risk management should focus on reducing the proportion of raw pork consumers among susceptible Thai population. Likewise, Takeuchi et al. (2017) advised that the food safety campaigns against *S. suis* should be continuous to maintain the intervention effectiveness.

Besides, we also evaluated the efficacy of the minimum recommended cooking conditions (70ºC for 10 min) provided in a *S. suis* controlling campaign by the National Institute of Health. The minimum recommended condition reduced the annual expected cases (2.7 cases per 100,000 persons). Additionally, the expected cases were greatly lower when extend the cooking time at 70 ºC a few more minutes (expected 0.2 and 0.04 cases per 100,000 persons for cooking time 13 min and 15 min, respectively). For this reason, we also suggested consumers to increase the cooking time in the food safety campaigns.

5.3 Limitations of this study

Sample collection in this study depended on co-operation of the government health offices and this affected sampling areas and sample size. The contamination patterns of *S. suis* in pig-to-pork and environmental samples in this study were limited to only in 4 production chains in Bangkok, where the case incidence was comparably lower than Northern and North-eastern regions. Further investigation in different areas are still needed to gain insight into the true nature of *S. suis* contamination, farm-to-market. In addition, most of the food stalls (stationary and mobile) were unregistered.

The samples from these unofficial retails were collected by convenient sampling resulted in smaller sample size comparing to other retail types.

The risk assessment model in this study had several constraints. Since the results from the experiment yielded no *S. suis* serotype 2 positive pork samples, this risk assessment model estimated the prevalence of *S. suis* serotype 2 in pork by previously published prevalence of *S. suis* serotype 2 in healthy pigs in Thailand. In addition, the cross-contamination factors were calculated from the experimental data of 4 slaughterhouses in Bangkok. More epidemiological studies on different slaughterhouses in different areas are needed.

The model used the information of closely related bacteria were used as the surrogates to describe the behavior of *S. suis* in pork and its infectivity when those of *S. suis* were not available. In addition, the growth model of *Streptococcus iniae* used in exposure assessment (Zhou et al., 2008) might not properly evaluate the nature of *S. suis* under freezing conditions.

The proportion of raw pork consumers in this model was estimated by integrating the proportion of raw food consumers in each region and the average daily pork consumption in Thai population (not classified by regions). Since the model did not consider the amount of pork consumption by regions of Thailand, the regional risk estimates from this model may not properly reflect the cultural difference in each region.

To reduce the uncertain variables and improve this risk assessment model, the following research gaps are required:

1. Mathematical growth models *S. suis* in pork production chain

2. Thermal inactivation parameters, *D*-value and *Z*-value, of *S. suis* in pork

3. A dose-response relationship of *S. suis* illness in humans

4. Proportions of pathogenic *S. suis* strains in pig, carcasses, or pork

5. Additional data on prevalence and concentration of *S. suis* and its serotype in pigs, carcasses, and pork products at farm, abattoir, and retail stages

6. Assessment of hygienic practices from more slaughterhouses to calculate the cross-contamination factors

7. Updated data on amount of pork consumption and/or proportions of raw pork consumers by regions of Thailand.

5.4 Conclusions

S. suis contaminated pigs, carcasses, pork products and environment along the pork production chains. The prevalence of *S. suis* in pig-to-pork samples was 11.4 % (56/492) and in environmental samples was 5.2% (25/480). Prevalence of S. suis in pork was 0.7% (1/144) with average concentration 4.38 log cfu/g. No *S. suis* serotype 2 was isolated from pork in this study. Workers at abattoirs are at risk of infections from direct contact to the bacteria, and might contribute the cross-contamination of *S. suis* in pig carcasses, or even pork. *S. suis* contaminated the retailed pork from both traditional and modern trades. The risk of *S. suis* illness was low with proper cooking conditions. The risk management should focus on reducing raw pork consumers, especially, in the regions that frequently consume raw pork preparations.

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APPENDIX

APPENDIX A

Formulas and recipes of media and reagents

1. Blood agar

    1.1 Blood agar containing sheep blood[1]

(1A)	Columbia blood agar base (Difco)	22.0	g
	Distilled water	475.0	ml
	Defibrinated sheep blood[2]	25.0	ml

    1.2 Blood agar containing sheep blood and streptococcus selective supplement[1]

(1A)	Columbia blood agar base (Difco)	22.0	g
	Distilled water	473.0	ml
	Defibrinated sheep blood[2]	25.0	ml
(1B)	Streptococcus selective supplement (Oxoid) SR0126[2]	2.0	ml

(1A) Columbia blood agar base (Difco)

    Approximate formula per liter

Pancreatic digest of casein	10.0	g
Proteose peptone no. 3	5.0	g
Yeast extract	5.0	g
Beef heart, infusion from 500 g	3.0	g
Corn starch	1.0	g
Sodium chloride	5.0	g
Agar	15.0	g

(1B) Streptococcus selective supplement (Oxoid) SR0126

    Vial contents (Dissolved in 2.0 ml sterilized distilled water)

Colistin sulphate	5.0	mg
Oxolinic acid	2.5	mg

2. Buffered peptone water[1]

(2A)	Buffered peptone water (Difco)	20.0	g
	Distilled water	1000.0	ml

(2A) Brain heart infusion broth (Oxoid)

Approximate formula per liter

Peptone	10.0	g
Sodium chloride	5.0	g
Disodium phosphate	3.5	g
Monopotassium phosphate	1.5	g

3. Brain heart infusion broth

3.1 Brain heart infusion broth[1]

(3A)	Brain heart infusion broth (Oxoid)	37.0	g
	Distilled water	1000.0	ml

3.2 Brain heart infusion broth with 6.5% sodium chloride[1]

(3A)	Brain heart infusion broth (Oxoid)	37.0	g
	Sodium chloride	6.0	g
	Distilled water	1000.0	ml

(3A) Brain heart infusion broth (Oxoid)

Approximate formula per liter

Brain infusion solids	12.5	g
Beef heart infusion solids	5.0	g
Proteose peptone	10.0	g
Glucose	2.0	g
Sodium chloride	5.0	g
Disodium phosphate	2.5	g

4. Starch agar[1]

(4A)	Starch agar (Himedia)	25.0	g
	Distilled water	1000.0	ml

(4A) Starch agar (Himedia)

Approximate formula per liter

Meat extract	3.0	g
Peptic digest of animal tissue	5.0	g
Soluble starch	2.0	g
Agar	15.0	g

Notes

[1] Heated to completely dissolve and then sterilized by autoclaving at 121 ºC for 15 minutes.

[2] Aseptically added to sterilized (1A) Columbia blood agar base at approximate 50 ºC.

VITA

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