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Mortality and outcomes of *Streptococcus suis* infection and economic burden in Thailand

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Abstract

Background

Streptococcus suis (S.suis) is a zoonotic bacterial pathogen mainly found in pigs which can cause serious infection in human. It is known to cause harm mainly on men of working age. The disease is endemic in Southeast Asia which can result in death and long-term complications. Meningitis, septicaemia, and infective endocarditis are most frequent clinical manifestations in which deafness and vestibular dysfunction are the most common complications among survivors. These sequelae are usually irreversible despite aggressive treatment upon occurrence of hearing loss symptoms. This may potentially result in significant health and economic burden as well as physical and emotional distress.

In Thailand, *S. suis* infection is an important health issue and one of the most common causes of bacterial meningitis with a high prevalence in the northern region due to cultural food habits involving consumption of raw pork. To date, much uncertainty remains regarding the understanding of S*. suis* clinical manifestations and treatment responses as well as gaps between different settings. The disease epidemiology in Thailand is not fully understood and epidemiological study to identify predicting factors for mortality and sequelae and the information concerning the burden of disease is still lacking. Therefore, this project was designed in order to explore insights on risk factors, clinical manifestations and outcomes of *S. suis* infection as well as to quantify the burden of disease and its economic impact on years of life lost, quality of life and work productivity in Thailand.

Methods

This dissertation consists of four main studies: (1) A systematic review and meta-analysis to identify the risk factors of *S. suis* infection, (2) A retrospective review of the 13-year data at Chiang Mai University Hospital (CMUH) during 2005-2018 to study on clinical manifestations, outcomes and potential risk factors of the disease mortality, (3) A clinical risk scoring system development to help physicians in identifying *S.suis* patients with high risk of hearing loss, and (4) A decision-analytic Markov model with life-table modelling to quantify the burden of disease and its impact on health and

productivity in Thailand. STATA version 14.2 was employed for all quantitative analyses.

Results

S. suis patients were generally healthy adult males in their middle-age before acquiring the infection. The mean age ranged between 37 to 63 years. The main risk factors associated with acquiring *S. suis* infection including eating raw/undercooked pork, pigs or raw pork exposure, male sex and pig-related occupation. The combined odd ratios for studies using community control groups and non-*S. suis* sepsis as controls respectively were 4.63 (95% CI 2.94-7.29) and 78.00 (95% CI 10.38-585.87) for raw pork consumption, 4.01 (95% CI 2.61-6.15) and 3.03 (95% CI 1.61-5.68) for exposure to pigs or pork, 11.47, (95% CI 5.68-23.14) and 3.07 (95% CI 1.81-5.18) for pig-related occupation and 3.56 (95% CI 2.18-5.80) and 5.84 (95% CI 2.76-12.36) for male sex.

Of 133 patients with culture-proven *S.suis* infection identified in the 13-year retrospective cohort study during 2005-2018, there were 92 males and 41 females. Septicaemia (55.64%) was the most common clinical manifestation followed by meningitis (37.59%) and infective endocarditis (25.56%). The overall mortality rate was 12.03% (n=16). According to the multivariate analysis, the independent risk factors for mortality were prolonged bacteremia ≥ 6 days, septic shock, and direct bilirubin > 1.5 mg/dl.

Significant predictors for *S. suis* hearing loss were meningitis, raw pork consumption, and vertigo. An easy-to-use risk score to promote early diagnosis and detection of *S.suis* infected patients who are prone to hearing loss in primary settings was developed. The predictive score ranged from 0-4 and correctly classified 81.95% patients as being at risk of *S.suis* hearing loss. The model showed good power of prediction and calibration.

According to the results from a decision-analytic Markov with life-table modelling, it was estimated that 312 people in Thailand acquired *S.suis* infection in 2019. With simulated follow-up from the age of 51 years until death or age 100 years, this cohort incurred 769 years of life lost (14% of predicted years of life lived had they not been infected), 826 quality-adjusted life years (QALYs) lost (21%) and 793 productivity-

adjusted life years (PALYs) (15%) lost. These equated to an average of 2.5 years of life, 2.6 QALYs and 2.5 PALYs lost per person. In terms of broader economic impact, the loss of PALYs was estimated to cost 346 million Thai baht (US$11.3 million) in lost gross domestic product (GDP), which equated to 1.1 million Thai baht (US$ 36,033) per person.

Conclusions

S. suis is not infrequent in Northern, Thailand, where the cultural food habit of raw pork eating is still practiced. Under diagnosis and lack of disease awareness are important issues resulting in a low number of cases reported. Early detection and prompt antibiotic therapy are essential to improve disease outcomes and complications. It is important that patients with predisposing factors should receive immediate and sufficient treatment to prevent long-term complications particularly sensorineural hearing loss (SNHL). The disease imposes significant health and economic burden on quality of life and productivity in Thai population. This highlights the importance of public health awareness programs for disease prevention and control. The findings from this Ph.D. project would be useful for clinicians in their routine practice as well as public health and policy makers regarding decisions and public health awareness programs planning for disease control and prevention.

Declaration

This thesis is an original work of my research and contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the report.

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Date: 6 March 2020…….

Publications during enrolment

Journal articles

1. **Rayanakorn A**, Goh B-H, Lee L-H, Khan TM, Saokaew S. Risk factors for *Streptococcus suis* infection: A systematic review and meta-analysis. Sci Rep. 2018;8(1):13358. doi: 10.1038/s41598-018-31598-w

2. **Rayanakorn A**, Katip W, Lee LH, Oberdorfer P. Endophthalmitis with bilateral deafness from disseminated *Streptococcus suis* infection. BMJ Case Reports. 2019;12(2). doi: 10.1136/bcr-2018-228501.

3. **Rayanakorn A**, Katip W, Goh BH, Oberdorfer P, Lee LH. Clinical Manifestations and Risk Factors of *Streptococcus suis* Mortality Among Northern Thai Population: Retrospective 13-Year Cohort Study. Infection and Drug Resistance. 2019;Volume 12:3955-65. doi: 10.2147/IDR.S233326.

4. **Rayanakorn A**, Katip W, Goh BH, Oberdorfer P, Lee LH. A risk scoring system for predicting *Streptococcus suis* hearing loss: A 13-year retrospective cohort study. PloS one. 2020;15(2):e0228488. doi: 10.1371/journal.pone.0228488.

5. **Rayanakorn A**, Ademi Z, Liew D, Lee LH. Burden of disease and productivity impact of *Streptococcus suis* infection in Thailand (Manuscript submitted for peer-review)

6. **Rayanakorn A**, Ser H-L, Pusparajah P, Chan K-G, Goh BH, Khan TM, et al. Comparative efficacy of antibiotic(s) alone or in combination of corticosteroids in adults with acute bacterial meningitis: A systematic review and network meta-analysis. PloS one. 2020;15(5):e0232947. doi: 10.1371/journal.pone.0232947.

Abstracts/Proceedings

1. **Rayanakorn A**, Hooi-Leng Ser, Priyia Pusparajah, Bey Hing Goh, Tahir Mehmood Khan, Surasak Saokaew, Learn Han Lee, A Network meta-analysis of randomized controlled trials for acute bacterial meningitis in adult population. Presented at International Conference of Pharmacoepidemiology, Mid-Year Meeting 2019, 2019 April 6-9; Rome. Abstract 13.

2. **Rayanakorn A**, Katip W, Goh BH, Oberdorfer P, Lee LH, Prediction score for *Streptococcus suis* hearing loss: a neglected disease in need of increased public health awareness. Presented at International Conference of Public Health, 2019 July 10-12; Kuala Lumpur. Abstract no. 392 (Awarded the Session's Best Presenter: Health Services Management) (https://publichealthconference.co/accepted-abstract/).

3. **Rayanakorn A**, Ademi Z, Liew D, Lee LH. PIN65 Estimating the lifetime economic burden of *Streptococcus suis* and its productivity impact in Thailand. Value in Health. 2020;23:S179. doi: https://doi.org/10.1016/j.jval.2020.04.530

Thesis including published works declaration

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

This thesis includes **three (*3)*** original papers published in peer reviewed journals and **one (*1)*** submitted publication. The core theme of the thesis is **mortality and outcomes of *Streptococcus suis* infection and its economic burden in Thailand.** The ideas, development and writing up of all the papers in the thesis were the principal responsibility of myself, the student, working within the **Jeffrey Cheah School of Medicine and Health Sciences** under the supervision of **Dr. Lee Learn Han.**

The inclusion of co-authors reflects the fact that the work came from active collaboration between researchers and acknowledges input into team-based research.

In the case of **Chapter 3-6** my contribution to the work involved the following:

Thesis Chapter	**Publication Title**	**Status** *(published, in press, accepted or returned for revision, submitted)*	**Nature and % of student contribution**	**Co-author name(s) Nature and % of Co-author's contribution***	**Co-author(s), Monash student Y/N***
3	Risk factors for *Streptococcus suis* infection: A systematic review and meta-analysis	*Published*	65% Formulating concept, collecting data, data analysis, drafting and writing of manuscript	1) Bey-Hing Goh, input into manuscript 8% 2) Learn-Han Lee, input into manuscript 8% 3) Tahir Mehmood Khan, input into manuscript 9% 4) Surasak Saokaew, input into manuscript 10%	1) N 2) N 3) N 4) N
4	Clinical manifestations and risk factors of *Streptococcus suis* mortality among northern Thai population	*Published*	70% Formulating concept, collecting data, data analysis, drafting and writing of manuscript	1) Wasan Katip, input into manuscript 10% 2) Bey-Hing Goh, input into manuscript 5% 3) Peninnah Oberdorfer, into manuscript 10% 4) Learn-Han Lee, input into manuscript 5%	1) N 2) N 3) N 4) N
5	A risk scoring system for predicting *Streptococcus suis* hearing loss: A 13-year retrospective cohort study.	*Published*	80% Formulating concept, collecting data, data analysis, drafting and writing of manuscript	1) Wasan Katip, input into manuscript 5% 2) Bey-Hing Goh, input into manuscript 5% 3) Peninnah Oberdorfer, into manuscript 5% 4) Learn-Han Lee, input into manuscript 5%	1) N 2) N 3) N 4) N

6	Burden of disease and productivity impact of *Streptococcus suis* infection in Thailand	*Submitted*	60% Formulating concept, collecting data, data analysis, drafting and writing of manuscript	1) Zanfina Ademi, input into manuscript 15% 2) Danny Liew, input into manuscript 15% 3) Learn-Han Lee, input into manuscript 10%	1) N 2) N 3) N

I have not renumbered sections of submitted or published papers in order to generate a consistent presentation within the thesis.

Student name: Ajaree Rayanakorn

Student signature: **Date:** 6 March 2020

I hereby certify that the above declaration correctly reflects the nature and extent of the student's and co-authors' contributions to this work. In instances where I am not the responsible author I have consulted with the responsible author to agree on the respective contributions of the authors.

Main Supervisor name: Dr. Lee Learn Han

Main Supervisor signature: **Date:** 7 March 2020

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List of abbreviations

AIDs	Acquired immune deficiency syndrome
ALD	Alcoholic liver disease
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
AuROC	Area under the Receiver Operating Curve
BMECs	Brain microvascular endothelial cells
BUN	Blood urea nitrogen
CFR	Case fatality rate
CI	Confidence interval
CMUH	Chiang Mai University Hospital
CNS	Central nervous system
CPR	Clinical prediction rule
CPS	Capsular polysaccharide
CRFs	Case Record Forms
CSF	Cerebrospinal fluid
DALYs	Disability-adjusted life years
DIC	Disseminated intravascular coagulation
DM	Diabetes Mellitus
EF	the extracellular factor
ES	Effect size
GCS	Glasgow coma scale
GDP	Gross domestic product
Hb	Hemoglobin
Hct	Hematocrit
HIV	Human immunodeficiency virus infection
HL	Hearing loss

HNs	Hospital numbers
IE	Infective endocarditis
MALDI-TOF	Matrix-Assisted Laser Desorption Ionization Time of Flight
MIC	Minimal Inhibitory Concentration
MUHREC	the Monash University Human Research Ethics Committee
MLST	Multilocus sequence typing
MRP	Muraminidase-released protein
NOS	the Newcastle-Ottawa Scale
OPD	Outpatient department
OR	Odds ratio
PRRS	Porcine Reproductive and Respiratory Syndrome
RCTs	Randomised controlled trials
RoB2.0	the revised tool to assess Risk of Bias in randomized trial
RT-PCR	Real-time polymerase reaction
RWE	Real-world evidence
SAPs	The simplified Acute Physiology Score
SBE	Subacute endocarditis
SD	Standard deviation
SLE	Systemic Lupus Erythematosus
SNHL	Sensorineural hearing loss
S. suis	*Streptococcus suis*
ST	Sequence type
STSS	*Streptococcus suis* toxic shock syndrome
TSS	Toxic shock syndrome
PALYs	Productivity-adjusted life years
PGS	Penicillin G
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses

QALYs	Quality-adjusted life years
VD	Vestibular dysfunction
VHD	Valvular heart disease
YLL	Years of life live

Chapter 1:

Introduction and research overview

1.1 Background and significance of the study

Streptococcus suis (S.suis) is a zoonotic disease mainly in pigs which can cause severe infection in human. The disease usually affects adults and elderly mainly males at their middle-age *(1)*. In Thailand, *S. suis* infection is an important health issue and one of the most common cause of adult bacterial meningitis *(2)* with a high prevalence in the northern region due to cultural food habits involving consumption of raw pork. According to the country Bureau of Epidemiology, Department of Disease Control, there were 338 and 374 *S.suis* cases reported with 29 and 31 deaths in 2018 and 2019 (from 1 January to 11 December 2019) respectively with majority of cases from northern Thailand (71%) *(3, 4)*. Lack of disease awareness, and misdiagnosis are major causes of delayed treatment and long-term complications. To date, much uncertainty remains regarding the understanding of *S. suis* clinical manifestations and treatment responses as well as gaps between different geographical settings *(5)*. The disease epidemiology in Thailand is not fully understood and epidemiological study to identify predicting factors for mortality and sequelae is limited. The information concerning the burden of disease is still lacking and no previous research has estimated the health and economic impact due to *S.suis* infection on work and productivity in Thailand.

To address all these major issues and unmet needs, this Ph.D thesis aimed to provide increased understanding about *S.suis* infection, clinical manifestations and outcomes and quantify the burden of disease and its economic impact in Thailand. A systematic review and meta-analysis was performed to identify potential risk factors associated with *S.suis* infection. Following that a retrospective cohort study to study on clinical manifestations and outcomes of *S. suis* infection among patients in northern Thailand would be carried out in order to determine potential risk factors associated with the disease mortality and its sequelae particularly hearing loss. Additionally, a decision-analytic Markov model with life-table modelling would be conducted to quantify *S.suis* burden of disease and its impact on health and productivity in Thailand. The research project has been approved by the Monash University Human Research Ethics Committee (MUHREC; Project Number 12225) and Research Ethics Committee, Faculty of Medicine, Chiang Mai University, Thailand (Research ID 5141).

The findings from this study would provide important insights about the disease epidemiology, clinical manifestations, and economic burden which would be useful for improved treatment, early detection, and informing decisions for disease control and prevention which have yet to be available.

1.2 General and study specific objectives

The ultimate goal of the project is to explore insights on risk factors for mortality/morbidity and outcomes of *S.suis* infection as well as its economic impact on health and productivity in Thailand.

This study has four specific objectives as follows:

1) To identify potential risk factors associated with *S.suis* infection.
2) To provide insights on clinical manifestations and predicting factors for *S.suis* mortality.
3) To develop a risk scoring system to promote early detection of *S.suis* hearing loss.
4) To quantify the burden of disease and economic impact of *S.suis* infection in Thailand in terms of health and productivity.

Based on the study objectives, the research questions that this study will elucidate are as follows:

1) What are potential risk factors in acquiring *S.suis* infection?
2) What are predictive factors that affect *S.suis* mortality and the main features/clinical manifestations of the disease?
3) What are predictors of *S.suis* hearing loss?
4) What are the burden of *S.suis* infection and its economic impact in Thailand?

1.3 Research overview

The study would be carried out in three phases as follows:

(1) A systematic review and meta-analysis to identify the potential factors associated with *S.suis* infection as well as provide an update on evidences regarding clinical presentations and outcomes of *S.suis* infection,

(2) A retrospective review of the 13-year data from May 2005 to December 2018 at Chiang Mai University Hospital (CMUH) in which there are 2 components as follows:

2.1 A 13-year retrospective cohort study to explore risk factors associated with *S.suis* infection.

2.2 A risk scoring system development to promote early diagnosis and detection of *S.suis* infected patients who potentially develop hearing loss in primary setting.

Some findings derived from the second phase including epidemiological data and acute treatment cost would be utilised for input parameters in the final study, a decision-analytic Markov with life-table modelling.

(3) A decision-analytic Markov model to quantify the burden of disease and its economic impact in Thailand in terms of years of life, quality-adjusted life years (QALYs), and productivity-adjusted life years (PALYs) lost which is a novel measure that adjusts years of life lived for productivity loss attributable to disease.

The framework describing each phase is illustrated in **Figure 1.**

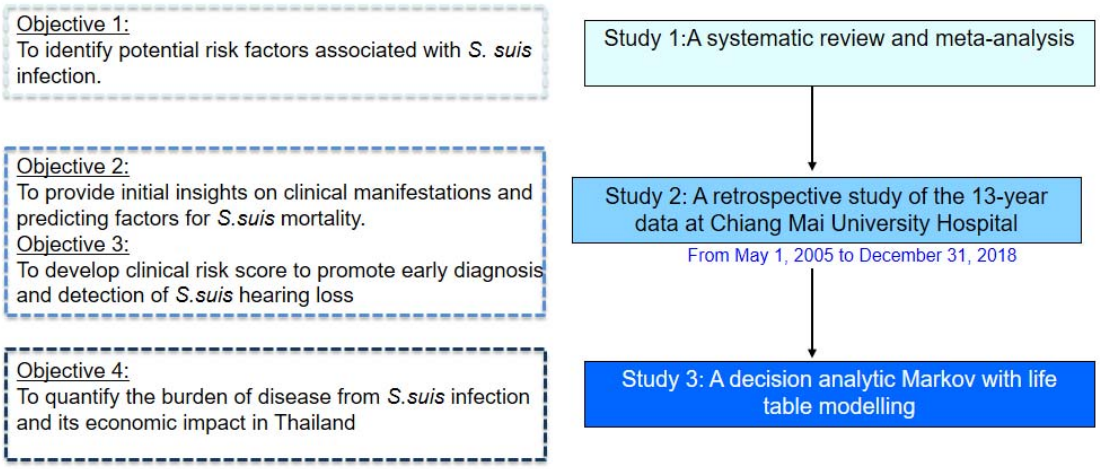


Figure 1 Overview of research project

Figure 1. provides an overview of the research project and study specific objectives. The investigations and research findings would be presented in the following Chapters with brief descriptions below.

I. In **Chapter 2**, provides a literature review with background information on *Streptococcus suis* infection including disease epidemiology, clinical manifestations and the burden of disease.

II. In **Chapter 3**, a systematic review and meta-analysis was performed to identify potential risk factors in acquiring *S.suis* infection in human and provide an update on evidences regarding clinical presentations and outcomes of the disease.

III. In **Chapter 4**, a 13-year retrospective cohort study during 2005-2018 was carried out to study on clinical manifestations and risk factors of *S.suis* mortality.

IV. In **Chapter 5**, a clinical risk scoring system to identify high risk *S.suis* hearing loss patients was developed. Procedures involving model development and internal validation were also explained.

V. In **Chapter 6**, a decision-analytic Markov with life-table modelling to examine the burden of disease and productivity impact of *S.suis* infection in Thailand was conducted in which some input parameters in the model were derived from the findings in Chapter 4.

VI. Last **Chapter 7**, summarises key findings of the research project, their implications and recommendations for future research.

Chapter 2:

Literature review

2.1 *Streptococcus suis* characteristics and epidemiology

Streptococcus suis (*S. suis*) is an encapsulated gram-positive cocci alpha-hemolytic bacterium mainly in pigs which can cause serious infections in human including meningitis, septicaemia, infective endocarditis (IE) and many others *(6-8)*. The bacteria is often found in pairs or sometimes in short chains *(9)* and can be classified into 29 serotypes based on a serological reaction against its capsular polysaccharides (CPS) *(10, 11)*. Serotype 2 strains are the most prominent causing infection in human *(2)*. Most *S.suis* strains form gray-whitish, alpha haemolytic colonies in bovine or sheep blood agar after 24 hours incubation at 37 ^{o}C *(9)*. These growth characteristics are similar with other *Streptococcus* or *Streptococcus-like* species which can make it overlooked and mixed with other bacterial species leading to misidentification *(12, 13)*.

S. suis was considered to be “rare” and occupational hazard until the disease outbreak in Sichuan, China involving 215 patients with 38 deaths *(14)*. Since then, the number of *S. suis* cases reported has notably increased to be over 700 cases in 2009 *(15)* and reached over 1,600 cases reported worldwide from the recent update *(5)*. The highest prevalence was in Southeast Asia region where there is a high rate of swine consumption *(6)*. According to global cumulative incidence from 1968 since the first human case reported *(16)* up to 2012, more than one third of *S.suis* infection were from the Southeast Asia region (36%) *(5)* (Figure 2.1). Majority of increased cases are originated from Thailand and Vietnam, making both countries the highest disease prevalence stratum globally *(5)*. Thailand is the country with the highest cumulative prevalence (8.21 cases/million population *(5)* and the second highest number of cases reported according to a recent review, accounting for 11% of all cases reported globally *(17)*. However, these numbers are likely underestimated as majority of cases are largely misdiagnosed and not reported *(9)*.

S.suis infection has been predominantly found among adults and elderly particularly middle-age males *(1)*. Cases are usually healthy adults before acquiring the infection whereas paediatric infection is rare *(1)*. The absence of the disease in children might be due to the lack of exposure to pigs and other relevant risk factors associated with infection among this population *(1)*.

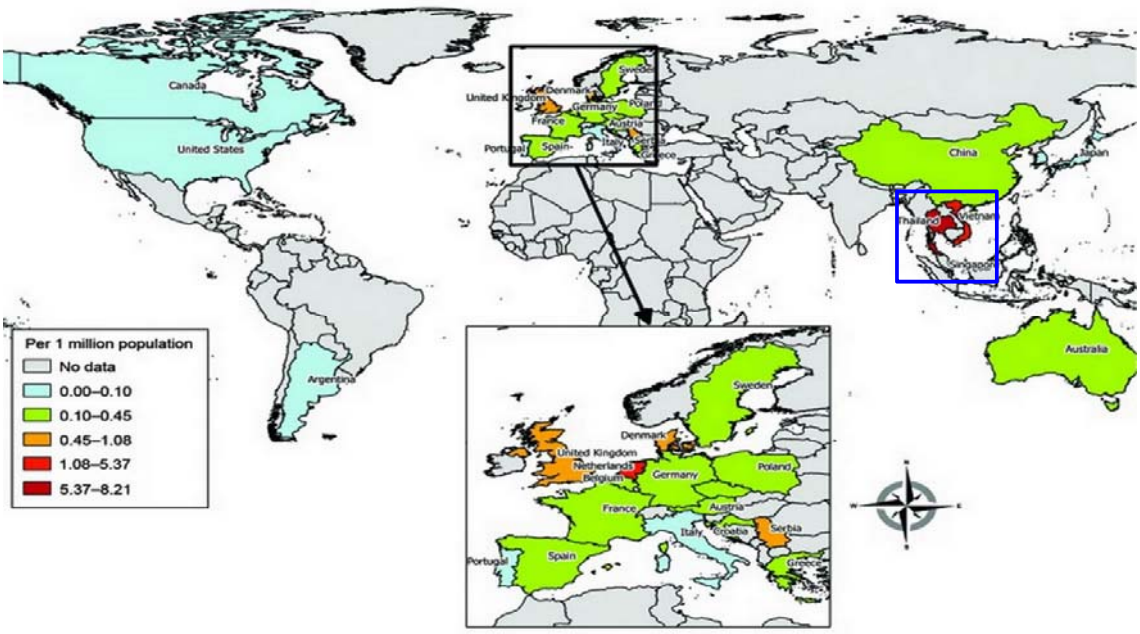


Figure 2.1 Global cumulative prevalence of *Streptococcus suis* infection up to 2012 (Adapted from *(5)*).

2.2 *Streptococcus suis* virulence mechanism and pathogenesis

The classification of *S.suis* strains is based on antigenic composition of polysaccharide capsule by serological typing while the pathogen genetic diversity can be classified by sequence type (ST) based on multilocus sequence typing (MLST) *(18)*. Initially, *S. suis* strains were classified into 35 serotypes *(18)*. However, serotypes 32 and 34 were later identified to be from the *Streptococcus orisratti* species *(19)* and a recent investigation identified a marked discrepancy of the molecular genetic profiles between serotypes 20, 22, 26 and other *S.suis* strains *(20)*. Thus, *S.suis* strains can be classified into 29 serotypes *(18)*. Serotype 2 is the most prominent accounting for around 75% of human infections and is considered to be the most virulent *(18)*.

Many virulence factors of *S.suis* infection have been identified. However, the understanding of the disease pathogenesis remains limited. The most important virulence factors have been widely studied include the capsular polysaccharide (CPS), the muraminidase-released protein (MRP), the extracellular factor (EF), and suilysin or the haemolysin *(21, 22)*.

The capsular polysaccharide (CPS) has been widely recognised as a virulence factor in protecting the bacteria against immune system. The adherence ability of bacterial capsule to escape the immune mechanism and dissemination within the host or the "modified Trojan horse" hypothesis emphasises the important role of *S.suis* capsule in pathogenesis *(22)*. However, the CPS alone may not be sufficient causing infection. *In vivo* and *in vitro* experiments suggested correlation between the absence of CPS with increased phagocytosis or bacterial killing via phagocytic cells with spontaneous clearance from blood circulation *(11, 22)*. This indicates that resistance to bacterial clearance is multifactorial and does not solely depend on CPS production *(11, 23)*.

Expression of the muraminidase-released protein (MRP), the extracellular factor (EF) protein was found in most serotype 2 *S.suis* strain isolates belonging to ST1 from earlier studies *(24, 25)*. MRP and EF were originally reported to be associated with *S.suis* serotype 2 strains virulence *(26)*. Strains associated with the disease virulence in the 2005 China outbreak were also found to produce MRP, EF and suilysin *(14)*. A recent study revealed that MRP was probably involved in bacterial survival in the host's blood circulation through human fibrinogen-MRP interaction *(11, 27)*. However, this is not the case for serotype 2 from other ST background *(28)*.

Suilysin or the haemolysin is *S.suis* produced toxin with cytotoxic properties *(29)*. The toxin can trigger transmembrane pores forming and lysis of epithelial membrane to enable bacterial dissemination *(29)*. However, suilysin may not be the only responsible factor of *S.suis* dissemination via epithelial barrier *(30)*. A few infection models studies demonstrated that suilysin was not needed for *S.suis* virulence *(30, 31)*.

S.suis is a widely heterogonous species which complicates in characterisation of potential virulence factors *(11)*. Discrepancies in characteristics were noted across strains and settings. More studies to characterise *S.suis* serotype 2 or full-genome sequencing would be helpful to elucidate virulence discrepancies observed within and between different strains *(11)*. A better understanding of *S.suis* virulence factors would be instrumental for future vaccine development against *S.suis* infection and valuable added information of this sophisticated zoonotic pathogen.

2.3 Transmission of *Streptococcus suis* infection

Pigs are usual reservoir of the disease and the main route of entry is through intranasal infection at the upper respiratory tract whereas human can be infected via pig exposure through skin abrasion or ingestion of contaminated pork products *(9, 11)*. After breaching into the human's subcutaneous mucosa, the pathogen can disseminate into the bloodstream and invade different organs in the body causing localised infection particularly arthritis and systematic infections including septicaemia and endocarditis *(11)*. Through the acidic property of its polysaccharide capsule *(19)*, it can also penetrate through the blood-brain barrier entering the central nervous system (CNS) and lead to severe meningitis resulting in irreversible complications *(11, 18)* (Figure 2.2). This cytotoxic ability of *S.suis* is believed to be the action of suilysin, the pathogen's virulence factor to brain microvascular endothelial cells (BMECs) which enables the bacteria to cross the blood-cerebrospinal fluid barrier *(18)*.

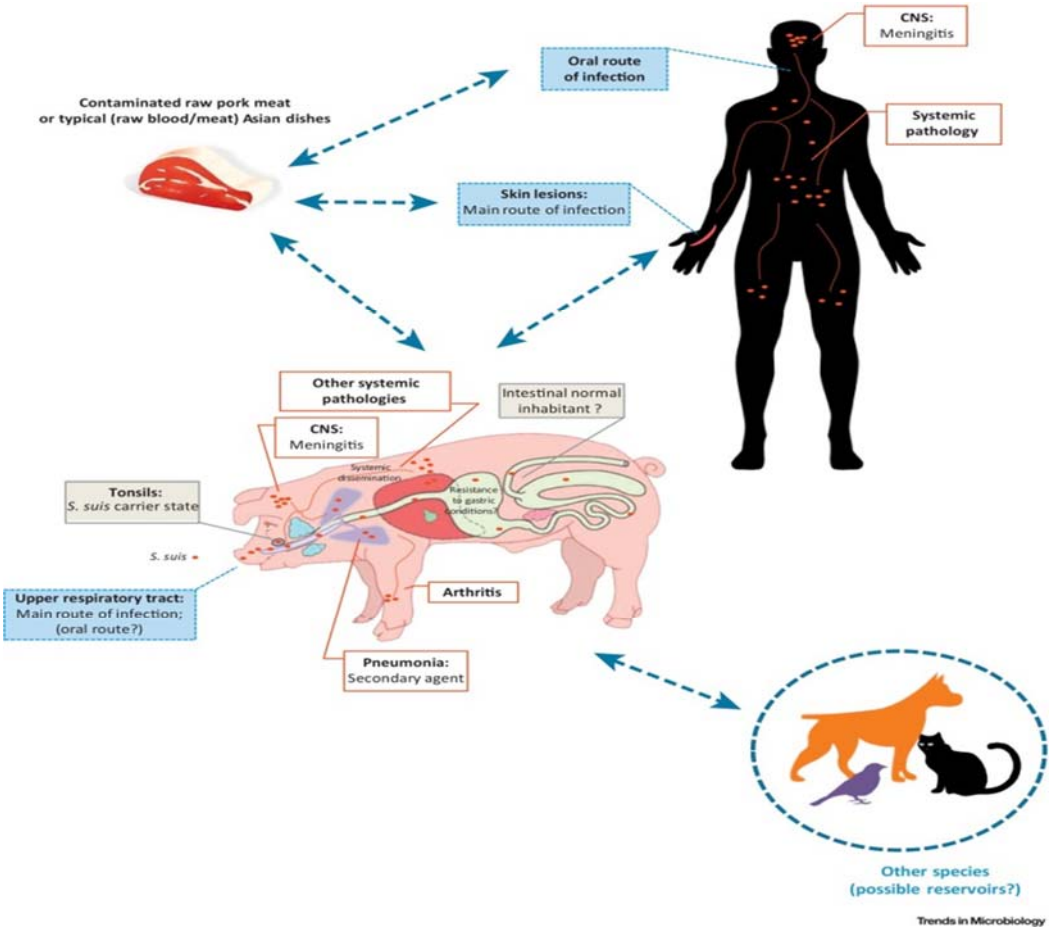


Figure 2.2 Pathogenesis of *S. suis* induced disease *(11)*.

2.4 *Streptococcus suis* clinical manifestations

S.suis is an emerging zoonosis and a leading cause of adult bacterial meningitis in many developing countries. Meningitis was the most frequent clinical manifestation, followed by sepsis (25%), arthritis (12.9%) and infective endocarditis (IE) (12.4%) whereas endophthalmitis (4.6%) and spondylodiscites (3.7%) were considered to be rare *(5)*. About two thirds of *S.suis* infected patients would develop meningitis syndrome (68%) in which deafness and vestibular dysfunction were the most common complications found among survivors and usually permanent *(5)*. A relative high number of IE was found in case series in Thailand in which aortic involvement was the most common vegetation site found *(32, 33)*. The disease predominantly affects middle-age men *(1, 5)* who are the working population. In Thailand *S.suis* meningitis is the second most common cause of adult *streptococcal* bacterial meningitis *(34)*. Lack of disease awareness and limited diagnostic capacities can lead to *S.suis* cases underestimation and misidentification *(5, 9)*. Misdiagnosis is common and the infections were often reported as *viridans streptococci* in primary cultures *(5)*. In Thailand, it was found that up to 70% of viridian streptococcal bacterial infections detected were later discovered to be *S.suis* infections in subsequent visits *(35)*. This led to inadequate and delayed treatment resulting in poor disease outcomes and long-term complications.

According to previous systematic review and meta-analysis, *S.suis* case fatality rate (CFR) was 12.8% (95%CI 9.0-18.0%) *(5)*. Although the mortality rate among *S.suis* meningitis cases (0-33.3%) *(2, 14, 33, 36-55)* is considerably lower than those caused by other agents *(56, 57)*, the rates of neurological and other sequelae found among *S.suis* meningitis survivors seem to be higher than other bacterial meningitis according to a previous meta-analysis *(58)*. Hearing loss was the most common sequelae found (33.9%), followed by multiple impairments (19.7%) in bacterial meningitis with majority of cases concentrated in the Africa and Southeast Asia regions *(58)*.

2.5 Risk factors associated with *S.suis* infection

While pig-related occupation is a main risk factor for human *S.suis* infection, pig exposure is not present in all cases of *S.suis* infection *(6)*. In Western countries, *S. suis* infection normally occurs among certain risk population particularly farmers and abattoir works involving meat processing *(34, 36)* whereas there were less than 50%

of occupational exposure cases documented in Asian countries *(37, 38)*. A lower proportion rate of occupational exposure to pigs were found in Thailand and Vietnam among *S.suis* infected patients *(5)*. Local dishes involving raw pork, pig's blood and internal organs including intestines and stomach could be potential sources of infection in Thailand and Vietnam *(48, 59, 60)*. This reflects that the risk of infection may be among general population *(61)* and other risk factors such as raw or partially cooked pork consumption habit may play an important part of infection in Asia *(5)*. In Northern, Thailand, the eating practice of raw pork dish flecked with herb and chili called *"Larb Dib"* is common. This typical local dish is usually consumed together with alcoholic beverage during social events *(32)*.

In the Sichuan outbreak, *S.suis* cases were found to be associated with backyard slaughtering of pigs *(14)*. Exposure to and consumption of infected animals were the main causes of the infection *(62)*. There was no infection from human to human identified and none of the healthcare workers involved in the outbreak were clinically infected *(63)*.

2.6 The burden of disease and economic impact

Streptococcus suis infection has been recognised as the cause of substantial loss in swine industry *(64)*. In human, the only previous study on burden of disease and economic impact of *S.suis* infection in Vietnam estimated *S.suis* burden of disease in term of disability-adjusted life years (DALYs) *(65)*. The DALYs estimated ranges from 1,437-1,866 from 2011-2014 and the mean direct cost per episode was US$1635 (95%CI 1352-1923) reflecting a large economic impact *(65)*. However, there was no estimation on long-term treatment in the model including follow up of audiological assessment and major clinical presentations including infective endocarditis (IE) and cardiac complication after IE (Figure 2.3). This indicates that an updated study with a robust disease model to allow more complex disease consequences may be needed to address the disease long-term complications.

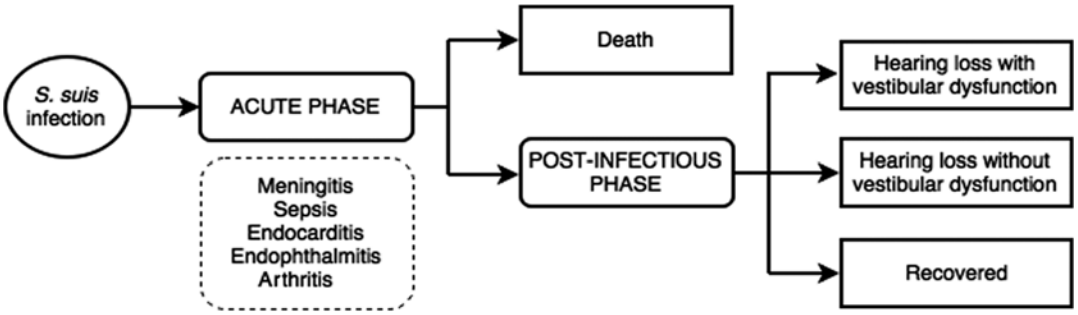


Figure 2.3 Outcome tree for *S. suis* infection in humans summarising different states from acute phase to clinical outcomes of the disease of *S.suis* economic impact study in Vietnam *(65)*.

2.7 *Streptococcus suis* infection treatment in human

Similar to other bacterial infections, treatment for *Streptococcus suis* infection is mainly based on antibiotic treatment. *S. suis* is generally susceptible to beta-lactam antibiotics (Penicillin G, cefotaxime, ceftriaxone, ceftiofur), vancomycin and chloramphenicol *(66)* and resistant to tetracyclines and erythromycin *(38)*. Empirical therapy with ceftriaxone (2 gram every 12 hours for 14 days) with or without vancomycin is generally recommended in *S.suis* meningitis treatment *(15)*. Successful treatment with intravenous penicillin G (24 million units per day for at least 10 days) has been observed *(67)* whereas experience with other therapies is limited *(15)*. Relapse in *S.suis* meningitis after 2 weeks treatment with penicillin or ceftriaxone with response to prolonged treatment up to 6 weeks was reported in some patients *(8)*. Longer treatment period up to 4 weeks with penicillin and gentamicin would be needed in patients with infective endocarditis *(68)*. Effective acute treatment is crucial for disease outcomes.

The benefit of adjunctive corticosteroids in bacterial meningitis in reducing the risk of mortality and sequelae is still controversial *(69)*. In a randomised, double-blind, placebo controlled trial in adult bacterial meningitis in Vietnam of which *S.suis* was the causative pathogen in more than half of patients included, adjunctive dexamethasone (15 minutes before antibiotic administration) was found to be associated with a lower risk of mortality, and disability including hearing loss at 6 months *(38)*. However, the effect of corticosteroids in hearing loss reduction could not be demonstrated in two other studies *(2, 48)*.

Chapter 3:

Systematic review and meta-analysis on risk factors of human *Streptococcus suis* infection

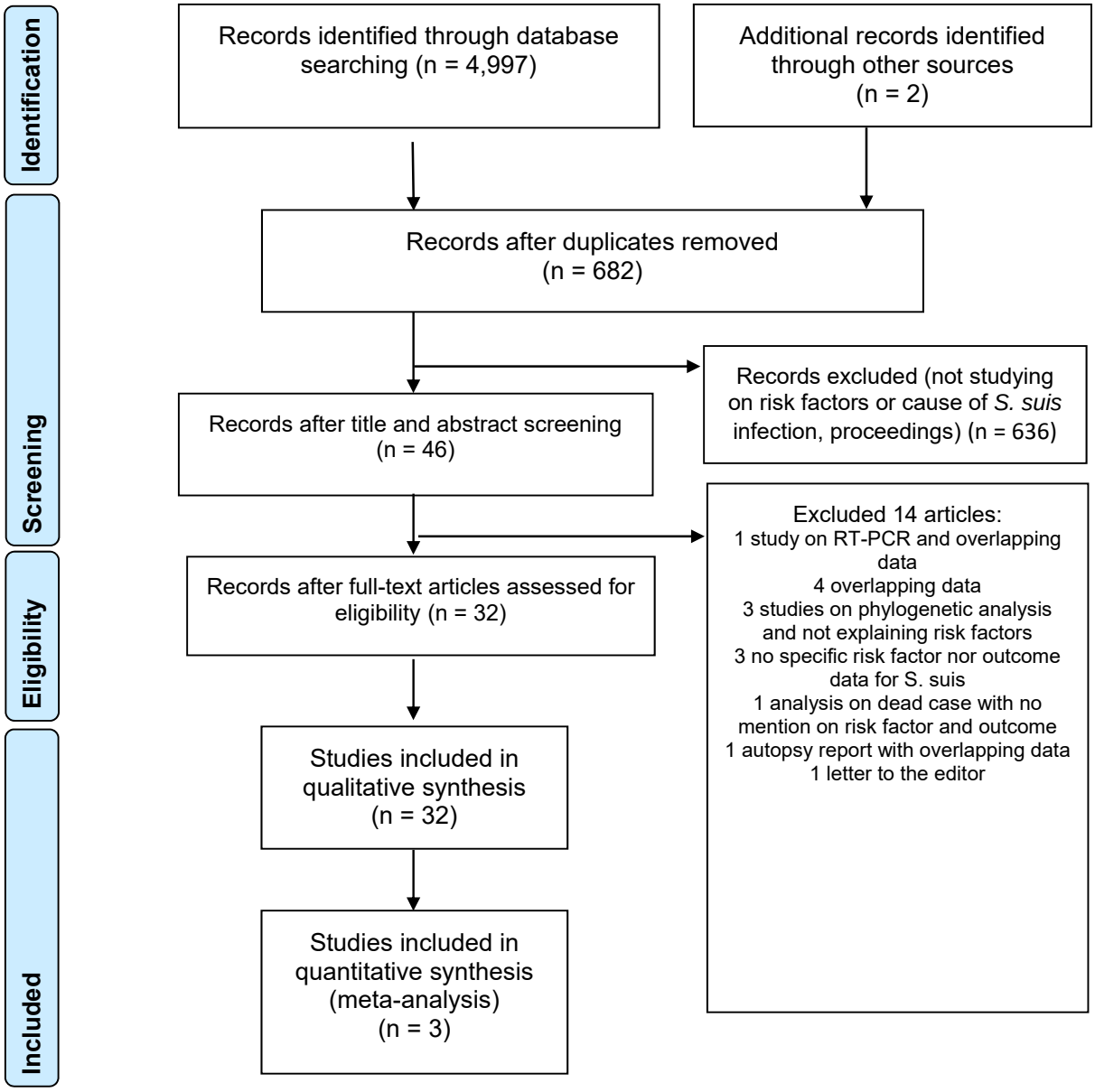
Despite growing evidences and research in *Streptococcus suis (S.suis)* infection, there has been no sign of reduction in the disease incidence especially in Asian countries *(34)*. Human behaviours and unknown or different sources of disease transmission have complicated the issue and development of disease control and prevention. In this Chapter, a systematic review and meta-analysis was conducted to identify the risk factors in acquiring *Streptococcus suis (S.suis)* infection and update evidences on treatment and outcomes of the disease. Part of this chapter has been published in a peer-reviewed journal according to below citation:

Rayanakorn A, Goh B-H, Lee L-H, Khan TM, Saokaew S. Risk factors for *Streptococcus suis* infection: A systematic review and meta-analysis. Sci Rep. 2018;8(1):13358. doi: 10.1038/s41598-018-31598-w

3.1 Summary of Chapter 3

A systematic review and meta-analysis was conducted to investigate on *S. suis* infection risk factors in humans. A total of 4,999 articles were identified in the initial searches from eight databases (n=4,997) and other sources (n=2). There were 682 records remaining after removing duplicates in which 636 citations that were proceedings or did not contain risk factors were excluded upon title and abstract screening. There were 32 articles included in systematic review *(2, 14, 33, 37-39, 41-46, 48, 49, 51-55, 59, 70-81)* and 3 case-control studies *(38, 43, 82)* in the meta-analysis after full texts evaluation. The PRISMA flow chart describing the study selection process was shown in Figure 3.1.

Figure 3.1 PRISMA flow chart of study selection process



The main risk factors associated with acquiring *S. suis* infection including eating raw/undercooked pork, pigs or raw pork exposure, male sex and pig-related occupation *(1)*. Meningitis was the most common clinical manifestation, and deafness was the most common sequelae found among survivors followed by vestibular dysfunction. Infective endocarditis was also noted as among the most common clinical presentations associated with a high mortality rate in a few studies. Meta-analyses categorized by type of control groups (community control, and non-*S. suis* sepsis) were done among 850 participants in 3 studies. The combined odd ratios for studies using community control groups and non-*S. Suis* sepsis as controls respectively were 4.63 (95% CI 2.94-7.29) and 78.00 (95% CI 10.38-585.87) for raw pork consumption, 4.01 (95% CI 2.61-6.15) and 3.03 (95% CI 1.61-5.68) for exposure to pigs or pork, 11.47, (95% CI 5.68-23.14) and 3.07 (95% CI 1.81-5.18) for pig-related occupation and 3.56 (95% CI 2.18-5.80) and 5.84 (95% CI 2.76-12.36) for male sex. The results were found to be significantly associated with *S. suis* infection and there was non-significant heterogeneity. History of skin injury and underlying diseases were noted only a small percentage in most studies.

The study emphasises the importance of prompt and appropriate treatment in patients with suggestive *S.suis* clinical symptoms and predisposing factors as well as public health interventions to enhance understanding about the disease.

A brief description of the study methodology according to the published article *(1)* can be summarised as the following.

3.2 Methods

The study reporting methodology was done according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) statement *(83)*. The study protocol has been registered in PROSPERO under protocol number CRD42018083596 (Appendix 1).

Search strategy and study selection

A number of relevant electronic databases were systematically searched including CINAHL plus, Cochrane, EMBASE, Global Health, Grey literature, Ovid Medline, PubMed, and Science Direct. The MeSH terms used were “Streptococcus suis” OR

“Streptococcus suis AND “infection” limited in human with no time nor language restriction.

The primary outcomes were risk factors associated with *Streptococcus suis* infection. The secondary outcomes were clinical presentations and outcomes of the disease. Articles were included if there were risk factors with or without clinical characteristics or outcomes of *Streptococcus suis* infection in human in which the cause of the disease was explained and at least 4 patients were described. Review, systematic review and meta-analysis articles as well as publications reporting overlapping data with the included articles were excluded. The references cited in the identified articles were also reviewed and judged to be included in case they deemed relevant. The final searched was done on September 18, 2017.

Data Extraction

The inclusion criteria were confirmed by two reviewers. The data was searched, screened and extracted by one reviewer and confirmed by another reviewer. The consultation process was employed in case of doubts or disagreements to reach a consensus between reviewers and all authors.

For articles containing ambiguous data, two email attempts to the corresponding authors were carried out for clarification. The studies were excluded for analyses if there was no response received. In case the study had primary data published elsewhere, the previous publications were also checked and verified. Alternatively, an attempt to obtain clarification from the first or corresponding author were made.

Risk of bias assessment

The quality of nonrandomized studies included were assessed according to the Newcastle-Ottawa Scale (NOS) *(84)*. The randomized controlled study *(38, 85)* was assessed using a revised tool to assess risk of bias in randomized trial (RoB 2.0) which was based on the Cochrane Collaboration Approach *(86)*. Information including predisposing factors, patient demographics, clinical manifestations, treatment and outcomes were extracted.

Meta-analysis

Meta-analysis for risk factors was carried out for case-control studies using STATA 14.2 (College Station, Texas, USA). The analyses by type of control groups (community control, and non-S. suis sepsis) were done as this variation could potentially be the source of clinical heterogeneity. Results for the association between risk behaviours and *streptococcus suis* infection were pooled using random-effects model in order to account for heterogeneity *(87, 88)*.

Forest plots were used to display the effect sizes (ES) from each study with their relevant 95% confidence intervals' and overall estimated ES. Heterogeneity was tested using I2 and Q statistics *(87, 88)*.

Four main predisposing factors were defined in analyses: (1) Exposure to pigs or pork, defined as history or recalled of exposure with pigs or pork before illness without slaughtering, (2) Pig-related occupation includes farmer, butcher, abattoir worker, seller of raw pork, (3) Consumption of raw pork, defined as consumption of raw or partially cooked pork including swine materials, and (4) Male sex. In case the number of the defined category was not provided, the relevant number which could be assumed to be similar or in closest category would be utilised. Community controls were selected as control group in order to derive the same population who actually would have been cases if the outcome was present except in the retrospective case-control study which was designed to have only hospital control group *(78)*.

3.3 Publication associated with Chapter 3 (Scientific Reports, IF: 4.525)

Rayanakorn A, Goh B-H, Lee L-H, Khan TM, Saokaew S. Risk factors for *Streptococcus suis* infection: A systematic review and meta-analysis. Sci Rep. 2018 2018/09/06;8(1):13358. doi: 10.1038/s41598-018-31598-w

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Risk factors for *Streptococcus suis* infection: A systematic review and meta-analysis

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Streptococcus suis **(*S. suis*) is a gram-positive bacterial pathogen in pigs which can cause serious infections in human including meningitis, and septicaemia resulting in serious complications. There were discrepancies between different data and little is known concerning associated risk factors of *S. suis*. A systematic review and meta-analysis was conducted to investigate on S. *suis* infection risk factors in human. We searched eight relevant databases using the MeSH terms "Streptococcus suis" OR "Streptococcus suis AND infection" limited in human with no time nor language restriction. Out of 4,999 articles identified, 32 and 3 studies were included for systematic review and meta-analysis respectively with a total of 1,454 *Streptococcus suis* cases reported. *S. suis* patients were generally adult males and the elderly. The mean age ranged between 37 to 63 years. Meningitis was the most common clinical manifestation, and deafness was the most common sequelae found among survivors followed by vestibular dysfunction. Infective endocarditis was also noted as among the most common clinical presentations associated with high mortality rate in a few studies. Meta-analyses categorized by type of control groups (community control, and non-*S. suis* sepsis) were done among 850 participants in 3 studies. The combined odd ratios for studies using community control groups and non-*S. Suis* sepsis as controls respectively were 4.63 (95% CI 2.94–7.29) and 78.00 (95% CI 10.38–585.87) for raw pork consumption, 4.01 (95% CI 2.61–6.15) and 3.03 (95% CI 1.61–5.68) for exposure to pigs or pork, 11.47, (95% CI 5.68–23.14) and 3.07 (95% CI 1.81–5.18) for pig-related occupation and 3.56 (95% CI 2.18–5.80) and 5.84 (95% CI 2.76–12.36) for male sex. The results were found to be significantly associated with *S. suis* infection and there was non-significant heterogeneity. History of skin injury and underlying diseases were noted only a small percentage in most studies. Setting up an effective screening protocol and public health interventions would be effective to enhance understanding about the disease.**

Streptococcus suis (*S. suis*) is a gram-positive bacterial pathogen in pigs which can cause serious infections in human including meningitis, septicaemia, and others[1–3]. The number of *S. suis* cases has notably increased during the past few years with the highest prevalence rate in Southeast Asia region where there is a high rate of swine consumption[1]. Majority of increased cases are originated from Thailand and Vietnam, making both countries the highest disease prevalence stratum globally[4].

About two thirds of *S. Suis* infected patients developing meningitis syndrome in which deafness and vestibular dysfunction were the most common complications found among survivors[4]. Although the case fatality rate among *S. suis* meningitis cases is lower than those caused by other agents[5,6], the rates of neurological and other sequelae found among *S. suis* meningitis survivors seem to be higher than other bacterial meningitis according to a recent meta-analysis[7]. Hearing loss was the most common sequelae found (33.9%), followed by multiple impairments (19.7%) in bacterial meningitis with majority of cases concentrated in the Africa and Southeast Asian regions[7].

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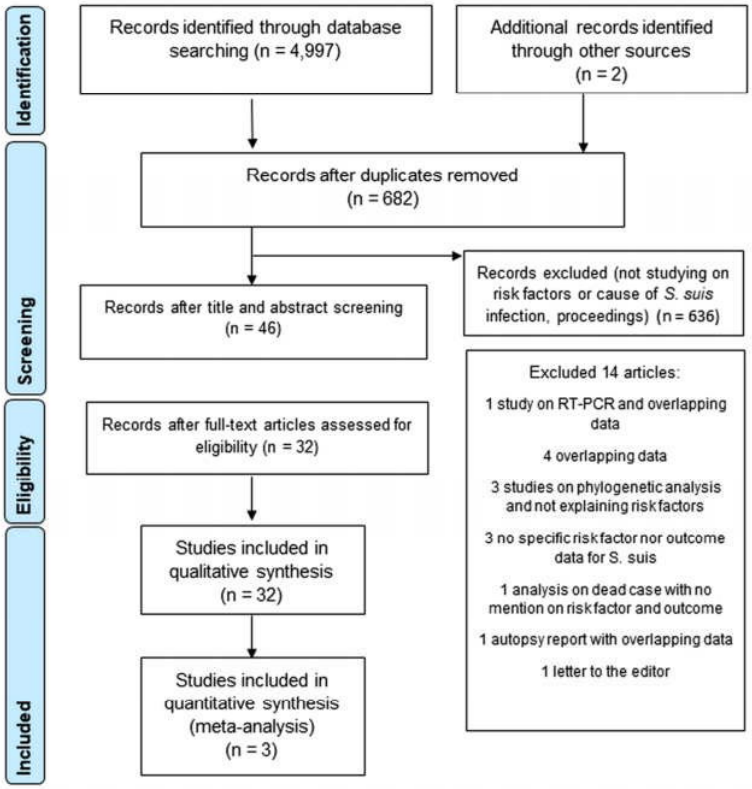

Figure 1. PRISMA flow chart of study selection process.

While pig-related occupation is a main risk factor for human *S. suis* infection, pig exposure is not present in all cases of *S. suis* infection[1]. In Western countries, *S. suis* infection normally occurs among certain risk population particularly farmers and abattoir works involving meat processing[8,9] whereas there were less than 50% of occupational exposure cases documented in Asian countries[10,11]. A lower proportion rates of occupational exposure to pigs were found in Thailand and Vietnam among *S. suis* infected patients[4]. This reflects that the risk of infection may be among general population[12] and other risk factors such as raw or partially cooked pork consumption habit may play an important part of infection in Asia[4].

Up to date, there have been no systematic reviews that comprehensively investigate on *Streptococcus suis* infection risk factors in human. This systematic review and meta-analysis aims to identify potential risk factors associated with *S. suis* infection as well as provides an update on evidences regarding clinical presentations and outcomes of the disease.

Results

Study selection. A total of 4,999 articles were identified in the initial searches from eight databases (n = 4,997) and other sources (n = 2). There were 682 records remaining after removing duplicates in which 636 citations that were proceedings or did not contain risk factors were excluded upon title and abstract screening. There were 32 articles included in systematic review[5,9–11,13–40] and 3 case-control studies[19,37,38] in the meta-analysis after full texts evaluation. The PRISMA flow chart describing the study selection process was shown in Fig. 1.

Study characteristics. The key study characteristics were shown in supplementary appendix Table S1. Included studies with different study designs were conducted in 9 distinctive countries. Among these, there were 1 randomized double-blind, placebo-controlled trial[10], 3 case-control studies in which there were 1 matched case-control[37], 1 retrospective case-control[38] and 1 prospective case-control studies[19], 28 descriptive studies including 3 public health surveillance studies[27,40,41], 2 outbreak investigations[36,39] and 1 epidemiological analysis in China[23], a population-based study on a food safety campaign[30] and a retrospective cohort identifying risk factor for *S. suis* mortality[34], 3 retrospective reviews[16,20,21] and 17 case reports or case series[9,11,13–15,17,18,22,24–26,28,29,31–33,35]. There were 27 articles in English and 5 in other languages; 3 in Chinese[23,24,37], and 1 each in Croatian[17] and Thai[40].

Majorities of studies were from Asia mainly Thailand, China, Hong Kong and Vietnam. Fourteen studies were from Thailand[16,18,20,21,26–31,33,34,39,40], four each were from China[23,24,36,37], Hong Kong[11,15,22,25] and Vietnam[10,19,35,38], two studies were from the Netherlands[9,13], and one study each from Japan[14], Serbia[17], the UK[32] and Togo[41]. Two out of the four articles from China were from epidemiological investigation in Sichuan outbreak in 2005[36,37].

Study	SELECTION				COMPARABILITY		EXPOSURE	
	1. Is the case definition adequate?	2. Representative of the cases	3. Selection of controls	4. Definition of Controls	1. Comparability of Cases and Controls on the Basis of the Design or Analysis	1. Ascertainment of Exposure	2. Same method of ascertainment for cases and controls	3. Non-Response Rate
Yu *et al.*[37]			+				+	
Ho *et al.*[19]	+		+	++	+	+	+	
Huong *et al.*[38]	+	+		+		+	+	

Table 1. Results of critical appraisal of included case-control studies based on the Newcastle-Ottawa Scale (NOS).

The year that the studies were published varied between 1983 and 2017. The number of patients included in each study ranged from 4 to 215 patients.

Risk of bias assessment. The results on risk of bias assessment for the three case-control studies using the Newcastle Ottawa Scale (NOS) were in Table 1. The results showed diverse quality among three studies. Based on overall assessment in terms of "selection", "comparability", and "ascertainment of exposure", there was only one study that attained a high score[19] whereas one study each may be classified as moderate[38] and low quality[37].

Both "selection" and "exposure" were generally quite weak across the studies reviewed. Two studies used community control groups[19,37] while one study had non-*S. suis* sepsis diagnosed patients as controls[38]. The definition of cases was explained sufficiently among studies with high and moderate score attainment[19,38] but representativeness of the cases was stated only in one study[38].

Only one study achieved a high "comparability" quality assessment score[19]. Neither of the two remaining studies adjusted for confounders[37,38]. Among these, one study used medical record for exposure ascertainment[38] whereas the other used a questionnaire without blinding the interviewers to case and control status[37]. None of the studies provided the information concerning non-response rate or addressed on the issue.

The included randomized controlled study was low risk of bias based on RoB 2.0[10]. Support for the judgement was provided in supplementary appendix Table S2.

Patient characteristics. Among 32 included studies, a total of 1,454 *Streptococcus suis* cases were reported. Majority of patients were men, comprising more than two-thirds of *S. suis* cases except in the study by Kerdsin *et al.* (2009) in which there was a relatively higher number of female patients compared to other studies[21]. Majority of cases were Asian particularly from Thailand, Vietnam and China whereas minority were patients from European countries where cases were largely occupation related. There were only 15 African patients derived from a population-based surveillance study in Togo[41]

S. suis patients were generally healthy adults before acquiring the infection. The mean age ranged between 37 to 63 years. A lower mean age was noted in 2 studies[16,28]. Mean age was reported in most studies[9,11,13–16,18,20,22–24,26,29–35,38] whereas 6 studies reported the value in median[10,21,25,27,36,39]. Neither mean nor median age were reported in 3 studies[17,37,41] (see supplementary appendix Table S1. Study key characteristics).

Study population were mainly *S. suis* meningitis identified from studies done in bacterial meningitis patients[9,10,13,15,19,22,23,28,29,35,41] while the rest were patients diagnosed with *S. suis* infection[11,14,16–18,20,21,24–27,31–34,38–40,42]. Diagnosis was based on either standard bacterial culture or real-time polymerase chain reaction (RT-PCR) in most studies. However, *S. suis* probable or suspected cases defined as cases with compatible clinical illness without laboratory confirmation were also included in 3 studies[36,37,39]. Most human *S. suis* infections were caused by serotype 2 strain. An occurrence of serotype 14 infections were sporadically reported mainly from northern, Thailand[21] whereas there were very few number of serotype 14 isolates identified in Vietnam[10]. Serotype 4 strain and untypeable serotype were considered to be rare[9].

Risk factors. Risk factors associated with acquiring *S. suis* infection included raw pork consumption, pig-related occupation, pigs or pork exposure, alcohol drinking, skin injury especially during pork exposure and underlying diseases contributing to immunocompromised conditions (supplementary appendix Table S1.). Although transmission by skin abrasion was believed to be the main route of infection, history of skin injury during exposure or before infection was noted only in some studies (9.5–100%) in which majority had a small percentage[9,11,14,23–25,31,32,36].

Varying results were noted between studies concerning risk factors of the disease. Exposure to pigs or pork and related occupation were the major risk factors found in a number of studies[14,23,37,38,41] whereas raw pork consumption or pig exposure was not present in around two-third of patients in other studies[10,16,28,31,33,35]. A high frequency of raw pork consumption was found among Thai patients especially in northern Thailand[26,39,40]. Although alcohol drinking was rarely reported in previous studies, a relatively high number of alcohol consumption was found in some studies from Thailand[26,29,40].

Despite similar study design, the three case-control studies included in the meta-analysis demonstrated different features. The prospective case-control study conducted in Vietnam included patients with invasive *S. suis* infection as cases and two control groups; an unmatched hospital control group and a matched community control group by residency and age within a 10 years range, at a ratio of 1:3[19] whereas the retrospective case-control study from the same country recruited *S. suis* infection patients as cases and non-*S. suis* sepsis diagnosed patients as controls[38]. A matched case-control study in Sichuan, China included *S. suis* infected patients in case group and individuals who had exposure with cases within 1 week prior to diagnosis as controls at a ratio of at least 1:1[37].

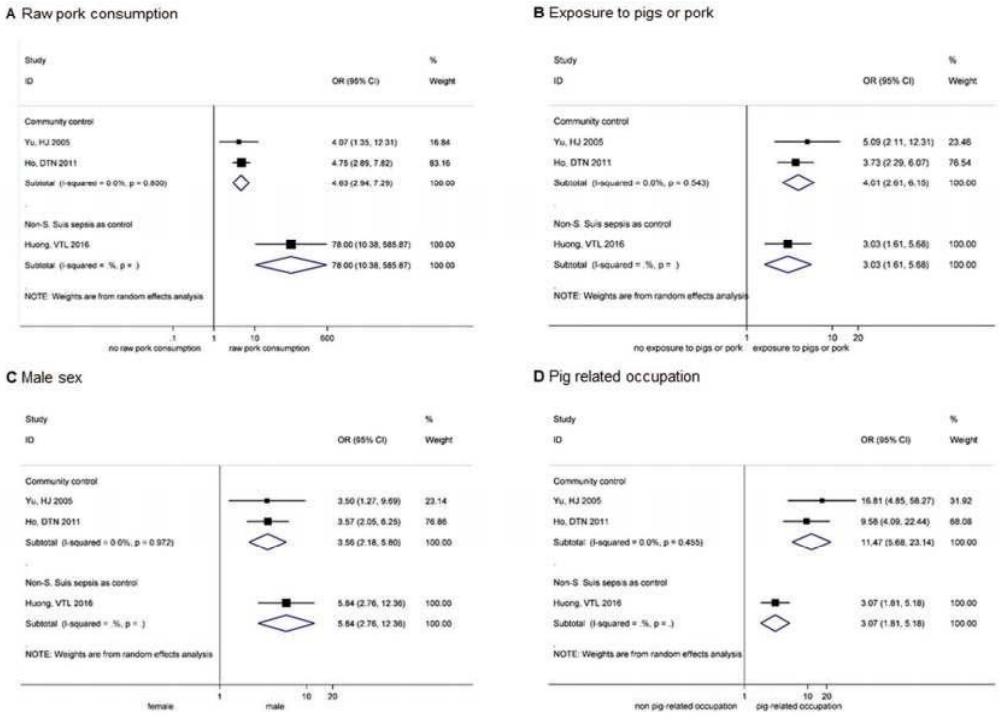

Figure 2. Risk factors of Streptococcus suis infection; Raw pork consumption (**A**), Exposure to pigs or pork (**B**), Male sex (**C**), and Pig related occupation (**D**). (**B**) *Note:* Individuals living in PRRS district or area adjacent to PRRS and those involved in pork cleaning, cutting and processing were used as proximate numbers for population exposed to pigs or pork in Huong *et al.* and Yu *et al.*[37,38] respectively. (**D**) *Note:* In Yu *et al.*, the number of those involved in slaughtering was used to represent pig-related occupation individuals[37].

A standardized questionnaire was used to investigate on predisposing factors in two studies[19,38]. However, only in the prospective case-control study, the interviewers were blinded to case and control status[19]. In the matched case-control, the interviewers were not blinded and only 15 out of 29 patients were interviewed face-to-face whereas the rest were unconscious, and the questionnaires were responded by their relatives[37]. The medical records were used for the other study[38].

Different case and control definitions were used among studies. A confirmed *S. suis* case was generally defined as an admitted patient with confirmed *S. suis* infection either by blood/CSF culture or real-time polymerase chain reaction (RT-PCR) in 2 studies[19,38] whereas a case was defined as *S. suis* case confirmed by either laboratory or clinical diagnosis in one study[37].

Community controls definition was quite similar in two case-control studies[19,37] except they were randomly identified and matched by age in one study[19] while one study only had hospital control group defined as confirmed non-*S. suis* sepsis patients during admission[38].

Meta-analysis. A total of 850 participants among 3 included case-control studies were analyzed by type of control groups (community controls and non-*S. suis* sepsis diagnosed cases). Major risk factors including raw pork consumption, exposure to pigs or pork, pig-related occupation and male sex were found to be significantly associated with *S. suis* infection according to all meta-analyses. Some proximate numbers were used due to different categorization of predisposing factors. The number of individuals living in Porcine Reproductive and Respiratory Syndrome (PRRS) district or adjacent area and those involved in pork cleaning, cutting and processing were used to represent population exposed to pigs or pork in the matched case-control and the retrospective case-control studies respectively[37,38]. For pig-related occupation, the number of those involved in slaughtering was used to represent pig-related occupation individuals under justification that most participants were farmers who were usually involved in slaughtering activity. The random-effects meta-analysis results on risk factors associated with *S. suis* infection were presented in Fig. 2A–D.

Raw pork consumption was significantly higher among cases than controls and much more pronounced in study by Houng *et al.* in which the control group was from non-*S. Suis* sepsis cases [pooled OR 4.63, 95% CI 2.94–7.29 and OR 78.00 95% CI 10.38–585.87, respectively] (Fig. 2A)[38]. Conversely, the overall estimate was stronger among studies with controls derived from community [pooled OR 11.47, 95% CI 5.68–23.14] for pig-related occupation whereas a weaker positive association was noted when the control group were non-*S. Suis* sepsis patients [OR 3.07 95% CI 1.81–5.18 (Fig. 2D). There was no significant heterogeneity observed between studies ($I^2 = 0.0\%$, $p = 0.80$ and $I^2 = 0.0\%$, $p = 0.455$ respectively).

The proportion of men was remarkably greater among cases in both analyses by type of control group [pooled OR 3.56, 95% CI 2.18–5.80 and OR 5.84 95% CI 2.76–12.36, respectively] (Fig. 2C)[19,37,38] and was nearly 6 times higher in cases than controls in the study with controls drawn from hospital non-*S. suis* sepsis patients [OR 5.84 95% CI 2.76–12.36][38].

Pigs or pork exposure was around 3 to 4 times higher in cases than controls among studies with controls drawn from hospital non-*S. Suis* sepsis diagnosed patients and community respectively [OR 3.03, 95% CI 1.61–5.68 and pooled OR 4.01, 95% CI 2.61–6.15, respectively] (Fig. 2B)[19,37,38]. The results were consistent among all analyses and there was non-significant heterogeneity. It was seen that the studies with community controls[19,37] generally showed more precise values compared with the study with controls drawn from non-*S. Suis* sepsis cases[38].

Clinical manifestations and outcomes. Meningitis was the most frequent clinical presentation, followed by septicaemia and arthritis in which an occurrence of cases subsequently developed sepsis arthritis were also reported (supplementary appendix Table S1). The spectrum of signs and symptoms of presentations were quiet similar across studies. Majority of meningitis patients developed classic meningitis symptoms including severe headache, high fever, neck stiffness and a change in mental status[9]. Petechiae or other skin abnormalities were present in few studies ranging between 3% to 7% among *S. suis* meningitis[9,10,22].

Endocarditis was usually less common whereas endopthalmitis and spondylodiscitis were[38] considered to be rare manifestations. In contrast, it was found that infective endocarditis was among or the most common clinical presentations found in two case series despite no underlying heart disease in most patients included[11,33]. The most frequent vegetation site found was aortic involvement[33]. The proportion of cases who developed toxic shock syndrome (TSS) was quite small approximately 2–28% in majority of studies[10,11,18,34–36,39] except in 2 epidemiological studies in China (62% and 50%)[24,37]. Toxic shock syndrome (TSS) and subacute endocarditis (SBE) were found to be associated with high mortality rate according to a series of 43 patients from Thailand, 80% and 50% among patients with TSS and SBE respectively[18]. TSS cases were younger with shorter incubation period, a lower total serum protein and antibody levels compared to non-TSS patients[18].

Incubation period was provided in 14 studies, the median time from exposure to onset ranged between 1 to 4.8 days[9–11,16–18,24,25,27,29,30,34,36,41]. Most infections occurred during the summer months[11,15,22,25,35,36] or the rainy season[20,26,33].

The disease mortality rate was low compared to meningitis caused by other agents (0–33.3%)[9–11,14,16–18,20–22,24–36,38,39,41]. However, deafness incidence was high in majority of studies and largely sequelae from meningitis syndrome (7–93%)[9–11,13–18,20–22,24–26,28–35,38,40,41]. Hearing loss was usually permanent once it already started even after successful meningitis treatment[22]. Vestibular dysfunction or ataxia was also common (8–80%)[11,17,24,26,29,32,35] and present in half of meningitis cases in a case series[26] whereas visual loss was noted in a few studies (4–60%)[17,35,41].

Deaths were mainly resulted from other complications rather than meningitis including multiple organ failure[33], disseminated intravascular coagulation (DIC)[22,31], bacterial peritonitis, sepsis and infective endocarditis[31]. Relapse rate was small and normally successfully treated with continuation of penicillin or combination therapy[9].

Treatments. Most *S. suis* isolates were sensitive to penicillin or cephalosporins[10,11,14,15,22,25,29,33,41]. Treatment with high dose intravenous Penicillin G were highly effective in majority of patients[22]. The mean of the minimum inhibitory concentration (MIC) for penicillin ranged from 0.015 to 0.06 mg/mL[11,14,15,22,31,33]. Tetracycline and macrolide resistance was common[10,11,14,25,41] whereas few cases with multiple antimicrobial resistance was noted[31].

Mean treatment duration ranged from 7 to 42 days[10,11,13,16,20,21,27,29–31,34–36,38]. Longer treatment duration was usually needed in case of complications including meningitis, spondylocitis and endocarditis[31,33]. Combination regimen including penicillin or cephalosporin plus aminoglycoside was found to be effective in treating infective endocarditis[33].

Adjuvant therapy with dexamethasone was found to reduce the risk of hearing loss and neurological complications according to the randomized double-blind, placebo-controlled trial included[10]. In contrast, the effect of steroid against hearing loss protection could not be established according to the two included case series[26,35].

Discussion

S. suis infection were predominantly found in adult male patients and the elderly[22]. The absence of pediatric infections was probably due to a lack of exposure to associated risk factors among children. A relative high proportion of male cases can be explained by the fact that the disease is occupation related. The more likelihood of exposure to pigs and predisposing factors such as raw pork consumption, slaughtering activity and alcohol use has posed men to be more prone to infection through their risk behaviors. However, more female patients were also identified despite no history of pigs or pork exposure[21]. According to the meta-analysis by type of control group of the included case control studies, pig-related occupation, exposure to pig or pork, male sex and raw pork consumption are significantly associated with *S. suis* infection[19,37,38]. However, a definite history of contact with pigs or pork could be elicited only in few number of patients[22]. Unknown skin lesions may contribute to the entry of organism[9,22]. The nature of recalled bias or missing data in retrospective studies included was also partly responsible for this finding. Indirect exposure was as well possible due to widely availability of wet markets in Asian countries. The possibility of oral route transmission has been raised which might explain the diarrhea found in some cases[11]. It was seen that raw pork consumption was significantly associated with *S. Suis* infection and the proportion of cases with raw pork consumption was substantially higher than controls drawn from non-*S. Suis* sepsis patients in study by Houng *et al.*[38]. The notably higher number was potentially subject to information bias as doctors would have more likely asked patients with *S. suis* infection concerning this particular exposure compared to others. Cultural food habits involving consumption of raw pork and pig's blood with alcohol drink could be a reason of the high frequency of infection among Thai population[18,26]. According to a study on impact of a

food safety campaign in the Phayao Province in northern Thailand, the disease incidence significantly declined after the first two years upon campaign implementation. However, the infection rate rose again in the third year which implied the existence of deep-rooted cultural behavior of raw pork consumption and the need of an effective public health education program to eliminate the risk of infection[42].

Other key characteristics that make patients vulnerable to infection include splenectomy[10,19,32], alcohol drinking or alcoholic liver disease[9,11,18–20,25–29,31–35,38,40], concurrent diabetes[11,18,19,25–28,33,34], renal or pulmonary tuberculosis[11,25], cancer[9,25,29,33], heart disease[16,18,27,29,31,33], on corticosteroid[29] which contribute to immunocompromised condition. There were around 50% and 80% of *S. suis* patients with underlying diseases found in two case series[18,33]. A high disease burden of rheumatic heart disease in Asia[43] combined with pig exposure which is an established risk factor may contribute to the high rate of infection in this region. Although there was no *S. suis* infection in pregnancy reported in literatures, according to the included retrospective cohort study, one of the dead cases was a 40-year old pregnant woman with 2-month-gestational age who presented with septic shock, DIC and died within 24 hours of admission[34]. This suggests that pregnancy may cause a patient to be in a more susceptible condition resulting in fatal outcome.

Meningitis is the most common clinical characteristic found in *S. suis* infection and is usually accompanied with hearing loss. This concomitant morbidity warrants the need of close monitoring and early adequate care in meningitis patients[34]. However, it should be noted that most studies included were done in meningitis population. The invasive nature of *S. suis* serotype 2 which is the major serotype found in human infections may be relevant. The polysaccharide capsule containing sialic acid feature makes the organism become highly invasive in entering to blood stream and penetration to blood-brain barrier[44].

Deafness appears to be the most common sequelae found among survivors. The mechanism of hearing loss is probable from *S. suis* invasion to the perilymph via cochlear aqueduct resulting in suppurative labyrinthitis according to animal experiments[45]. The benefit of dexamethasone treatment in hearing loss prevention was demonstrated in the included randomized control trial study[10]. However, inconsistent findings were shown in the two case series[26,35]. This was probably due to a small number of patients and varying degree of deafness severity[26,35]. Notwithstanding this contradiction, it should be justified to use corticosteroid as an adjunctive therapy based on the high rate of hearing impairment in *S. suis* meningitis.

The high frequency of STSS and fatality rate was found in the China outbreak[36], but, by contrast a lower proportion of STSS and mortality was reported in the outbreak investigation in Thailand[39]. The marked contrast in clinical outcome and severity could be due to the different main risk factors. In the Sichuan outbreak, pig slaughtering and widespread of porcine disease were the leading causes whereas consumption of raw pork was the major risk factor in the Thailand outbreak[36,39]. STSS was a significant risk factor of mortality with a rapid onset[18,34]. There were significant shorter incubation period and hospital stay among patients with STSS in which majority died within 24–72 hours upon admission[18].

The disease mortality was low. The number of *S. suis* meningitis case-fatality rate was lower compared to other meningitis among the same age group population[5,46,47]. This might be due to most *S. suis* infected individuals were healthy adults with less frequent predisposing conditions whereas other bacterial meningitis patients usually presented with underlying diseases[47] which have been correlated with a poor prognosis[9].

The number of *S. suis* cases appears to be higher during the summer or rainy seasons. The high occurrence of infection in the hot and humid weather is believed to be a precipitating factor that triggers more stress on pigs during transportation[15]. Apart from that the condition also enable the organism to proliferate in pig carcasses increasing infectivity during contaminated meat exposure[15].

To the best of our knowledge, this is the first systematic review and meta-analysis with the primary aim to comprehensively explore on the risk factors acquiring *Streptococcus suis* infection in human whereas the previous systematic review[48] focused only among studies on *S. Suis* meningitis. We searched extensively in 8 major databases and included all studies with at least 4 *S. Suis* infection cases with no time nor language restrictions while the previous systematic review included only studies written in West-European languages published between January 1, 1980 and August 1, 2015 and described at least 5 adult *S. suis* meningitis patients in whom at least one described clinical characteristic[48]. A rigorous quality assessment was done for both included observational and randomized controlled studies as well as comprehensive critical appraisals on all 32 studies included.

Some limitations of this review can be noted. The studies included in the review were largely descriptive and very diverse in terms of study designs and quality resulting in a small number of studies could be utilized for meta-analysis. The variability in the methodology and quality of the three case-control studies particularly the different types of control group caused some challenges whether they were combinable. However, we took consideration of this potential clinical heterogeneity and performed meta-analysis by control group. There was no significant heterogeneity seen in our analyses and the odd ratios were towards the same direction in favoring increasing risk of *S. suis* infection in cases than controls. The studies with community controls were generally showed more precise results[19,37] compared with values from the study with controls obtained from hospital[38] which might be due to larger sample size and selection of controls. Given the report with at least 4 cases of the inclusion criteria, there may be underreported cases. However, with the fact that the data is highly heterogeneous especially in different population and the primary outcome of interest is risk factors associated with the infection. All the main risk factors should have been identified based on the study inclusion criteria. In addition, majority of articles reviewed were retrospective studies which could have been potentially to recall bias and missing data. Finally, some proximate numbers were used in meta-analysis on risk factors. However, these could be the closest estimated numbers to be drawn according to the defined definition based on the authors' judgement under this limitation.

Conclusion

S. suis infection is not uncommon. The low number of cases reported were largely due to under diagnosis and unawareness of the disease. The organism is often misidentified by clinicians resulting in delay or inadequate treatment. It is important that patients with suggestive *S. suis* clinical symptoms with predisposing risk factors should receive adequate care while waiting for laboratory confirmation despite negative bacterial culture either due to misidentification or previous antibiotic administration. Developing a screening protocol would be useful to aid the treatment decision. Once a clear clinical picture is identified, the diagnosis should not be too difficult. The immediate treatment with penicillin or antimicrobial that the pathogen is susceptible to before development of complications particularly deafness would be essential in preventing long term mortality and morbidity.

In an absence of vaccination, the best control measure is to prevent the disease transmission. Public health interventions including a food safety campaign would be effective to enhance understanding about the disease especially in settings where there is a strong relationship between raw pork consumption and traditional culture.

Methods

The study reporting methodology was done according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) statement[49]. The study protocol has been registered in PROSPERO under protocol number CRD42018083596 (https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=83596).

Search strategy and study selection. A number of relevant electronic databases were systematically searched including CINAHL plus, Cochrane, EMBASE, Global Health, Grey literature, Ovid Medline, PubMed, and Science Direct. The MeSH terms used were "Streptococcus suis" OR "Streptococcus suis AND "infection" limited in human with no time nor language restriction.

The primary outcomes were risk factors associated with *Streptococcus suis* infection. The secondary outcomes were clinical presentations and outcomes of the disease. Articles were included if there were risk factors with or without clinical characteristics or outcomes of *Streptococcus suis* infection in human in which the cause of the disease was explained and at least 4 patients was described. Review, systematic review and meta-analysis articles as well as publications reporting overlapping data with the included articles were excluded. The references cited in the identified articles were also reviewed and judged to be included in case they deemed relevant. The final searched was done on September 18, 2017.

Data Extraction. The inclusion criteria were confirmed by AR and TMK. The data was searched, screened and extracted by one reviewer (AR) and confirmed by either BHG, LHL, TMK or SS for articles in English and Thai. For studies in other languages, BHG extracted the data from articles in Chinese with confirmation by LHL. The data from the article in Croatian language was extracted by TMK and confirmed by a native speaker together with the English abstract. The search strings used can be referred to Appendix 1. The consultation process was employed in case of doubts or disagreements to reach a consensus between reviewers and all authors.

For articles containing ambiguous data, two email attempts to the corresponding authors were carried out for clarification. The studies were excluded for analyses if there was no response received. In case the study had primary data published elsewhere, the previous publications were also checked and verified. Alternatively, an attempt to obtain clarification from the first or corresponding author would be made.

Risk of bias assessment. The quality of nonrandomized studies included were assessed according to the Newcastle-Ottawa Scale (NOS)[50]. The randomized controlled study[10,51] was assessed using a revised tool to assess risk of bias in randomized trial (RoB 2.0) which was based on the Cochrane Collaboration Approach[52]. The main study previously published[51] was also referred to where there was no information mentioned in the included article[10].

Information including predisposing factors, patient demographics, clinical manifestations, treatment and outcomes were extracted.

Meta-analysis. Meta-analysis for risk factors was carried out for case-control studies using STATA 14.2 (College Station, Texas, USA). The analyses by type of control groups (community control, and non-*S. suis* sepsis) were done as this variation could potentially be the source of clinical heterogeneity. Results for the association between risk behaviors and *streptococcus suis* infection were pooled using random-effects model in order to account for heterogeneity[53,54].

Forest plots were used to display the effect sizes (ES) from each study with their relevant 95% confidence intervals' and overall estimated ES. Heterogeneity was tested using I^2 and Q statistics[53,54].

Four main predisposing factors were defined in analyses: (1) Exposure to pigs or pork, defined as history or recalled of exposure with pigs or pork before illness without slaughtering, (2) Pig-related occupation includes farmer, butcher, abattoir worker, seller of raw pork, (3) Consumption of raw pork, defined as consumption of raw or partially cooked pork including swine materials, and (4) Male sex. In case the number of the defined category was not provided, the relevant number which could be assumed to be similar or in closest category would be utilized. Community controls were selected as control group in order to derive the same population who actually would have been cases if the outcome was present except in the retrospective case-control study which was designed to have only hospital control group[38].

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Author Contributions

A.R. conducted searching, screening and data extraction which were confirmed by B.H.G., L.H.L., T.M.K. or S.S., B.H.G. extracted the data from articles in Chinese with confirmation by L.H.L., T.M.K. extracted the article in Croatian language and checked with a native speaker together with the English abstract. A.R. drafted the manuscript, created the tables and figures with support from T.M.K. and S.S. The manuscript was reviewed and approved by all authors.

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Supplementary appendix

Risk factors for *Streptococcus suis* infection: A systematic review and meta-analysis

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Supplementary Content

Appendix 1: The search strings used

EMBASE (Ovid)

streptococcus suis.mp. [mp=ti, ot, ab, sh, hw, kw, tx, ct]
limit 1 to humans
("streptococcus suis" and streptococcus suis and infection).mp. [mp=ti, ot, ab, sh, hw, kw, tx, ct]
limit 3 to humans

PubMed

("streptococcus suis"[MeSH Terms] OR ("streptococcus"[All Fields] AND "suis"[All Fields]) OR "streptococcus suis"[All Fields]) OR (("streptococcus suis"[MeSH Terms] OR ("streptococcus"[All Fields] AND "suis"[All Fields]) OR "streptococcus suis"[All Fields]) AND ("infection"[MeSH Terms] OR "infection"[All Fields])) AND "humans"[MeSH Terms]

CINAHL plus (via EBSCO)

Streptococcus suis OR (Streptococcus suis AND infection) AND humans

Medline (Ovid)

(streptococcus suis or streptococcus suis and infection).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
limit 1 to humans

Science Direct

(streptococcus suis) or (streptococcus suis and infection), limit to humans

Cochrane (via Ovid)

(("streptococcus suis" or streptococcus suis) and infection).mp. [mp=ti, ot, ab, sh, hw, kw, tx, ct]
limit 1 to humans

Global Health

((streptococcus suis) OR (Streptococcus suis and infection) AND (humans))

Grey literature (Greynet)

Streptococcus suis OR "Streptococcus suis AND infection"

Table S1. Study key characteristics

Study	Country	Study design	Sample size	Mean age (yr) (range)	Male (%)	Major risk factors %				Main clinical presentations (%)	Treatment	Outcomes (%)
						Recent exposure with pigs/pork[a]	Related occupation[b]	Raw pork consumption [c]	Others			
Mai et al. 2008 [10]	Vietnam	A randomized, double-blind, placebo-controlled trial	151 *Note:* 76 received dexamethaxone, 72 received placeo, 3 received neither All patients initially received Cetriaxone 2 g q 12 h	46.5* (19-84)	77.5	33.1	8.6	NR	2 splenectomy	100 Meningitis¶	100 ceftriaxone 2 g q 12 h 50.33 Dexamethasone	2.6 Death Complete recovery: Dexa gr 36.8% vs. placebo gr. 38.7% 66.4 Hearing loss: 34.4 temporary, 41 permanent
Khin Thi & Chan 1985 [22]	Hong Kong	Case series	30	58.7 (23-84)	66.7	23.3	NR	NR	NR	86.67 Meningitis 26.67 Diarrhea 16.67 Arthristis 13.33 Sepsis 6.67 DIC & petechiae 3.33 Endopthalmitis	Penicillin G	23.3 Death 50 Hearing loss
Kay, R et. al. 1995 [45]	Hong Kong	Case reports & case series	25	55 (20-75)	64	NR	60	NR	16 Skin injury up to 16 dys before admission 12 Alcohol drinking 8 Concurrent DM 4 Renal tuberculosis	84 Meningitis 24 Arthritis 4 Sepsis 4 SBE 4 Septic shock	IV Penicillin G IV Penicillin G+gentamicin	4 Death 64 Hearing loss 40 Vestibular dysfunction 44 Vertigo/ ataxia

Study	Country	Study design	Sample size	Mean age (yr) (range)	Male (%)	Major risk factors %				Main clinical presentations (%)	Treatment	Outcomes (%)
						Recent exposure with pigs/pork[a]	Related occupation[b]	Raw pork consumption [c]	Others			
Dragojlovic et. al. 2005 [17]	Serbia	Case series	5	NR (22-63)	100	100	NR	NR	NR	100 Meningitis 20 Sepsis 20 SBE 20 Endothalmitis 20 Respiratory failure 20 Thrombocytopenia	2nd and 3rd generation Cephalosporins (80%) Aminoglycosides (20%)	No death 20 Complete recovery 80 Recovery with permanent hearing loss
Yu, HJ et al. 2005 [37]	China	A matched case-control study	29/147 (cases/ controls)	NR (36-72)	82.8/ 57.8 (cases/ controls)	72.4/34 (21/50) *Note:* Calculated from individuals involved in cleaning, cutting, processing of raw pork	89.66/34 (26/50) *Note:* Calculated from individuals involved in pig slaughtering	86.2/60.5 (25/89)	NR	27.6 Meningitis 10.34 Sepsis 62 STSS	NR	NR
Yu, H et al. 2006 [36]	China	Outbreak investigation report	215**	54* (26-82)	84	28	96 *Note:* All were farmers.	0	65 Sick pig/goat slaughtering 48 Skin injury during exposure	48 Meningitis 28 STSS 24 Sepsis	NR	18.14 Death

Study	Country	Study design	Sample size	Mean age (yr) (range)	Male (%)	Major risk factors %				Main clinical presentations (%)	Treatment	Outcomes (%)
						Recent exposure with pigs/pork[a]	Related occupation[b]	Raw pork consumption [c]	Others			
Fongcum et. al. 2009 [18]	Thailand	Case series	43 *Note*: 10 patients from retrospective review during the outbreak were excluded from clinical presentation and outcomes	50	92.5	7	NR	88.7	83 Underlying diseases	37.21 Meningitis 27.91 Sepsis 23.26 STSS 9.3 SBE 2.33 Spondylodiscitis	NR	27.9 Death 46.5 Complete recovery 18.6 Permanent deafness 7 Disability
Wertheim et. al. 2009 [35]	Vietnam	Case series	50	48 (17-78)	88	10	70	6	26 Alcohol drinking	100 fever 88 Neck stiffness 84 Kernig's sign 92 headache 46 confusion 16 Respiratory failure 14 Skin rash 12 STSS	Ceftriaxone in combination with ampicillin 52% received corticosteroid	6 Death 52 Complete recovery 42 Recovery with sequelae 38 Hearing loss 4 Paralysis 4 Loss of vision 4 Dysarthria with gait ataxia

Study	Country	Study design	Sample size	Mean age (yr) (range)	Male (%)	Major risk factors %				Main clinical presentations (%)	Treatment	Outcomes (%)
						Recent exposure with pigs/pork[a]	Related occupation[b]	Raw pork consumption [c]	Others			
Kong et. al. 2009 [23]	China	Epidemiological analysis	4	47.3	100	100	50	NR	100 Skin injury	100 Meningitis¶	NR	100 Fever 100 Headache 100 Meningeal irritation 50 Vomiting 25 each Coma, cramping and chilling
Ho et. al. 2011 [19]	Vietnam	Prospective case-control study	101/303/ 300 (cases/ hospital controls/ community controls)	50 (41-59)/ 27 (20-40)/ 50 (41-60)	82.2/ 66.7/ 56.3	45.5/12.9 /18.3 (46/39/55)	20.8/2.6/2.7 (21/8/8)	47.5/21.8/16 (48/66/48)	32.7/5.9/3.7 Skin injury 13.9/5.9/6.7 Alcoholism 3/1/1.3 DM 1/0/0 Splenectomy	NR	NR	NR
Huong et. al. 2016 [38]	Vietnam	Retrospective case-control study	90/183 (cases/ controls) *Note:* Cases: *S.Suis* case patients Controls: non-*S.suis* sepsis controls	48.5 (46.2-50.8)/ 50.6 (48.2-53.1)	90/ 60.7	83.3/62. 3 (75/114) *Note:* Derived from those living in PRRS district or area adjacent to PRRS^ district	64.4/37.2 (58/68)	30/0.5 (27/1)	31.1/21.3 Alcoholism	86.7/0 Neck stiffness 0/3.3 SBE, p=0.09 5.7/29.5 STSS, p=0.001 22.2/19. Skin rash 16.7/0.5 Purpura fulminans, p<0.001 15.9/28.4 ARF, p = 0.025 5.7/8.7 ALF 5.7/12.6 ARDS, p=0.08 4.5/2.7 Coagulopathy	Corticosteroid received 75%/11.5%, p= 0.001	6.6/25.6 Death 28.9/4.4 Recovery with sequelae 16.7/0 Hearing loss 8.9/0 Tinnitus

Study	Country	Study design	Sample size	Mean age (yr) (range)	Male (%)	Major risk factors %				Main clinical presentations (%)	Treatment	Outcomes (%)
						Recent exposure with pigs/pork[a]	Related occupation[b]	Raw pork consumption [c]	Others			
Tall et al. 2016 [41]	Togo	A public health surveillance study	15	NR (5-≥50)	80	80	66.7	93.3 *Note*: Pork consumption, 9 patients at least once per week	20 Pig famers involving slaughtering	100 Meningitis¶	NR	7 Death 13.33 Complete recovery 80 Recovery with sequelae 67 Hearing loss 42 Visual impairment 17 Paralysis
Takeuchi et. al. 2017 [42]	Thailand	A descriptive study on food safety campaign	71 *Note:* Before the campaign: 31 After the campaign: 41	56.7	71.8	18.3	NR	78.9	NR	53.5 Meningitis	NR	9.9 Death 29.6 Hearing loss
Arends et. al. 1988 [9]	The Netherlands	Case series	30^^	49 (21-76	86.7	6.7	83	NR	63.3 Skin injury 6.7 Skull fracture/ operation 6.7 Carcinoma 3.3 Alcohol abuse 3.3 Zollinger-Ellison syndrome 3.3 Cerebral confusion	100 Meningitis¶ 6.7 Shock 6.7 Petechiae 3.3 Macular bleeding/ arthritis	Ampicillin/ Penicillin	6.7 Death 6.7 Relapse *Note:* Penicillin MIC: 0.04 mg/mL and successfully treated with penicillin continuation 54 Hearing loss

Study	Country	Study design	Sample size	Mean age (yr) (range)	Male (%)	Major risk factors %				Main clinical presentations (%)	Treatment	Outcomes (%)
						Recent exposure with pigs/pork[a]	Related occupation[b]	Raw pork consumption [c]	Others			
Walsh et. al. 1992 [32]	The UK	Case series	35	47.3 (19-87)	97.1	NR	82.9	NR	14 Skin injury 2.9 Drinking alcohol 2.9 Splenectomy	85 Meningitis classic triad¶¶ 60 Cellulitis 53 Arthritis 5.7 Meningitis 2.9 Endophthalmitis	Penicillin G, Gentamicin, Choramphenical	13 Death 57.1 Recovered with sequelae 50 Hearing loss 30 Vertigo and ataxia 2.9 Relapse
Donsakul et. al. 2003 [16]	Thailand	A retrospective review	8	39.5 (19-75)	87.5	25	NR	NR	25 Ventricular septal defect	76 Meningitis 25 Endocarditis 12.5 Arthritis	IV Penicillin (7/9) 18-24mU/dy, 2-6 wks or Ampicillin (1/8) 12 g/dy, 4 wks	37.5 Complete recovery 62.5 Recovery with sequelae 62.5 Hearing loss
Suankratay et. al. 2004 [29]	Thailand	Case reports and case series	12	49.5 (27-75)	75	41.7	25	NR	75 Alcohol drinking 16.7 On corticosteriod 33.3 Underlying disease: - 8.3 Urinary bladder cancer - 8.3 RHD - 8.3 Cerebrovascular accident - 8.3 Chronic arterial insufficiency	83.3 Meningitis 66.7 Meningitis classic triad¶¶ 66.7 Skin and soft tissue infection 41.7 Severe myalgia 16.7 Shock 16.7 Sepsis 16.7 Arthritis	IV Penicillin G IV Cefotaxime	8.3 Death 58.3 Hearing loss 8.3 Vestibular dysfunction 8.3 Cerebritis/ ventriculitis 8.3 Relapse

Study	Country	Study design	Sample size	Mean age (yr) (range)	Male (%)	Major risk factors %				Main clinical presentations (%)	Treatment	Outcomes (%)
						Recent exposure with pigs/pork[a]	Related occupation[b]	Raw pork consumption [c]	Others			
Chang et. al. 2006 [14]	Japan	Case series	7	53.7 (47-58)	57.1	100	NR	NR	71.4 Skin injury	71.4 Meningitis 57.1 DIC 42.9 Sepsis 42.9 Arthritis 28.6 Endophthalmitis 14.3 Epidural abscess	NR *Note*: All *S. suis* isolates were susceptible to penicillin, cefotaxime, ciprofloxacin	14.3 Death 71.4 Deafness
Wangkaew et. al. 2006 [33]	Thailand	Case series	41	51 (27-77)	78	7.3 *Note:* Hx of raw beef consumption	4.9	24.4	34.1 Alcohol drinking 7.3 Heart diseases 4.9 Concurrent DM 2.4 Stomach cancer	39 Infective endocarditis 31.7 Meningitis 24.4 Sepsis 2.4 Spondylodiscitis 2.4 Endophthalmitis	Penicillin/ Cephalosporin+ Aminoglycoside *Note:* In infective endocarditis patients IV Cefotaxime Ceftriaxone IV Penicillin G	19.5 Death 30 Hearing loss
Lin et. al. 2007 [24]	China	Case series	6	54.5	100	NR	100	NR	83.3 Skin injury 33.3 Slaughtering	50 Meningitis 50 STSS	IV Penicillin G	33.3 Death 33.3 Complete recovery 33.3 Recovered with sequelae: - 16.7 slow response - 16.7 Hearing loss and vestibular dysfunction

Study	Country	Study design	Sample size	Mean age (yr) (range)	Male (%)	Major risk factors %				Main clinical presentations (%)	Treatment	Outcomes (%)
						Recent exposure with pigs/pork[a]	Related occupation[b]	Raw pork consumption [c]	Others			
Beek et. al. 2008 [13]	The Netherlands	Case reports	4	51 (33-64)	75	NR	100	NR	NR	100 Meningitis¶ 25 Seizure 25 Focal neurologic deficits	Amoxicillin or penicillin 14 days (3/4) Ceftriaxone and stepped down to penicillin 14 days (1/4)	25 Complete recovery 75 Permanent hearing loss
Ma et. al. 2008 [25]	Hong Kong	Case series	21	61.1 (26-89) *Note:* Median :62 years	86	43	24	NR	9.5 Skin injury 29 Underlying disease: - 14.3 DM - 4.8 Alcoholism - 4.8 Pulmonary tuberculosis - 4.8 Breast carcinoma	48 Meningitis 38 Sepsis 14 Endocarditis 9.5 Arthritis	Penicillin	5 Death 19 Hearing loss
Rusmeechan et. al. 2008 [28]	Thailand	Case series	41	37 (21-80)	68.3	NR	NR	NR	20 Alcoholism 12 DM	100 Meningitis¶ 95 Meningitis classic triad¶¶ 54 Abnormal mental status 2.4 Hemiparesis 29 Ataxia	Penicillin or 3rd generation cephalosporins	93 Hearing loss No death

Study	Country	Study design	Sample size	Mean age (yr) (range)	Male (%)	Major risk factors %				Main clinical presentations (%)	Treatment	Outcomes (%)
						Recent exposure with pigs/pork[a]	Related occupation[b]	Raw pork consumption [c]	Others			
Wangsom boonsiri et.al.2008 [34]	Thailand	A descriptive study (Retrospective cohort study)	66	52.9	68.2	NR	6	59	11 Alcoholic liver disease 6 DM	52 Meningitis 27 Sepsis 12 STSS 8 Endocarditis 1.5 Septic arthritis	Penicillin or ceftriaxone	17 Death 29 Hearing loss 6 DIC 5 CHF 3 Intracerebral hemorrhage 1.5 Endophthalmitis 1.5 Subdural empyema 1.5 Peritonitis 1.5 Intervertebral discitis
Kerdsin et.al. 2009 [21]	Thailand	A retrospective review	12	62.9* (40-79)	58.3	NR	0	16.7	NR	58.3 Meningitis 25 Septic arthritis 16.7 Sepsis	NR	41.7 Hearing loss 8.3 Acute respiratory distress syndrome 8.3 Extradural and subdural abscess No death

Study	Country	Study design	Sample size	Mean age (yr) (range)	Male (%)	Major risk factors %				Main clinical presentations (%)	Treatment	Outcomes (%)
						Recent exposure with pigs/pork[a]	Related occupation[b]	Raw pork consumption [c]	Others			
Navacharoen et. al. 2009 [26]	Thailand	Case series	40	55 (27-82)	75	25	52	62.5	49 Alcohol drinking 25 DM 5 Cirrhosis 5 HT 2.5 hyperlipidaemia	47.5 Meningitis 27.5 Sepsis 25 Endocarditis	NR	20 Death 27.5 Hearing loss: - 15 Permanent hearing loss 50 Vestibular dysfunction 2.5 Visual impairment
Kerdsin et. al. 2011 [20]	Thailand	A retrospective review	158	56.6 (18-98)	72.8	7	NR	32.9	21 Alcohol abuse	58.9 Meningitis 35.4 Sepsis 3.2 Septic arthritis 1.9 Infective endocarditis 0.6 Bacterial pneumonia 17.1 Diarrhea 22.2 Altered consciousness 5.7 STSS	IV Antibiotics e.g. ceftriaxone Corticosteroid received 2.5%	9.5 Death 21.5 Hearing loss

Study	Country	Study design	Sample size	Mean age (yr) (range)	Male (%)	Major risk factors %				Main clinical presentations (%)	Treatment	Outcomes (%)
						Recent exposure with pigs/pork[a]	Related occupation[b]	Raw pork consumption [c]	Others			
Praphasiri et. al. 2015[27]	Thailand	A public health surveillance study	38	50* (23-73)	73.7	26.3	33	65.8 *Note:* Included 13 patients involved in slaughtering who ate pork but could not recall how the meat was processed.	53 Drinking alcohol 12 Underlying disease: - 10.5 HT - 7.9 DM - 7.9 Alcoholism - 2.6 Heart disease - 2.6 Gout	63.2 Meningitis 26.3 Sepsis 11 Arthritis	IV Cefotaxime	31.6 Permanent deafness 68 Complete recovery No death
Vilaichone et. al. 2002[31]	Thailand	Case series	17	46.2 (1 mth-75 yrs)	64.7	17.7	2	5.8	11.8 Skin injury 17.7 Alcoholics 5.9 Congenital hydrocephalus 23.5 RHD	52.9 Meningitis 11.8 Sepsis 25.5 Infective endocarditis 5.9 Pneumonia 5.9 Spontaneous bacterial peritonitis	IV Pen G+gentamicin IV cloxacillin +gentamicin IV Cefotaxime IV Cetriazone and metronidazole	17.6 Hearing loss: - 11.8 Permanent deafness - 5.8 Temporary hearing loss
Chau et. al. 1983 [15]	Hong Kong	Case series	8	47.4 (24-71)	87.5	NR	100	NR	NR	100 Meningitis¶ 50 Arthitis	IV Penicillin G	87.5 Hearing loss Note: 1 patient had no data 1 Loss of balance

Study	Country	Study design	Sample size	Mean age (yr) (range)	Male (%)	Major risk factors %				Main clinical presentations (%)	Treatment	Outcomes (%)
						Recent exposure with pigs/pork[a]	Related occupation[b]	Raw pork consumption [c]	Others			
Khadthasrima et. al. [39]	Thailand	Outbreak investigation report	50 *Note:* 29 laboratory confirmed, 21 suspected cases	49* (10-77)	56	NR	NR	NR *Note:* All 9 cases interviewed reported consumption of raw pork	NR	2 STSS 100 Fever >80 Myalgia 60 Headache >20 Nausea/ Vomiting 20 Diarrhea 10 Meningitis 10 Altered consciousness < 10 Neck stiffness 5 Ecchymosis <5 Seizure <5 Arthralgia	NR	96 Hospitalized 14 ICU (Intensive care Unit) 6 Death 5 Hearing loss
Thayawiwat et. al. 2012 [40]	Thailand	A public health surveillance study	31	49.7 (21-70)	80.6	3.2	NR	71	83.9 Alcohol drinking	93.5 Fever 77.4 Headache 64.5 Myalgia 48.8 Neck stiffness 48.4 Nausea/ vomiting	NR	51.6 Hearing loss

[a] History/recalled of exposure with pigs or pork before illness without slaughtering; [b] Related occupation includes farmer, butcher, abattoir worker, seller of raw pork; [c] Consumption of undercooked pork including raw pig blood, intestine and other internal organs; * Median age; ** Among 215 cases; 149 probable cases, 66 confirmed cases. A probable case referred to a compatible clinical illness without laboratory evidence. A confirmed case was defined as a compatible clinical illness with *S. suis* isolated verified from a normal sterile site despite the exposure; ^ PRRS: Porcine Reproductive and Respiratory Syndrome; ^^ Case series of Dutch and non-Dutch population. Non-Dutch data was excluded as the information was derived from literature; ¶ Study in bacterial meningitis patients; ¶¶ Classic triad signs of meningitis: fever defined as body temperature ≥ 38.5 Degree Celsius, neck stiffness, photophobia or a change in mental status defined as a Glasgow Coma Score < 14; NR: Not reported; ALF: Acute liver failure; ARF: Acute renal failure; ARDS: Acute Respiratory Distress Syndrome; DM: Diabetes Mellitus; DIC: Disseminated intravascular coagulation; HT: Hypertension; RHD: Rheumatic Heart Disease; STSS: Streptococcal Toxic Shock Syndrome; SBE: Subacute bacterial endo

Table S2: Risk of bias assessment (RoB2.0): detailed notes for randomized, double-blind, placebo-controlled trial* [10,51]

Domain	Signalling questions	Rating	Description/Support for judgement
1. Bias arising from the randomization process	1.1 Was the allocation sequence random?	Yes	Quote: "A computer-generated sequence of random numbers was used to assign treatment in blocks of 100 patients. If a patient met the entry criteria, the attending physician instructed a nurse to open a numbered envelope containing instructions to give either active drug or placebo". P. 2432 (Mai, NT et. al. 2007) Quote: "All patients, the physicians who enrolled them, and study investigators were unaware of the treatment assignments until the last patient had completed follow-up" P. 2432 (Mai, NT et. al. 2007)
	1.2 Was the allocation sequence concealed until participants were recruited and assigned to interventions?	Yes	
	1.3 Were there baseline imbalances that suggest a problem with the randomization process?	No	Quote: "The study medication was given 15 minutes before the administration of antibiotics, although some patients may have had prior antibiotic treatment". P. 2432 (Mai, NT et. al. 2007) "There were no significant difference in the baseline characteristics between the study groups", P. 2435 (Mai, NT et. al. 2007) *Comment: Chance imbalances are not bias.
	Risk of bias judgement		
	Optional: What is the predicted direction of bias arising from the randomization process?	Low	
2. Bias due to deviations from intended interventions	2.1. Were participants aware of their assigned intervention during the trial?	No	Quote: "All patients, the physicians who enrolled them, and study investigators were unaware of the treatment assignments until the last patient had completed follow-up" P. 2432 (Mai, NT et. al. 2007)
	2.2. Were carers and trial personnel aware of participants' assigned intervention during the trial?	No	
	2.3. If Y/PY/NI to 2.1 or 2.2: Were there deviations from the intended intervention beyond what would be expected in usual practice?		
	2.4. If Y/PY to 2.3: Were these deviations from intended intervention unbalanced between groups *and* likely to have affected the outcome?		
	2.5 Were any participants analysed in a group different from the one to which they were assigned?	Yes	ITT approach was employed in the trial analysis. Quote: Dexamethasone gr "2 were loss to FU, 1 discontinued dexamethasone"; placebo gr, 4 were loss to FU after 1 M, Figure 1 P. 2432 (Mai, NT et. al. 2007) Quote: "The study drug was withdrawn in one patient in each study group after 3 days because of bleeding in the upper gastrointestinal tract". P. 2439 (Mai, NT et. al. 2007)
	2.6 If Y/PY/NI to 2.5: Was there potential for a substantial impact (on the estimated effect of intervention) of analysing participants in the wrong group?	PN	Comment: 76 received Dexamethaxone, 72 received placeo, 3 received neither. All patients initially received Cetriaxone 2 g q 12 h (Mai, NT., et al. 2008)
Domain	**Signalling questions**	**Rating**	**Description/Support for judgement**

	Risk of bias judgement		
	Optional: What is the predicted direction of bias due to deviations from intended interventions?	Low	
3. Bias due to missing outcome data	3.1 Were outcome data available for all, or nearly all, participants randomized?	Yes	There was low number of loss to follow up according to Figure 1. Enrollment and Outcomes P. 2432 (Mai, NT et. al. 2007). Outcomes were available in all 151 *S. suis* patients (Mai, NT., et al. 2008)
	3.2 If N/PN/NI to 3.1: Are the proportions of missing outcome data and reasons for missing outcome data similar across intervention groups?		
	3.3 If N/PN/NI to 3.1: Is there evidence that results were robust to the presence of missing outcome data?		
	Risk of bias judgement		
	Optional: What is the predicted direction of bias due to missing outcome data?	Low	
4. Bias in measurement of the outcome	4.1 Were outcome assessors aware of the intervention received by study participants?	No	
	4.2 If Y/PY/NI to 4.1: Was the assessment of the outcome likely to be influenced by knowledge of intervention received?		
	Risk of bias judgement		
	Optional: What is the predicted direction of bias due to measurement of the outcome?	Low	
5. Bias in selection of the reported result	Are the reported outcome data likely to have been selected, on the basis of the results, from... 5.1. ... multiple outcome measurements (e.g. scales, definitions, time points) within the outcome domain?	Yes	
	5.2 ... multiple analyses of the data?	PN	
Risk of bias judgement	Low / High / Some concerns	Low	
Optional: What is the predicted direction of bias due to selection of the reported result?	Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable	Favours experimental	
Overall bias	**Risk of bias judgement**	Low	
	Optional:		

*The included article [10] does not contain information for all domains. Therefore, the information from the main study previously published [51] was also referred to for quality assessment.

Chapter 4:

Clinical manifestations and risk factors of *Streptococcus suis* mortality among northern Thai population

4.1 Summary of Chapter 4

With the highest cumulative prevalence of *S.suis* infection in Southeast Asia with majority of cases from Thailand (8.21 cases/million population) followed by Vietnam (5.40 cases/million population), the disease has caused substantial loss in swine industry and deaths in human *(5, 89)*. Despite the losses and deaths, there is no reduction in the disease incidence, this life-threatening infection is still largely neglected. The disease epidemiology in Thailand is not fully understood and epidemiological study to identify predicting factors for *S.suis* mortality is still limited. Although there were a few studies on *S. suis* mortality in this region *(33, 55)* their statistical significances could not be confirmed by multivariate analyses due to insufficient sample size. Therefore, a 13-year retrospective cohort study in Chiang Mai, Thailand during 2005-2018 was conducted to explore risk factors associated with *S.suis* mortality and provide an update evidence on the disease outcomes.

Study setting

A retrospective review during 13-year period from 2005-2018 which was the longest period with available medical and microbiological records of *S.suis* infected patients admitted at Chiang Mai University Hospital (CMUH), northern Thailand was carried out. CMUH is located in Chiang Mai, the provincial capital in northern Thailand where there is the highest number of investigational reports of *S.suis* infection in the country *(63)*. It is the largest hospital in northern of Thailand and the fourth largest hospital in the country where most patients in the region are from northern 17 provinces (Table 4.1 and Figure 4.1) are referred to for tertiary care. The study has been approved by the Monash University Human Research Ethics Committee (MUHREC; Project Number 12225) and Research Ethics Committee, Faculty of Medicine, Chiang Mai University, Thailand (Research ID 5141) (Appendix 3 and 4).

Table 4.1 List of Northern provinces of Thailand as of 2018 (National Statistics Office (NSO). [cited 2019 22 July]; Available from: http://www.nso.go.th/sites/2014/nsopublic)

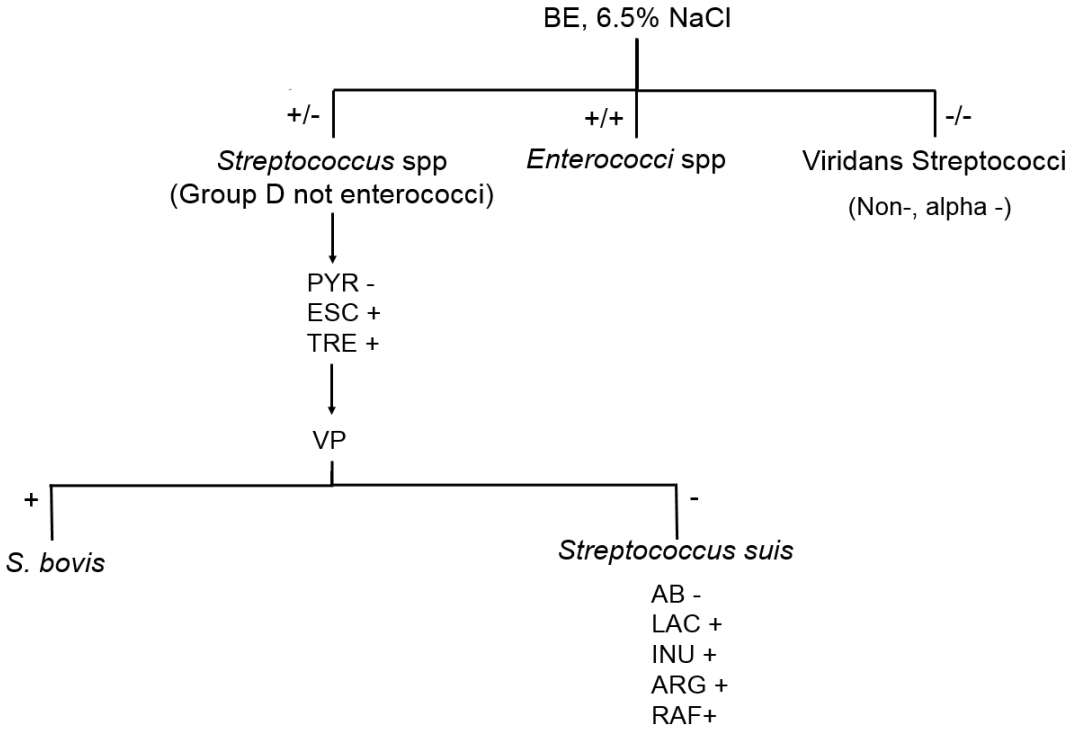
Upper Northern Thailand	Lower Northern Thailand
Chiang Rai	Tak
Chiang Mai	Kamphaeng Phet
Nan	Nakhon Sawan
Phayao	Phetchabun
Phrae	Phichit
Mae Hong Son	Phitsanulok
Lumphun	Sukhothai
Lampang	Uthai Thani
Uttaradit	

Figure 4.1 Northern Thailand provinces (adapted from NordNordWest. Thailand location map. 2009 [cited 2019 6 December]; Available from: https://commons.wikimedia.org/wiki/File:Thailand_location_map.svg).
Note: The content is free to copy and redistribute or adapt for any purpose.

Study participants

S.suis cases were identified by the hospital microbiology laboratory data who provided the list of patients with culture-proven *S.suis* positive with their hospital numbers (HNs). *S.suis* isolates were confirmed by biochemical methods (Figure 4.2) and Matrix-Assisted Laser Desorption Ionization Time of Flight (MALDI-TOF) Mass Spectrometry. All cases with confirmed positive *S.suis* either by cerebrospinal fluid (CSF) or hemoculture admitted at CMUH from 2005-2018 were included (Figure 4.3).

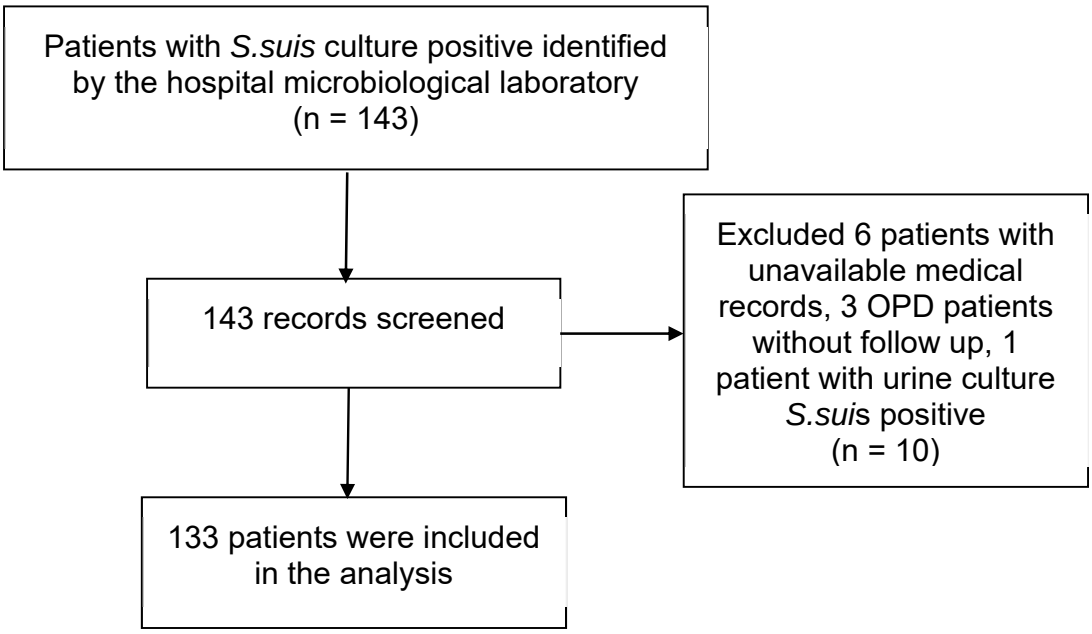
Figure 4.2 Biochemical test protocol for identification of *Streptococcus suis* strains



Streptococcus suis strains is alpha-hemolysis in sheep blood agar with no growth in broth with 6.5% NaCl and positive in esculine, trehalose reactions, negative in Voges-Proskauer, pyrrolidonyl arylamidase with following biochemical characteristics: acid fermentation of lactose, inulin, raffinose broth, hydrolysis of L-arginine, no acid fermentation of arabinose.

Abbreviations: BE, bile esculin, PYR, pyrrolidonyl arylamidase, ESC, esculin, TRE, D-Trehalose, VP, Voges-Proskauer, AB, arabinose, LAC, D-Lactose, INU, inulin, ARG, L-arginine, RAF, D-raffinose

Figure 4.3 Patients identification and selection



OPD, Outpatient department

Clinical manifestations and outcomes

Of 133 patients with culture-proven *S.suis* infection identified, there were 92 males and 41 females (Table 4.2). Septicaemia (55.64%) was the most common clinical manifestation followed by meningitis (37.59%) and infective endocarditis (25.56%). The overall mortality rate was 12.03% (n=16).

Potential risk factors for mortality

Potential risk factors of mortality were identified using univariate and multivariate logistic regression. The univariate logistic regression was performed for clinical characteristics and laboratory data among dead and survived patients to estimate odds ratios (OR) for potential risk factors associated with *S. suis* mortality. A two-tail, with p-value less than 0.05 would be considered to be statistically significant. Any variables with $P < 0.05$ in the univariate analysis will be carried forward in multivariate logistic regression to analyze the prognostic indicators for *S.suis* mortality. Potential collinearity was also checked before building the predictive model. According to the multivariate analysis, the independent risk factors for mortality were prolonged bacteremia ≥ 6 days, septic shock, and direct bilirubin > 1.5 mg/dl.

Table 4.2 Patient characteristics, major clinical manifestations and outcomes

Characteristics	Total (n=133)
Age (year) (mean±SD)	56.47± 13.68
Male	92 (69.17%)
Major clinical manifestation	
- Acute meningitis	50 (37.59%)
- Septicaemia	74 (55.64%)
- Septic shock	20 (15.04%)
- IE	34 (25.56%)
- Arthritis	9 (6.77%)
- Infective spondylodiscitis	12 (9.02%)
Outcomes	
- Recovered	70 (52.73%)
- Recovered with sequelae	44 (33.08%)
- Not recovered	1 (0.75%)
- Death	16 (12.03%)

IE, Infective endocarditis

Note: Treatment and outcomes were unavailable in 1 patient.

To our knowledge, this is the largest series focusing on risk factors of *S.suis* mortality in Thailand. Close patients monitoring on mortality risk parameters (prolonged bacteremia ≥ 6 days, septic shock, and a high level of direct bilirubin > 1.5 mg/dL) and supportive fluid resuscitation are essential to improve patient outcomes. The study identified a number of significant predictors of *S. suis* mortality which would be useful in clinical practice and future research to improve patient cares.

4.2 Publication associated with Chapter 4 (Infection and Drug Resistance, IF: 3.000)

Rayanakorn A, Katip W, Goh BH, Oberdorfer P, Lee LH. Clinical Manifestations and Risk Factors of *Streptococcus suis* Mortality Among Northern Thai Population: Retrospective 13-Year Cohort Study. Infection and Drug Resistance. 2019 12/01;Volume 12:3955-65. https://doi.org/10.2147/IDR.S233326

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ORIGINAL RESEARCH

Clinical Manifestations and Risk Factors of *Streptococcus suis* Mortality Among Northern Thai Population: Retrospective 13-Year Cohort Study

This article was published in the following Dove Press journal:
Infection and Drug Resistance

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Purpose: *Streptococcus suis (S. suis*) is an emerging zoonotic disease mainly in pigs, causing serious infections in humans with high prevalence in Southeast Asia. Despite a relatively high mortality rate, there are limited data regarding the risk factors of this life-threatening infection. Therefore, a 13-year retrospective cohort study in Chiang Mai, Thailand during 2005–2018 was conducted to explore risk factors associated with *S. suis* mortality and to update the outcomes of the disease.

Patients and methods: *S. suis* positive cases were derived from those with positive *S. suis* isolates from microbiological culture results and Matrix-Assisted Laser Desorption Ionization Time of Flight (MALDI-TOF). Potential risk factors of mortality were identified using univariate and multivariate logistic regression.

Results: Of 133 patients with culture-proven *S. suis* infection identified, there were 92 males and 41 females. The mean age was 56.47 years. Septicemia (55.64%) was the most common clinical manifestation followed by meningitis (37.59%) and infective endocarditis (25.56%). Alcohol drinking and raw pork consumption were documented in 66 (49.62%) and 49 (36.84%) cases respectively. The overall mortality rate was 12.03% (n=16). According to the multivariate analysis, the independent risk factors for mortality were prolonged bacteremia ≥ 6 days (OR = 43.57, 95% CI = 2.46–772.80, P =0.010), septic shock (OR = 13.34, 95% CI = 1.63–109.03, P =0.016), and direct bilirubin > 1.5 mg/dL (OR = 12.86, 95% CI = 1.91–86.59, P =0.009).

Conclusion: *S. suis* is not infrequent in Northern Thailand, where the cultural food habit of raw pork eating is still practiced. To the best of our knowledge, this is the largest series focusing on risk factors of *S. suis* mortality which has been conducted in Thailand. Prolonged bacteremia ≥ 6 days, septic shock, and direct bilirubin > 1.5 mg/dL were strong predictors associated with *S. suis* mortality. The mortality risk factors identified may be further utilized in clinical practice and future research to improve patient outcomes.

Keywords: *Streptococcus suis*, *S. suis*, risk factor, *S. suis* infection, mortality, Thailand

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Introduction

Streptococcus suis (S. suis) is a gram positive diplococci alpha-hemolytic bacteria mainly found in pigs. The pathogen has been classified into 29 serotypes[1] of which serotype 2 is the most prominent cause of infection in humans[2] contributing to meningitis, septicemia, infective endocarditis and other serious complications. The disease is considered to be an occupational hazard via percutaneous exposure in Western countries[3,4] whereas oral route transmission through ingestion of raw/undercooked pork including fresh pig's blood, intestines, and other internal organs

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have been noted in the Asia region.[5–8] A recent systematic review and meta-analysis demonstrated the main risk factors in acquiring *S. suis* infection including eating raw/undercooked pork, exposure to pigs or raw pork, male sex, and pig-related occupation.[9]

There is a high prevalence rate in Southeast Asia, particularly in Thailand and Vietnam.[10] In Thailand, *S. suis* infection is an important health issue and one of the most common causes of bacterial meningitis with a high prevalence rate in the northern region due to cultural food habits involving raw pork consumption. To date, much uncertainty remains regarding the understanding of *S. suis* clinical manifestations and treatment responses as well as gaps between different geographical settings. The disease epidemiology in Thailand is not fully understood and epidemiological study to identify predicting factors for mortality is still limited. Although there have been a few studies on *S. suis* mortality in this region,[11,12] their statistical significances could not be confirmed by multivariate analyses.

The highest number of investigational reports of *S. suis* infection in Thailand was from Chiang Mai,[13] the provincial capital and the largest province in northern Thailand. Since the previous two-year series of 41 *S. suis* patients conducted in 2000 at Chiang Mai University Hospital (CMUH),[11] there has not been any further study on this life-threatening infection in this setting. Here, we conducted a single-center retrospective cohort study during a 13-year period to determine potential risk factors of *S. suis* mortality and to update the evidence concerning clinical manifestations, outcomes, and treatment of the disease.

Materials and Methods

Study Design and Setting

A retrospective review, during a 13-year period, of records of *S. suis* infected patients admitted at CMUH, a 1400-bed tertiary teaching hospital from May 2005 to December 2018, was conducted. CMUH is the fourth largest hospital in the country and the largest hospital in northern Thailand where most of cases in northern region from 17 provinces are referred to for tertiary care (Table S1).

Study Participants

S. suis positive cases were identified from the hospital microbiology laboratory data which provided the list of patients with positive *S. suis* culture and their hospital numbers (HNs). *S. suis* isolates were cultured on sheep blood agar at 37°C and were subsequently confirmed by biochemical method (Figure S1) and Matrix-Assisted Laser Desorption Ionization Time of Flight (MALDI-TOF) Mass Spectrometry. All confirmed *S. suis* cases with *S. suis* positive culture either from blood or cerebrospinal fluid (CSF) and compatible clinical presentations admitted at CMUH with available medical records were collected and reviewed through the hospital databases (Figure 1).

Clinical Data and Definitions

S. suis case was defined as those with *S. suis* positive culture from sterile site (CSF or hemoculture) with compatible clinical presentations. Time to microbiological cure is an objective measure which was defined as the duration from the date with positive cultures obtained until the date of negative cultures from the same site of *S. suis* isolate. In case the repeated culture was not done to show microbiological success, the date of discharge or resolution of clinical symptoms or discontinuation of antibiotics was used. The same principle was applied for dead cases with available repeated culture results, otherwise this was regarded as treatment failure. E-test method was employed to identify the minimum inhibitory concentration (MIC) of penicillin and ceftriaxone.

Echocardiography was used in the assessment of endocarditis and detection of vegetation. An audiogram was used to diagnose and monitor the degree of hearing ability.

Data Collection

Study data were collected and managed using REDCap electronic data capture tools hosted at Research Electronic Data Capture, Monash University Malaysia, a secure, web-based

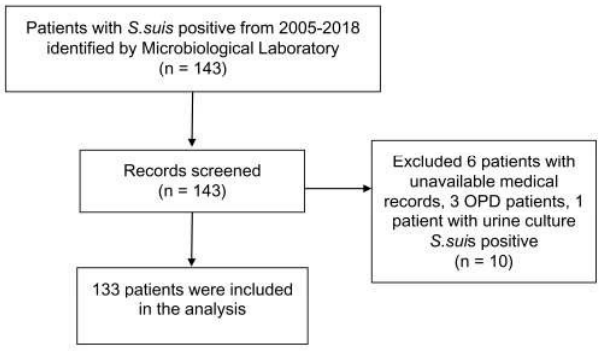

Figure 1 Patients' identification and selection.
Abbreviation: OPD, Outpatient department.

application designed to support data capture for research studies.[14] Data including patient characteristics, pigs/pork exposure, medical history, clinical presentations, outcomes, laboratory results, treatment and antimicrobial susceptibility were collected and analyzed. Any clinical discrepancy was reviewed and discussed within the team to reach the consensus.

The study was approved by the Research Ethics Committee, Faculty of Medicine, Chiang Mai University (IRB no.010/2018) and Monash University Human Research Ethics Committee (MUHREC) (Project no.12225). Patient names, personal and other traceable information that could be used to identify the person were omitted and treated as confidential in all processes of data collection and management.

Data Analysis

Statistical analysis was done by STATA 14.2 (College Station, Texas, USA). Demographic and clinical data were initially analyzed descriptively. Variables of interest included age, gender, Glasgow coma scale (GCS) score < 8,[15] underlying diseases (alcoholic liver disease, valvular heart disease (VHD), diabetes mellitus (DM)), meningitis, septicemia, septic shock, infective endocarditis, receiving corticosteroids, elevated liver functions, and hypoalbuminemia were explored for their associations with *S. suis* mortality. Parameters' selection was based on authors' clinical viewpoints and previous studies on *S. suis* mortality.[11,12] The univariate logistic regression was performed for clinical characteristics and laboratory data among dead and survivors to estimate odds ratios (OR) for potential risk factors associated with *S. suis* mortality. A two-tail, with p-value less than 0.05 was considered statistically significant. Any variables with $P < 0.05$ in the univariate analysis were carried forward in multivariate logistic regression to analyze the prognostic indicators for *S. suis* mortality. Potential collinearity was also checked before building the predictive model.

Results

Demographics and Clinical Characteristics

One hundred and thirty-three patients with culture-proven *S. suis* infection were identified. There were 92 males and 41 females. All patients were from different provinces in Northern Thailand. Majority of patients were from Chiang Mai (n=93, 70%), followed by Lumphun (n=18, 13.53%), and Chiang Rai (n=4, 3%). There were three and two patients each from Lampang, Maehongson, Prae, and Phayao, Sukhothai respectively. There was one patient each from Uttradit and Phetchabun, while there were three patients whose home address information was unavailable.

The mean age was 56.47 years ranging between 9–90 years. Most patients were middle-aged men. There was only one pediatric case which was a 9-year old boy with a medical history of congenital rheumatic heart disease (RHD) who previously underwent surgery for aortic coarctation, patent ductus arteriosus (PDA) division and ventricular septal defect (VSD) closure. All cases generally had good health status (SAPS II 27.05± 13.89) and consciousness (GCS 12.65± 3.15) during admission. Alcohol drinking and raw pork consumption were documented in 66 (49.62%) and 49 (36.84%) of included patients respectively. There were five cases (3.76%) which reported recent pig or pork exposure, 3 patients (2.26%) had occupation related to pigs, and 2 patients (1.50%) had history of skin injury. The information on patient demographics and clinical characteristics were summarized in Table 1.

About one third of patients had underlying valvular heart disease (VHD) (n=44, 33.08%). There were 27 patients (20.30%) with medical history of spondylodiscites, 26 (19.55%) with diabetes mellitus (DM), 21 (15.79%) with systemic lupus erythematosus (SLE), and 16 (12.03%) reported alcoholic liver disease (ALD). Two patients each (1.50%) were noted to have history of cancer and splenectomy, and human immunodeficiency virus (HIV) infection. The mean duration from exposure to onset and admission were about 8 days (7.67± 11.66 days) and 14 days (13.55± 19.21 days) respectively (Table 1).

The highest number of patients admitted was in the year 2005 (n=22), 2009 (n=21), and 2012 (n=14) (Figure 2). The number of *S. suis* cases admitted was quite high during summer and rainy season from April to June, and in August and October respectively (Figures 3A and B).

Clinical Manifestations and Outcomes

Septicemia (n=74, 55.64%) was the most common clinical manifestation found followed by meningitis (n=50, 37.59%) in which neck stiffness was recognized among the majority of cases. Infective endocarditis (IE) ranked third, involving 34 patients (25.56%) of which 31 had underlying VHD and 20 patients had undergone valve replacement. The most common vegetation site was aortic valve (n=26, 19.55%), followed by mitral valve (n=19, 14.29%) and tricuspid valve (n=4, 3.01%). There were 1 and 4 patients with three and two vegetation sites detected

Table 1 Patient Characteristics

Characteristics	Total (n=133)
Age (years) (mean±SD)	56.47 ± 13.68
Male	92 (69.17%)
Female	41 (30.83%)
GCS	12.65 ± 3.15
SAPS II	27.05 ± 13.89
Relevant social behavior	
• Consumption of raw pork	49 (36.84%)
• Recent contact with pigs/pork exposure	5 (3.76%)
• Pig-related occupation	3 (2.26%)
• Skin injury	2 (1.50%)
• Alcohol drinking	66 (49.62%)
• No	24 (18.05%)
Underlying diseases	
• DM	26 (19.55%)
• ALD	16 (12.03%)
• Splenectomy	2 (1.50%)
• VHD	44 (33.08%)
• Cancer	2 (1.50%)
• Corticosteroids use	3 (2.26%)
• HIV/AIDS	2 (1.50%)
• Spondylodiscites	27 (20.30%)
• SLE	21 (15.79%)
Exposure to onset (days) (mean±SD)†	7.67 ± 11.66
Time from exposure to admission (days) (mean±SD)‡	13.55 ± 19.21

Notes: †Available data from 37 patients; ‡available data from 40 patients.
Abbreviations: GCS, Glasgow coma scale; SAPS II, The Simplified Acute Physiology Score; DM, Diabetes Mellitus; ALD, Alcoholic liver disease; HIV, Human immunodeficiency virus infection; AIDS, Acquired immune deficiency syndrome; SLE, Systemic lupus erythematosus; VHD, Valvular heart disease.

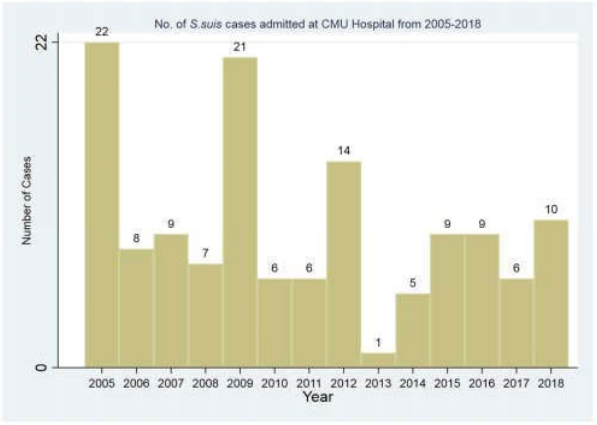

Figure 2 Number of *S. suis* cases admitted at CMUH from 2005–2018.

respectively (1 patient with aortic, tricuspid and mitral valve, 3 patients with aortic and mitral valve and 1 patient with tricuspid and mitral valve). Thirty-seven patients (27.82%) experienced diarrhea and twenty-seven patients (20.03%) had vomiting while only 10 patients (7.52%) presented with vertigo. Septic shock and infective spondylodiscites were recognized in 20 (15.04%) and 12 patients (9.02%) respectively whereby 8 out of 12 had underlying disease of spondylodiscites. The key clinical manifestations and outcomes were summarized in Table 2.

The overall case fatality rate was 12.03% (n=16). The main causes of death were septic shock (9/16, 56.25%) and metabolic acidosis (7/16, 43.75%). Of 16 deaths, 7 developed metabolic acidosis in which 2 had multi-organ failure and 1 patient each had HIV positive, and hepatic encephalopathy. Of 5 patient deaths with underlying alcoholic liver disease; 1 developed liver cirrhosis with diffused hematoma and suspected portal vein thrombosis; 2 had hepatic encephalopathy; 1 had underlying coronary artery disease from steroid abuse and developed chronic subdural hematoma septicemia and end-stage renal disease (ESRD); 1 had medical history of hepatocellular carcinoma (HCC) and spondylodiscites. There were 2 HIV infected patients. The first one was a 52-year old man who died within a day of admission from severe metabolic acidosis. The other one was a 25-year old female who was admitted with diarrhea and shock. She later presented with septicemia, pharyngeal candidiasis, herpes zoster infection at right side of face and expired due to acute renal failure and electrolyte imbalance. One patient died from rupture of infected abdominal aortic aneurysm, and cardiac arrest. One patient with history of post ST-Elevation Myocardial Infarction (STEMI) had infective endocarditis and died from pulmonary edema with respiratory failure. Among 2 patient deaths with no relevant medical history nor risk factors; one patient was a carpenter and admitted with shortness of breath in left side of chest, then developed massive hemoptysis and died shortly on day 3; the other presented with septicemia and mottled skin.

Approximately half of patients, including the 9-year-old boy with congenital rheumatic heart disease, recovered from infection (52.73%), whereas 44 patients (33.08%) had sequelae. Hearing loss was the most prominent complication found followed by vestibular dysfunction. Out of 42 (31.58%) affected with hearing loss, 13 had mild sensory neural hearing loss (SNHL); 7 had moderate SNHL; 4 had severe SNHL; 12 had profound SNHL in which one

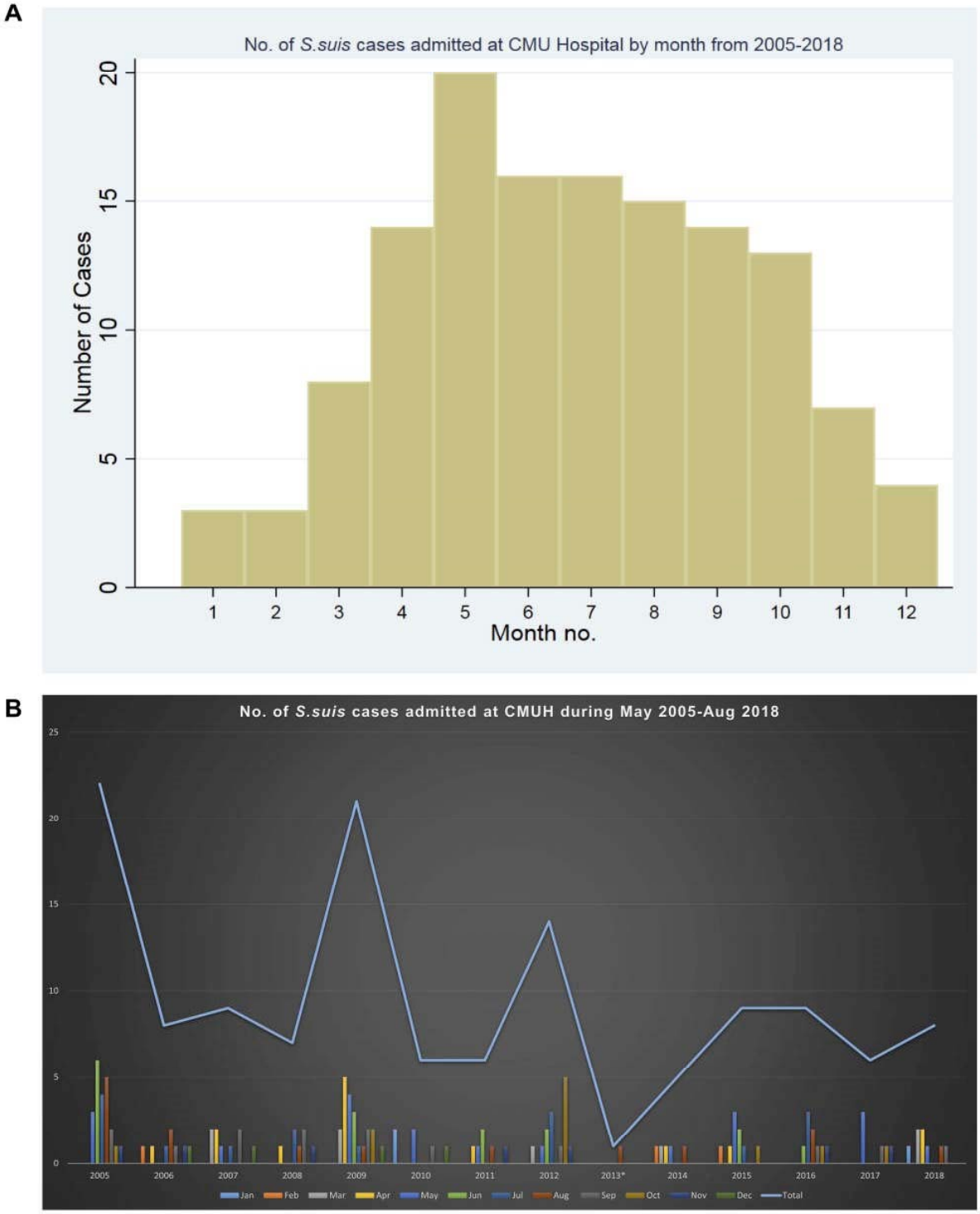

Figure 3 (**A** and **B**) Number of *S. suis* cases admitted at CMUH by month from 2005–2018.

Table 2 Clinical Manifestations and Outcomes

Characteristics	Total (n=133)
Signs and symptoms	
• Diarrhea	37 (27.82%)
• Vomiting	27 (20.03%)
• Vertigo	10 (7.52%)
• Neck stiffness	47 (35.34%)
Major clinical manifestation	
• Acute meningitis	50 (37.59%)
• Septicemia	74 (55.64%)
• Septic shock	20 (15.04%)
• IE	34 (25.56%)
• Aortic valve	26 (19.55%)
• Mitral valve	19 (14.29%)
• Tricuspid valve	4 (3.01%)
• Pulmonary valve	2 (1.50%)
• Aortic, mitral	3 (2.26%)
• Tricuspid, mitral	1 (0.75%)
• Aortic, tricuspid, mitral	1 (0.75%)
• Arthritis	9 (6.77%)
• Infective spondylodiscitis	12 (9.02%)
Complications	
• Any hearing loss	42 (31.58%)
• Mild SNHL	13 (9.77%)
• Moderate SNHL	7 (5.26%)
• Severe SHNL	4 (3.01%)
• Profound SNHL	12 (9.02%)
• Valve replacement	20 (15.04%)
• Vestibular dysfunction	30 (22.56%)
• Endophthalmitis	2 (1.50%)
Outcomes	
• Recovered	70 (52.73%)
• Recovered with sequelae	44 (33.08%)
• Not recovered	1 (0.75%)
• Death	16 (12.03%)
Mean duration of admission (days)	18.18 ± 17.09
Main antibiotic regimen use	
• Ceftriaxone	47 (36%)
• Ceftriaxone+Others	49 (37%)
• PGS/Ampicillin	10 (7%)
• PGS/Ampicillin+Others	5 (4%)
• Others	21 (16%)
Received dexamethasone	27 (20.30%)
• Time to microbiological cure (days) ±SD	8.82 ± 14.04

Note: Treatment and outcomes were unavailable in 1 patient.
Abbreviations: IE, Infective endocarditis; SNHL, Sensorineural hearing loss; PGS, Penicillin G, Others include vancomycin in combination with gentamicin and levofloxacin, cefotaxime in combination with vancomycin or metronidazole, ciprofloxacin, clindamycin, and doxycycline.

patient also developed Bell's palsy while the others had no information specified concerning the degree of hearing impairment. There were 30 (22.56%) and 2 (1.5%) patients affected by vestibular dysfunction and endophthalmitis respectively. One patient who experienced both meningitis and septicemia also developed Bell's palsy on the left side. The mean duration of admission was about 18 days.

Treatments

The empirical antibiotics prescribed varied, including gentamicin, ciprofloxacin, ceftriaxone, and penicillin antibiotics. The main antibiotic regimens prescribed were ceftriaxone, followed by ceftriaxone in combination with ampicillin. Other regimens included vancomycin in combination with gentamicin and levofloxacin, cefotaxime in combination with vancomycin or metronidazole, ciprofloxacin, clindamycin, and doxycycline. Ceftriaxone as adjunct with dexamethasone was used in 12 patients (9.4%), whereas Penicillin G (PGS) or ampicillin was administered in 10 patients (7%). Only 27 (20.3%) patients received adjunctive dexamethasone (Table 2 and Figure 4). The average time to microbiological cure was around 9 days. The minimum inhibitory concentration (MIC) of penicillin and ceftriaxone was carried out in 57 and 53 patients respectively. *S. suis* isolates were generally susceptible to penicillin and ceftriaxone. The mean MIC for penicillin and ceftriaxone were 0.16 µg/mL and 0.24 µg/mL respectively (Table 3).

Potential Risk Factors for Mortality

The estimated odds ratios (ORs) for potential risk factors of *S. suis* fatality among deaths vs survivors were presented in Table 5. From the univariate analysis, independent risk factors of mortality included GCS score < 8, SAPS II score > 50, time to microbiological cure ≥ 6 days, ALD, presence of acute meningitis, septic shock, total bilirubin > 2.5 mg/dL, direct bilirubin > 1.5 mg/dL, BUN > 28 mg/dL, creatinine > 1.8 mg/dL, bicarbonate < 18 mmol/, albumin < 3.5 g/dL (Table 4).

In the multivariate analysis, the remaining risk factors for mortality were time to microbiological cure ≥ 6 days, septic shock, and direct bilirubin > 1.5 mg/dL (Table 5). Forward and backward stepwise multivariate logistic regression was also performed. According to the analyses, significant risk factors associated with *S. suis* mortality identified were similar, with low albumin level below 3.5 g/dL as addition. (Table S2 shows significant predictors of *S. suis* mortality).

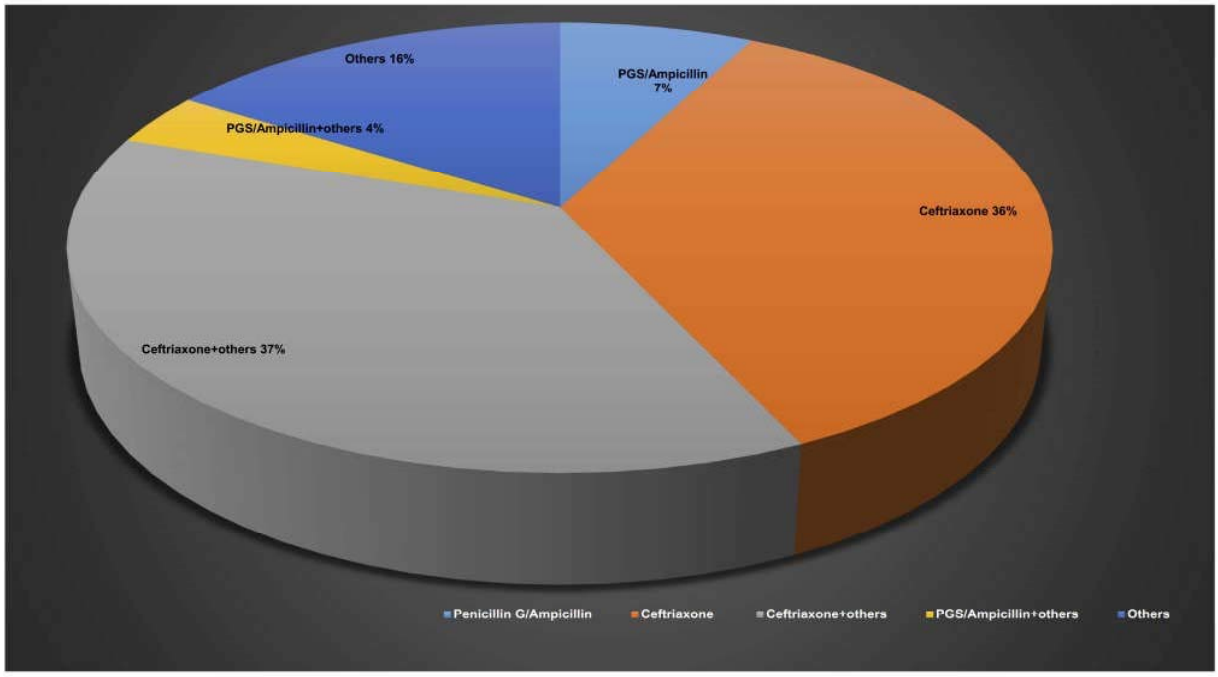

Figure 4 Main antibiotic therapy use.

Discussion

In this 13-year retrospective cohort study, we identified a number of potential risk factors for *S. suis* mortality. The remaining prognostic indicators from multivariate analysis were time to microbiological cure ≥ 6 days, septic shock, and direct bilirubin > 1.5 mg/dL. The risk factors identified are straight-forward in terms of clinical explanations.

Mortality from 72 hrs to first ten days could be a reliable indicator from antimicrobial treatment failure.[16] Observations of patients whether they received effective antibiotic treatment have shown relatively lower mortality during that interval.[17]

Hyperbilirubinemia reflects impact from liver damage which could be potentially caused by systemic infection.[18,19] This is consistent with other clinical symptoms including febrile illnesses, elevated liver enzymes, and low albumin levels caused by septicemia.

Hypoalbuminemia could potentially be a predictor of *S. suis* mortality. Although the association was not statistically significant in the full model analysis, the p-value was very near significant level (0.054), which may be due to limited sample size. Nevertheless, in forward and backward stepwise multivariate logistic regression, low serum albumin level <3.5 g/dL was significantly associated with *S. suis* mortality.

The findings were generally consistent with the previous studies.[5,11,12] Septic shock was a major risk factor of mortality in both our study and two previous series.[5,12] This clinical event could result in liver dysfunction through hemodynamic changes contributing to elevated serum creatinine, bilirubin and liver enzymes.[20] Consistently, this variable was also a significant predictor of *S. suis* mortality in two previous studies.[11,12] Early diagnosis, prompt antibiotic therapy, close patient monitoring regarding these risk parameters, and supportive fluid resuscitation are essential to improve clinical outcomes. Daily physical examination and serial microbiological work-up may be necessary in non-responsive cases.

A history of raw pork consumption was recognized in more than one third of included patients despite the fact of retrospective nature, which is in contrast with the previous study in the same setting whereby this correlation could not be demonstrated.[11] Interestingly, a history of alcohol drinking was documented in nearly half of all *S. suis* cases. This reflects the northern Thai culture involving *"Larb Lu"* a famous northern raw pork dish flecked with herb and chili consumption together with alcohol drinking during social events/gatherings. Although alcohol drinking is not a risk factor, heavy drinking and alcoholic liver disease could potentially result in immunocompromised condition which makes patients more susceptible to infection. Skin injury, pig-related occupation, recent exposure to pigs/raw pork were documented only in small percentages of patients.

Table 3 Laboratory Investigations

Characteristics	Total
Total bilirubin (0.00–1.20 mg/dL) (n=113)	1.78 ± 3.33
Direct bilirubin (>,=0.30mg/dL) (n=113)	0.89 ± 2.01
WBCs count (cells/cu.mm) (5000–10,000) (n=129)	19,897.21 ± 37,555.65
Total protein (6.6–8.7g/dL) (n=113)	6.65 ± 0.94
Platelet count (cells/cu.mm) (n=130)	221,983 ± 143,810
Albumin (3.2–5.2 g/dL) (n=113)	3.12 ± 0.59
Globulin (3.1–3.5 g/dL) (n=113)	3.53 ± 0.80
BUN (6–20 mg/dL) (n=129)	25.64 ± 22.71
AST (0–40 U/L) (n=113)	100.31 ± 161.64
ALT (0–41 U/L) (n=113)	62.23 ± 66.93
Hb (g/dL) (10–15) (n=130)	11.58 ± 2.58
Hct (%) (40–50) (n=130)	35.20 ± 7.876
Microbiological results: • Mean MIC to penicillin (μg/mL) ±SD • Mean MIC to ceftriaxone (μg/mL) ±SD	 0.16 ± 0.16 0.24 ± 0.27

Abbreviations: WBC, white blood cells; BUN, Blood Urea Nitrogen; AST, Aspartate Aminotransferase; ALT, Alanine Aminotransferase; Hb, Hemoglobin; Hct, Hematocrit; MIC, Minimal Inhibitory Concentration.

This is different from the reports from Western countries that showed *S. suis* is a disease among occupations involving swine contact.[3,4,21]

Similar to previous studies,[2,3,5,7,8,11,12,22–34] most *S. suis* patients were middle-aged men. The rarity of the disease in children was probably due to the lack of exposure to risk factors in acquiring the infection in pediatric population. In our study, there was only one pediatric case with medical history of congenital rheumatic heart disease without any predisposing risk factors. The underlying disease would probably make the patient more susceptible to infection. The frequent occurrence of the disease in summer and rainy season is consistent with previous findings[2,5,6,8,11,23,35–37] that these weather conditions precipitate the pathogen's infectivity and proliferation.[35] A relatively high number of cases in April may be related to Songkran's festival or Thai New Year which is one of the biggest social events among locals. The overall mortality rate (12%) was comparatively lower than findings from previous studies (19%).[11,12] The relatively low mortality rate in this study might be explained by the generally good health status (low SAPS II score) of included patients during admission.

Discrepancies regarding clinical manifestations were noted among studies despite the fact that they were conducted within the same region. In the study by Wangkaew et al (2006),[11] IE was the most common clinical manifestation while only 8% of IE cases were found in another hospital setting in the lower northern region of Thailand.[12] According to our results, the most common clinical presentations found were septicemia, meningitis, diarrhea, and IE. The observation is still similar to previous reports in terms of spectrum of presentations in which meningitis and septicemia were still among the most prominent. Septicemia was usually found concomitantly with either meningitis or IE and one of the major causes of complications including septic shock, multi-organ failure and DIC (Disseminated Intravascular Coagulation). This indicates that supportive treatment is also important apart from timely and adequate antibiotic treatment. The relatively high proportion of patients with diarrhea symptoms suggests an oral route transmission which is consistent with the considerably high number of raw pork and alcohol consumption.

The most common underlying diseases were VHD, spondylodiscites, DM, SLE, and ALD in which ALD was also a significant risk factor of disease mortality in the univariate analysis. These underlying medical conditions could lead to immunocompromised conditions and make patients more susceptible to infection. The fact that nearly all patients with IE were found with history of VHD may suggest the association of IE with this underlying condition.

To the best of our knowledge, this 13-year retrospective cohort study was probably the largest series focusing on risk factors of *S. suis* mortality and clinical outcomes conducted in Thailand. The longer duration and higher number of included cases enabled us to demonstrate several significant factors of the disease fatality and to see a better clinical picture of this neglected life-threatening infection. The inclusion of all *S. suis* culture-proven positive patients regardless of their ages, unlike previous studies which usually included only adult population,[11,12,22] may provide more insights on the burden of the disease. In addition, some significant risk factors of mortality were also confirmed in the multivariate analyses which could not be demonstrated in the previous studies due to small sample size. Nonetheless, there are some limitations to be noted. The number of cases included may not represent the true incidence within the region due to the retrospective nature which could potentially lead to missing or incomplete data. As only admitted patients with available medical records were included, outpatients or few patients

Table 4 Clinical Characteristics of *S. Suis* Infected Patients for Mortality

Characteristics	Total	Died (n=16, 12.03%)		Survived (n=117, 87.97%)		OR	95% CI	p-value
		n	%	n	%			
Age (years) (mean±SD)	56.47± 13.68	58.25±15.61	NA	56.23±13.46	NA	1.01	0.97–1.05	0.578
Male sex	92 (69.17%)	11	68.75	81	69.23	0.98	0.32–3.02	0.969
GCS < 8	NA	9.92± 3.95	NA	13.06± 2.83	NA	9.42	2.08–42.55	0.006
SAPS II > 50	NA	46.56± 17.64	NA	24.34±10.87	NA	21.97	5.40–89.42	<0.001
Time to microbiological cure ≥ 6 days	NA	6.33± 3.44	NA	8.96± 14.39	NA	6.21	1.35–28.55	0.005
Underlying disease								
• VHD	44 (33.08%)	5	11.36	39	88.64	0.91	0.30–2.80	0.868
• ALD	16 (12.03%)	5	31.25	11	68.75	4.38	1.29–14.93	0.026
• DM	26 (19.55%)	4	15.38	22	84.62	1.43	0.42–4.89	0.568
Major clinical manifestations								
• Acute meningitis	50 (37.59%)	2	4	48	96	0.21	0.04–0.95	0.018
• Septicemia	74 (55.64%)	12	16.22	62	83.78	2.66	0.81–8.73	0.088
• Septic shock	20 (15.27%)	9	45.00	11	55.00	12.39	3.86–39.79	<0.001
• IE	34 (25.56%)	3	8.82	31	91.18	0.64	0.17–2.40	0.493
Received dexamethasone	27 (20.30%)	1	3.740	26	96.30	0.23	0.03–1.85	0.096
Total bilirubin >2.5 mg/dL	1.78±3.33	4.90±8.19	NA	1.30±1.17	NA	3.90	1.33–11.41	0.013
Direct bilirubin >1.5mg/dL	0.89±2.01	2.74±4.89	NA	0.60±0.76	NA	5.30	1.77–15.88	0.002
WBC count (cells/cu.mm)	19,897±37,555	15,117± 8512.37	NA	20,574±39,981	NA	1.0	1.00–1.00	0.472
AST >300 U/L	100.31± 161.64	179.53±252.09	NA	88.18±140.98	NA	2.0	0.67–6.01	0.228
ALT >300 U/L	62.23± 66.93	84.33± 110	NA	58.84±57.78	NA	1.44	0.42–4.89	0.568
BUN >28 mg/dL	25.64± 22.71	38.25± 42.83	NA	23.86±17.83	NA	4.24	1.43–12.61	0.008
Creatinine > 1.8 mg/dL	1.80± 2.50	3.01± 4.97	NA	1.63± 1.90	NA	4.73	1.60–13.96	0.005
Bicarbonate < 18 mmol/L	21.44± 4.39	18.81± 5.86	NA	21.82±4.03	NA	4.91	1.60–15.04	0.007
Phosphorus > 4.5 mg/dL	3.75± 1.90	5.27± 3.00	NA	3.48± 1.50	NA	1.91	0.67–5.50	0.225
Albumin < 3.5 g/dL	3.12± 0.59	2.75± 0.53	NA	3.17± 0.58	NA	5.04	1.10–23.21	0.015
Globulin > 4 g/dL	3.53± 0.80	3.99± 0.87	NA	3.46± 0.76	NA	2.48	0.86–7.14	0.092

Table 5 Significant Predictors of *S. Suis* Mortality

Predictors	Univariate Analysis			Multivariable Analysis		
	OR	95% CI	p-value	Adjusted OR	95% CI	p-value
GCS <8	9.42	2.08–42.55	0.0058	1.71	0.10–28.50	0.709
Time to microbiological cure ≥ 6 days	6.21	1.35–28.55	0.0052	**43.57**	**2.46–772.80**	**0.010**
ALD	4.38	1.29–14.93	0.0262	2.24	0.32–15.84	0.417
Acute meningitis	0.21	0.045–0.95	0.0176	0.24	0.03–2.33	0.236
Septic shock	12.39	3.86–39.79	<0.001	**13.34**	**1.63–109.03**	**0.016**
Direct bilirubin >1.5 mg/dL	5.30	1.77–15.88	0.0024	**12.86**	**1.91–86.59**	**0.009**
Creatinine >1.8 mg/dL	4.73	1.60–13.96	0.0050	0.41	0.01–6.49	0.414
Bicarbonate <18 mmol/L	4.91	1.60–15.04	0.0073	3.00	0.12–73.62	0.500
Albumin <3.5 g/dL	5.04	1.10–23.21	0.0149	10.97	0.96–125.81	0.054

Note: All significant predictors, their 95% confidence intervals and p-value are indicated in bold.

whose medical data could not be located or had been destroyed were not accounted for in the findings. However, this number was quite small and should not have affected the results. *S. suis* is a generally serious and life-threatening infection which requires hospitalization. Therefore, majority of *S. suis* cases should have been captured in our study. The

unavailability of *S. suis* serotyping at the hospital made it impossible to gather the genotypic profile, yet it could be assumed that most infections should be from serotype 2 based on the previous genotyping study.[37,38] In addition, the selection of only positive hemoculture or CSF cases may have led to us missing some clinical presentations such as ecchymosis, petechiae and other skin abnormalities. However, we believe that this selection bias would indicate positive cases from true infection. Finally, the uniqueness of local traditional culture of raw pork consumption in northern Thailand may have affected the findings regarding mortality and outcomes of *S. suis* infection in this setting. Therefore, the findings may not be applicable in the settings where there is no existence of this eating behavior.

Whereas *S. suis* is considered to be uncommon and an occupational hazard in the Western region, this disease is considerably common in Asian countries, especially in Southeast Asia. This warrants the need for continuous educational or public health awareness programs for sustainable results. The incidence is largely under-reported and serotyping detection is usually not routinely available in hospital settings. Development of surveillance and strengthening of laboratory networks are essential to monitor the extent of the current disease situation. The clinical risk factors identified from this study may be further utilized in future research, including a mortality risk score development to improve patient outcomes.

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Author Contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

Disclosure

All authors declare no conflict of interest in this work.

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Supplementary appendix

Clinical manifestations and risk factors of *Streptococcus suis* mortality among northern Thai population: retrospective 13-year cohort study

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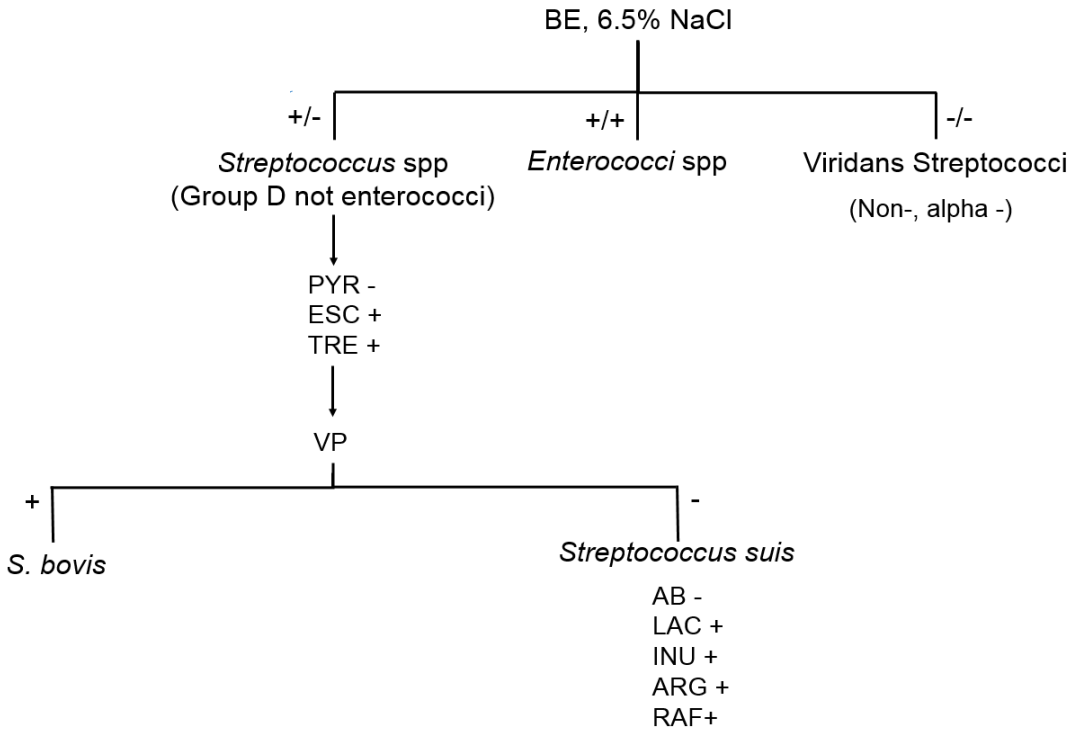
Table S1 List of Northern provinces of Thailand as of 2018 (National Statistics Office (NSO). [cited 2019 22 July]; Available from: http://www.nso.go.th/sites/2014/nsopublic)

Upper Northern Thailand	Lower Northern Thailand
Chiang Rai	Tak
Chiang Mai	Kamphaeng Phet
Nan	Nakhon Sawan
Phayao	Phetchabun
Phrae	Phichit
Mae Hong Son	Phitsanulok
Lumphun	Sukhothai
Lampang	Uthai Thani
Uttaradit	

Figure S1 Biochemical test protocol for identification of *Streptococcus suis* strains



Streptococcus suis strains is alpha-hemolysis in sheep blood agar with no growth in broth with 6.5% NaCl and positive in esculine, trehalose reactions, negative in Voges-Proskauer, pyrrolidonyl arylamidase with following biochemical characteristics: acid fermentation of lactose, inulin, raffinose broth, hydrolysis of L-arginine, no acid fermentation of arabinose.

Abbreviations: BE, bile esculin, PYR, pyrrolidonyl arylamidase, ESC, esculin, TRE, D-Trehalose, VP, Voges-Proskauer, AB, arabinose, LAC, D-Lactose, INU, inulin, ARG, L-arginine, RAF, D-raffinose

Table S2 Significant predictors of *S. suis* mortality

Predictors	Multivariable analysis			Multivariable analysis		
	Adjusted OR	95% CI	p-value	Adjusted[a] OR	95% CI	p-value
GCS <8	1.71	0.10-28.50	0.709	-	-	-
Time to microbiological cure > 6 days	**43.57**	2.46-772.80	**0.010**	**33.19**	**2.82-386.10**	**0.005**
ALD	2.24	0.32-15.84	0.417	-	-	-
Acute meningitis	0.24	0.03-2.33	0.236	-	-	-
Septic shock	**13.34**	1.63-109.03	**0.016**	**13.61**	**2.57-72.00**	**0.002**
Direct bilirubin >1.5 mg/dl	**12.86**	1.91-86.59	**0.009**	**17.06**	**2.73-106.47**	**0.002**
Creatinine >1.8 mg/dl	0.41	0.01-6.49	0.414	-	-	-
Bicarbonate <18 mmol/L	3.00	0.12-73.62	0.500	-	-	-
Albumin <3.5 g/dl	10.97	0.96-125.81	0.054	**23.46**	**2.44-225.92**	**0.006**

Note: Significant predictors were indicated in bold.
[a] Forward and backward stepwise logistic regression with p-value <0.05

Chapter 5:

A risk scoring system for predicting *Streptococcus suis* hearing loss

5.1 Summary of Chapter 5

Meningitis is the most common clinical manifestation from *S.suis* infection in which sensorineural hearing loss (SNHL) is the most common and debilitating complication among surviving patients *(5)*. This complication is usually irreversible despite adequate treatment upon occurrence of any hearing loss sign and symptom. This may cause significant physical and emotional distress as well as economic constraint to the nation considering that the infection is mainly among working age men.

According to prior studies, the most likely underlying mechanism of hearing loss is *suppurative labytinthitis* caused by bacterial invasion in the inner ear through cochlear aqueduct *(90, 91)*. This process was found to evolve at the acute stage of meningitis and could progress to permanent hearing loss if meningitis had not been promptly treated sufficiently *(91)*. In the 13-year retrospective cohort study we conducted, hearing loss was the most common complication accounting for nearly one-third of *S.suis* patients (N=42, 31.58%) *(32)*. This sequela was usually permanent and mainly bilateral. The audiometry screening was not routinely performed until patients' compliant which may be too late to prevent or reverse the significant deterioration of hearing loss disorder *(32)*. To our knowledge, a prediction model on *S.suis* hearing loss has not been developed. Therefore, the objective of this chapter is to develop an easy-to-use risk score to promote early diagnosis and detection of *S.suis* infected patients who are prone to hearing loss in primary settings.

A risk scoring system to predict the risk of hearing loss among *S.suis* infected patients with the ultimate aim to aid clinicians in identifying patients at high risk in primary settings was developed. Univariate and multivariate logistic regressions were carried out to develop a parsimonious predictive model. A simple clinical scoring system was derived from the coefficients of significant predictors. Area under the receiver operator characteristic curve (AuROC) was identified to confirm the model discriminative ability. Bootstrap resampling technique with 1000-fold bootstrapping was employed for internal validation. Among 133 patients, the incidence of hearing loss was 31.6% (n=42). Significant predictors for *S. suis* hearing loss in the final predictive model were meningitis, raw pork consumption, and vertigo. The predictive score ranged from 0-4 and correctly classified 81.95% patients as being at risk of *S.suis* hearing loss. The model showed good prediction power (AuROC: 0.859; 95%CI 0.785-0.933). The mean

AuROC from internal bootstrap was 0.860 (95%CI 0.716-0.953) suggesting good calibration.

Overall, it is an important study into the area of prognosis and outcomes of *S.suis* infection in trying to develop a tool to support clinicians in managing the disease and the impact of its sequelae in surviving patients. The strength of the study is a large sample size compared to other previous studies on *S.suis* infection in human *(33, 55)*. Although the result suggested good power of prediction and good calibration, generalisability should be done with caution and future research to validate this clinical risk score developed is needed.

5.2 Development and validation of *S.suis* hearing loss predictive model

5.2.1 Introduction

Clinical prediction rule (CPR) has been increasing applied to complement clinical decisions in modern medicine *(92, 93)*. The aim is to estimate the probability of occurrence of interested events/outcomes in particular individuals/patients using their demographics and clinical characteristics which should be measurable and available in routine practice. Three or more predictors are normally included in CPRs *(94)* to assist clinicians' decision-making in managing the disease and improving patient outcomes. The ideal CPR should be simple and easy to be applied in routine practice as well as able to accurately classify potentially diseased patients from non-disease patients.

In this context, we developed an easy-to-use CPRs or risk score to predict the risk of *S.suis* hearing loss using the data from a 13-year retrospective cohort study (Chapter 4) which is real-world evidence (RWE) and more generalisable compared to randomised controlled trials (RCTs). The seven elements employed in developing the prediction model include: (1) model derivation and potential variables examination; (2) predictors coding/categorization; (3) selection of predictors/model specification; (4) scoring system creation/model estimation; (5) assessing model performance; (6) model validation and; (7) model presentation *(95, 96)*. STATA 14.2 (College Station, Texas, USA) was used in all statistical analyses. Each step of model development was summarised as follows except the final step: (7) model presentation and its

application which should be considered for future research after evaluating its external validity.

5.2.2 Model development

The development phase aims to derive parsimonious model that can classify patients who potentially develop *S.suis* hearing loss from non-hearing loss patients. Patient demographics and clinical characteristics were initially analysed descriptively to identify variables associated with an increased risk of *S.suis* hearing loss.

1) Model derivation and potential variables examination

Univariate analysis was performed to compare potential predictors between hearing loss and non-hearing loss patients. For continuous variables, Student's t test was used in case of normal distribution whereas Mann-Whitney-Wilcoxon test was employed if the data is not normally distributed. Chi-square test or Fisher's exact test was used for categorical variables. We performed both complete case analysis and multiple imputation using predictive mean matching with 20 iterations for missing Glasgow Coma Scale (GCS) values *(97)*.

2) Predictors coding/categorization

Variables are defined as risk factors associated with an increased risk of *S.suis* hearing loss. Continuous variables were categorized into dichotomous variables to simplify application. The cut point for creatinine was 2.0 mg/dL which was around 1.5 to 2 times compared to the mean value among *S.suis* hearing loss cases whereas 4.0 mmol/L was the cut-off point for serum potassium.

Logistic regression among 133 patients, in which 42 patients experienced sensorineural hearing loss (SNHL) based on audiometry screening suggested an association between *S.suis* hearing loss with raw pork consumption ($p<0.001$), valvular heart disease (VHD) ($p=0.009$), alcoholic liver disease (ALD) ($p=0.093$), acute meningitis ($p<0.001$), neck stiffness ($p<0.001$), infective endocarditis (IE) ($p=0.005$), vomiting ($p=0.019$), vertigo ($p=0.011$) and lower level of serum creatinine ($p=0.018$) and potassium ($p=0.002$) (Table 5.1). Variables with p-value less or equal to 0.10 would be carried forward in multivariate analysis. Potential collinearity was also explored before building the predictive model. GCS variable remained a non-significant predictor in both complete case analysis and multiple imputation data (Table 5.2).

Table 5.1 Clinical characteristics of *S. suis* infected patients for hearing loss

Characteristics	Hearing loss (n=42) N (%)	Non-hearing loss (n=91) N (%)	p-value
Demographics:			
- **Age (year) (mean±SD)**	54.62±12.12	57.33±14.33	0.290
- **Male**	29 (69.05)	63 (69.23)	0.568
- **Raw pork consumption**	25 (59.05)	24 (26.37)	<0.001
- **Alcohol drinking**	25 (59.52)	41 (45.05)	0.138
Baseline characteristics:			
GCS †	13.09±2.28	12.42± 3.52	0.318
Microbiological results:			
- **Time to microbiological cure**	9.86±11.07	8.33±15.29	0.526
- **Mean MIC to penicillin (µg/mL) ‡**	0.14±0.18	0.17±0.15	0.106
- **Mean MIC to ceftriaxone (µg/mL) ‡‡**	0.14±0.10	0.29±0.31	0.388
Underlying disease			
- **Valvular heart disease**	7 (16.67)	37 (40.66)	0.009
- **ALD**	2 (4.76)	14 (15.38)	0.093
- **DM**	5 (11.90)	21 (23.08)	0.162
- **Spondylodiscites**	6 (14.29)	21 (23.08)	0.354
Major clinical manifestations			
- **Acute meningitis**	34 (80.95)	16 (17.58)	<0.001
- **Neck stiffness**	31 (73.81)	16 (17.58)	<0.001
- **Septicaemia**	23 (54.76)	51 (56.04)	1.000
- **IE**	4 (9.52)	30 (32.97)	0.005
- **Vomiting**	14 (33.33)	13 (14.29)	0.019
- **Vertigo**	7 (16.67)	3 (3.30)	0.011
Receiving steroids	22 (53.66)	1 (1.10)	0.600
Laboratory findings:			
- **CSF protein††**	277.12±220.25	341.39±256.93	0.419
- **CSF glucose‡‡**	31.69±9.70	30.72±21.55	0.173
- **Creatinine (mg/dl)**	1.18±0.75	2.09±2.94	0.018
- **Potassium (mmol/L)±**	3.52±0.43	3.93±0.77	0.002

ALD, Alcoholic liver disease; CSF, Cerebrospinal fluid; DM, Diabetes Mellitus; GCS, Glasgow coma scale; IE, Infective endocarditis; MIC, Minimal Inhibitory Concentration

† Data available in 101 patients; ‡ Data available in 57 patients; ‡‡ Data available in 53 patients; †† Data available in 52 patients; ± Data available in 127 patients

Table 5.2 Clinical characteristics of *S. suis* infected patients for hearing loss based on imputed GCS

Characteristics	Hearing loss (n=42) N (%)	Non-hearing loss (n=91) N (%)	p-value
Demographics:			
- **Age (year) (mean±SD)**	54.62±12.12	57.33±14.33	0.290
- **Male**	29 (69.05)	63 (69.23)	0.568
- **Raw pork consumption**	25 (59.05)	24 (26.37)	<0.001
- **Alcohol drinking**	25 (59.52)	41 (45.05)	0.138
Baseline characteristics:			
GCS † (Hearing loss n=41)	13.39±0.37	12.76±0.34	0.268
Microbiological results:			
- **Time to microbiological cure**	9.86±11.07	8.33±15.29	0.526
- **Mean MIC to penicillin (mcg/mL) ‡**	0.14±0.18	0.17±0.15	0.106
- **Mean MIC to ceftriaxone (mcg/mL) ‡‡**	0.14±0.10	0.29±0.31	0.388
Underlying disease			
- **Valvular heart disease**	7 (16.67)	37 (40.66)	0.009
- **ALD**	2 (4.76)	14 (15.38)	0.093
- **DM**	5 (11.90)	21 (23.08)	0.162
- **Spondylodiscites**	6 (14.29)	21 (23.08)	0.354
Major clinical manifestations			
- **Acute meningitis**	34 (80.95)	16 (17.58)	<0.001
- **Neck stiffness**	31 (73.81)	16 (17.58)	<0.001
- **Septicaemia**	23 (54.76)	51 (56.04)	1.000
- **IE**	4 (9.52)	30 (32.97)	0.005
- **Vomiting**	14 (33.33)	13 (14.29)	0.019
- **Vertigo**	7 (16.67)	3 (3.30)	0.011
Receiving steroids	22 (53.66)	1 (1.10)	0.600
Laboratory findings:			
- **CSF protein††**	277.12±220.25	341.39±256.93	0.419
- **CSF glucose‡‡**	31.69±9.70	30.72±21.55	0.173
- **Creatinine (mg/dl)**	1.18±0.75	2.09±2.94	0.018
- **Potassium (mmol/L)±**	3.52±0.43	3.93±0.77	0.002

ALD, Alcoholic liver disease; CSF, Cerebrospinal fluid; DM, Diabetes Mellitus; GCS, Glasgow coma scale; IE, Infective endocarditis; MIC, Minimal Inhibitory Concentration
† Data was imputed in 31/32 missing GCS values due to there was one missing parameter to estimate GCS in 1 patient.

3) Selection of predictors/model specification

Two predictors were removed after checking on collinearity including neck stiffness and VHD whereas eight predictors (raw pork consumption, ALD, acute meningitis, IE, vomiting, vertigo, a low level of serum creatinine and potassium) were included in the stepwise forward logistic regression which is a widely used selection method to reduce the number of candidate predictors *(95)*. Predictors that remained significant at p-value ≤ 0.10 were included in the final parsimonious model which were meningitis, raw pork consumption and vertigo.

4) Scoring system creation/model estimation

The final parsimonious model at significant level ≤ 0.1 which provided the optimal area under the receiver operating characteristic curve (AuROC) was selected. The item scores were generated by dividing significant coefficients with the smallest coefficients. The clinical predictive score ranged from 0-4. The risk of *S. suis* hearing loss increases as the score increases (Table 5.3). The score cut-off point was identified based on the score distribution into four quartile groups which reflects LR+ of 1, 2, 3.9 and 13.5. Patients were classified into four groups according to the risk of low, moderate, high and very high risk. The score cut-off point at 3 out of 4 produced the optimum sensitivity and specificity with a sensitivity of 80.95% and 79.12% respectively. The classification, sensitivity, specificity, LR+ and LR- were presented in Table 5.4. The findings imply that medical attention is already required among patients who have been classified at moderate to high risk with the cut-off score at 2 to 3 out of 4. Overall, 81.95% patients were correctly identified as being at risk or not being at risk of *S.suis* hearing loss.

Table 5.3 Derived item scores from multivariable logistic regression (n=133)

Predictors	OR[a]	95%CI	p-Value	ROC Area (95%CI)	Beta coefficient (intercept = -53.11)	Score
Meningitis						
No	1.00	reference	-		-	0
Yes	16.64	6.25-44.30	<0.001	0.82 (0.75-0.89)	2.812	2
Raw pork consumption						
No	1.00	reference	-		-	0
Yes	3.52	1.32-9.42	0.012	0.67 (0.58-0.75)	1.26	1
Vertigo						
No	1.00	reference	-		-	0
Yes	5.06	0.74-34.41	0.097	0.57 (0.51-0.63)	1.62	1

[a] Model for Multivariable logistic regression using backward stepwise logistic regression

Table 5.4 Risk stratification of prediction values of *S.suis* hearing loss score

Risk classification	Scores	Outcomes		Sensitivity (%)	Specificity (%)	Correctly classified (%)	LR+ (95%CI)	LR- (95%CI)
		Hearing loss	Non-hearing loss					
Low	1	18	39	100	0	31.58	1.00	0
Moderate	2	15	8	88.10	51.14	66.92	2.06	0.21
High	3	19	5	80.95	79.12	79.70	3.88	0.24
Very high	4	24	5	59.52	95.60	84.21	13.54	0.42

5) Assessing model performance

Measures for assessing model performance include calibration and discrimination *(95)*. Calibration suggests the closeness between predicted and observed values whereas discrimination or C statistics which is identical to the area under the receiver operating characteristic curve (AuROC) reflects the model ability to distinguish diseased from non-disease patients *(95)*. The calibration plot (Figure 5.1) and Hosmer-Lemeshow goodness-of-fit test indicated good model calibration (chi-square = 3.13, p-value 0.372) (Table 5.5). The scoring system indicated a good performance in *S.suis* hearing loss prediction (AuROC 0.87; 95% CI 0.80-0.95) (Figure 5.2).

Figure 5.1 Calibration plot between predicted vs. observed probability of *S.suis* hearing loss

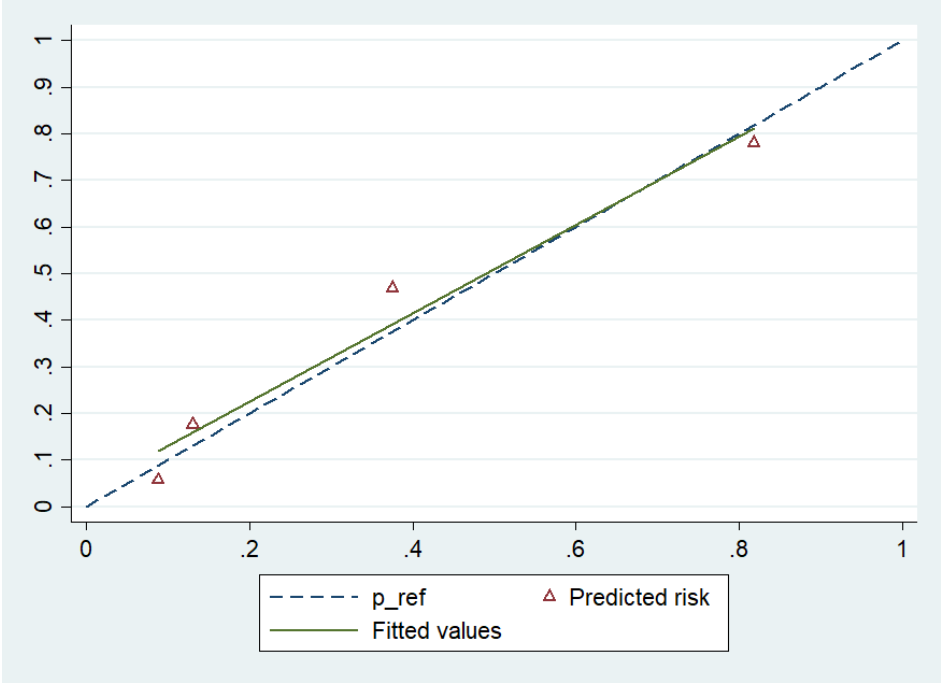
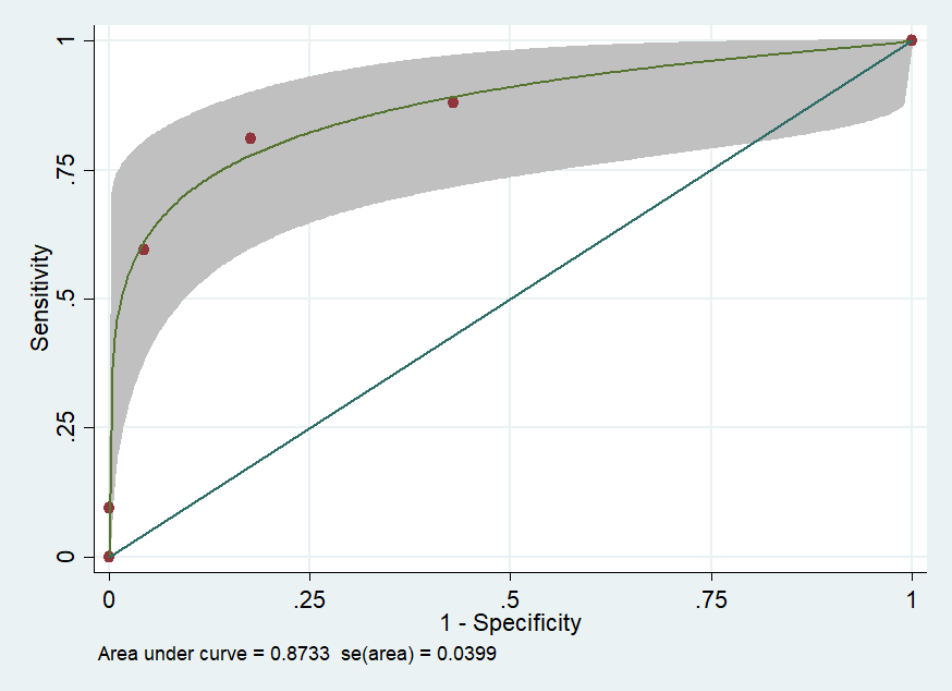


Table 5.5 Hosmer-Lemeshow good-ness-of-fit test

Group	Probabilities	Obs_1	Exp_1	Obs_0	Exp_0	Total
4	0.0571	5	3.3	52	53.7	57
6	0.1759	3	4.0	20	19.0	23
7	0.5018	9	11.2	15	12.8	24
9	0.7803	18	17.2	4	4.8	22
10	0.9473	7	6.3	0	0.7	7

Number of observations = 133
Number of groups = 5
Hosmer-Lemeshow chi2(3) = 3.13
Prob > chi2 = 0.3721

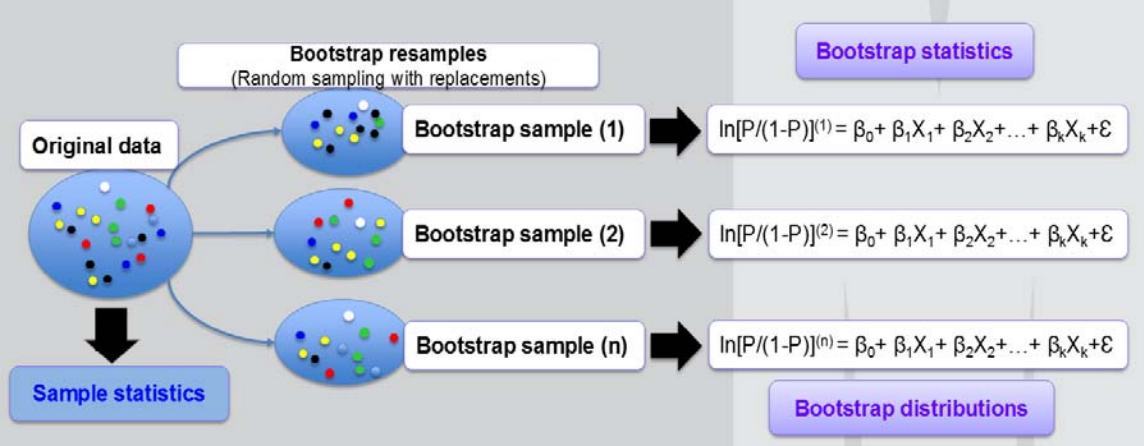
Figure 5.2 Area under the receiver-operator characteristic curve (AuROC) for the scoring system to predict *S.suis* hearing loss with 95% confidence interval.



6) Model Validation

Bootstrapping with 1000-fold was performed for internal validation. Bootstrapping is a resampling technique which is usually applied when there is a limited sample size with a small number of events *(98)*. The technique relies on random sampling with replacement from a single original data *(98)*. For each resampling a smaller sample which is called “bootstrapped” or “bootstrap sample” from the original data are repeatedly drawn with replacement *(99, 100)* (Figure 5.3).

Figure 5.3 Resampling method with replacement or bootstrap



For each resampling data set, a logistic regression would be constructed (bootstrap statistics) and calibration coefficient and C statistics would be estimated. With this regards, Somers' D which is a rank statistics for measurement of association for ordinal variables *(101)* was applied to assess the extent of bias in calibration. The correlation coefficients between observed and expected values in the original data set called "D origin" and each bootstrap called "D boot" or Somers' D coefficient were estimated. The difference between each two coefficients (D origin – D boot) or "optimism" were calculated in order to estimate the mean optimism (D_0) for the whole bootstrap replicates. The lesser the difference or closer to zero indicates low bias and good calibration in the model *(98)*.

The mean optimism (D_0) result from statistical analysis in STATA 14.2 was illustrated below as highlighted:

```
. list D_org bias_D bs_correctedD in 1

     +------------------------------+
     |   D_org      bias_D   bs_cor~D |
     |------------------------------|
  1. | .718472   -.0021508   .7206228 |
     +------------------------------+
```

According to the result, the Somer'D coefficient which is an estimated correlation between the observed and predicted value of all bootstrap data (D boot) and the

original data (D origin) were 0.721 (95%CI 0.432-0.905) and 0.718 (95%CI 0.570-0.867) respectively. The average bias or optimism was -0.0022 (95%CI -0.002 to -0.002) suggesting low bias and good calibration.

The same principle was applied for discrimination coefficient or C statistics. The difference between C statistics from the original data and each bootstrap or C_0 were calculated. Then mean C_0 and bias or corrected discrimination coefficient were estimated.

The statistical analysis results were as follows:

```
. sum bias* roc

    Variable |       Obs        Mean    Std. Dev.       Min        Max
-------------+--------------------------------------------------------
      bias_D |       998   -.0021508           0  -.0021508  -.0021508
   bias_roc |       998   -.0011114           0  -.0011114  -.0011114
         roc |     1,000    .8603114    .0381388   .7158921   .9526316

. sum bias_roc

    Variable |       Obs        Mean    Std. Dev.       Min        Max
-------------+--------------------------------------------------------
   bias_roc |       998   -.0011114           0  -.0011114  -.0011114
```

The average C statistic from bootstraps was 0.860 (95%CI 0.716-0.953) with estimated bias of -0.001 (95%CI -0.001 to -0.001) suggesting low discrimination bias and high precision of the model.

5.2.3 Conclusion

To our knowledge, this is the first risk scoring system development of *S.suis* hearing loss. The model showed good calibration and discrimination despite the lack of external validation. The clinical risk score developed might be useful to aid clinicians in identifying patients who are likely to develop hearing loss from *S.suis* infection after

evaluating its external validity. Future research to validate this risk score in other settings should be considered before application.

5.3 Publication associated with Chapter 5 (PLoS One, IF: 2.776)

Rayanakorn A, Katip W, Goh BH, Oberdorfer P, Lee LH. A risk scoring system for predicting *Streptococcus suis* hearing loss: A 13-year retrospective cohort study. PLOS ONE. 2020;15(2):e0228488. https://doi.org/10.1371/journal.pone.0228488

Figure 5.4 Striking still image for publication (raw pork dish)

PLOS ONE

RESEARCH ARTICLE

A risk scoring system for predicting *Streptococcus suis* hearing loss: A 13-year retrospective cohort study

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Abstract

Background

Streptococcus suis (S.suis) is an emerging zoonosis disease with a high prevalence in Southeast Asia. There are over 1,500 cases reported globally in which majority of cases are from Thailand followed by Vietnam. The disease leads to meningitis in human with sensorineural hearing loss (SNHL) as the most common complication suffered by the patients. Early diagnosis and treatment is important to prevent severe neurological complication. In this study, we aim to develop an easy-to-use risk score to promote early diagnosis and detection of *S.suis* in patients who potentially develop hearing loss.

Methods

Data from a retrospective review of 13-year *S.suis* patient records in a tertiary hospital in Chiang Mai, Northern, Thailand was obtained. Univariate and multivariate logistic regressions were employed to develop a predictive model. The clinical risk score was constructed from the coefficients of significant predictors. Area under the receiver operator characteristic curve (AuROC) was identified to verify the model discriminative performance. Bootstrap technique with 1000-fold bootstrapping was used for internal validation.

Key Results

Among 133 patients, the incidence of hearing loss was 31.6% (n = 42). Significant predictors for *S. suis* hearing loss were meningitis, raw pork consumption, and vertigo. The predictive score ranged from 0–4 and correctly classified 81.95% patients as being at risk of *S.suis* hearing loss. The model showed good power of prediction (AuROC: 0.859; 95%CI 0.785–0.933) and calibration (AuROC: 0.860; 95%CI 0.716–0.953).

Competing interests: The authors have declared that no competing interests exist.

Conclusions

To our best knowledge, this is the first risk scoring system development for *S.suis* hearing loss. We identified meningitis, raw pork consumption and vertigo as the main risk factors of *S.suis* hearing loss. Future studies are needed to optimize the developed scoring system and investigate its external validity before recommendation for use in clinical practice.

Introduction

Streptococcus suis (S.suis) is a gram-positive alpha-hemolytic bacterium mainly in pigs which can causes serious infection in human with a high prevalence in Southeast Asia. There were over 1,500 *S.suis* cases reported worldwide, with the highest incidence in Thailand (> 600 cases) [1, 2]. About two thirds of *S.suis* infected patients would develop meningitis in which sensorineural hearing loss (SNHL) is the most common complication among most survivors [2]. SNHL is usually irreversible despite adequate treatment upon the sign of hearing loss occurs. Majority of patients experience persistent hearing loss upon acquiring the infection which is mainly bilateral [3, 4]. This may cause physical and emotional distress as well as constraint the economics of a nation.

Streptococcus suis SNHL is poorly diagnosed because patients do not undergo audiometry screening until they complaint of severe hearing disorder. By then, the prognosis of the disease is severe with slim percentage of recovery. Prior animal experiments and electrophysiological studies in human revealed that the underlying mechanism of hearing loss is probably from bacterial invasion through cochlear aqueduct, which causes suppurative labyrinthitis [5, 6]. This inflammatory disorder in the inner ear has been identified as the responsible site of deafness and meningitis auditory lesion [6, 7]. SNHL was found to evolve at the early stage of meningitis and could progress to permanent hearing loss if meningitis had not been promptly treated appropriately [6]. Therefore, early diagnosis and immediate treatment are essential to reduce detrimental consequences mainly SNHL from *S.suis* infection. This study aims to develop an easy-to-use risk score to promote early diagnosis and detection of *S.suis* infected patients who are prone to hearing loss in primary settings.

Methods

Study design and setting

The data was collected as a part of a retrospective cohort study over 13-year period at Chiang Mai University Hospital (CMUH) [8], a 1400-bed tertiary teaching hospital. The hospital is the largest in northern, Thailand and the fourth largest hospital in the country where most of patients in northern region from 17 provinces are referred to for tertiary care (see S1 Table). Cultural eating habit of raw pork dishes and fermented raw pork is commonly practiced in northern, Thailand. This deep-rooted cultural eating behavior is a major route of the disease transmission and contributing factor of a high prevalence of the disease in this region.

Study population and data collection

S. suis positive cases were identified based on the microbiology laboratory data available and hospital numbers (HNs) admitted from May 2005 to December 2018. *S. suis* cases were confirmed by blood or cerebrospinal fluid (CSF). All confirmed *S.suis* cases with available medical records were included in this analysis (Fig 1) [8].

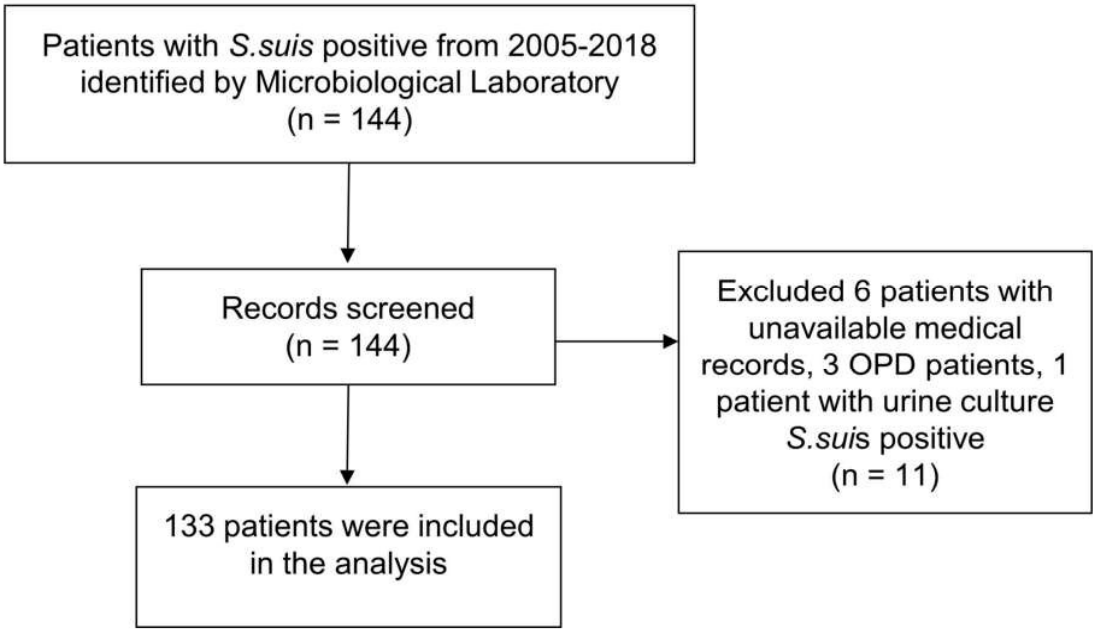

Fig 1. Patients identification and selection.

https://doi.org/10.1371/journal.pone.0228488.g001

The data collected included patient demographics, clinical characteristics and manifestations, outcomes and treatments. Patient data were collected and managed using REDCap electronic data capture tools hosted at Research Electronic Data Capture, Monash University Malaysia, a secure, web-based application designed to support data capture for research studies [9]. Patient information collected was anonymous and treated as confidential throughout the process of data collection and management.

Outcomes

S. suis meningitis was confirmed by cerebrospinal fluid (CSF) culture with compatible clinical presentation. An audiogram was used to diagnose and monitor the degree of hearing ability. The degree of hearing loss was assessed by otorhinolaryngologists based on individual patients' hearing thresholds in decibel (dB) at different frequencies. The level of hearing loss was classified into mild, moderate, severe and profound. Presbycusis or any pre-existing hearing loss would be excluded. Endocarditis and site of vegetation was assessed by echocardiography.

Ethical considerations

The study was approved by the Research Ethics Committee, Faculty of Medicine, Chiang Mai University (IRB no.010/2018) and Monash University Human Research Ethics Committee (MUHREC) (Project no.12225). Informed consent forms were not required in this retrospective review. The study reporting was in accordance to the Transparent Reporting of a Multivariable Prediction Model for Individual Prognosis or Diagnosis (TRIPOD) statement [10] (S1 TRIPOD Checklist).

Statistical analysis

Demographics and clinical characteristics were initially analyzed descriptively. Variables are defined as risk factors associated with an increased risk of *S.suis* hearing loss. Univariate

analysis was performed to compare potential predictors between hearing loss and non-hearing loss patients. For continuous variables, Student's t test was used in case of normal distribution whereas Mann-Whitney-Wilcoxon test was employed if the data is not normally distributed. Chi-square test or Fisher's exact test was used for categorical variables. We performed both complete case analysis and multiple imputation using predictive mean matching with 20 iterations for missing Glasgow Coma Scale (GCS) values [11]. Variables with p-value less or equal to 0.10 would be carried forward in multivariate analysis. Potential collinearity was also explored before building the predictive model. Forward step-wise logistic regression was utilized to identify significant predictors for *S.suis* hearing loss. Predictors that remained significant at p-value ≤ 0.10 would be included in the final parsimonious model.

Risk score development and internal validation

A clinical scoring system was developed by transforming regression coefficients into item scores. Each item score was generated by dividing with the smallest coefficient in the model and rounding the number to the closest integer [12]. The model discrimination was determined from the area under the receiver-operator characteristic curve (AuROC) or the concordance (C) statistics. An area under the curve (AUC) of 1.0 reflects a perfect discrimination performance whereas an AUC of 0.5 implies a completely no discriminative ability [13]. Calibration of the final model was tested using Hosmer-Lemeshow good-ness-of-fit test and the calibration plot portraying predicted vs. observed probability of *S.suis* hearing loss was illustrated. The risk stratification was done according to scores distribution into four quartile groups. The classification performances including sensitivity, specificity, positive (LR+) and negative likelihood ratios (LR-) were also calculated. A bootstrap with 1,000 replications technique was used for internal validation to correct for optimism [14]. All statistical analyses were done by STATA 14.2 (College Station, Texas, USA).

Results

One hundred and thirty-three patients with *S.suis* infection were included in this analysis, majority were males (67.2%). Table 1 summarizes the patient demographics and medical history. More than one-third of patients had a history of raw pork consumption and nearly half of the patients were regular alcohol drinkers. Valvular heart disease was the most common underlying disease followed by Diabetes Mellitus (DM) and spondylodiscites. A total of 42 patients (31.58%) experienced SNHL from *S.suis* infection in which 13 (9.77%) were mild SNHL, 7 (5.26%) were moderate SNHL, 4 (3.01%) were severe SNHL and 12 (9.02%) were profound SNHL. All SNHL still persisted based on audiometry upon discharge and latest follow-up visit up to December 2018.

Univariate analysis suggested association between *S.suis* hearing loss with raw pork consumption ($p<0.001$), valvular heart disease (VHD) ($p = 0.009$), alcoholic liver disease (ALD) ($p = 0.093$), acute meningitis ($p<0.001$), neck stiffness ($p<0.001$), infective endocarditis (IE) ($p = 0.005$), vomiting ($p = 0.019$), vertigo ($p = 0.011$) and lower level of serum creatinine ($p = 0.018$) and potassium ($p = 0.002$) (Table 2). Continuous variables were categorized into two groups to simplify application. For creatinine, the cut point was 2.0 mg/dL which was around 1.5 to 2 times compared to the mean value among *S.suis* hearing loss cases whereas 4.0 mmol/L was the cut-off point for serum potassium. GCS variable remained a non-significant predictor in both complete case analysis and multiple imputation data (S2 Table).

Two predictors were removed after checking on collinearity which were neck stiffness and VHD whereas eight predictors (raw pork consumption, ALD, acute meningitis, IE, vomiting, vertigo, a low level of serum creatinine and potassium) were included in the stepwise forward

Table 1. Patient characteristics.

Characteristics	Total (n = 133)
Age (year) (mean±SD)	56.47± 13.68
Male	92 (69.17%)
GCS	12.65± 3.15
Risk behaviors	
• Consumption of raw pork	49 (36.84%)
• Recent contact with pigs/pork exposure	5 (3.76%)
• Pig related occupation	3 (2.26%)
• Skin injury	2 (1.50%)
• Alcohol drinking	66 (49.62%)
• No	24 (18.05%)
Underlying diseases	
• DM	26 (19.55%)
• ALD	16 (12.03%)
• Splenectomy	2 (1.50%)
• Valvular heart disease	44 (33.08%)
• Cancer	2 (1.50%)
• Corticosteroid use	3 (2.26%)
• HIV/AIDS	2 (1.50%)
• Spondylodiscites	27 (20.30%)
• SLE	21 (15.79%)
Exposure to onset (days) † (mean±SD)	7.67± 11.66
Time from exposure to admission (days) (mean±SD) ‡	13.55± 19.21

GCS, Glasgow coma scale; DM, Diabetes Mellitus; ALD, Alcoholic liver disease; HIV, Human immunodeficiency virus infection; AIDS, Acquired immune deficiency syndrome; SLE, Systemic lupus erythematosus

Note

†Available data from 37 patients

‡ Available data from 40 patients

https://doi.org/10.1371/journal.pone.0228488.t001

logistic regression. There were three predictors remained in the model at p-value ≤ 0.1 which were meningitis, raw pork consumption and vertigo. The final parsimonious model at significant level ≤ 0.1 which provided optimal the area under the receiver operating characteristic curve (AuROC) was selected. The scoring system indicated a good performance in *S.suis* hearing loss prediction (AuROC 0.87; 95% CI 0.80–0.95) (Fig 2).

The C statistics or AuROC was 0.86 (95% CI 0.78–0.93) suggesting acceptable discrimination from *S.suis* hearing loss from non-hearing loss. The calibration plot and Hosmer-Lemeshow goodness-of-fit test indicated good model calibration (chi-square = 3.13, p-value 0.372), see S1 Fig and S3 Table.

The item scores were generated by dividing significant coefficients with the smallest coefficients. The clinical predictive score ranged from 0–4. The risk of *S. suis* hearing loss increases as the score increases (Table 3). The score cut-off point was identified based on the score distribution into four quartile groups which reflects LR+ of 1, 2, 3.9 and 13.5. Patients were classified into four groups according to the risk of low, moderate, high and very high risk. The score cut-off point at 3 out of 4 produced the optimum sensitivity and specificity with a sensitivity of 80.95% and 79.12% respectively. The classification, sensitivity, specificity, LR+ and LR- were presented in Table 4. Overall, 81.95% patients were correctly identified as being at risk or not being at risk of *S.suis* hearing loss.

Table 2. Clinical characteristics of *S. suis* infected patients for hearing loss.

Characteristics	Hearing loss (n = 42)	Non-hearing loss (n = 91)	p-value
	N (%)	N (%)	
Demographics:			
• Age (year) (mean±SD)	54.62±12.12	57.33±14.33	0.290
• Male	29 (69.05)	63 (69.23)	0.568
• Raw pork consumption	25 (59.05)	24 (26.37)	<0.001
• Alcohol drinking	25 (59.52)	41 (45.05)	0.138
Baseline characteristics:			
GCS †	13.09±2.28	12.42± 3.52	0.318
Microbiological results:			
• Time to microbiological cure	9.86±11.07	8.33±15.29	0.526
• Mean MIC to penicillin (μg/mL) ‡	0.14±0.18	0.17±0.15	0.106
• Mean MIC to ceftriaxone (μg/mL) ‡‡	0.14±0.10	0.29±0.31	0.388
Underlying disease			
• Valvular heart disease	7 (16.67)	37 (40.66)	0.009
• ALD	2 (4.76)	14 (15.38)	0.093
• DM	5 (11.90)	21 (23.08)	0.162
• Spondylodiscites	6 (14.29)	21 (23.08)	0.354
Major clinical manifestations			
• Acute meningitis	34 (80.95)	16 (17.58)	<0.001
• Neck stiffness	31 (73.81)	16 (17.58)	<0.001
• Septicaemia	23 (54.76)	51 (56.04)	1.000
• IE	4 (9.52)	30 (32.97)	0.005
• Vomiting	14 (33.33)	13 (14.29)	0.019
• Vertigo	7 (16.67)	3 (3.30)	0.011
Receiving steroids	22 (53.66)	1 (1.10)	0.600
Laboratory findings:			
• CSF protein††	277.12±220.25	341.39±256.93	0.419
• CSF glucose‡‡	31.69±9.70	30.72±21.55	0.173
• Creatinine (mg/dl)	1.18±0.75	2.09±2.94	0.018
• Potassium (mmol/L)±	3.52±0.43	3.93±0.77	0.002

ALD, Alcoholic liver disease; CSF, Cerebrospinal fluid; DM, Diabetes Mellitus; GCS, Glasgow coma scale; IE, Infective endocarditis; MIC, Minimal Inhibitory Concentration

† Data available in 101 patients

‡ Data available in 57 patients

‡‡ Data available in 53 patients

†† Data available in 52 patients

± Data available in 127 patients

https://doi.org/10.1371/journal.pone.0228488.t002

A bootstrap of 1000 sampling with replacement was performed for internal validation. The Somer'D coefficient which is an estimated correlation between the observed and predicted value of all boostrap data (D_{boot}) and the original data (D_{origin}) were 0.721 (95%CI 0.432–0.905) and 0.718 (95%CI 0.570–0.867) respectively. The average bias was -0.0022 (95%CI -0.002 to—0.002) indicating low bias and good calibration. The average C statistic from bootstraps was 0.860 (95%CI 0.716–0.953) with estimated bias of -0.001 (95%CI -0.001 to -0.001). The AuROC was 0.860 (95%CI 0.716–0.953).

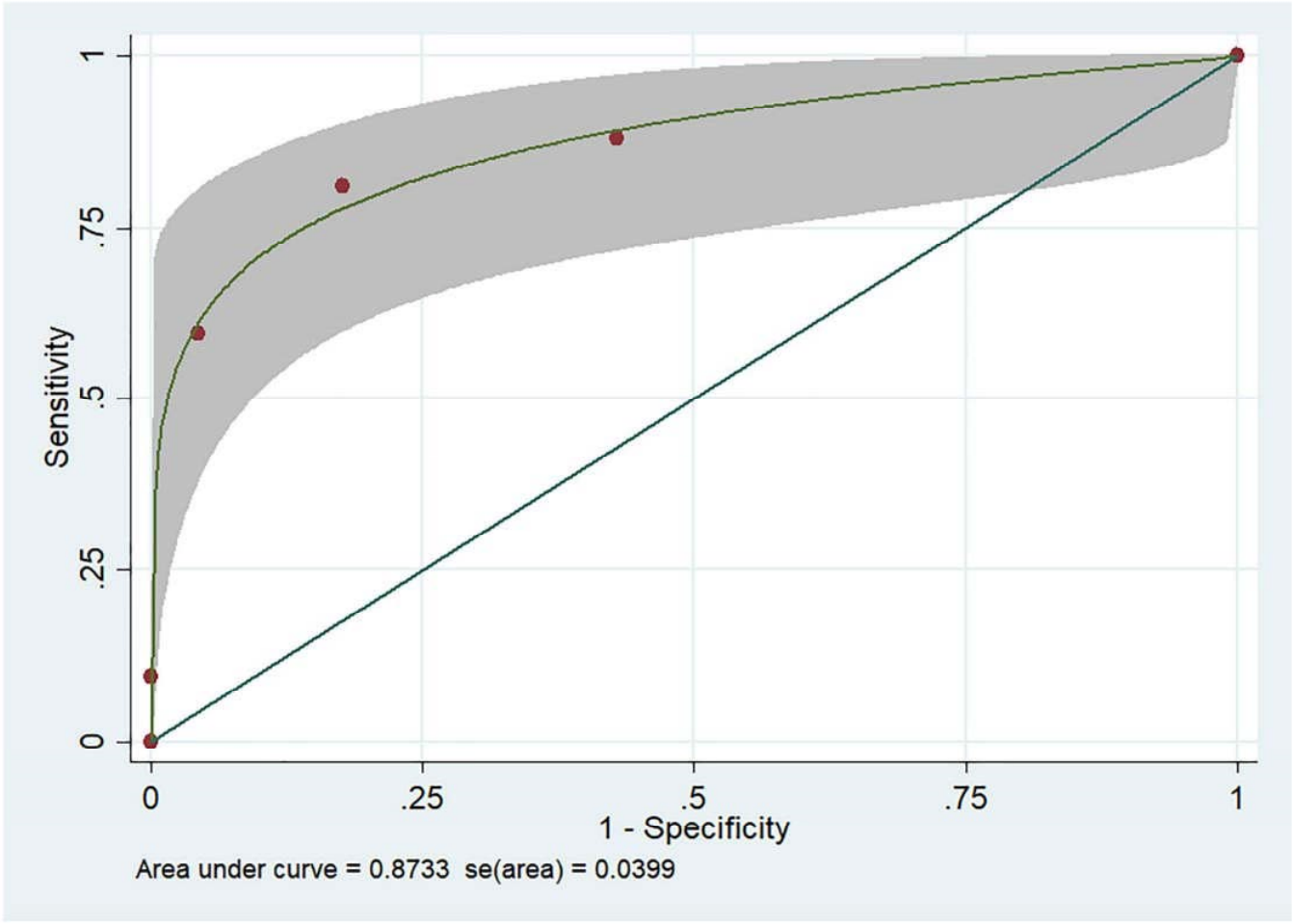

Fig 2. Area under the receiver-operator characteristic curve (AuROC) for the scoring system to predict *S.suis* hearing loss with 95% confidence interval.

https://doi.org/10.1371/journal.pone.0228488.g002

Table 3. Derived item scores from multivariable logistic regression (n = 133).

Predictors	OR[a]	95%CI	p-Value	ROC Area (95%CI)	Beta coefficient (intercept = -53.11)	Score
Meningitis						
No	1.00	reference	-		-	0
Yes	16.64	6.25–44.30	<0.001	0.82 (0.75–0.89)	2.812	2
Raw pork consumption						
No	1.00	reference	-		-	0
Yes	3.52	1.32–9.42	0.012	0.67 (0.58–0.75)	1.26	1
Vertigo						
No	1.00	reference	-		-	0
Yes	5.06	0.74–34.41	0.097	0.57 (0.51–0.63)	1.62	1

[a] Model for Multivariable logistic regression using backward stepwise logistic regression

https://doi.org/10.1371/journal.pone.0228488.t003

Table 4. Risk stratification of prediction values of *S.suis* hearing loss score.

Risk classification	Scores	Outcomes		Sensitivity (%)	Specificity (%)	Correctly classified (%)	LR+ (95%CI)	LR- (95%CI)
		Hearing loss	Non-hearing loss					
Low	1	18	39	100	0	31.58	1.00	0
Moderate	2	15	8	88.10	51.14	66.92	2.06	0.21
High	3	19	5	80.95	79.12	79.70	3.88	0.24
Very high	4	24	5	59.52	95.60	84.21	13.54	0.42

https://doi.org/10.1371/journal.pone.0228488.t004

Discussion

To our best knowledge, this is the first risk scoring system development for *S.suis* hearing loss. We identified meningitis, raw pork consumption and vertigo as the main risk factors of *S.suis* hearing loss. The data was derived from the real setting in routine practice upon admission at Chiang Mai University Hospital (CMUH). Usually these data are available and do not require any invasive laboratory procedure. After evaluating its external validity, this simple scoring system might be useful to assess patients with *S.suis* hearing loss in hospital and primary care settings.

The mechanism of SNHL which is a remarkable consequence from acquiring *S.suis* infection in up to 50% of cases is assumed to be caused by the direct infection at the cochlea. The pathogen invasion through the cochlea aqueduct is believed to be from its exotoxin lytic action [7]. Presence of vertigo could be attributed from vestibular damage which may result in vestibular dysfunction. The benefit of steroids in reducing the likelihood of hearing loss has been established in pediatric meningitis but remained uncertain in adult population [15]. From our observation, audiometry test was generally performed upon patients' compliant or presentation of hearing loss symptoms which may be too late to prevent the sequelae. Timely diagnosis and immediate antibiotic treatment before occurrence or sign of hearing loss symptoms are essential to prevent or reduce damage particularly SNHL caused by *S.suis* infection. This study is the first attempt to develop a risk score for predicting *S.suis* hearing loss as a tool to support clinicians in managing the disease and reduce the impact from its sequelae. The strength of the study is a reasonably large sample size of *S.suis* patients compared to previous studies [16, 17]. However, a number of limitations can be noted in our study. Missing data and recall bias may have arisen due to retrospective nature. Considering *S.suis* as a 'rare' disease, the data from over 13-year period was captured to get a high sample size. With this regards, the treatment and management may have changed over the years. However, audiometry was routinely used to diagnose SNHL at this setting throughout the whole study period and *S.suis* infection is still generally susceptible to common antibiotics such as penicillin and ceftriaxone. Therefore, this might not have substantially affected the results of the study. In addition, there were few predictors included in the model in which history of raw pork consumption may be potentially to be affected by a recall bias. As our main purpose was to develop a simple clinical prediction tool which is easy to be applied in primary setting, having these predictors (meningitis, raw pork consumption and vertigo) which are available upon patients' presentation should be practical to assist with initial detection of potential hearing loss cases. Raw pork consumption was known to be a potential factor in acquiring the disease whereas documentation on medical history of such cases was limited. However, we still could identify more than one-third of patients with documented medical history of raw pork consumption whereas there were many fewer cases with this medical history could be confirmed in the previous retrospective study at the same setting [16]. An extensive medical record review to capture all relevant information may have contributed to this finding. Nevertheless, as traditional culture involving raw pork

consumption is a well-known risk behavior among northern Thai population, doctors might have more likely asked patients about this risk behavior which might have been potentially subject to information bias. Therefore, generalizability of the finding should be done with caution. With limited study sample size at one tertiary hospital, the external validation of the model would not be possible. We contacted investigators who had previously conducted studies in *S.suis* patients for the purpose of external validation but we did not receive any response. According to the rule of thumb from a simulation study, the number of event per variable (EPV) of 10 or more was required to prevent bias in the regression coefficients [11]. In our study, there were 42 *S.suis* patients with hearing loss as convenience sample and there were three significant risk factors confirmed in the final model. Therefore, this should be considered to be sufficiently powered. Future studies to optimize such tool and investigation on its external validity should be mandated before implementation. However, robust statistical methods including the bootstrap of 1000 replications for internal validation were performed. The results demonstrated very close Somer's D coefficients and very low bias between original and bootstrap data indicating good model calibration. The benefits of corticosteroids use in preventing SNHL from *S.suis* dexamethasone with antibiotics could not be determined in our study due to a relatively few number of patients received adjuvant corticosteroids with antibiotics. Apart from that most adjuvant corticosteroids were administered among those with moderate to profound SNHL after clinical features of hearing loss presentation. Finally, it should be noted that the participants included in the study were mainly from Northern, Thailand where traditional raw pork eating is practiced. This may limit generalizability in other settings especially where raw pork consumption is uncommon.

In conclusion, after external validation, our simple clinical risk score developed might be useful to aid clinicians in identifying patients who are likely to develop hearing loss from *S.suis* infection. Although this tool cannot replace clinical judgement, physicians can look upon these clinical characteristics in patients (meningitis, raw pork consumption and vertigo) for early detection of potential *S.suis* hearing loss cases and administrate immediate treatment to avoid long-term complications.

With an absence of vaccination for *S.suis* prevention, timely diagnosis and immediate antibiotic treatment before occurrence or sign of hearing loss symptoms are essential to prevent or reduce damage particularly SNHL caused by *S.suis* infection. Additionally, increased awareness among clinicians and microbiologists on *S.suis* infection as an emerging zoonosis is important to foster early disease detection, management, and prevention. Public awareness program and food safety campaign should also be considered for disease control and prevention.

Supporting information

S1 TRIPOD Checklist. Prediction Model Development [10].
(PDF)

S1 Checklist. STROBE checklist.
(PDF)

S1 Table. List of Northern provinces of Thailand as of 2018.
(DOCX)

S2 Table. Clinical characteristics of *S. suis* infected patients for hearing loss based on imputed GCS.
(DOCX)

S3 Table. Hosmer-Lemeshow good-ness-of-fit test.
(DOCX)

S1 Fig. Calibration plot between predicted vs. observed probability of *S. suis* hearing loss.
(TIF)

S2 Fig. Discrimination of *S. suis* hearing loss based on *S. suis* hearing loss scores. Blue grey bars: non-hearing loss cases (N = 91); Red bars: hearing loss cases (N = 42).
(TIF)

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Author Contributions

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Data curation: Ajaree Rayanakorn.

Formal analysis: Ajaree Rayanakorn, Wasan Katip, Peninnah Oberdorfer.

Methodology: Ajaree Rayanakorn, Wasan Katip, Peninnah Oberdorfer.

Supervision: Bey Hing Goh, Peninnah Oberdorfer, Learn Han Lee.

Visualization: Ajaree Rayanakorn.

Writing – original draft: Ajaree Rayanakorn.

Writing – review & editing: Ajaree Rayanakorn, Wasan Katip, Bey Hing Goh, Peninnah Oberdorfer, Learn Han Lee.

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Supporting information

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(TIF)

Section/Topic	Item	Checklist Item	Page
Title and abstract			
Title	1	Identify the study as developing and/or validating a multivariable prediction model, the target population, and the outcome to be predicted.	1
Abstract	2	Provide a summary of objectives, study design, setting, participants, sample size, predictors, outcome, statistical analysis, results, and conclusions.	3
Introduction			
Background and objectives	3a	Explain the medical context (including whether diagnostic or prognostic) and rationale for developing or validating the multivariable prediction model, including references to existing models.	4-5
	3b	Specify the objectives, including whether the study describes the development or validation of the model or both.	4-5
Methods			
Source of data	4a	Describe the study design or source of data (e.g., randomized trial, cohort, or registry data), separately for the development and validation data sets, if applicable.	5-6
	4b	Specify the key study dates, including start of accrual; end of accrual; and, if applicable, end of follow-up.	5, 8
Participants	5a	Specify key elements of the study setting (e.g., primary care, secondary care, general population) including number and location of centres.	5-6
	5b	Describe eligibility criteria for participants.	5
	5c	Give details of treatments received, if relevant.	N/A
Outcome	6a	Clearly define the outcome that is predicted by the prediction model, including how and when assessed.	6-7
	6b	Report any actions to blind assessment of the outcome to be predicted.	N/A
Predictors	7a	Clearly define all predictors used in developing or validating the multivariable prediction model, including how and when they were measured.	6-7
	7b	Report any actions to blind assessment of predictors for the outcome and other predictors.	N/A
Sample size	8	Explain how the study size was arrived at.	12
Missing data	9	Describe how missing data were handled (e.g., complete-case analysis, single imputation, multiple imputation) with details of any imputation method.	7
Statistical analysis methods	10a	Describe how predictors were handled in the analyses.	6-7
	10b	Specify type of model, all model-building procedures (including any predictor selection), and method for internal validation.	7
	10d	Specify all measures used to assess model performance and, if relevant, to compare multiple models.	7
Risk groups	11	Provide details on how risk groups were created, if done.	N/A
Results			
Participants	13a	Describe the flow of participants through the study, including the number of participants with and without the outcome and, if applicable, a summary of the follow-up time. A diagram may be helpful.	5
	13b	Describe the characteristics of the participants (basic demographics, clinical features, available predictors), including the number of participants with missing data for predictors and outcome.	8
Model development	14a	Specify the number of participants and outcome events in each analysis.	8
	14b	If done, report the unadjusted association between each candidate predictor and outcome.	N/A
Model specification	15a	Present the full prediction model to allow predictions for individuals (i.e., all regression coefficients, and model intercept or baseline survival at a given time point).	18
	15b	Explain how to the use the prediction model.	9
Model performance	16	Report performance measures (with CIs) for the prediction model.	9
Discussion			
Limitations	18	Discuss any limitations of the study (such as nonrepresentative sample, few events per predictor, missing data).	11-12
Interpretation	19b	Give an overall interpretation of the results, considering objectives, limitations, and results from similar studies, and other relevant evidence.	12-13
Implications	20	Discuss the potential clinical use of the model and implications for future research.	12-13
Other information			
Supplementary information	21	Provide information about the availability of supplementary resources, such as study protocol, Web calculator, and data sets.	15
Funding	22	Give the source of funding and the role of the funders for the present study.	N/A

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No.	Recommendation	Page No.	Relevant text from manuscript
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1; Title page,	"A risk scoring system for predicting *Streptococcus suis* hearing loss: A 13-Year Retrospective Cohort Study"
			3; Abstract	"Data from a retrospective review of 13-year *S.suis* patient records in a tertiary hospital in Chiang Mai, Northern, Thailand was obtained."
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3; Abstract	"Methods Data from a retrospective review of 13-year *S.suis* patient records in a tertiary hospital in Chiang Mai, Northern, Thailand was obtained. Univariate and multivariate logistic regressions were employed to develop a predictive model. The clinical risk score was constructed from the coefficients of significant predictors. Area under the receiver operator characteristic curve (AuROC) was identified to verify the model discriminative performance. Bootstrap technique with 1000-fold bootstrapping was used for internal validation. Key Results Among 133 patients, the incidence of hearing loss was 31.6% (n=42). Significant predictors for *S. suis* hearing loss were meningitis, raw pork consumption, and vertigo. The

				predictive score ranged from 0-4 and correctly classified 81.95% patients as being at risk of *S.suis* hearing loss. The model showed good power of prediction (AuROC: 0.859; 95%CI 0.785-0.933) and calibration (AuROC: 0.860; 95%CI 0.716-0.953)."
Introduction				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5; Introduction	"*Streptococcus suis* SNHL is poorly diagnosed because patients do not undergo audiometry screening until they complaint of severe hearing disorder. By then, the prognosis of the disease is severe with slim percentage of recovery." "SNHL was found to evolve at the early stage of meningitis and could progress to permanent hearing loss if meningitis had not been promptly treated appropriately [6]. Therefore, early diagnosis and immediate treatment are essential to reduce detrimental consequences mainly SNHL from *S.suis* infection."
Objectives	3	State specific objectives, including any prespecified hypotheses	5; Introduction	"This study aims to develop an easy-to-use risk score to promote early diagnosis and detection of *S.suis* infected patients who are prone to hearing loss in primary settings."
Methods				

Study design	4	Present key elements of study design early in the paper	5: Methods, Study design and setting	"The data was collected as a part of a retrospective cohort study over 13-year period at Chiang Mai University Hospital (CMUH)."
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5: Methods, Study design and setting	"The hospital is the largest in northern, Thailand and the fourth largest hospital in the country where most of patients in northern region from 17 provinces are referred to for tertiary care."
			6; Methods, Study population and data collection	"*S. suis* positive cases were identified based on the microbiology laboratory data available and hospital numbers (HNs) admitted from May 2005 to December 2018". "The data collected included patient demographics, clinical characteristics and manifestations, outcomes and treatments."
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	6; Methods, Study population and data collection	"*S. suis* positive cases were identified based on the microbiology laboratory data available and hospital numbers (HNs) admitted from May 2005 to December 2018. *S. suis* cases were confirmed by blood or cerebrospinal fluid (CSF). All confirmed *S.suis* cases with available medical records were included in this analysis (Figure 1 Patient identification and selection) [8]."
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	N/A	

Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6; Methods, Outcomes	"*S. suis* meningitis was confirmed by cerebrospinal fluid (CSF) culture with compatible clinical presentation. An audiogram was used to diagnose and monitor the degree of hearing ability. The degree of hearing loss was assessed by otorhinolaryngologists based on individual patients' hearing thresholds in decibel (dB) at different frequencies. The level of hearing loss was classified into mild, moderate, severe and profound. Presbycusis or any pre-existing hearing loss would be excluded. Endocarditis and site of vegetation was assessed by echocardiography."
			7; Methods, Statistical analysis	"Variables are defined as risk factors associated with an increased risk of *S.suis* hearing loss." "Variables with p-value less or equal to 0.10 would be carried forward in multivariate analysis. Potential collinearity was also explored before building the predictive model. Forward step-wise logistic regression was utilized to identify significant predictors for *S.suis* hearing loss. Predictors that remained significant at p-value ≤ 0.10 would be included in the final parsimonious model."
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6; Methods, Outcomes	"An audiogram was used to diagnose and monitor the degree of hearing ability. The degree

				of hearing loss was assessed by otorhinolaryngologists based on individual patients' hearing thresholds in decibel (dB) at different frequencies. The level of hearing loss was classified into mild, moderate, severe and profound. Presbycusis or any pre-existing hearing loss would be excluded".
			7, Methods, statistical analysis	"Univariate analysis was performed to compare potential predictors between hearing loss and non-hearing loss patients. For continuous variables, Student's t test was used in case of normal distribution whereas Mann-Whitney-Wilcoxon test was employed if the data is not normally distributed. Chi-square test or Fisher's exact test was used for categorical variables. We performed both complete case analysis and multiple imputation using predictive mean matching with 20 iterations for missing Glasgow Coma Scale (GCS) values [11]."
Bias	9	Describe any efforts to address potential sources of bias	7; Methods, Statistical analysis	"Potential collinearity was also explored before building the predictive model".
			8; Methods, Risk score development and internal validation	"A bootstrap with 1,000 replications technique was used for internal validation to correct for optimism [14]."
			11-12; Discussion	"An extensive medical record review to capture all relevant

			12; Discussion	information may have contributed to this finding". "We contacted investigators who had previously conducted studies in *S.suis* patients for the purpose of external validation but we did not receive any response."
Study size	10	Explain how the study size was arrived at	12; Discussion	"According to the rule of thumb from a simulation study, the number of event per variable (EPV) of 10 or more was required to prevent bias in the regression coefficients [11]. In our study, there were 42 *S.suis* patients with hearing loss as convenience sample and there were three significant risk factors confirmed in the final model."
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	9; Results	"Continuous variables were categorized into two groups to simplify application. For creatinine, the cut point was 2.0 mg/dL which was around 1.5 to 2 times compared to the mean value among *S.suis* hearing loss cases whereas 4.0 mmol/L was the cut-off point for serum potassium."
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8-9; Results	"Univariate analysis suggested association between *S.suis* hearing loss with raw pork consumption ($p<0.001$), valvular heart disease (VHD) ($p=0.009$), alcoholic liver disease (ALD) ($p=0.093$), acute meningitis ($p<0.001$), neck stiffness

	(p<0.001), infective endocarditis (IE) (p=0.005), vomiting (p=0.019), vertigo (p=0.011) and lower level of serum creatinine (p=0.018) and potassium (p=0.002) (Table 2). Continuous variables were categorized into two groups to simplify application. For creatinine, the cut point was 2.0 mg/dL which was around 1.5 to 2 times compared to the mean value among *S.suis* hearing loss cases whereas 4.0 mmol/L was the cut-off point for serum potassium. GCS variable remained a non-significant predictor in both complete case analysis and multiple imputation data (Table S2 Clinical characteristics of *S. suis* infected patients for hearing loss based on imputed GCS). Two predictors were removed after checking on collinearity which were neck stiffness and VHD whereas eight predictors (raw pork consumption, ALD, acute meningitis, IE, vomiting, vertigo, a low level of serum creatinine and potassium) were included in the stepwise forward logistic regression. There were three predictors remained in the model at p-value ≤ 0.1 which were meningitis, raw pork consumption and vertigo. The final parsimonious model at significant level ≤ 0.1 which provided optimal the area under the receiver operating

				characteristic curve (AuROC) was selected."
		(*b*) Describe any methods used to examine subgroups and interactions	N/A	
		(*c*) Explain how missing data were addressed	7; Methods, Statistical analysis	"We performed both complete case analysis and multiple imputation using predictive mean matching with 20 iterations for missing Glasgow Coma Scale (GCS) values [11]."
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	9; Results	"GCS variable remained a non-significant predictor in both complete case analysis and multiple imputation data (Table S2 Clinical characteristics of S. suis infected patients for hearing loss based on imputed GCS)."
		(*e*) Describe any sensitivity analyses	N/A	
Results				
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	6; Methods, Study population and data collection	"*S. suis* positive cases were identified based on the microbiology laboratory data available and hospital numbers (HNs) admitted from May 2005 to December 2018. *S. suis* cases were confirmed by blood or cerebrospinal fluid (CSF). All confirmed *S.suis* cases with available medical records were included in this analysis (Figure 1 Patient identification and selection)."
			8; Results	"One hundred and thirty-three patients with *S.suis* infection were included in this analysis, majority were males (67.2%)."
		(b) Give reasons for non-participation at each stage	N/A	
		(c) Consider use of a flow diagram	N/A	

Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	5; Methods, Study design and setting	"The data was collected as a part of a retrospective cohort study over 13-year period at Chiang Mai University Hospital (CMUH) [8], a 1400-bed tertiary teaching hospital. The hospital is the largest in northern, Thailand and the fourth largest hospital in the country where most of patients in northern region from 17 provinces are referred to for tertiary care (see Table S1 List of Northern provinces of Thailand as of 2018). Cultural eating habit of raw pork dishes and fermented raw pork is commonly practiced in northern, Thailand. This deep-rooted cultural eating behavior is a major route of the disease transmission and contributing factor of a high prevalence of the disease in this region."
		(b) Indicate number of participants with missing data for each variable of interest	17; Table 2 Clinical characteristics of *S. suis* infected patients for hearing loss	"† Data available in 101 patients; ‡ Data available in 57 patients; ‡‡ Data available in 53 patients; †† Data available in 52 patients; ± Data available in 127 patients"
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	8; Results	"All SNHL still persisted based on audiometry upon discharge and latest follow-up visit up to December 2018."
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	8; Results	"More than one-third of patients had a history of raw pork consumption and nearly half of the patients were regular alcohol drinkers. Valvular heart disease was the most common underlying disease followed by

				Diabetes Mellitus (DM) and spondylodiscites. A total of 42 patients (31.58%) experienced SNHL from S.suis infection in which 13 (9.77%) were mild SNHL, 7 (5.26%) were moderate SNHL, 4 (3.01%) were severe SNHL and 12 (9.02%) were profound SNHL. All SNHL still persisted based on audiometry upon discharge and latest follow-up visit up to December 2018."
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	N/A	
		Cross-sectional study—Report numbers of outcome events or summary measures	N/A	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	N/A	
		(*b*) Report category boundaries when continuous variables were categorized	9; Results	"Continuous variables were categorized into two groups to simplify application. For creatinine, the cut point was 2.0 mg/dL which was around 1.5 to 2 times compared to the mean value among S.suis hearing loss cases whereas 4.0 mmol/L was the cut-off point for serum potassium."
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	9; Results Supporting information; Table S2	"GCS variable remained a non-significant predictor in both complete case analysis and multiple imputation data (Table S2 Clinical characteristics of S. suis infected patients for hearing loss based on imputed GCS)."
Discussion				

Key results	18	Summarise key results with reference to study objectives	10;Discussion	"To our best knowledge, this is the first risk scoring system development for *S.suis* hearing loss. We identified meningitis, raw pork consumption and vertigo as the main risk factors of *S.suis* hearing loss. The data was derived from the real setting in routine practice upon admission at Chiang Mai University Hospital (CMUH). Usually these data are available and do not require any invasive laboratory procedure. After evaluating its external validity, this simple scoring system might be useful to assess patients with *S.suis* hearing loss in hospital and primary care settings."
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	11;Discussion	"However, a number of limitations can be noted in our study. Missing data and recall bias may have arisen due to retrospective nature……"
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12-13; Discussion	"In conclusion, after external validation, our simple clinical risk score developed might be useful to aid clinicians in identifying patients who are likely to develop hearing loss from *S.suis* infection. Although

				this tool cannot replace clinical judgement, physicians can look upon these clinical characteristics in patients (meningitis, raw pork consumption and vertigo) for early detection of potential *S.suis* hearing loss cases and administrate immediate treatment to avoid long-term complications."
Generalisability	21	Discuss the generalisability (external validity) of the study results	12; Discussion	"Nevertheless, as traditional culture involving raw pork consumption is a well-known risk behavior among northern Thai population, doctors might have more likely asked patients about this risk behavior which might have been potentially subject to information bias. Therefore, generalizability of the finding should be done with caution." "Finally, it should be noted that the participants included in the study were mainly from Northern, Thailand where traditional raw pork eating is practiced. This may limit generalizability in other settings

				especially where raw pork consumption is uncommon."
Other information				
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	N/A	

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

S1 Table. List of Northern provinces of Thailand as of 2018 (National Statistics Office (NSO). [cited 2019 22 July]; Available from: http://www.nso.go.th/sites/2014/nsopublic)

Upper Northern Thailand	Lower Northern Thailand
Chiang Rai	Tak
Chiang Mai	Kamphaeng Phet
Nan	Nakhon Sawan
Phayao	Phetchabun
Phrae	Phichit
Mae Hong Son	Phitsanulok
Lumphun	Sukhothai
Lampang	Uthai Thani
Uttaradit	

S2 Table. Clinical characteristics of *S. suis* infected patients for hearing loss based on imputed GCS

Characteristics	Hearing loss (n=42) N (%)	Non-hearing loss (n=91) N (%)	p-value
Demographics:			
- **Age (year) (mean±SD)**	54.62±12.12	57.33±14.33	0.290
- **Male**	29 (69.05)	63 (69.23)	0.568
- **Raw pork consumption**	25 (59.05)	24 (26.37)	<0.001
- **Alcohol drinking**	25 (59.52)	41 (45.05)	0.138
Baseline characteristics:			
GCS † (Hearing loss n=41)	13.39±0.37	12.76±0.34	0.268
Microbiological results:			
- **Time to microbiological cure**	9.86±11.07	8.33±15.29	0.526
- **Mean MIC to penicillin (mcg/mL) ‡**	0.14±0.18	0.17±0.15	0.106
- **Mean MIC to ceftriaxone (mcg/mL) ‡‡**	0.14±0.10	0.29±0.31	0.388
Underlying disease			
- **Valvular heart disease**	7 (16.67)	37 (40.66)	0.009
- **ALD**	2 (4.76)	14 (15.38)	0.093
- **DM**	5 (11.90)	21 (23.08)	0.162
- **Spondylodiscites**	6 (14.29)	21 (23.08)	0.354
Major clinical manifestations			
- **Acute meningitis**	34 (80.95)	16 (17.58)	<0.001
- **Neck stiffness**	31 (73.81)	16 (17.58)	<0.001
- **Septicaemia**	23 (54.76)	51 (56.04)	1.000
- **IE**	4 (9.52)	30 (32.97)	0.005
- **Vomiting**	14 (33.33)	13 (14.29)	0.019
- **Vertigo**	7 (16.67)	3 (3.30)	0.011
Receiving steroids	22 (53.66)	1 (1.10)	0.600
Laboratory findings:			
- **CSF protein††**	277.12±220.25	341.39±256.93	0.419
- **CSF glucose‡‡**	31.69±9.70	30.72±21.55	0.173
- **Creatinine (mg/dl)**	1.18±0.75	2.09±2.94	0.018
- **Potassium (mmol/L)±**	3.52±0.43	3.93±0.77	0.002

ALD, Alcoholic liver disease; CSF, Cerebrospinal fluid; DM, Diabetes Mellitus; GCS, Glasgow coma scale; IE, Infective endocarditis; MIC, Minimal Inhibitory Concentration

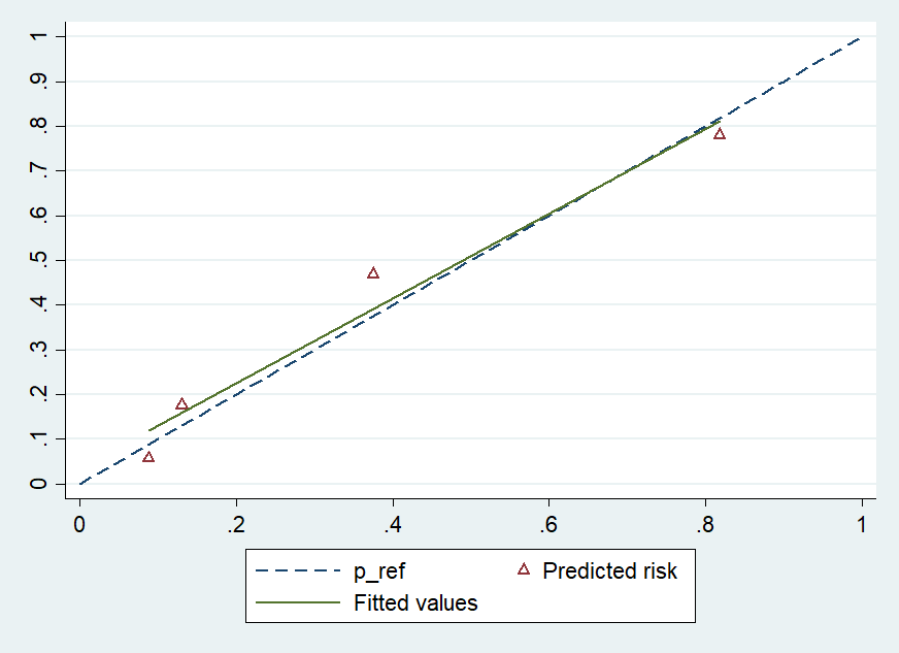
† Data was imputed in 31/32 missing GCS values due to there was one missing parameter to estimate GCS in 1 patient.

S3 Table. Hosmer-Lemeshow good-ness-of-fit test

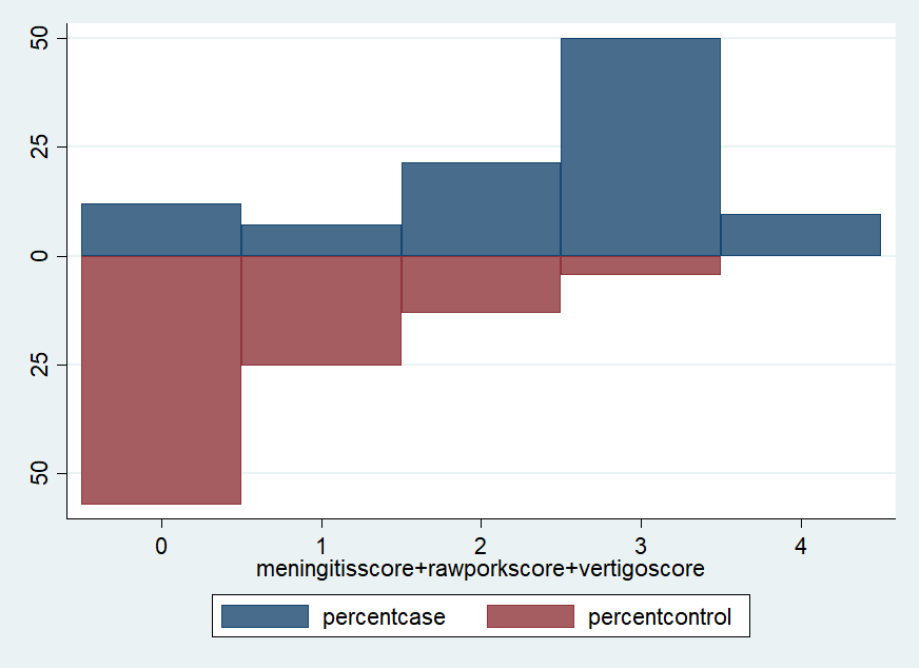
Group	Probabilities	Obs_1	Exp_1	Obs_0	Exp_0	Total
4	0.0571	5	3.3	52	53.7	57
6	0.1759	3	4.0	20	19.0	23
7	0.5018	9	11.2	15	12.8	24
9	0.7803	18	17.2	4	4.8	22
10	0.9473	7	6.3	0	0.7	7

Number of observations = 133
Number of groups = 5
Hosmer-Lemeshow chi2(3) = 3.13
Prob > chi2 = 0.3721

S1 Fig. Calibration plot between predicted vs. observed probability of *S. suis* hearing loss.



S2 Fig. Discrimination of *S.suis* hearing loss based on *S.suis* hearing loss scores. Blue grey bars: non-hearing loss cases (N = 91); Red bars: hearing loss cases (N = 42)



Chapter 6:

Burden of disease and productivity impact of *Streptococcus suis* infection in Thailand

6.1 Summary of Chapter 6

Streptococcus suis (S.suis) infection is serious and potentially lethal in human especially males of working age *(1, 5)*. The major clinical presentations include meningitis, septicaemia, and infective endocarditis in which a large number of surviving patients would develop long-term complications *(1, 5, 33, 55, 102)*. Long-term complications among survivors especially sensorineural hearing loss with or without vestibular dysfunction and valvular heart disease may result in significant economic burden. To our knowledge, no previous research has estimated the burden of this disease and its economic impact in Thailand. Therefore, this Chapter aims to quantify the burden of disease from *S.suis* infection and its economic impact on years of life, quality of life and work productivity in Thailand.

The quality-adjusted life years (QALYs) and productivity-adjusted life years (PALYs) were used to estimate the burden of disease. QALYs is a common health outcome measure in years life lived in perfect health gain with inclusion of both quality and quantity *(103, 104)*. QALYs is generated by multiplying the number years of life lived by a health related quality of life (HRQL) or utility weights *(105)*. A utility score ranges from 0 to 1 where the score of 1 represents a perfect health whereas “0” was equivalent to death *(105)*.

- QALYs = The number of years life lived * Utility weights

QALYs has been widely used in economic evaluations and comparisons across diseases *(104)*. However, its limitations include its failure to capture wider effects on economic impact and social consequences in terms of work productivity loss from the disease *(106)*. As *S.suis* infection affects mainly men of working age and there was no previous study estimating the disease impact on loss productivity, quantifying the burden of disease in term of economic cost to the country would be essential in addition the data in the burden of disease for effective disease control and prevention.

PALYs is conceptually similar to QALYs, but accounts on work productivity loss attributable to the disease instead of quality of life *(107)*. It is a novel measure that help to quantify the impact from the disease on work productivity *(108)*. PALYs is calculated by multiplying the number of life years lived by productivity indices instead

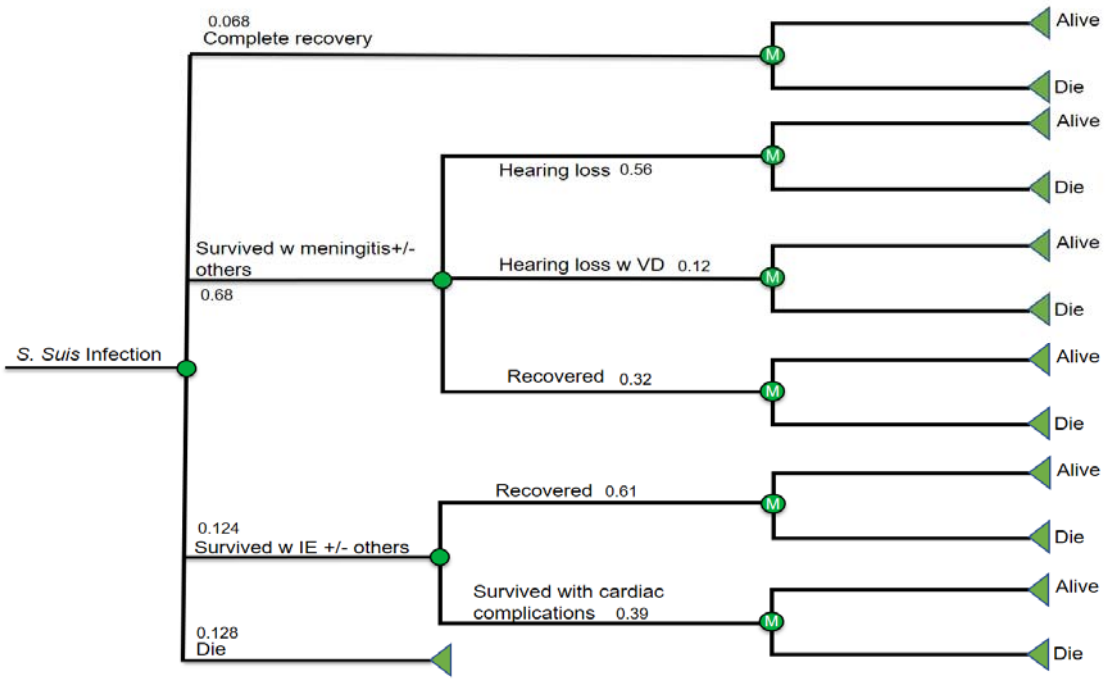
of utility *(108)*. Productivity indices were estimated based on absenteeism (absence from work due to sickness) and presenteeism (reduced efficiency at work) *(109)*.

- PALYs = The number of years life lived * productivity indices

A decision-analytic Markov model to reflect *S. suis* infection and its major complications: death, meningitis and infective endocarditis was constructed (Figure 6.1). The impact of the disease in Thailand in terms of life lost, quality-adjusted life years (QALYs) lost, and productivity-adjusted life years (PALYs) lost, a novel measure estimating productivity impact were examined. Predicted incident cases of *S.suis* infection in 2019 (N=312) was estimated based on the annual incidence data for 2015-2018 reported by the Thailand Bureau of Epidemiology at the Ministry of Public Health *(110)*. In 2019, it was estimated that 312 people in Thailand acquired *S.suis* infection. With simulated follow-up from the age of 51 years until death or age 100 years, this cohort incurred 769 years of life lost (14% of predicted years of life lived had they not been infected), 826 QALYs lost (21%) and 793 PALYs (15%) lost. These equated to an average of 2.46 years of life, 2.64 QALYs and 2.54 PALYs lost per person. In terms of broader economic impact, the loss of PALYs was estimated to cost 346 million Thai baht (US$11.3 million) in lost gross domestic product (GDP), which equated to 1.1 million Thai baht (US$ 36,033) per person.

In conclusion, *S.suis* infection imposes a significant economic burden both in terms of health and productivity. Public health awareness programs and interventions could potentially improve awareness among Thai population and healthcare policy makers in order to reduce the number of new cases and consequently save substantial costs in a long term.

Figure 6.1 A decision tree and Markov model with relevant health states from *S.suis* infection



6.2 Publications associated with Chapter 6

6.2.1 Manuscript associated with Chapter 6 (PLOS Neglected Tropical Diseases, IF: 4.487)

Rayanakorn A, Ademi Z, Liew D, Lee LH. Burden of disease and productivity impact of *Streptococcus suis* infection in Thailand. PLOS NTD 2020 (submitted).

Burden of disease and productivity impact of *Streptococcus suis* infection in Thailand

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Keywords: *Streptococcus suis*; cost; productivity; quality of life; *S.suis*; Thailand

Word count: Abstract 355, Body text 3,846

Abstract

Background

Streptoccocus suis (S.suis) infection is a neglected zoonosis disease in humans mainly affects men of working age. We estimated the health and economic burden of *S.suis* infection in Thailand in terms of years of life lost, quality-adjusted life years (QALYs) lost, and productivity-adjusted life years (PALYs) lost which is a novel measure that adjusts years of life lived for productivity loss attributable to disease.

Methods

A decision-analytic Markov model was developed to simulate the impact of *S. suis* infection and its major complications: death, meningitis and infective endocarditis among Thai people in 2019 with starting age of 51 years. Transition probabilities, and inputs pertaining to costs, utilities and productivity impairment associated with long-term complications were derived from published sources. A lifetime time horizon with follow-up until death or age 100 years was adopted. The simulation was repeated assuming that the cohort had not been infected with *S.suis*. The differences between the two set of model outputs in years of life, QALYs, and PALYs lived reflected the impact *of S.suis* infection. An annual discount rate of 3% was applied to both costs and outcomes. One-way sensitivity analyses and Monte Carlo simulation modeling technique using 10,000 iterations were performed to assess the impact of uncertainty in the model.

Key Results

This cohort incurred 769 (95% uncertainty interval [UI]: 695 to 841) years of life lost (14% of predicted years of life lived if infection had not occurred), 826 (95% UI: 568 to 1,098) QALYs lost (21%) and 793 (95%UI: 717 to 867) PALYs (15%) lost. These equated to an average of 2.46 years of life, 2.64 QALYs and 2.54 PALYs lost per person. The loss in PALYs was associated with a loss of 346 (95% UI: 240 to 461) million Thai baht (US$11.3 million) in GDP, which equated to 1.1 million Thai baht (US$ 36,033) lost per person.

Conclusions

S.suis infection imposes a significant economic burden both in terms of health and productivity. Further research to investigate the effectiveness of public health awareness programs and disease control interventions should be mandated to provide a clearer picture for decision making in public health strategies and resource allocations.

Author summary

Streptoccocus suis (S.suis) infection is a potentially lethal zoonotic disease in humans. In the present study, we sought to estimate the impact of the disease in Thailand in terms of years of life lost, quality-adjusted life years (QALYs) lost, and productivity-adjusted life years (PALYs) lost. A decision-analytic Markov model was developed to simulate the impact of *S.suis* infection and its major complications among Thai people. In 2019, it was estimated that the infection incurred 769 years of life lost (14% of predicted years of life lived if infection had not occurred), 826 QALYs lost (21%) and 793 PALYs (15%) lost. These equated to an average of 2.5 years of life, 2.6 QALYs and 2.5 PALYs lost per person. The loss in PALYs was associated with a loss of 346 million Thai baht (US$11.3 million) in GDP, which equated to 1.1 million Thai baht (US$ 36,033) lost per person. The findings call for increased public health awareness and comprehensive efforts to control and prevent the disease.

Introduction

Streptococcus suis (S.suis) is a gram positive alpha-hemolytic bacteria whose natural host is usually the pig. It can cause serious infection in humans through contact with pigs or pig meat, especially via the ingestion of uncooked pork. Meningitis, septicaemia and infective endocarditis are major clinical manifestations of *S.suis* infection, and case fatality is 13% [1]. Long-term complications among survivors include sensorineural hearing loss with or without vestibular dysfunction and valvular heart disease. The highest prevalence of *S.suis* infection is in South East Asia, notably Thailand (0.487 per 100,000) [2] and Vietnam (0.249 per 100,000) [3]. Globally, there are more than 1,600 reported cases [1]. The disease predominantly affects males from young adulthood to middle age [1, 4]. This may be due to their risk behaviors including raw pork consumption, exposure to pigs or raw pork through their occupations and slaughtering activity [4]. While the disease mainly affects farmers or abattoir workers in western countries, it was found that ingestion of raw or undercooked

pork is an important risk factor in Asia, especially in Thailand and Vietnam, where raw pork consumption is traditionally practiced.

To our knowledge, no previous research has estimated the health and economic burden of *S.suis* infection in Thailand. Our study aimed to quantify this burden in terms of years of life, quality-adjusted life years (QALYs), and productivity-adjusted life years (PALYs) lost, as well as impact on gross domestic product (GDP).

Methods

Model description

The model comprised two parts (Figure 1). The first was a decision analytic tree which reflected the first 30 days of *S.suis* infection, over which time subjects could: i) completely recover without any long-term sequelae; ii) survive meningitis (with or without other complications); iii) survive infective endocarditis (IE, with or without other complications except meningitis); or iv) die. The second part of the model consisted of a Markov model with seven long-term health states: i) completely recovered; ii) post meningitis without complications; iii) post meningitis with long-term hearing loss but no vestibular dysfunction; iv) post meningitis with long-term hearing loss and vestibular dysfunction; v) post IE without long-term complications; vi) post IE with long-term complications; and vii) dead. Vestibular dysfunction without hearing loss and endopthalmitis are rare, and therefore were not considered. Cycle lengths in the Markov model were one year.

For all the living health states, only two transition states were considered: stay alive and die. We assumed that those who survived meningitis, regardless of whether or not they had hearing loss or vestibular dysfunction, had the same annual risks of dying as the sex-and-age matched general population. The same assumption was applied to those who suffered IE without long-term complications. By contrast, those who suffered IE with long-term complications had a 2.2-fold higher risk of dying compared to the general population [5].

The model population comprised 312 Thais (239 males and 73 females) predicted to develop *S.suis* infection in 2019. The baseline age was assumed to be 51 years, which is the mean age of onset of acute infection [1]. The cohort was stratified by sex and followed up until age 100 years, which means

the time horizon was 49 years. An annual discount rate of 3% was applied to both costs and outcomes in accordance with Thai Health Technology Assessment (HTA) guidelines [6].

To estimate the impact of the disease, the model simulation was repeated assuming that the cohort did not contract *S.suis* infection. Differences in the results of these two simulations in terms of years of life, QALYs and PALYs reflected the burden attributable to *S.suis* infection. PALYs are a novel measure that adjust years of life lived for productivity loss attributable to the disease in the same way that QALYs adjust years if life lived for impaired quality of life [7].

Data sources

Epidemiological data

Key input parameters and their sources are summarized in Table 1.

Demographic and mortality data for the general Thai population were obtained from the Country Office of Insurance Commission [8]. Predicted incident cases of *S.suis* infection in 2019 (N=312) was estimated based on the annual incidence data for 2015-2018 reported by the Thailand Bureau of Epidemiology at the Ministry of Public Health [2]. The proportion of males (76.6%) was based on a meta-analysis [3]. Case fatality during the acute infection and likelihoods of developing meningitis and IE were derived from a systematic review and meta-analysis [1]. Among subjects who developed meningitis, the probability of long-term hearing loss with and without vestibular dysfunction were obtained from a retrospective study in Northern Thailand [9]. Among subjects who developed IE, the probability of long-term cardiac complications was based on a systematic review and meta-analysis [10]. The relative risk of death among subjects with post IE patients with long-term cardiac complications compared to general population (2.2) was based on a Swedish cohort study [5]. No equivalent Thai data were available.

Table 1 Key input parameters

Parameters	Base-case value	Ranges	Distributions	Source
Total population of Thailand in 2018 (million)	68.416	-	Fixed	Thailand Board of Investment. Thailand in Brief: Demographic.[11]
Annual disease incidence 2019 (per 100,000 population)	0.457	0.420-0.493	Fixed	Bureau of Epidemiology, Department of Disease Control, MoPH, Thailand*.[2]

Age of onset of acute infection (years)	51.4	49.5-53.2	Fixed	A systematic review and meta-analysis.[1]
Transitional probabilities				
Case fatality rate	0.128	0.09-0.180	Uniform	A systematic review and meta-analysis.[1]
Infective endocarditis	0.124	0.067-0.219	Uniform	A systematic review and meta-analysis.[1]
Meningitis	0.680	0.589-0.758	Uniform	A systematic review and meta-analysis.[1]
Meningitis-hearing loss	0.381	0.310-0.478	Uniform	Retrospective cohort study [9].
Markov				
Probability of developing hearing loss	0.680	0.544-0.816	Uniform	Retrospective cohort study [9].
Probability of developing hearing loss with VD	0.120	0.096-0.144	Uniform	Retrospective cohort study [9].
Probability of developing complications from IE	0.048	0.040-0.057	Uniform	A systematic review and meta-analysis.[10]
Probability of dying after IE	2.20	2.00-2.30	Uniform	A nationwide cohort study.[5]
Acute treatment cost*				
IPD per episode (THB)	124,675	11,337-40,4863	Gamma	Office of medical records and statistics, CMU Hospital
Chronic treatment cost				
IE after 1 month within 1st year (THB)	55,785	44628-66942	Gamma	Thai Acute Coronary Syndrome (ACS) registry.[12]
IE following years (THB)	15,934	12748-19121	Gamma	Thai Acute Coronary Syndrome (ACS) registry.[12]
Hearing aids reimbursement (THB)	12,769	10215-15323	Gamma	National Health Security Office, Thailand.[13]
Audiometry (THB)	220	176-264	Gamma	Case series and Standard Cost List for Health Economic evaluation in Thailand.[6, 14]
CT Temporal bone (THB)	7,344	5875-8813	Gamma	Case series and Standard Cost List for Health Economic evaluation in Thailand.[6, 14]
Health Utilities (Quality of Life Estimates)				
Hearing loss with or without VD	0.58	0.34-0.81	Beta	A multi-center, prospective study.[15]
Infective endocarditis (IE)	0.67	0.40-0.94	Beta	A single-center, prospective study.[16]
PALYs				
Productivity index for no hearing loss	0.96	-		A national longitudinal study.[17]
Productivity index for hearing loss	0.95	0.72-0.96	Uniform	A national longitudinal study.[17]
Productivity index for hearing loss with VD	0.92	0.72-0.96	Uniform	A cross-sectional survey.[18]
Productivity index for IE	0.94	0.45-0.96	Uniform	A coronary heart disease model development study.[19]

CT, computed tomography; IE, infective endocarditis; PALYs, productivity-adjusted life years; THB, Thai baht; VD, vestibular dysfunction

Note: * Extrapolated from the recent 4-year data from 2015-2018 using logistic regression; ** Direct medical care costs i.e. Medication, laboratory tests, X-ray, hospitalization

Cost parameters

Treatment costs for the acute *S.suis* infection were based on average inpatient expenses per *S.suis* episode at Chiang Mai University Hospital, Northern Thailand from 2005 to 2018. These unpublished data were provided by the hospital's office of medical records and statistics and based on 130 consecutive patients. Local cost data on IE or rheumatic heart disease are lacking. Therefore, data from Thai Acute Coronary Syndrome (ACS) registry which is considered to be the most comprehensive cost study in Thailand was used to estimate chronic treatment costs of IE [12]. The cost for non-fatal myocardial infarction (MI) one month after the event for the first year and the following years were used as surrogate costs of IE. The cost of hearing aids were based on the maximum reimbursement rate per device under universal health coverage scheme according to the Thailand National Health Security Office [13]. The costs for routine hearing test and computed tomography (CT) of temporal bone were obtained from Thailand's standard costing list [20]. The number of testes conducted during follow-up was based on a case series of 40 adult *S.suis* infected patients in northern Thailand [14]. Cost data were inflated to year 2019 values applying consumer price index for medical care [21]. The average market exchange rate in Quarter 3, 2019 was $US1= 30.7123 Thai baht (THB) [22].

Utilities and productivity indices

Country-specific health utility data was obtained from the European Quality of life five dimension (EQ-5D) index using the European visual analogue scale (VAS) value set for Thailand by the EuroQol Group [23]. The EQ-5D is one of the most widely used multi attribute utility instruments for measuring health related quality of life, stratified into five dimensions: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression [23]. The utility index is measured on a scale between 0 (complete equity in health) to 1 (complete inequity in health) [23]. In a similar way, productivity indices account for proportional reduction in work productivity, and were calculated

based on presenteeism and absenteeism data reported by Nachtegaal et al. (2012) [17]. The study surveyed 1,295 working adults and compared the differences between sick leave taken by those with hearing impairment and their hearing able peers. The average amount of sick leave taken among those with insufficient and poor hearing ability was 4.3 days in four months (extrapolated to 12.9 days in one year), whereas the number of sick days taken by those who were in 'good' hearing test category was 3.1 days in four months (9.3 days per year). Findings from a cross sectional study of patients with bilateral and unilateral vestibular dysfunction by Sun et al (2014) [18] were used to estimate productivity loss attributable to hearing loss with vestibular dysfunction (productivity index = 0.9240). An assumption of 14 working days loss from IE was made based on the short-term disability benefits of 14 days leave among patients with coronary heart disease imputed in a model development study [19] as a conservative estimate. In the absence of the data concerning presenteeism in IE, only absenteeism was accounted in all productivity indices calculations for the sake of consistency. The equation with regards to productivity index calculation for each group is presented below [24]:

Productivity index = (full working year – absent days)/full working years

The number of working days in a years was estimated to be 250 days for full-time employment based on five working days per week and ten days of public holidays.

To estimate PALYs lived due to *S.suis* infection, each year lived in the labor force by the cohort was multiplied by a productivity index. We assumed that the economic value of each PALY was equivalent to annual gross domestic product (GDP) per worker, which equated to THB 436,039 ($US 13,955). This was derived from total Thailand GDP in 2018 (THB 16,318,033 million or $US 522,167.14) [25] divided by the estimated equivalent full-time (EFT) Thai workers in 2018 (n=37,418,710) [26, 27]. Owing to the scarcity of data on the part-time workforce participation and the predominance of full-time employment in Thailand, all employees were assumed to be in full-time employment.

Sensitivity analyses

One-way sensitivity analyses were undertaken to assess the impact of uncertainty by varying key input parameters one at a time (Table 1). The ranges of probability of developing of meningitis with

hearing loss and costs were set ±20% from the mean values. The upper and lower ranges for productivity indices were according to decreasing and increasing estimates of absenteeism. The working days lost from bilateral vestibular dysfunction was set as the lower bonds for productivity indices calculation for hearing loss with and without vestibular dysfunction [18], whereas the maximum working days lost (137 days) was applied for the productivity index for IE estimation [19]. The annual discount rates for both costs and benefits were varied to 0% and 6% [6].

We also performed probabilistic sensitivity analysis (PSA), to understand join uncertainty through Monte Carlo simulation using 10,000 iterations. The @RISK software version 8.0 for Excel was employed which allows multiple recalculations each time using a different set of random values for each parameter according to the defined distributions [28]. The uniform distribution was applied to the incidence, and transitional probabilities related to the disease and productivity indices, gamma distribution for costs, and beta for utilities. The 95% confidence interval (CI) of uncertainty ranges for the output variables attributable to the burden of disease were calculated.

Results

It was estimated that *S.suis* affected 312 Thai people per annum based on the country's population number in 2018 [27] and the 2019 annual incidence. There were more males infected than females (76.6% vs.23.4%). The base-case results are presented in Table 2.

Table 2 Base-case analyses results, with uncertainty intervals

	Male		Female		Total		Difference (%)	Lost per person
	Infection	No infection	Infection	No infection	Infection	No infection		
YLLs	5,482	6,376	1,680	1,954	7,162	8,329	1,167 (14.01)	3.74
Disc 3%	3,707	4,296	1,136	1,316	4,844	5,612	769 (13.71)	2.46
Overall difference (uncertainty ranges 95% CI)							695 (12.38) to 841 (14.99)	
QALYs	3,422	4,352	1,049	1,334	4,471	5,686	1,214 (21.37)	3.89
Disc 3%	2,323	2,955	712	906	3,035	3,861	826 (21.39)	2.64
Overall difference (uncertainty ranges 95% CI)							588 (15.23) to 1,098 (28.44)	
PALYs	5,219	6,139	1,600	1,881	6,818	8,020	1,202 (14.98)	3.85
Disc 3%	3,529	4,136	1,082	1,267	4,611	5,404	793 (14.67)	2.54

Overall difference (uncertainty ranges 95% CI)							717 (13.27) to 867 (16.04)	
Total treatment cost (Thai baht)	35,813,161	0	10,949,289	0	46,762,449	0	-46,762,449	-149,661
Disc 3%	34,804,660	0	10,639,082	0	45,443,742	0	-45,443,742	-145,441
Overall difference (uncertainty ranges 95% CI)							-55,222,281 to -38,966,206	
Broader economic cost (million) (Thai baht)	2,276	2,677	697	820	2,973.29	3,497	524	1.68
Disc 3% (million)	1,539	1,804	472	553	2,011	2,356	346	1.11
Overall difference (uncertainty ranges 95% CI)							239,717,748 to 461,379,424	
Net cost (Thai baht)	2,2340	2,677	687	820	2,927	3,497	570,760,094	1.83
Disc 3%	1,504	1,804	461	553	1,965	2,356	391,277,289	1.25
Overall difference (uncertainty ranges 95% CI)							281,879,015 to 509,098,159	

YLLs, years of life lived; Disc, discount rate; QALYs, quality-adjusted life years; PALYs, productivity-adjusted life years, CI, confidence interval

Years of life lost to *S.suis* infection

The total discounted years of life lost to *S.suis* infection among the cohort was 588.64 (13.7% of total years of life lived) among the 239 males and 180.18 (13.7%) among the 73 females. This equated to 2.46 and 2.47 years of life lost per male and female, respectively.

Quality-adjusted life years lost to *S.suis* infection

The total discounted QALYs lost to *S.suis* infection among the cohort was 632.04 (21.4% of total QALYs lived) among the 239 males and 193.55 (21.4%) among the 73 females. This equated to 2.64 and 2.65 QALYs lost per male and female, respectively.

Productivity-adjusted life years lost to *S.suis* infection

The total discounted PALYs lost to *S.suis* infection among the cohort was 607.08 (14.7% of total PALYs lived) among the 239 males and 185.84 (14.7%) among the 73 females. This equated to 2.54 and 2.55 QALYs lost per male and female, respectively.

In terms of broader economic costs, the PALYs lost to *S.suis* equated to 346 million Thai baht (US$11.3 million) lost in GDP, which equated to an average of 1.1 million baht lost (US$36,033) GDP loss per person.

Sensitivity analyses

Tornado diagrams were used to display one-way sensitivity analyses for QALYs and PALYs (Figures 2 and 3). The modelled results were most sensitive to productivity indices, utilities and discount rates. Variation in discount rate caused the largest variation in estimated QALYs lost to *S.suis* infection, from -133.1% to 74.1%, followed by hearing loss utilities, which resulted in -74.9% to 74.9% change in QALY.

Variation in discount rate also led to the greatest variation in estimated PALYs lost to *S.suis* infection, from -189.1% to 118.3%. Variation in case fatality and productivity index for hearing loss caused changes in PALYs lost from -25.9% to 35.4% and -3.9% to 60.5%, respectively. The other parameters exerted minimal impact on QALYs and PALYs lost.

The PSA indicates that the results were robust (Table 2). Overall burden due to *S.suis* infection ranged from 695 to 841 years of life , 588 to 1,098 QALYs, and 717 to 867 PALYs lost. The lost in GDP were estimated between 239.7 to 461.4 million Thai baht whereas the total net costs varied between 281.9 to 509.1 million Thai baht annually across the 10,000 simulations. Similarly, a slight variation in 95% uncertainty interval of total treatment cost was observed ranging between -55.2 to -39.0 million Thai baht.

Discussion

The study highlights the impact of *S.suis* infection on the years of life, QALYs, and PALYs lived in Thailand. Among cohorts of Thai population with *S.suis* infection followed over a lifetime, it was predicted that the infection would lead to 769 years of life lost (14%), 826 QALYs lost (21.4%) and 793 PALYs lost (14.7%). This was equivalent to 2.46 years of life lost, 2.64 QALYs and 2.54 years productivity lost per person. A 14.7% loss of PALYs was also associated with a significant economic impact of 346 million Thai baht (US$11.3 million) loss in GDP. The years of life, QALYs, and PALYs lost were greater among men due to a higher prevalence of the disease in male population. The results are consistent with estimates from sensitivity analyses reflecting the robustness of the model.

S.suis infection causes a significant health and economic burden in Southeast Asia particularly Thailand and Vietnam. Meningitis is the most common clinical presentation which leads to hearing

loss and vestibular dysfunction among most survivors [1]. A relatively high number of infective endocarditis (IE) as a major clinical manifestation was also reported in Thailand [9, 29, 30]. The disease affects mainly middle-age working men population [1, 4]. The previous study in Vietnam showed a large burden of disease disproportionately distributed towards working-age men and substantial economic impact from *S.suis* corresponding 1,437 disability-adjusted life years (DALYs) lost with the annual direct cost US$370,000 in 2014 [3]. Despite the disease being largely under-reported, the number of cases are likely to increase. According to the recent report from Bureau of Epidemiology, Department of Disease Control, Ministry of Health, Thailand, there were 337 *S.suis* cases with 28 deaths from 1 January to 23 October 2019 [31] which is already more than the individuals with infection in 2019 predicted in our model.

Our study is the first to quantify the impact of *S.suis* infection in terms of PALYs, which is a novel measure that account for productivity loss attributable to the disease. To our knowledge, there is only one previous study on economic impact of *S.suis* infection conducted in Vietnam [3]. However, the burden of disease was estimated in term of DALYs without estimation of long-term treatment such as follow-up audiological assessments and hearing aids and some major clinical manifestations and complications including infective endocarditis and cardiac complications after IE were not accounted. As the disease is regarded as being “rare” and largely under-reported, this information would provide important insights on the disease long-term outcome and economic consequences which is far beyond the acute infection to policy makers in planning for disease control and prevention.

The model was sensitive to productivity indices, utilities and discount rate which implies the wide ranges of disease sequelae, spectrum of clinical manifestations, and productivity attributed to the disease particularly hearing impairment with or without vestibular dysfunction. The lowest and highest ends of the 95% CI uncertainty range for YLLs, QALYs, PALYs and total net cost differed modestly from the base case analyses which suggest robustness of the model.

Food safety campaigns or public health interventions to raise the disease awareness are potentially effective for disease control and prevention. According to a study on impact of a food safety campaign in Phayao province in northern, Thailand, the disease incidence was markedly reduced

during the first two years after the program implementation but started to rise again in the third year [32]. This emphasizes the existence of deep-rooted cultural behavior of raw pork consumption which is a major cause of infection and the need for continuous effective public health interventions to improve awareness among Thai population and healthcare policy makers. Raw pork eating practice was also identified as one of significant predictors (raw pork eating, meningitis, and vestibular dysfunction) of *S.suis* hearing loss which is a major sequelae among most surviving patients [33]. Considering significant economic burden incurred from *S.suis* infection which is far more than acute infection treatment, reducing incidence of new cases can potentially save costs substantially in a long term.

There are a number of limitations from our study that warrant mention. Our direct medical cost estimate was based on the hospital charges at a tertiary hospital (Chiang Mai University Hospital) in Northern, Thailand. Therefore, this may not represent treatment cost in primary settings. However, *S.suis* infection is usually severe and life-threatening in which majority of cases would require tertiary care. The same utility for hearing loss and hearing loss with vestibular dysfunction was used in our model for base case estimate due to limited data available. However, even with highly conservative assumptions, the results showed significant economic impact from *S.suis* infection. Some cost parameters including cochlear implant, rehabilitation relating to vestibular disorder were not included due to a lack of reliable information. According to the previous finding by Health Intervention and Technology Assessment Program (HiTAP), Thailand, cochlear implant is not a cost effective intervention in any group of patients with deafness [34]. The estimated cost per patient was as high as nearly 100,000 THB (US$ 31,811) in the first year and 30,000 THB (US$ 977) in subsequent years [35]. Therefore, it is unlikely that this intervention will be reimbursed in Thailand in the near future. Using life-table modeling which is a simple and commonly used tool, the age-specific mortality and RR of dying from IE would be constant over time which is a well-known limitation of the “life table assumption”. Nonetheless, this approach was applied to both individuals with infection and no infection and the disease impact after the acute infection is unlikely to change tremendously. Therefore, this should not have resulted in significant change of our estimates and conclusion. In addition, local data was not available for some input parameters including productivity indices of

S.suis infection in Thai population. In the absence of presenteeism data for IE, only absenteeism was used in the productivity indices calculation. This may have led to underestimation of productivity indices and economic impact. Finally, the simulated model assumed that GDP was stable rather than increasing over time throughout the follow-up which is not likely to be the case. This would also have led to underestimation of the economic impact from *S.suis* infection. Notwithstanding with these limitations, the overall conclusion of the study would be unlikely to change.

In conclusion, *S.suis* infection imposes a significant economic burden both in terms of health and productivity. Future research to investigate the effectiveness of public health awareness programs and disease control interventions should be carried out to provide a clearer picture to assist decision making in public health strategies and resource allocations.

Author contributions

AR and DL conceived, designed the study and developed the model. AR, ZA, DL made substantial contributions to analysis and interpretation of the data. All authors (AR, ZA, DL, LLH) contributed substantially to drafting and revision of the manuscript for important intellectual content. AR drafted the manuscript. ZA, DL, and LLH contributed to study supervision.

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Conflict of interest

All authors declare no competing interests.

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Figure 1 A decision tree and Markov model of clinical manifestations from *S.suis* infection

Figure 2 One way sensitivity analysis-QALYs

Figure 3 One way sensitivity analysis-PALYs

Figure 1 A decision tree and Markov model of clinical manifestations from *S.suis* infection

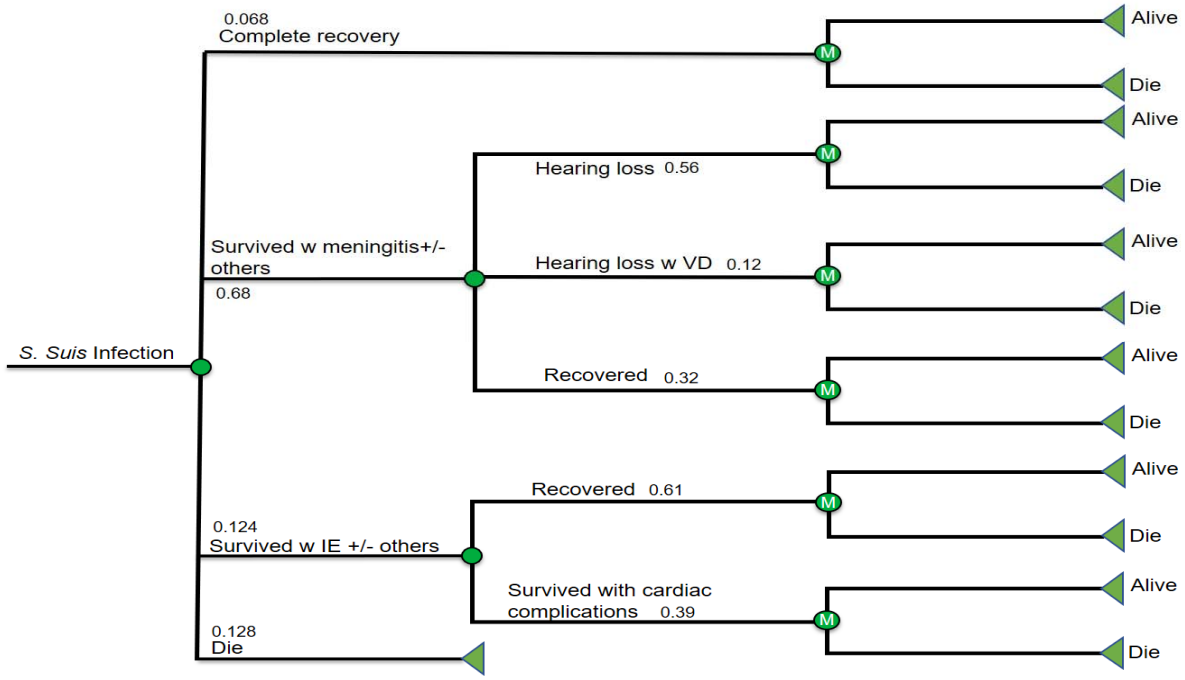


Figure 2 One way sensitivity analysis-QALYs

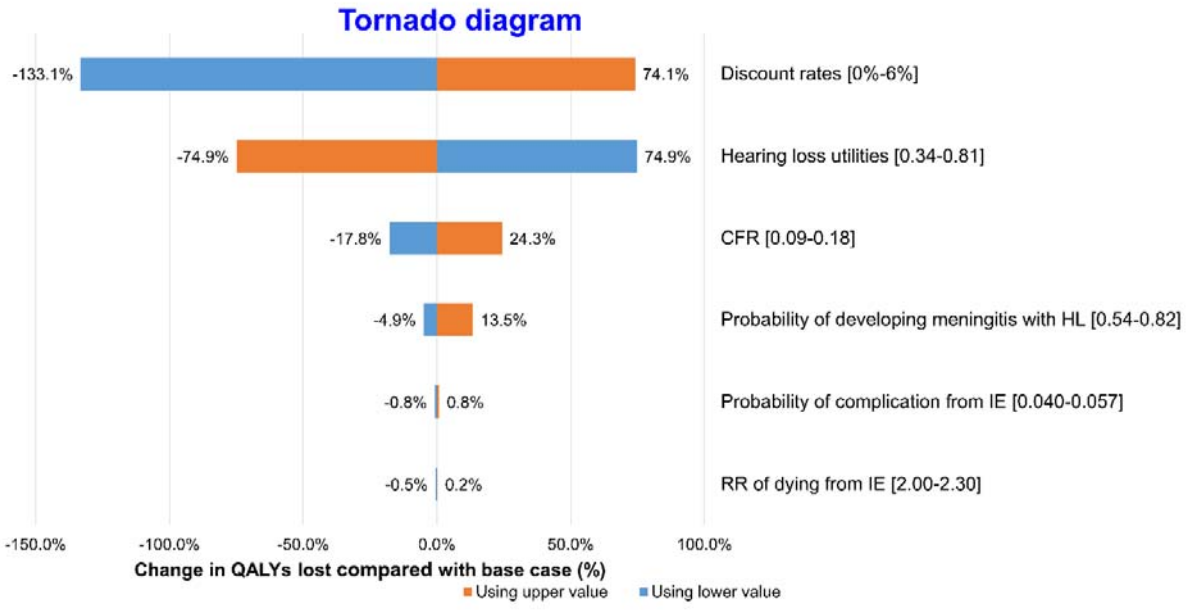
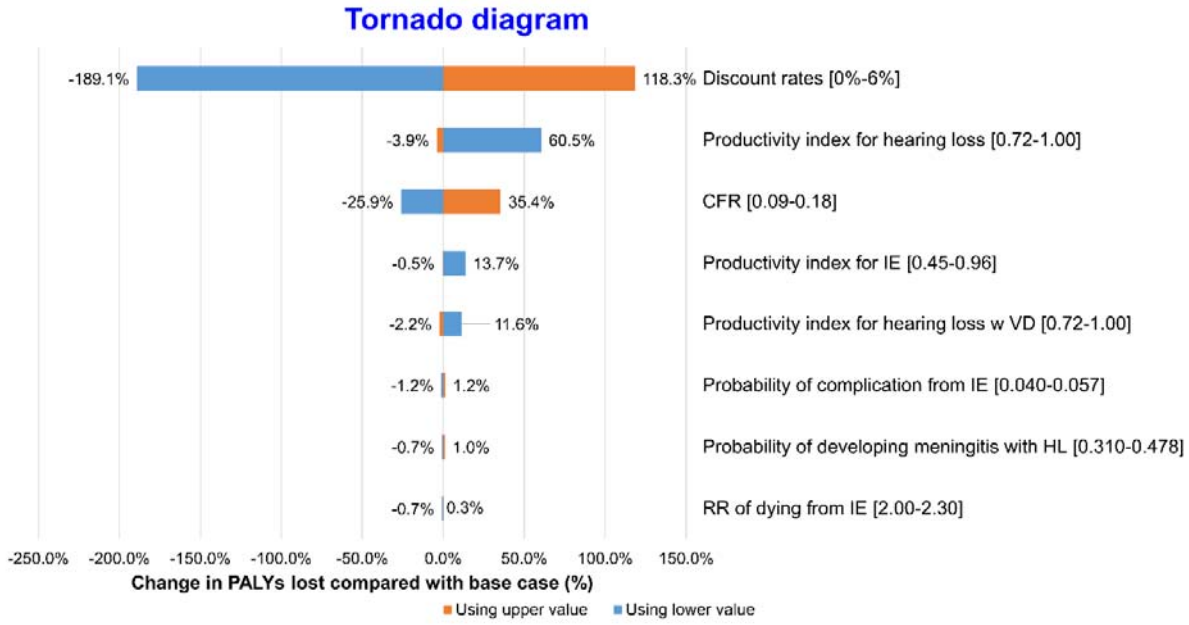


Figure 3 One way sensitivity analysis-PALYs



Chapter 7:

Discussion and conclusions

7. Chapter 7: Discussion and conclusion

This last Chapter summarises the main findings of this research project as well as their implications and recommendations for relevant policies, interventions and future research to be emphasised. The contents of Chapter 7 can be summarised as follows:

7.1 Main findings of the research

7.2 Limitations and strengths

7.3 Conclusions and future directions

7.1 Main findings of the research

The ultimate goal of the study was to explore insights on risk factor of *S.suis* mortality and hearing loss as well as to quantify the burden of the disease and its economic impact in Thailand. This research consists of three main studies to address four main objectives which are inter-related. The graphical summary of the project is illustrated as below:

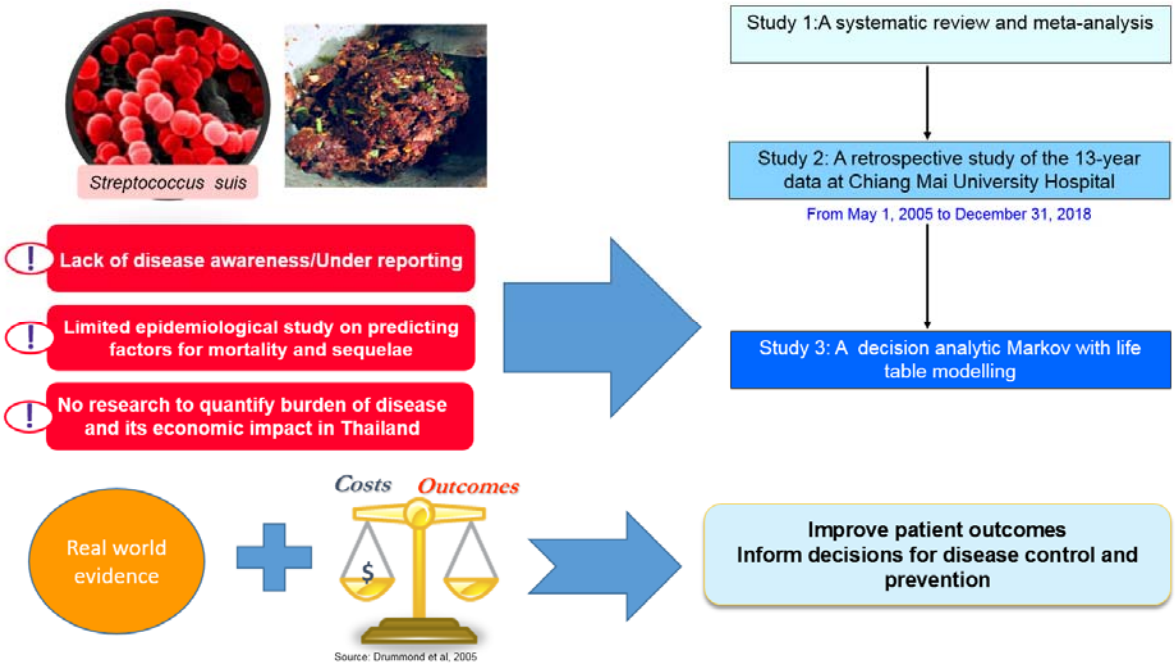


Figure 7.1 Graphical summary of the project

The followings provide a summary of key findings respective to each specific research question.

Research Question 1:

What are potential risk factors in acquiring *S.suis* infection?

As shown from our systematic review and meta-analysis (Chapter 3), major risk factors associated with *S.suis* infection include raw pork consumption, exposure to pigs or pork, male sex, and pig-related occupation (Figure 7.1A-D).

Raw pork consumption was common in studies from in studies from Vietnam and Thailand *(33, 42, 55, 60, 78, 111)* whereas those with occupation related to pigs are certain risk population in Western countries *(34, 112)*. This reflects on the traditional culture involving raw pork consumption in Asia countries. A higher prevalence of the disease in males can be explained by the fact that the disease is occupation related and their risky behaviours e.g. slaughtering activities, raw pork consumption and alcohol drinking which have posed them to be more susceptible to infection.

Figure 7.1.1A-D Meta-analysis results on risk factors associated with *S.suis* infection

Figure 7.1.1A Raw pork consumption

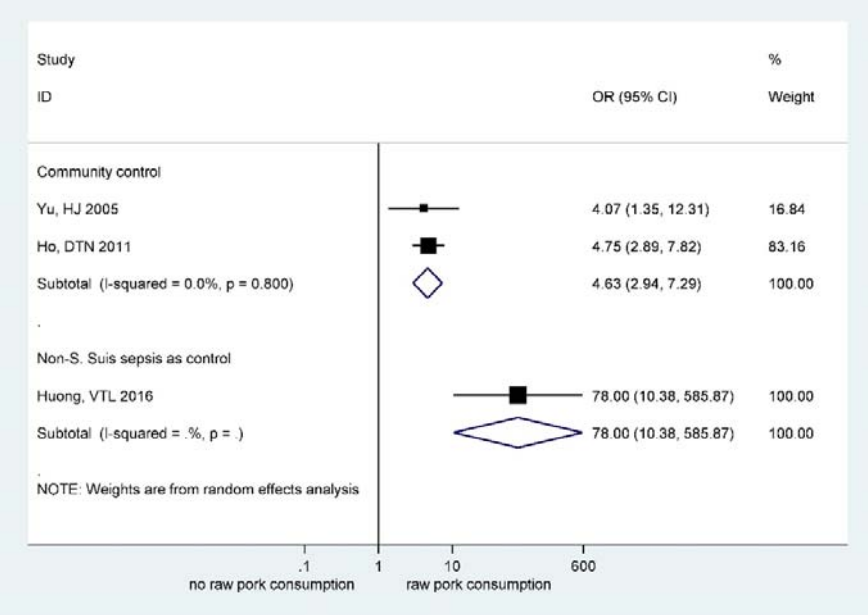


Figure 7.1.1B Exposure to pigs or pork

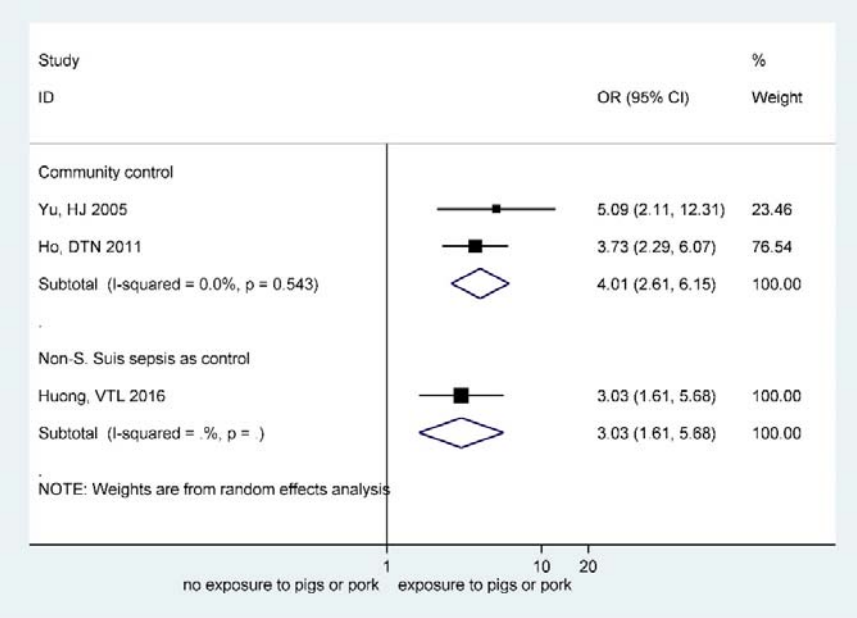


Figure 7.1.1C Male sex

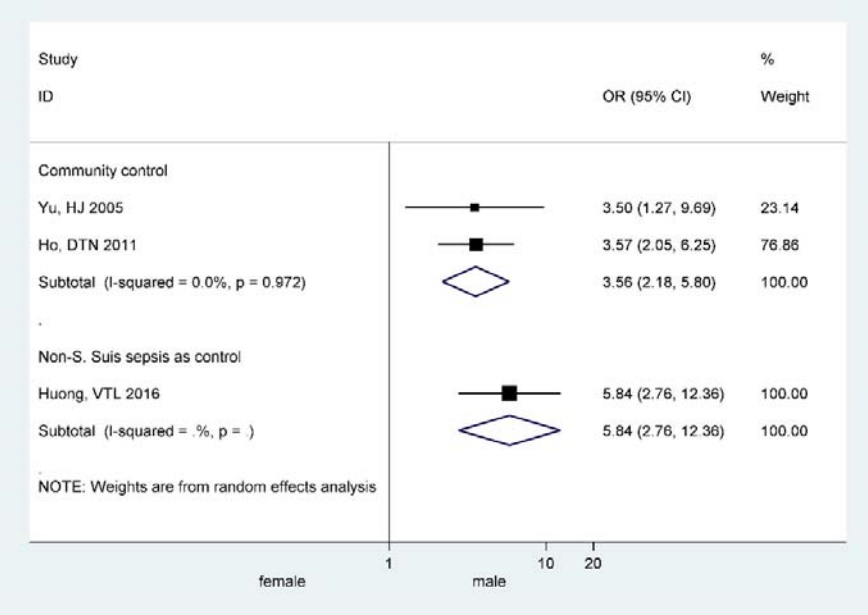
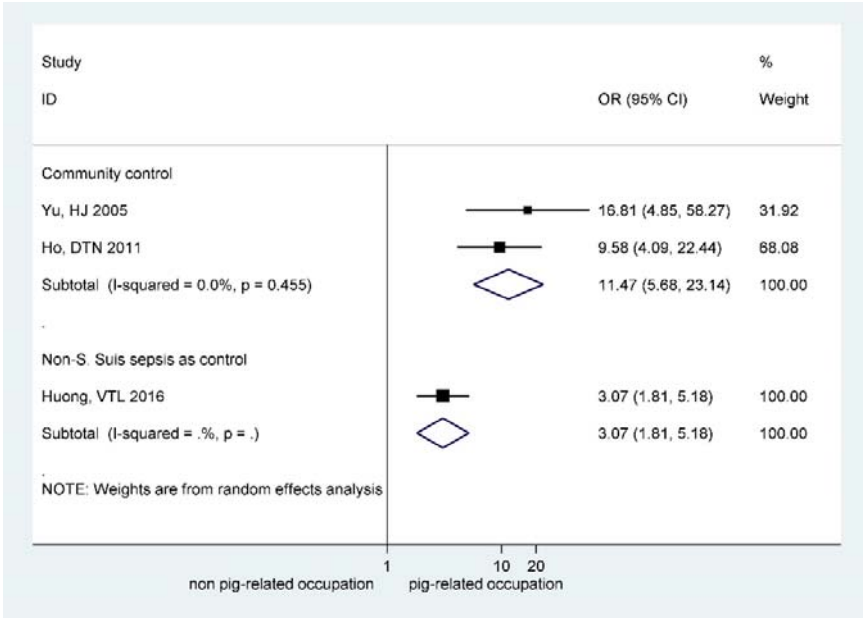


Figure 7.1.1D Pig-related occupation



Research Question 2:

What are predictive factors that affect *S.suis* mortality and the main features/clinical manifestation of the disease?

We conducted a 13-year retrospective cohort study and identified a number of risk factors of *S.suis* mortality (Chapter 4). From multivariate analysis, time to microbiological cure ≥ 6 days, septic shock, and direct bilirubin > 1.5 mg/dl were significant risk factors associated with *S.suis* mortality (Table 7.2.1). Forward and backward stepwise multivariate logistic regression was also performed and similar predictors were identified with low albumin level below 3.5 g/dl as additional predictor (Figure 7.2.2).

Table 7.2.1Significant predictors of *S.suis* mortality

Predictors	**Univariate analysis**			**Multivariable analysis**		
	OR	**95% CI**	**p-value**	**Adjusted OR**	**95% CI**	**p-value**
GCS <8	9.42	2.08-42.55	0.0058	1.71	0.10-28.50	0.709
Time to microbiological cure ≥ 6 days	6.21	1.35-28.55	0.0052	**43.57**	**2.46-772.80**	**0.010**
ALD	4.38	1.29-14.93	0.0262	2.24	0.32-15.84	0.417
Acute meningitis	0.21	0.045-0.95	0.0176	0.24	0.03-2.33	0.236
Septic shock	12.39	3.86-39.79	<0.001	**13.34**	**1.63-109.03**	**0.016**
Direct bilirubin >1.5 mg/dl	5.30	1.77-15.88	0.0024	**12.86**	**1.91-86.59**	**0.009**
Creatinine >1.8 mg/dl	4.73	1.60-13.96	0.0050	0.41	0.01-6.49	0.414
Bicarbonate <18 mmol/L	4.91	1.60-15.04	0.0073	3.00	0.12-73.62	0.500
Albumin <3.5 g/dl	5.04	1.10-23.21	0.0149	10.97	0.96-125.81	0.054

Table 7.2.2 Significant predictors of *S.suis* mortality using forward and backward stepwise logistic regression

Predictors	Multivariable analysis			Multivariable analysis		
	Adjusted OR	95% CI	p-value	Adjusted[a] OR	95% CI	p-value
GCS <8	1.71	0.10-28.50	0.709	-	-	-
Time to microbiological cure > 6 days	**43.57**	2.46-772.80	**0.010**	**33.19**	**2.82-386.10**	**0.005**
ALD	2.24	0.32-15.84	0.417	-	-	-
Acute meningitis	0.24	0.03-2.33	0.236	-	-	-
Septic shock	**13.34**	1.63-109.03	**0.016**	**13.61**	**2.57-72.00**	**0.002**
Direct bilirubin >1.5 mg/dl	**12.86**	1.91-86.59	**0.009**	**17.06**	**2.73-106.47**	**0.002**
Creatinine >1.8 mg/dl	0.41	0.01-6.49	0.414	-	-	-
Bicarbonate <18 mmol/L	3.00	0.12-73.62	0.500	-	-	-
Albumin <3.5 g/dl	10.97	0.96-125.81	0.054	**23.46**	**2.44-225.92**	**0.006**

Note: Significant predictors were indicated in bold.

[a] Forward and backward stepwise logistic regression with p-value <0.05

Antimicrobial treatment failure could be a reliable factor causing mortality especially during the first 72 hours to ten days *(113)*. Close patient monitoring, and receiving effective antibiotic treatment, was probably responsible for the relatively low number of deaths during the period *(114)*. Hyperbilirubinemia accentuates an impact from liver damage which might be the result from systematic infection *(115, 116)*. This is consistent with clinical symptoms found in the study patients caused by septicaemia including febrile illnesses, elevated liver enzymes, and low albumin level.

Hypoalbuminemia could potentially be a predictor of *S.suis* mortality from forward and backward stepwise logistic regression. Although the association was not statistically significant in the full model analysis, its p-value was very close to significant level (0.054) (Table 7.2.1). Limited sample size might have led to this insignificant result.

Among 133 culture-proven *S.suis* patients identified, septicaemia (55.64%) was the most common clinical presentation followed by meningitis (37.59%) and infective endocarditis (22.56%) *(102)*. More than one third of *S.suis* infected patients had a history of raw pork consumption and more than half of cases reported alcohol drinking *(102)*. Raw pork dish which is called *"Larb Dib"* in northern Thailand is usually consumed together with alcohol

drinks during social or gathering events. This indicates that traditional culture of raw pork consumption plays an important role of the disease transmission.

Research Question 3:

What are predictors of *S.suis* hearing loss?

From multivariable model among 133 *S.suis* patients, significant predictors of *S.suis* hearing loss were meningitis, raw pork consumption and vertigo (Table 7.3.1). A risk scoring system to predict the risk of *S.suis* hearing loss was developed (Chapter 5). The model demonstrated acceptable performance (AuROC: 0.86; 95%CI 0.79-0.93) and stability (AuROC: 0.86; 95%CI 0.72-0.95). The predictive score ranged from 0-4 and correctly classified 81.95% patients as being at risk of *S.suis* hearing loss (Table 7.3.2). The scoring system suggested a good performance in predicting *S.suis* hearing loss (AuROC 0.87; 95% CI 0.80-0.95) (Figure 7.3.1).

Table 7.3.1 Derived item scores from multivariable logistic regression (n=133)

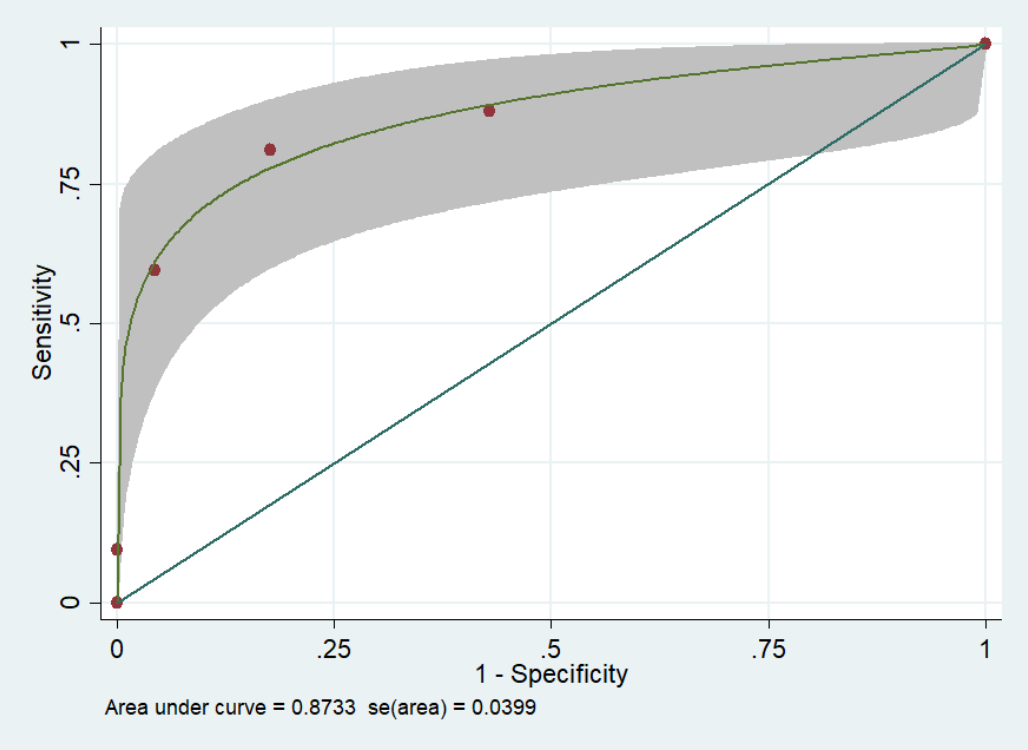
Predictors	OR[a]	95%CI	p-Value	ROC Area (95%CI)	Beta coefficient (intercept = -53.11)	Score
Meningitis						
No	1.00	reference	-		-	0
Yes	16.64	6.25-44.30	<0.001	0.82 (0.75-0.89)	2.812	2
Raw pork consumption						
No	1.00	reference	-		-	0
Yes	3.52	1.32-9.42	0.012	0.67 (0.58-0.75)	1.26	1
Vertigo						
No	1.00	reference	-		-	0
Yes	5.06	0.74-34.41	0.097	0.57 (0.51-0.63)	1.62	1

[a] Model for Multivariable logistic regression using backward stepwise logistic regression

Table 7.3.2 Risk stratification of prediction values of *S.suis* hearing loss score

Risk classification	Scores	Outcomes		Sensitivity (%)	Specificity (%)	Correctly classified (%)	LR+ (95%CI)	LR- (95%CI)
		Hearing loss	Non-hearing loss					
Low	1	18	39	100	0	31.58	1.00	0
Moderate	2	15	8	88.10	51.14	66.92	2.06	0.21
High	3	19	5	80.95	79.12	79.70	3.88	0.24
Very high	4	24	5	59.52	95.60	84.21	13.54	0.42

Figure 7.3.1 Area under the receiver-operator characteristic curve (AuROC) for the scoring system to predict *S.suis* hearing loss with 95% confidence interval



Research Question 4:

What are the burden of disease and economic impact of *S.suis* infection in Thailand?

According to a decision-analytic Markov model to estimate the burden of disease from *S.suis* infection and its major complications in Thailand, it was estimated that there were 312 people acquired *S.suis* infection in 2019. With simulated follow-up from the age of 51 years until death with an annual discount rate of 3% applied, this cohort incurred 769 years of life lost (14% of predicted years of life lived had they not been infected), 826 QALYs lost (21%) and 793 PALYs (15%) lost. These equated to an average of 2.5 years of life lost, 2.6 QALYs and 2.5 PALYs per person (Table 7.4.1). In terms of broader economic impact, the loss of PALYs was estimated to cost 346 million Thai baht (US$11.1 million) in lost gross domestic product (GDP), which was equivalent to 1.1 million Thai baht (US$ 35,413) per person. The burden of disease and its economic impact were greater in men due to a higher disease prevalence in this population. The results reflect substantial cost from the infection which is far beyond acute infection treatment.

Table 7.4.1 Base-case analyses results

	Male		Female		Total		Difference (%)	Lost per person
	Infection	No infection	Infection	No infection	Infection	No infection		
YLLs	5,482	6,376	1,680	1,954	7,162	8,329	1,167 (14.01)	3.74
Disc 3%	3,707	4,296	1,136	1,316	4,844	5,612	769 (13.71)	2.46
QALYs	3,422	4,352	1,049	1,334	4,471	5,686	1,214 (21.36)	3.89
Disc 3%	2,323	2,955	712	906	3,035	3,861	826 (21.38)	2.64
PALYs	5,219	6,139	1,600	1,881	6,818	8,020	1,202 (14.98)	3.85
Disc 3%	3,529	4,136	1,082	1,267	4,611	5,404	793 (14.67)	2.54
Total treatment cost	35,813,161	0	10,949,289	0	46,762,449	0	-46,762,449	-149,661
Disc 3%	34,804,660	0	10,639,082	0	45,443,742	0	-45,443,742	-145,441
Broader economic cost (million)	2,276	2,677	697	820	2,973.29	3,497	524	1.68
Disc 3% (million)	1,539	1,804	472	553	2,011	2,356	346	1.11
Net cost	2,2340	2,677	687	820	2,927	3,497	571	1.83
Disc 3%	1,504	1,804	461	553	1,965	2,356	391	1.25

YLLs, years of life lived; Disc, discount rate; QALYs, quality-adjusted life years; PALYs, productivity-adjusted life years

7.2 Strengths and limitations of the research

This research project has a number of strengths and limitations. Other than what mentioned in each Chapter of this thesis, major strengths and limitations that are worthwhile mentioning can be summarised as follows:

1. Our systematic review and meta-analysis was exhaustive. The searches were done in eight major databases with no time or language restrictions. However, the results suggest scarcity and heterogeneity of data on *S.suis* infection. The population included in meta-analysis from the three case-control studies were different in terms of demographics and characteristics. Therefore, the meta-analysis by type of control group (community controls and non-*S.suis* sepsis diagnosed cases) was performed. All major risk factors including raw pork consumption, exposure to pigs or pork, pig-related occupation and male sex were found to be significantly associated with *S. suis* infection and there was no significant heterogeneity observed between studies according to the analyses. The fact that most data were largely descriptive and

majority were retrospective studies warrant the need for increased scientific and research attention of this infection.

2. Our 13-year retrospective cohort study was one of the largest number of *S.suis* cases reported in the country which covered different types of clinical manifestations over a longer period compared to previous studies *(33, 55, 111)*. All *S.suis* culture-proven positive patients were included regardless of their age unlike previous studies which usually include only adult population *(33, 38, 55)*. However, due to the retrospective nature of the study, the information could be potentially subject to information bias. With a low prevalence of the disease and the lack of funding support for this research project, conducting a prospective study would not have been possible.
3. Considering that *S.suis* is a rare disease, a small sample size is acceptable but this could influence the ability of the results to inform medical decisions, not to mention of mixed data from 13 years and practices or procedures which might have changed over time. Nevertheless, audiometry was routinely used to diagnose hearing loss at the study setting throughout the whole study period and *S.suis* infection is still generally susceptible to common antibiotics such as penicillin and ceftriaxone. Therefore, it is unlikely that this led to any change of the conclusion of study.
4. Our risk scoring system development to predict the risk of *S.suis* hearing loss was an important study in the area of prognosis and probably the first attempt in identifying a tool to support clinicians in managing the disease impact on sequelae among surviving patients. Despite the model showed good power of prediction, acceptable performance, discrimination and internal validation, there was no external validation. In addition, generalisability of the findings may be highly uncertain as the study was conducted in northern, Thailand where traditional habit of raw pork consumption is practiced. Therefore, interpretation of findings should be done with caution.
5. A decision-analytic Markov modelling conducted was the first study to measure the impact from *S.suis* in term of PALYs (productivity-adjusted life years), which is a novel measure that accounts on the loss of work productivity attributable to the disease *(108)*. A decision tree and Markov model to reflect *S suis* infection and its major complications was carefully developed and comprehensively constructed. Nevertheless, in the absence of a number of local data, other relevant sources were used as input parameters. This included the data from Thai Acute Coronary Syndrome (ACS) registry which was used to estimate chronic treatment cost for infective endocarditis due unavailability of cost data in infective endocarditis or rheumatic heart disease. However, this was already considered to be the most

comprehensive cost study in Thailand. Also, presenteeism was not accounted in productivity indices calculation for proportional reduction in work productivity which could have led to underestimation on productivity indices and economic impact. However, even with highly conservative estimation, the results showed significant economic impact from *S.suis* infection.

7.3 Conclusions and future directions

Streptococcus suis (S.suis), the gram-positive, alpha-hemolytic bacterium whose natural host is mainly the pig can cause serious infection and long-term complications. The disease mainly affects adults and elderly with mean age ranging between 37-63 years *(1)*. Majority of patients were men which was probably due to their risky behaviours such as raw pork consumption, slaughtering activity and alcohol use which have posed them to be more susceptible to infection *(1, 32)*. Meningitis, septicaemia were most frequent clinical presentations in which hearing loss is the most common complication among surviving patients *(1, 32)*. A high number of *S.suis* patients with infective endocarditis was found in Thailand whose aortic involvement was the most common vegetation site found and long-term complications were common *(1, 32)*. Under-reporting and lack of disease awareness are major issues causing the disease to be largely neglected with poor disease outcomes. Timely diagnosis and immediate antibiotic treatment are essential to alleviate the impact of the disease and its complications particularly hearing loss which usually occurs among most surviving patients.

Despite growing of evidence and increased scientific attention in this neglected zoonotic disease, there has been little progress in disease control and prevention. From this research project findings, the impact from *S.suis* is far greater than acute infection and involved long-term consequences including premature mortality, sensorineural hearing loss and vestibular dysfunction. The disease imposes significant economic burden both in terms of health: 769 (14%) years of life lost or 2.5 years of life lost per person, 826 (21%) QALYs lost or 2.6 QALY per person, 793 (15%) PALY lost or 2.5 PALY per person, and productivity: 346 million Thai baht (US$11.3 million) lost in GDP or 1.1 million Thai baht (US$ 36,033) lost per person in Thai population even with highly conservative assumption. This research project highlights the importance of public health awareness programs targeting on behaviour changes to reduce the number of new cases which could potentially save high costs in a long term.

In spite of several limitations, this research project is an important contribution to the knowledge of this significant but little-studied disease. The systematic review and meta-analysis performed was rigorous and comprehensive and was the first study in which meta-analysis on risk factors of *S.suis* infection was done. The 13-year retrospective study conducted was one of the largest series in the country on *S.suis* patients. The study covered different types of clinical manifestations and epidemiological patterns over a longer period compared to previous studies *(33, 42, 55, 111)* with *S.suis* mortality risk factors confirmed by multivariate logistic regression. A risk scoring system on *S.suis* hearing loss developed was the first attempt in trying to find a tool for clinicians in managing and minimising the impact from the disease sequelae despite the lack of external validation. The final study, a decision-analytic Markov model with life-table modelling was the first study to quantify the health and economic burden of *S.suis* infection in Thailand and also the first model that applied PALYs (productivity-adjusted life years) to account on productivity loss attributable to this infection.

Overall, the research provides significant insights in the disease epidemiology, clinical presentations and its economic impact both in terms of health and productivity which would be useful for improved treatment, early diagnosis and detection to prevent or alleviate long-term complications and informing decisions on public health measures for disease control and prevention.

Based on the research findings and discussion, the following suggestions for future research could be summarised as follows:

1. The nature of retrospective study poses several limitations regarding the data quality, incomplete information and utility of analysis. Due to a low prevalence of the disease and a small sample size would impede statistical analysis, a multi-centre prospective study and future research collaborations would be essential to generate more precise evidences in this area.
2. Further research to optimize the risk scoring system to predict the risk of *S.suis* hearing loss and external validation are clearly needed. Although the model showed good power of prediction and calibration, internal calibration was done in the same population due to limited sample size. This limits generalisability and reliability of the model. Validation in different population would be essential to confirm the reliability of the model in other clinical settings.

3. Under reporting and misdiagnosis are common in *S.suis* infection. Apart from that serotyping detection is usually not routinely available in hospital settings. Therefore, development of surveillance and strengthening laboratory network are essential to monitor the extent of the disease and facilitate understanding on the pathogenesis and virulence of the disease as well as the association with clinical manifestations.
4. Further research to investigate on pathogenesis and mechanism of *S.suis* hearing loss and vestibular dysfunction is required. Sensorineural hearing loss is the most common complication from *S.suis* meningitis *(1, 33, 38, 55, 102)* and much more pronounced among *S.suis* surviving patients compared to other bacterial meningitis *(117)*. Better understanding of the pathogenesis and the site of auditory lesion would facilitate optimal treatment regimens and clinical management to reduce the impact from the disease sequelae.
5. Further research to identify effective treatment to minimise the impact from *S.suis* infection particularly hearing loss sequelae as well as the effectiveness of adjunctive corticosteroids treatment should be carried out. The benefits of adjunctive corticosteroids in reducing mortality and hearing loss has been established in paediatric meningitis but has yet been clearly confirmed in adult population *(82)*. As *S.suis* mainly affects healthy adults, its effectiveness in *S.suis* meningitis remains uncertain. This aspect could not be elucidated in our study due to variation of treatment regimens and small number of patients received adjunctive corticosteroids.
6. The fact that pigs are usually natural reservoir of *S.suis* infection and swine exposure is a major cause of the disease transmission highlights the significance of multi-disciplinary research between veterinary and human healthcare professionals. Under current clinical paradigm that veterinarians and clinicians usually work in parallel with little “cross-professional communication” *(62)*. The concept of “One Health” involving both animal and human healthcare workers should be considered for effective disease control and prevention.
7. Future studies to investigate the effectiveness and cost-effectiveness of *S.suis* disease control and prevention interventions should be mandated. According to the study on the impact from food safety campaign in Phayao Province, northern, Thailand, the program was effective and resulted in a significant decline of *S.suis* infection during the first two years after program implementation *(52)*. However, the disease incidence started to rise again in the third year *(52)*. This reflects on the deep-rooted cultural behaviour of raw pork eating among northern Thai population and emphasises the importance of continuous public health interventions targeting

behaviour changes to reduce incidences of new cases which can potentially save high costs in a long term.

This research project can serve as a pilot study that provides important insights, better understanding and economic burden of this zoonotic disease, but yet there is a long way to go.

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Appendices

Appendix 1 Study 1 Protocol registration in PROSPERO

PROSPERO
International prospective register of systematic reviews

NHS
National Institute for Health Research

Risk factors for Streptococcus suis infection: a systematic review and meta-analysis

Ajaree Rayanakorn, Bey Hing Goh, Learn-Han Lee, Tahir Mehmood Khan, Surasak Saokaew

Citation

Ajaree Rayanakorn, Bey Hing Goh, Learn-Han Lee, Tahir Mehmood Khan, Surasak Saokaew. Risk factors for Streptococcus suis infection: a systematic review and meta-analysis. PROSPERO 2018 CRD42018083596 Available from: http://www.crd.york.ac.uk/PROSPERO/display_record.php?ID=CRD42018083596

Review question

What predisposing factors are associated with the risk of Streptococcus suis infection?

Searches

A systematic search will be carried out in CINAHL Plus, The Cochrane Library, EMBASE, Global Health, the grey literature, Ovid MEDLINE, PubMed, and ScienceDirect.

The MeSH terms to be used will be: "Streptococcus suis" OR "Streptococcus suis AND infection".

The searches will be limited to those conducted in humans, but there will be no time or language restrictions imposed.

Additional search strategy information can be found in the attached PDF document (link provided below).

Types of study to be included

Inclusion criteria: studies including risk factors with or without clinical characteristics or outcomes for Streptococcus suis infection in humans, in which the cause of the disease has been explained and described in at least four patients.
Exclusion criteria: reviews, systematic reviews and meta-analyses, and studies which do not include any information about the risk factors or causes of the disease.

Condition or domain being studied

Streptococcus suis is a gram-positive bacterial pathogen of pigs which can cause serious infections in humans, including meningitis, septicaemia and many other conditions.
To date, there have been no systematic reviews that have comprehensively investigated Streptococcus suis infection risk factors in humans. This systematic review and meta-analysis therefore aims to identify the potential risk factors associated with infection, and to provide an update on the evidence regarding its clinical presentations and outcomes.

Participants/population

Inclusion criteria: individuals who have become infected with Streptococcus suis.

Exclusion criteria: patients infected with other bacterial pathogens.

Intervention(s), exposure(s)

Streptococcus suis infection.

Comparator(s)/control

Non-exposed groups (those not infected with S. suis).

Context

Studies in areas where there is pig rearing, raw pork consumption, or populations whose occupations involve exposure to pigs or to pork.

Main outcome(s)

Identification of the risk factors associated with acquiring Streptococcus suis infections.

Timing and effect measures

Measured as a percentage, RR, OR, as the total number in exposed and non-exposed groups or as event rates.

Additional outcome(s)

Clinical presentations and outcomes of S. suis infection.

Timing and effect measures

Measured as a percentage or as an event rate.

Data extraction (selection and coding)

Data will be extracted from the studies selected for inclusion in the review by one reviewer (AR), and further confirmed by other investigators, either BHG, LHL, TMK or SS. Any disagreements arising will be resolved through the consultation process, and the attainment of consensus between the reviewers and all authors. The information to be extracted will include: predisposing factors included, patient characteristics, clinical presentations, treatments and outcomes.

Risk of bias (quality) assessment

Quality assessment for the included studies will be performed independently by two reviewers (AR and TMK). The Newcastle-Ottawa Scale (NOS) will be used for non-randomized studies, and randomized controlled studies will be evaluated using a revised tool for the assessment of the risk of bias in randomized trials (RoB 2.0), which is based on the Cochrane Collaboration's approach.

Strategy for data synthesis

A meta-analysis for the risk factors will be used for case control or cohort studies, if the results can be pooled using Review Manager Software (RevMan version 5.3; Cochrane Collaboration), and a random-effects model will be used to pool the results on associated risk factors in order to account for any heterogeneity. Forest plots will be used the display the effect sizes (ES) for each study, with corresponding 95% CI and overall estimated ES, and the I^2 statistic will be used to test for levels of heterogeneity.

Analysis of subgroups or subsets

Four main predisposing factors will be investigated:

(1) Exposure to pigs or pork, defined as the history of, or the recollection of, the exposure to pigs or pork before illness without slaughtering;

(2) Pig-related occupations, including farmers, butchers and laborers in the swine industry; (3) The consumption of raw pork, defined as the consumption of undercooked pork, including swine-based materials; (4) Male sex

An analysis on each risk factor type will be performed, if possible.

Contact details for further information

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Organisational affiliation of the review

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www.up.ac.th

Review team members and their organisational affiliations

Ms Ajaree Rayanakorn. School of Pharmacy, Monash University Malaysia
Dr Bey Hing Goh. School of Pharmacy, Monash University Malaysia
Dr Learn-Han Lee. School of Pharmacy, Monash University Malaysia
Dr Tahir Mehmood Khan. School of Pharmacy, Monash University Malaysia
Assistant/Associate Professor Surasak Saokaew. School of Pharmaceutical Sciences, University of Phayao, Phayao, Thailand

Type and method of review
Epidemiologic, Meta-analysis, Systematic review

Anticipated or actual start date
18 September 2017

Anticipated completion date
31 March 2018

Funding sources/sponsors
None

Conflicts of interest

Language
(there is not an English language summary)

Country
Thailand, Malaysia

Stage of review
Review Completed published

Details of final report/publication(s)
Rayanakorn A, Goh B-H, Lee L-H, Khan TM, Saokaew S. Risk factors for Streptococcus suis infection: A systematic review and meta-analysis. Scientific Reports. 2018;8:13358. doi:10.1038/s41598-018-31598-w. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6127304/

Subject index terms status
Subject indexing assigned by CRD

Subject index terms
Agriculture; Farmers; Farms; Humans; Red Meat; Risk; Risk Factors; Streptococcal Infections; Streptococcus suis; Sus scrofa; Swine; Zoonoses

Date of registration in PROSPERO
09 January 2018

Date of publication of this version
29 October 2018

Details of any existing review of the same topic by the same authors

Stage of review at time of this submission

Stage	Started	Completed
Preliminary searches	Yes	Yes
Piloting of the study selection process	Yes	Yes
Formal screening of search results against eligibility criteria	Yes	Yes
Data extraction	Yes	Yes
Risk of bias (quality) assessment	Yes	Yes
Data analysis	Yes	Yes

Versions

09 January 2018
29 October 2018

Appendix 2 Study 1- Results of critical appraisal of included case-control studies based on the Newcastle-Ottawa Scale (NOS)

Study	SELECTION				COMPARABILITY	EXPOSURE		
	1. Is the case definition adequate?	2. Representative of the cases	3. Selection of controls	4. Definition of Controls	1. Comparability of Cases and Controls on the Basis of the Design or Analysis	1. Ascertainment of Exposure	2. Same method of ascertainment for cases and controls	3. Non-Response Rate
Yu et. al. 2005 (51)			+				+	
Ho et. al. 2011 (34)	+		+	++	+	+	+	
Huong et. al. 2016 (21)	+	+		+		+	+	

Appendix 3 Ethics Approval from Research Ethics Committee, Faculty of Medicine, Chiang Mai University

No. 010/2018

Certificate of Approval

Name of Ethics Committee : Research Ethics Committee 4,
Faculty of Medicine, Chiang Mai University

Address of Ethics Committee : 110 Intavaroros Rd., Amphoe Muang, Chiang Mai, Thailand 50200

Principal Investigator: Ajaree Rayanakorn.
Faculty of Pharmacy, Monash University.

Protocol title: A retrospective study to assess the clinical treatment, outcomes and antimicrobial susceptibility of Streptococcus suis infection among patients in northern Thailand.

STUDY CODE: NONE-2560-05141/ Research ID : 5141

Sponsor:-

Documents filed	Document reference
Research protocol	Version 1.0 date 17 November 2017
Case Record Form	Version 1.0 date 17 November 2017
Principal Investigator Curriculum vitae	Version date 22 November 2017

DECISION : [✓] By expedited review
[ ] By full committee meeting .....Date : .....

Opinion of the Ethics Committee/Institutional Review Board : PLS. CHECK ONE

✓ Approval

.....

Progress report submit every ☐ 3 months ☐ 6 months

☑ 1 year ☐ Other.....

Date of Approval: 10 January 2018 Expiration Date: 9 January 2019

This Ethics Committee is organized and operates according to GCPs and relevant international ethical guidelines, the applicable laws and regulations.

Signed : .....

(Emeritus Professor Panja Kulapongs, M.D.)

Chairperson, Faculty of Medicine

GENERAL CONDITION OF APPROVAL:

- Please submit the progress report at least once a year except where required more frequent by the REC.
- In particular, approval of this study must be renewed at least three months before the expiration date if work is to continue.
- Prior Research Ethics Committee approval is required before implementing any changes in the consent documents or protocol unless those changes are required urgently for the safely of subjects.
- Any event or new information that may affect the benefit/risk ratio of the study must be reported to the REC promptly
- Any protocol deviation/violation must be reported to the REC

Appendix 4 Ethics Approval from Research Ethics Committee, Faculty of Medicine, Chiang Mai University

MONASH University

Monash University Human Research Ethics Committee

Confirmation of Registration

Project Number: 12225
Project Title: Incidence and susceptibility to Streptococcus suis infection and economic burden in Thailand
Chief Investigator: Dr Lee Learn Han
Expiry Date: 31/01/2023

Terms:

1. Registration is valid whilst you hold a position at Monash University and approval at the primary HREC is current.
2. This notification does not constitute an HREC approval. It is the responsibility of the Chief Investigator to ensure that approval from the primary HREC continues for the duration of the research.
3. End of project: You should notify MUHREC at the conclusion of the project or if the project is discontinued before the expected date of completion.
4. Retention and storage of data: The Chief Investigator is responsible for the storage and retention of the original data pertaining to this project in accordance with the *Australian Code for the Responsible Conduct of Research*.

Thank you for your assistance.

Professor Nip Thomson

Chair, MUHREC

CC: Dr Goh Bey Hing, Dr Tahir Mehmood, Dr Surasak Saokaew, Miss Ajaree Rayanakorn

Appendix 5 Good Clinical Practice (GCP) Certificate

The Faculty of Pharmaceutical Medicine has accredited this course for 9 Continuing Professional Development credits.

GCP web-based training course designed and developed by

INFONETICA

Ashford and St. Peter's Hospitals NHS
NHS Trust

Certificate Of Achievement

This is to certify that
Ajaree Rayanakorn
of Monash University Malaysia

has successfully passed web-based examination covering all aspects of Good Clinical Practice as laid down in the International Conference on Harmonisation Guidelines, the EU GCP Directives (2001/20 & 2005/28) and the UK Clinical Trials Regulations 2004 (SI 1031) and amendments of 2006 (SI 1928 & 2984), 2008 (SI 941) and 2009 (SI 1164 & 3063)

16 November 2017
(Recommended renewal date: 16 November 2019)

Certificate No: 36426-9-13361

Dr Isaac John, Hon Lecturer
Course Director
Hon Lecturer, Royal Holloway University of London
Assistant Director Research & Development
Ashford & St Peter's Hospitals NHS Trust

Royal Holloway University of London

Endorsed by
Professor George Dickson
Head, School of Biological Sciences
Royal Holloway, University of London

DEMO

Appendix 6: Case Record Form (CRF)

Case Report Form

Study Title: A retrospective study to assess the clinical treatment, outcomes and antimicrobial susceptibility of *Streptococcus suis* infection among patients in Northern, Thailand

Subject number: |____|____|____|

Study Site: Faculty of Medicine, Chiang Mai University
Chiang Mai, 50200 Thailand

Table of Contents

PART A Evaluation Criteria EC

INITIAL |____|____| **DATE:** |__|__|__|__|__|__|
DD MM YY

Inclusion criteria (All points must be answered with 'yes')	Yes	No
1. Confirmed positive S. suis either by _____cerebrospinal fluid (CSF) or _____hemoculture	☐	☐

PART B BASELINE CHARACTERISTICS BL

1) Date of birth: |__|__|__|__|__|__|
DD MM YY

2) Gender: ☐ 1. Male ☐ 2. Female

3) Occupation: **…….**

4) Relevant medical history/ current underlying diseases:

Yes	No	
☐	☐	1. Splenectomy
☐	☐	2. Valvular heart disease
☐	☐	3. Cancer
☐	☐	4. Renal/pulmonary tuberculosis
☐	☐	5. Corticosteroid use
☐	☐	6. Alcoholic liver disease
☐	☐	7. Other, specify…….

5) Risk factors:

Yes	No	
☐	☐	1. Consumption of raw pork
☐	☐	2. Recent contact with pigs/pork products
☐	☐	3. Pig related occupation e.g. farmer
☐	☐	4. Skin injury
☐	☐	5. Concurrent DM
☐	☐	6. Others, specify……

6) Body weight: |__|__|.|__| kg **Height:** |__|__|__|.|__| cm

7) BMI: |__|__|.|__| kg/m^2

8) Admission date: |__|__|__|__|__|__|
DD MM YY

9) Discharge date: |__|__|__|__|__|__|

10) Duration at hospitals: ……Days

11) Time from exposure to onset ……Days

12) Glasgow Coma Scale Score ……..

PART C Clinical presentations CP

2) Clinical presentations:

☐ 1. Meningitis

Sign and symtoms……

CSF protein concentration mg%.....

CSF glucose concentration mg%.....

CSF pleocytosis/mm3.....

CSF leukocyte/mm3.....

☐ 2. Septicaemia

☐ 3. Arthritis

☐ 4. Endocarditis/SBE

☐ 5. Spondylodiscitis

☐ 6. Toxic shock syndrome (TSS)

☐ 6. Others, specify _____

3) Medication regimen (Part D):

PART D Medication Regimen MR

Medications and dosage	Daily dose											
	Before admission	**Day 0**	**Day1**	**Day2**	**Day3**	**Day4**	**Day5**	**Day6**	**Day7**	**Day8**	**Day9**	**Day10**
	__/__/__	__/__/__	__/__/__	__/__/__	__/__/__	__/__/__	__/__/__	__/__/__	__/__/__	__/__/__	__/__/__	__/__/__
1. Penicillin G IV												
Daily dose												
2. Ceftriaxone												
Daily dose												
3. Ampicillin												
Daily dosage												
4. IV Pen G+Gentamicin												
Daily dosage												
5.												
Daily dosage												
6.												
Daily dosage												

PART D (continue 1) Medication Regimen MR

Antimicrobial susceptibility profile:
Drug name:……, Susceptible/Resistance %.....

Medications and dosage	**Daily dose**											
	Day 11	**Day12**	**Day13**	**Day14**	**Day15**	**Day16**	**Day17**	**Day18**	**Day19**	**Day20**	**Day21**	**Day22**
	__/__/__	__/__/__	__/__/__	__/__/__	__/__/__	__/__/__	__/__/__	__/__/__	__/__/__	__/__/__	__/__/__	__/__/__
1. Penicillin G IV												
Daily dose												
2. Ceftriaxone												
Daily dose												
3. Ampicillin												
Daily dosage												
4. IV Pen G+Gentamicin												
Daily dosage												
5.												
Daily dosage												
6.												
Daily dosage												

MIC:
MIC50 mg/L:……MIC90 mg/L…….

PART E LABORATORY FINDINGS LAB

PART E (continue 1)

Data / Date	D-1	D0	D1	D2	D3	D4	D5	D6	D7	D8	D9
	__/__/__	__/__/__	__/__/__	__/__/__	__/__/__	__/__/__	__/__/__	__/__/__	__/__/__	__/__/__	__/__/__
BP (mmHg)	/	/	/	/	/	/	/	/	/	/	/
BW (kg)											
Complete blood count											
Hb (g/dl) (male 13-18), (female 12-16)											
Hct (%) (male 40-54), (female 37-47)											
WBC (cells/cu.mm) (5000-10000)											
Neutrophil (%) (40-74)											
Eosinophil (%) (0.0-7.0)											
Lymphocyte (%) (19.0-48.0)											
Monocyte (%) (3.0-9.0)											
Platelet (cells/cu.mm) (140000-450000)											
Glucose (74-109 mg/dl)											
Creatinine (mg/dl) (male 0.67-1.17), (female 0.51-0.95)											
Liver function test											
Total protein (6.0-8.5g/dl)											
Albumin (3.2-5.0 g/dl)											
Globulin (2.8-3.5 g/dl)											
Alkaline phos (23-98 U/L)											
AST (3-35 U/L)											
ALT (7-33 U/L)											

Data / Date	D10	D11	D12	D13	D14	D15	D16	D17	D18	D19	D20
	__/__/__	__/__/__	__/__/__	__/__/__	__/__/__	__/__/__	__/__/__	__/__/__	__/__/__	__/__/__	__/__/__
BP (mmHg)	/	/	/	/	/	/	/	/	/	/	/
BW (kg)											
Complete blood count											
Hb (g/dl) (male 13-18), (female 12-16)											
Hct (%) (male 40-54), (female 37-47)											
WBC (cells/cu.mm) (5000-10000)											
Neutrophil (%) (40-74)											
Eosinophil (%) (0.0-7.0)											
Lymphocyte (%) (19.0-48.0)											
Monocyte (%) (3.0-9.0)											
Platelet (cells/cu.mm) (140000-450000)											
Glucose (74-109 mg/dl)											
Creatinine (mg/dl) (male 0.67-1.17), (female 0.51-0.95)											
Liver function test											
Total protein (6.0-8.5g/dl)											
Albumin (3.2-5.0 g/dl)											
Globulin (2.8-3.5 g/dl)											
Alkaline phos (23-98 U/L)											
AST (3-35 U/L)											
ALT (7-33 U/L)											

PART E (continue 2)
PART E (continue 3)

Data / Date	D-1	D0	D1	D2	D3	D4	D5	D6	D7	D8	D9
	__/__/__	__/__/__	__/__/__	__/__/__	__/__/__	__/__/__	__/__/__	__/__/__	__/__/__	__/__/__	__/__/__
Liver function test (con't)											
Cholesterol (150-250 mg/dl)											
Total bilirubin (0.2-1.0 mg/dl)											
Direct bilirubin (0-0.2 mg/dl)											
Urine examination											
RBC (mid value)											
WBC (mid value)											
Albumin (0 to +4)											
Protein (g/d)											
Lipid profile											
Cholesterol (0-200 mg/dl)											
Triglyceride (0-200 mg/dl)											
LDL (0-100 mg/dl)											
HDL (>,=55 mg/dl)											

Study code SS01-2560 Project ID NONE-2560-05141 **Subject No.** |____|____|____|

PART F ADVERSE EVENT **AE**

Adverse event	Start date	End date	Intensity (1)	SAE criteria (2)	Causality Assessment (3)	Action Taken (4)
	|__|__|__|__|__|__|	|__|__|__|__|__|__|	|____|	|____|	|____|	|____|
	|__|__|__|__|__|__|	|__|__|__|__|__|__|	|____|	|____|	|____|	|____|
	|__|__|__|__|__|__|	|__|__|__|__|__|__|	|____|	|____|	|____|	|____|
	|__|__|__|__|__|__|	|__|__|__|__|__|__|	|____|	|____|	|____|	|____|
	|__|__|__|__|__|__|	|__|__|__|__|__|__|	|____|	|____|	|____|	|____|
	|__|__|__|__|__|__|	|__|__|__|__|__|__|	|____|	|____|	|____|	|____|
	|__|__|__|__|__|__|	|__|__|__|__|__|__|	|____|	|____|	|____|	|____|
	|__|__|__|__|__|__|	|__|__|__|__|__|__|	|____|	|____|	|____|	|____|
	|__|__|__|__|__|__|	|__|__|__|__|__|__|	|____|	|____|	|____|	|____|

(1) Intensity
1. Non-serious
2. Serious

(2) Serious adverse event criteria
1. Death
2. Life-threatening
3. Involved or prolonged inpatient hospitalization
4. Involved persistent or significant disability or incapacity
5. Congenital anomaly/birth defect
6. Other significant medical events

(3) Caussality assessment
1. Not suspected
2. Suspected

(4) Action taken
1. No theraphy required
2. Therapy required

PART F (continue 1) ADVERSE EVENT AE

Adverse event	Start date	End date	Intensity (1)	SAE criteria (2)	Causality Assessment (3)	Action Taken (4)
	|__|__|__|__|__|__|	|__|__|__|__|__|__|	|____|	|____|	|____|	|____|
	|__|__|__|__|__|__|	|__|__|__|__|__|__|	|____|	|____|	|____|	|____|
	|__|__|__|__|__|__|	|__|__|__|__|__|__|	|____|	|____|	|____|	|____|
	|__|__|__|__|__|__|	|__|__|__|__|__|__|	|____|	|____|	|____|	|____|
	|__|__|__|__|__|__|	|__|__|__|__|__|__|	|____|	|____|	|____|	|____|
	|__|__|__|__|__|__|	|__|__|__|__|__|__|	|____|	|____|	|____|	|____|
	|__|__|__|__|__|__|	|__|__|__|__|__|__|	|____|	|____|	|____|	|____|
	|__|__|__|__|__|__|	|__|__|__|__|__|__|	|____|	|____|	|____|	|____|
	|__|__|__|__|__|__|	|__|__|__|__|__|__|	|____|	|____|	|____|	|____|

(5) Intensity

3. Non-serious
4. Serious

(6) Serious adverse event criteria

7. Death
8. Life-threatening
9. Involved or prolonged inpatient hospitalization
10. Involved persistent or significant disability or incapacity
11. Congenital anomaly/birth defect
12. Other significant medical events

(7) Caussality assessment

3. Not suspected
4. Suspected

(8) Action taken

3. No theraphy required
4. Therapy required

PART G Outcomes measurement OM

1) Treatment outcomes:

☐ 1. Recovery

☐ 1.1 Complete recovery

☐ 1.2 Recovery with sequelae, specify…….

☐ 2. Relapse

Treatment regimen after relapse:

Medication……..dose……duration……days

Outcome……

☐ 3. Death

2) Sequelae:

☐ 1. Hearing loss

☐ 1.1 Temporary

☐ 1.2 Permanent, specify……...

☐ 2. Vestibular dysfunction

☐ 3. Visual impairment

☐ 4. Others, specify……

Appendix 7 Checklist: STROBE Checklist

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No.	Recommendation	Page No.	Relevant text from manuscript
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract		
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found		
Introduction				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported		
Objectives	3	State specific objectives, including any prespecified hypotheses		
Methods				
Study design	4	Present key elements of study design early in the paper		
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection		
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants		
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case		
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable		
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group		
Bias	9	Describe any efforts to address potential sources of bias		
Study size	10	Explain how the study size was arrived at		

Continued on next page

Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period

Continued on next page

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

Section/Topic		Checklist Item	Page
Title and abstract			
Title	1	Identify the study as developing and/or validating a multivariable prediction model, the target population, and the outcome to be predicted.	
Abstract	2	Provide a summary of objectives, study design, setting, participants, sample size, predictors, outcome, statistical analysis, results, and conclusions.	
Introduction			
Background and objectives	3a	Explain the medical context (including whether diagnostic or prognostic) and rationale for developing or validating the multivariable prediction model, including references to existing models.	
	3b	Specify the objectives, including whether the study describes the development or validation of the model or both.	
Methods			
Source of data	4a	Describe the study design or source of data (e.g., randomized trial, cohort, or registry data), separately for the development and validation data sets, if applicable.	
	4b	Specify the key study dates, including start of accrual; end of accrual; and, if applicable, end of follow-up.	
Participants	5a	Specify key elements of the study setting (e.g., primary care, secondary care, general population) including number and location of centres.	
	5b	Describe eligibility criteria for participants.	
	5c	Give details of treatments received, if relevant.	
Outcome	6a	Clearly define the outcome that is predicted by the prediction model, including how and when assessed.	
	6b	Report any actions to blind assessment of the outcome to be predicted.	
Predictors	7a	Clearly define all predictors used in developing or validating the multivariable prediction model, including how and when they were measured.	
	7b	Report any actions to blind assessment of predictors for the outcome and other predictors.	
Sample size	8	Explain how the study size was arrived at.	
Missing data	9	Describe how missing data were handled (e.g., complete-case analysis, single imputation, multiple imputation) with details of any imputation method.	
Statistical analysis methods	10a	Describe how predictors were handled in the analyses.	
	10b	Specify type of model, all model-building procedures (including any predictor selection), and method for internal validation.	
	10d	Specify all measures used to assess model performance and, if relevant, to compare multiple models.	
Risk groups	11	Provide details on how risk groups were created, if done.	
Results			
Participants	13a	Describe the flow of participants through the study, including the number of participants with and without the outcome and, if applicable, a summary of the follow-up time. A diagram may be helpful.	
	13b	Describe the characteristics of the participants (basic demographics, clinical features, available predictors), including the number of participants with missing data for predictors and outcome.	
Model development	14a	Specify the number of participants and outcome events in each analysis.	
	14b	If done, report the unadjusted association between each candidate predictor and outcome.	
Model specification	15a	Present the full prediction model to allow predictions for individuals (i.e., all regression coefficients, and model intercept or baseline survival at a given time point).	
	15b	Explain how to the use the prediction model.	
Model performance	16	Report performance measures (with CIs) for the prediction model.	
Discussion			
Limitations	18	Discuss any limitations of the study (such as nonrepresentative sample, few events per predictor, missing data).	
Interpretation	19b	Give an overall interpretation of the results, considering objectives, limitations, and results from similar studies, and other relevant evidence.	
Implications	20	Discuss the potential clinical use of the model and implications for future research.	
Other information			
Supplementary information	21	Provide information about the availability of supplementary resources, such as study protocol, Web calculator, and data sets.	
Funding	22	Give the source of funding and the role of the funders for the present study.	

We recommend using the TRIPOD Checklist in conjunction with the TRIPOD Explanation and Elaboration document.

Appendix 9 Certificate of Exemption from Research Ethics Committee, Faculty of Medicine, Chiang Mai University

No. EXEMPTION-5935/2561

Certificate of Exemption

Principal Investigator: Ajaree Rayanakorn.
Monash University Malaysia.
Address: Jalan Lagoon Selatan ,47500 Bandar Sunway ,Selangor Darul Ehsan,Malaysia.
Protocol title: Endophthalmitis with bilateral deafness from disseminated Streptococcus suis infection.
STUDY CODE: PED-2561-05935 Research ID: 5935
Sponsor: -

This research protocol complies with a research with exemption category.

It has been certified as exempt from Research Ethics Committee of Faculty of Medicine, Chiang Mai University

Date of Issue : 21 November 2018

This Ethics Committee is organized and operates according to GCPs and relevant international ethical guidelines, the applicable laws and regulations.

Signed : P. Kulapongs
(Emeritus Professor Panja Kulapongs, M.D.)
Chairperson, Faculty of Medicine

Note: Please submit the close study report to REC when complete the research protocol. The close study report must be generated in ROS system and can be downloaded documents from website http://www.medicine.cmu.ac.th/research/ethics/default.htm

Appendix 10 Case report publication (BMJ Case Report)

Rayanakorn A, Katip W, Lee LH, Oberdorfer P. Endophthalmitis with bilateral deafness from disseminated *Streptococcus suis* infection. BMJ Case Reports. 2019;12(2). DOI:10.1136/bcr-2018-228501

CASE REPORT

Endophthalmitis with bilateral deafness from disseminated *Streptococcus suis* infection

Ajaree Rayanakorn,[1] Wasan Katip,[2] Learn Han Lee,[1,3] Peninnah Oberdorfer[4]

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[2]Department of Pharmaceutical Care, Chiang Mai University, Chiang Mai, Thailand
[3]The Institute of Pharmaceutical Sciences, Lahore, Pakistan
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Dr Peninnah Oberdorfer, oberdorferp@gmail.com

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To cite: Rayanakorn A, Katip W, Lee LH, *et al*. *BMJ Case Rep* 2019;**12**:e228501. doi:10.1136/bcr-2018-228501

SUMMARY

Streptococcus suis is a Gram-positive cocci bacterium that are found mainly in pigs and can be transmitted to human through pigs or pork exposure. The disease is mainly found among occupations involving swine contact in western countries whereas in Asia the disease is usually contracted through raw pork consumption. In this case report, we present a case of a middle-aged Thai man who acquired the infection from raw pork consumption. He presented with endogenous endophthalmitis with infective spondylodiscitis, sepsis and meningitis and later developed blindness of the right eye and permanent bilateral hearing loss disseminated from *S. suis* infection. Our report suggests that *S. suis* infection be considered as a causative factor in patient presenting with established clinical symptoms and predisposing factors. Cultural habit of eating raw pork should be taken into account especially in Asian countries.

BACKGROUND

Streptococcus suis is an emerging zoonosis that is mainly found as normal flora in pigs.[1] The pathogen can be transmitted to human through exposure of pigs or consumption of raw pork[2] causing a number of serious infections.[3] The most common clinical manifestations found were meningitis (68%), followed by sepsis (25.0%) and arthritis (12.9%) whereas infective endocarditis (12.4%), endopthalmitis (4.6%) and spondylodiscitis (3.7%) were uncommon.[4] Sensorineural hearing loss (SNHL) (39%) was the most common complication from *S. suis* meningitis in which the disease is usually irreversible despite effective treatment.[4 5]

The disease is usually found prevalent among farmers, butchers and abattoir workers involving swine contact in western countries.[6 7] However, this was not always the case in Asian countries where pig-related occupation infection is less than half of the reported cases.[8 9] According to a recent systematic review and meta-analysis, raw pork consumption, exposure to pigs or raw pork, pig-related occupation and male sex are the significant risk factors of the infection.[10] This suggests that traditional food habit involving raw pork consumption probably plays an essential role in the disease infection in the Asian region.

Since the first *S. suis* infection in human related to meningitis and sepsis was reported in Denmark in 1968,[11] the disease has caused wide spread infection globally with more than 1,500 reported cases as of 2012.[12] In Thailand, *S. suis* infection is an important health problem with more than 500 cases reported up to 2017[10] and possibly the second causative agent of adult streptococcal meningitis.[13]

We therefore present a patient with endogenous endophthalmitis which is an uncommon clinical presentation, who later developed blindness of the right eye and permanent bilateral SNHL disseminated from *S. suis* infection.

CASE PRESENTATION

A 48-year-old educated, Thai male was admitted to Chiang Mai University Hospital (CMUH) with endogenous endopthalmitis infecting his right eye. He is a degree graduate and works as an airport security administration officer. He consumes two to three bottles of beer daily and often consumes raw fermented pork or *'naem'* (sour pork). He has a medical history of gout and spondylodiscitis. He refused taking any other medications except analgesics for his back pain. He did not travel to anywhere within the region and had no pig farming exposure prior to admission at the hospital.

Upon detailed consultation, the patient narrated that he often experienced lack of sleep due to work stress, fatigue and dizziness for the past few months before admission. Six days before admission, the patient reported to have consumed a northern-Thai style raw pork dish called *'larb dib'* at *Songkran* (the Thai new year) party at his office. The pork and ingredients were bought from wet markets in Sankampaeng and Saraphi Districts, Chiang Mai Province and cooked by his colleagues. According to the patient, he was the only one who fell sick after the party. Two days later, he developed a low-grade fever, neck, joint and waist pain and blurred vision at his right eye along with a decrease in hearing ability at his right ear. The symptoms got worse and he visited a local hospital where he underwent an X-ray for waist and neck bone. He received an unknown intravenous antibiotic for joint pain, then later progressed to have nausea, vomiting, hearing loss and redness of his right eye. He was then referred to CMUH.

At admission, the body temperature was 37.5°C with 24 hours peak temperature of 37.8°C, pulse rate of 90 beats per minute and a blood pressure of 130/80 mm Hg. His respiratory rate was 20 breaths per minute. A physical examination identified chemosis and periorbital swelling at the right eye and C-spine tenderness.

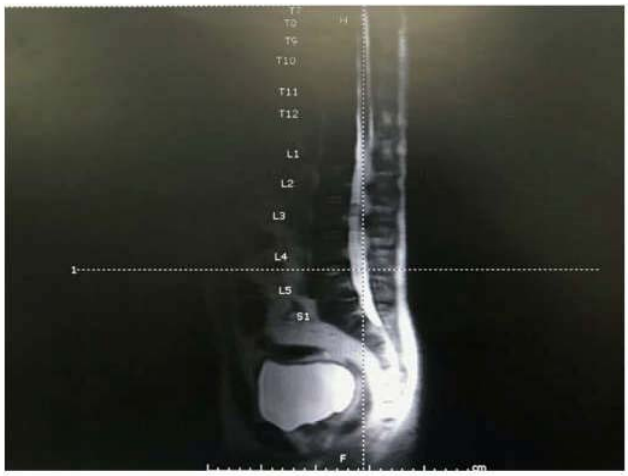

Figure 1 MRI of whole spine.

INVESTIGATIONS

A routine laboratory and microbiological culture were ordered. An MRI of the whole spine was done to rule out infective spondylodiscitis suggested spondylodiscitis at C4/5 level and left side of L5/S2 (figure 1). There was left paracentral disc extrusion with associated disc bulging and osteophyte causing mild spinal cord compression.

Assessment of visual acuity (VA) revealed hand movement (HM) at right VA and a VA of 6/16 on the left. There were marked injected conjunctiva, whitish, large keratic precipitate, and hypopyon uveitis in the right eye. The relative afferent pupil defect (RAPD) test showed a positive reverse RAPD. The left eye was normal. The patient was still alert and oriented despite the presence of febrile illness and neck stiffness.

The laboratory results showed no outstanding value except an elevated neutrophil (83.6%; normal range, 40%–75%), and erythrocyte sedimentation rate (65 mm/hour; normal range, 0–15 mm/hour), and a transient increase of alanine aminotransferase (65 U/L; normal range, 0–41 U/L), blood urea nitrogen (21 mg/dL; normal range, 6–20 mg/dL) and hyponatraemia (128 mmol/L; normal range, 136–145 mmol/L). Ultrasound abdomen was performed to rule out liver abscess. A serologic anti-HIV antibody testing was negative. Immunoglobulin and IgG subset analysis were not performed. A lumbar puncture was carried out 3 days after admission showing a turbid colour of the cerebrospinal fluid (CSF), an increased CSF protein level (88 mg/dL; normal range, 15–45 mg/dL) and decreased glucose level (15 mg/dL; normal range, 45–80 mg/dL). The CSF analysis showed white blood cell count $0.131x10^9$/L, red blood cell count $100x10^{12}$/L, polymorphonuclear 41%, mononuclear 60%. The Gram staining was negative.

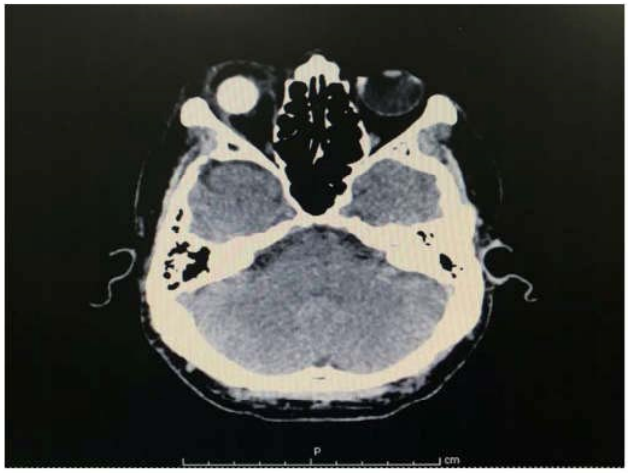

Figure 2 CT brain with contrast media (CM)

DIFFERENTIAL DIAGNOSIS

Three bottles of blood samples and vitreous from the right eye positively revealed *S. suis* infection after 3 days collections. The Gram strain result of vitreous at the right eye showed moderate quantity of Gram-positive coccobacilli. The minimum inhibitory concentrations were 0.094 µg/mL for both penicillin and ceftriaxone. The repeated microbiological culture was negative 2 days upon treatment initiation. Two days after admission, the right eye globe pathology report showed acute endophthalmitis with perforated cornea and vitreous haemorrhage. The audiogram was done at 5-day post admission suggesting irreversible bilateral SNHL disseminated from *S. suis* infection. The tympanic membrane was intact and external auditory canal remained normal.

TREATMENT

Fortified antibiotic ophthalmic solutions (ceftazidime 50 mg/mL at right eye at 1-hour interval and vancomycin 50 mg/mL at right eye at 1-hour interval) and intravenous ceftriaxone (2.0 g at 12-hour interval) and vancomycin (1.0 g at 8-hour interval) were empirically given to treat the infection. Intravenous hydration was administered to treat hypovolaemic hyponatraemia. Ceftriaxone treatment was continued after the microbiological culture confirmed of *S. suis* infection.

The right vitreous tapping, enucleation was performed. Conjunctival peritomy was done. Tenon was undermined and cut separately from sclera. The residual was cleaned and sutured by vicryl stitches. The optic nerve was cut to around 2 mm under the globe. Mull no. 16 and conformer were placed to fit in orbit. One day later, the patient developed a low-grade fever with a temperature of 38.2°C, and experienced a significant loss of hearing in both ears starting from left to right. The CT brain showed no evidence of brain abscess nor leptomeningeal enhancement. There was heterogeneous enhancing lesion adjacent to anterior aspect of right enucleation material and swelling of right extraocular muscles and right optic nerve which might be due to infectious process or postoperative change (figure 2). The condition remained stable without any complications.

After 15 day of admission, the patient recovered from *S. suis* meningitis, septicaemia and spondylodiscitis but suffered from loss of hearing and right eye vision. Ceftriaxone 2.0 g at 12-hour interval until 4-week treatment completion and levofloxacin 500 mg 1.5 tablets per oral for 2 months were prescribed for home medication. In addition, the patient was referred to a nearby local hospital for intravenous ceftriaxone injection.

OUTCOME AND FOLLOW-UP

At 3-month follow-up medical examination, the CT scanning of the temporal bone revealed mild to moderate degree of labyrinthitis ossificans involving both vestibules and all semicircular canals of both ears (figure 3). The basal turn of cochlear was more severe on the left side. Both facial and vestibulocochlear nerve were within normal limit. The videonystagmography at 4 months was done showing bilateral death labyrinths. The right cochlear implant was done at 7 months. A lead electrode was

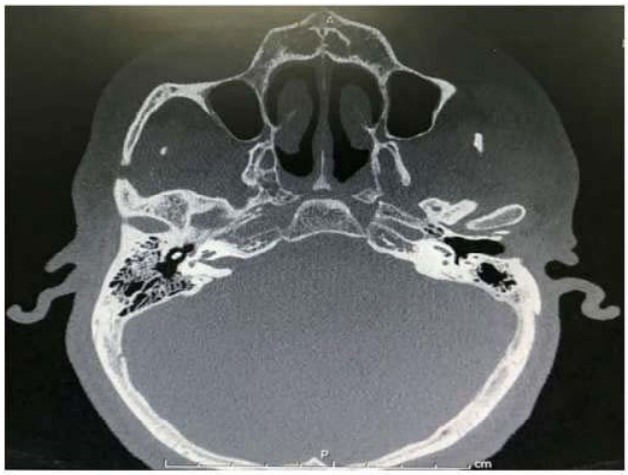

Figure 3 CT scan of temporal bone.

fully inserted into the scala tympani. Impedance/neural response imaging responses were detected. The patient was discharged in a stable condition after a 4-day admission.

DISCUSSION

S. suis is usually an occupational related disease affecting farmers, abattoir workers and butchers in western countries.[6 7] The first case of endopthalmitis secondary to *S. suis* infection was reported in 1978 by McLendon *et al*[14] in which a Caucasian man from the UK who worked at a pork pie factory developing deafness at the right ear and bilateral endophthalmitis was described.[14]

In our case, the patient was an office worker without any occupational exposure to pigs or raw pork. The disease transmission was presumably from raw pork consumption or '*Larb Dib*' he ate 6 days predated the admission. '*Larb*' is a northern Thai dish commonly eaten in raw form in northern Thailand. This emphasises the significant role of raw pork eating in *S. suis* infection. His physical weakness from sleep deprivation and underlying conditions were probably contributing factors resulting in a more susceptible condition to infection. His medical history of regular alcohol drinking was not surprising as raw pork is usually consumed together with alcohol drinks among Thai people especially northern Thai men. Consistently, a considerable large number of alcohol consumption was also noted in studies from Thailand.[13 15 16]

The patient established acute endopthalmitis and infective spondylodiscities in addition to meningitis and septicaemia which are common clinical manifestations of *S. suis*. He developed the symptom shortly after consuming raw pork together with back and joint pain implicating infective spondylodiscites. However, the cause of his illness was not identified until nearly a week after the onset when fortified antibiotic ophthalmic solutions were given. The urgent enucleation due to endopthalmitis with perforated cornea at his right eye was performed and, right blindness resulted.

According to a systematic review, the disease transmission from skin abrasion is believed to be the main route of pathogen entry, however this was noted only in some studies.[10] The presence of fever prior to the ocular symptom suggested that endopthalmitis could be disseminated from *S. suis* septicaemia in this patient.

The property of steroids in reducing inflammatory reactions at subarachnoid space which is a major process causing brain injury and neuronal dysfunction in acute bacterial meningitis has provided the rational of adjunctive corticosteroids use.[17 18] However, the benefit of steroids in reducing hearing loss has been established in paediatric meningitis but has yet to be clearly confirmed among adult population.[19] As *S.suis* infection is mainly found in adults and its effects in this population remains uncertain, using adjunctive dexamethasone may not have reversed the sequelae. In this patient, dexamethasone was not administered. The diagnosis was delayed in the primary setting and permanent SNHL as a consequence of *S. suis* meningitis seemed to be existed before admission. In our views, it still warrants the use of steroids to benefit the patient from its anti-inflammatory property in order to reduce inflammation. A comprehensive counselling session to emphasise on the risk of acquiring *S.suis* infection from raw or partially cooked pork including sour pork or '*Naem*' in raw form should have been mandated.

The high fatality rate in the China outbreak in 2005 involving 215 patients and 38 deaths has emphasised the importance of this zoonosis infection.[20] In this outbreak, the main cause of the widespread of infection was due to backyard slaughtering of sick pigs whereas the deep-rooted behaviour of raw pork consumption seemed to be the predominating cause of the infection in Thailand. Apart from laboratory confirmation, the virulence factors of *S.suis* isolates from the outbreak including *mrp, sly* and *ef* were also related to strains in Europe which are considered to be more virulent than strains identified in North America.[21]

Regardless of the rare incidence of endopthalmitis, physicians should be more aware of the clinical symptoms especially in patient with suggestive clinical presentations and predisposing risk factors of *S. suis* infection. In addition, *S.suis* could also be the causative pathogen causing septicaemia among recent travellers to the Southeast Asia region or endemic areas presenting with fever. A detailed patient interview and history taking to indicate the timelines, relevant exposures and all relevant information are essential for a clear clinical picture. In the absence of available *S. suis* vaccine, early and adequate treatment is crucial to alleviate the disease progress and clinical complications as well as long term neurological sequelae particularly deafness which usually occurs among most *S. suis* meningitis survivors.

Learning points

- *Streptococcus suis* is not uncommon in Asia population where there is an association between traditional eating habits and raw pork consumption, whereas farmers and abattoirs workers in western countries are certainly at high risk of *S. suis* infections.
- *S. suis* infection should be considered in patients presenting with established clinical symptoms and predisposing factors.
- Early detection and appropriate treatment are important to improve symptom outcomes and complications.
- Public health awareness programme is essential especially to those areas where traditional cultural habit of raw pork consumption is still practiced.
- There is limited data concerning *S. suis* epidemiology and molecular characteristics. Further research should be carried out to understand the disease epidemiology, clinical manifestations and treatment regimens.

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Contributors AR performed conception, designed the work, wrote the first draft of the manuscript and revisions with support from WK and PO. PO guided the interpretation of the clinical data and content. LHL, WK and PO critically reviewed the manuscript for intellectual content. All authors met the ICMJE criteria for authorship and approved the final version of the manuscript.

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Patient consent for publication Obtained.

Provenance and peer review Not commissioned; externally peer reviewed.

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Appendix 11 Patient consent form for case report publication

Source: Patient consent form. BMJ Author Hub [cited 2019 17 December]; Available from: https://authors.bmj.com/policies/patient-consent-and-confidentiality/

- **Appendix 11.1 Patient consent form English version**
- **Appendix 11.2 Patient consent form Thai version**

Consent form

For a patient's consent to publication of images and/or information about them in BMJ publications.

Name of patient: _____

Relationship to patient (if patient not signing this form): _____

*Description of the photo, image, text or other material (**Material)** about the patient. **A copy of the Material must be attached to this form**:* _____

Provisional title of article in which Material will be included: _____

_____

CONSENT

I_____ *[PRINT FULL NAME]* give my consent for the Material about me/the patient to appear in a BMJ publication.

I confirm that I: *(please tick boxes to confirm)*

- ☐ ***have seen the photo, image, text or other material about me/the patient***
- ☐ ***have read the article to be submitted to BMJ***
- ☐ ***am legally entitled to give this consent.***

I understand the following:

(1) The Material will be published without my/the patient's name attached, however I understand that complete anonymity cannot be guaranteed. It is possible that somebody somewhere - for example, somebody who looked after me/the patient or a relative - may recognise me/the patient.

(2) The Material may show or include details of my/the patient's medical condition or injury and any prognosis, treatment or surgery that I have/the patient has, had or may have in the future.

(3) The article may be published in a journal which is distributed worldwide. BMJ's publications go mainly to doctors and other healthcare professionals but are also seen by many others including academics, students and journalists.

(4) The article, including the Material, may be the subject of a press release, and may be linked to from social media and/or used in other promotional activities. Once published, the article will be placed on a BMJ website and may also be available on other websites.

(5) The text of the article will be edited for style, grammar and consistency before publication.

(6) I/the patient will not receive any financial benefit from publication of the article.

(7) The article may also be used in full or in part in other publications and products published by BMJ and/or by other publishers. This includes publication in English and in translation, in print, in digital formats, and in any other formats that may be used by BMJ or other publishers now and in the future. The article may appear in local editions of journals or other publications, published in the UK and overseas.

(8) I can revoke my consent at any time before publication, but once the article has been committed to publication ("gone to press") it will not be possible to revoke the consent.

(9) This consent form will be retained securely and in confidence by BMJ in accordance with the law, for no longer than necessary. Personal data provided in this form will be used and retained in accordance with BMJ's Privacy Policy available at https://www.bmj.com/company/your-privacy/.

Please tick box to confirm the following:

☐ Where this consent relates to an article in *BMJ Case Reports*, I have/the patient has had the opportunity to comment on the article and I am satisfied that the comments, if any, have been reflected in the article.

*Signed:*_____ *Print name:* _____

Address: _____ *Email address:* _____

_____ *Telephone no:* _____

If signing on behalf of the patient, please give the reason why the patient can't consent for themselves (e.g. patient is under 18 or has cognitive or intellectual impairment).

_____ *Date:* _____

☐ *If you are signing for a family or other group, please tick the box to confirm that all relevant members of the family or group have been informed.*

If the patient is under the age of 18 but has sufficient understanding of the consent process and its implications, they must also confirm their agreement:

*Signed:*_____ *Print name:* _____

Date of birth: _____ *Date:*_____

Details of person who has explained and administered the form to the patient or their representative (e.g. the corresponding author or other person who has the authority to obtain consent).

*Signed:*_____ *Print name:* _____

Position: _____ *Address:*_____

*Institution:*_____ _____

_____ _____

Email address: _____ *Telephone no:*_____

Date: _____

เอกสารแสดงความยินยอม

สำหรับความยินยอมของผู้ป่วยในการเผยแพร่ภาพและ/หรือข้อมูลเกี่ยวผู้ป่วยในเอกสารเผยแพร่ของ BMJ

ชื่อผู้ป่วย: _____

ความสัมพันธ์กับผู้ป่วย (หากผู้ป่วยมิได้เป็นผู้ลงนามในเอกสารแสดงความยินยอมนี้): _____

คำอธิบายเกี่ยวกับภาพถ่าย รูปภาพ ข้อความหรือข้อมูลอื่น ๆ ***(ข้อมูล)*** *เกี่ยวกับผู้ป่วย* ***ควรแนบสำเนาข้อมูลดังกล่าวมากับเอกสารแสดงความยินยอมฉบับนี้ :*** _____

ชื่อของบทความเฉพาะที่จะมีข้อมูลนี้รวมอยู่: _____

_____

การให้ความยินยอม

ข้าพเจ้า_____ *[ชื่อ นามสกุลตัวบรรจง]*
ยินยอมให้ใช้ข้อมูลเกี่ยวข้าพเจ้า/ผู้ป่วยในเอกสารเผยแพร่ของ BMJ ได้

ข้าพเจ้ายืนยันว่าข้าพเจ้า *(กรุณาทำเครื่องหมายเลือกช่องต่าง ๆ เพื่อยืนยัน)*

- ☐ ***ได้ดูภาพถ่าย รูปภาพ ข้อความหรือข้อมูลอื่น ๆ เกี่ยวกับข้าพเจ้า/ผู้ป่วยแล้ว***
- ☐ ***ได้อ่านบทความที่จะส่งให้แก่ BMJ แล้ว***
- ☐ ***มีสิทธิ์ตามกฎหมายที่จะให้ความยินยอมนี้***

ข้าพเจ้าเข้าใจในเรื่องดังต่อไปนี้:

(1) ข้อมูลดังกล่าวจะได้รับการเผยแพร่ โดยไม่มีชื่อของข้าพเจ้า/ผู้ป่วย อย่างไรก็ตามข้าพเจ้าเข้าใจว่าบริษัทไม่สามารถรับรองการเก็บรักษาเป็นความลับได้ทั้งหมด อาจเป็นไปได้ว่าอาจมีบางคนในบางแห่งจดจำข้าพเจ้า/ผู้ป่วยได้ ตัวอย่างเช่น บุคคลที่ดูแลข้าพเจ้า/ผู้ป่วยที่ดูแลข้าพเจ้า/ผู้ป่วย หรือญาติ

(2) ข้อมูลอาจแสดงหรือมีรายละเอียดเกี่ยวกับภาวะทางการแพทย์หรืออาการบาดเจ็บของข้าพเจ้า/ผู้ป่วย และการพยากรณ์โรค การรักษา หรือการผ่าตัดที่ข้าพเจ้า/ผู้ป่วยมี เคยมี หรืออาจมีในอนาคต

(3) บทความดังกล่าวอาจได้ในรับการตีพิมพ์ในวารสารที่เผยแพร่ไปทั่วโลก หลัก ๆ แล้วเอกสารตีพิมพ์เผยแพร่ของ BMJ จะถูกส่งให้แก่แพทย์และบุคลากรทางการแพทย์ แต่ยังมีบุคคลอื่น ๆ อีกได้แก่ นักวิชาการ นักศึกษาและนักข่าว

(4) บทความ รวมทั้งข้อมูลดังกล่าวอาจอยู่ในข่าวประชาสัมพันธ์ และอาจเชื่อมโยงกับสื่อสังคมออนไลน์ และ/หรือถูกนำไปใช้เพื่อกิจกรรมการส่งเสริมการขาย เมื่อมีการตีพิมพ์เผยแพร่แล้ว จะมีการประกาศบทความดังกล่าวไว้ในเว็บไซต์ของ BMJ และอาจมีเผยแพร่ในเว็บไซต์อื่น ๆ ด้วยเช่นกัน

(5) ข้อความของบทความจะได้รับการเรียบเรียงแก้ไขในส่วนของรูปแบบการเขียน ไวยากรณ์และความสอดคล้องก่อนการตีพิมพ์

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(7) นอกจากนี้ ยังอาจมีการใช้บทความทั้งหมดหรือบางส่วนในเอกสารและผลิตภัณฑ์อื่น ๆ ที่เผยแพร่โดย BMJ และ/หรือสำนักพิมพ์อื่น ๆ
ซึ่งรวมถึงการตีพิมพ์เป็นภาษาอังกฤษและในฉบับแปลในรูปแบบเอกสารสิ่งพิมพ์ รูปแบบดิจิทัล และรูปแบบอื่น ๆ ที่ BMJ หรือสำนักพิมพ์อื่น ๆ อาจใช้ในปัจจุบันและ
ในอนาคต บทความอาจปรากฏในวารสารท้องถิ่นหรือสิ่งพิมพ์อื่น ๆ ที่เผยแพร่ในสหราชอาณาจักร และในต่างประเทศ

(8) ข้าพเจ้าสามารถเพิกถอนความยินยอมของข้าพเจ้าเมื่อใดก็ได้ก่อนการตีพิมพ์เผยแพร่ แต่เมื่อบทความดังกล่าวได้รับการตีพิมพ์เผยแพร่แล้ว ("ส่งเข้ากระบวนการตีพิมพ์") จะไม่สามารถเพิกถอนความยินยอมได้

(9) เอกสารแสดงความยินยอมฉบับนี้จะได้รับการเก็บรักษาไว้อย่างปลอดภัยและเป็นความลับโดย BMJ โดยเป็นไปตามกฎหมายและจะไม่เก็บไว้เกินกว่าระยะเวลาที่จำเป็น

กรุณาทำเครื่องหมายเลือกช่องต่าง ๆ เพื่อยืนยันในเรื่องดังต่อไปนี้:

☐ ข้าพเจ้ายินยอมให้ BMJ จัดเก็บรายละเอียดข้อมูลที่อยู่ของข้าพเจ้า (รวมทั้งนอก EEA) เพื่อวัตถุประสงค์ในการติดต่อข้าพเจ้าในอนาคตเท่านั้น หากจำเป็น

☐ กรณีที่ความยินยอมนี้เกี่ยวข้องกับบทความใน*รายงานของผู้ป่วยของ BMJ* ข้าพเจ้า/ผู้ป่วยได้มีโอกาสในการให้ข้อเสนอแนะเกี่ยวกับบทความดังกล่าว และข้าพเจ้าพึงพอใจที่ข้อเสนอแนะดังกล่าวได้นำไปใช้ในบทความนี้ (หากมี)

*ลงนาม:*_____ *ชื่อตัวบรรจง:* _____

ที่อยู่: _____ *ที่อยู่อีเมล:* _____

_____ *หมายเลขโทรศัพท์:* _____

หากลงนามในนามของผู้ป่วย กรุณาระบุเหตุผลว่าเพราะเหตุใดผู้ป่วยจึงไม่ให้ความยินยอมด้วยตนเอง (เช่น ผู้ป่วยเสียชีวิต มีอายุต่ำกว่า 18 ปี หรือมีความบกพร่องในการรับรู้หรือสติปัญญา)

_____ ***วันที่:*** _____

☐ *หากคุณกำลังลงนามแทนครอบครัวหรือกลุ่มบุคคลอื่น ๆ*
กรุณาทำเครื่องหมายในช่องเพื่อยืนยันว่าสมาชิกในครอบครัวทั้งหมดหรือกลุ่มบุคคลดังกล่าวได้รับแจ้งให้ทราบข้อมูลแล้ว

หากผู้ป่วยเป็นเด็กที่มีอายุตั้งแต่ 7 ปีขึ้นไป เด็กจะต้องให้ความยินยอมด้วยตนเองด้วย:

*ลงนาม:*_____ *ชื่อตัวบรรจง:* _____

วันเดือนปีเกิด: _____ *วันที่:*_____

รายละเอียดของบุคคลที่ได้อธิบายและให้เอกสารแสดงความยินยอมฉบับนี้แก่ผู้ป่วยหรือตัวแทน (เช่น ผู้เขียนที่เกี่ยวข้อง หรือบุคคลอื่น ๆ ที่มีอำนาจในการรับความยินยอม)

*ลงนาม:*_____ *ชื่อตัวบรรจง:* _____

ตำแหน่ง: _____ *ที่อยู่:*_____

*สถาบัน:*_____ _____

_____ _____

ที่อยู่อีเมล: _____ *หมายเลขโทรศัพท์:*_____

วันที่: _____