รายงานวิจัยฉบับสมบูรณ์

โครงการวิจัย

สถานการณ์การแพรกระจายและการดื้อยาต้านจุลชีพ ของ *Streptococcus suis* ที่แยกได้จากสุกรในเขตจังหวัดภาคเหนือของประเทศไทย

(Situation and antimicrobial resistance of *Streptococcus suis* isolated from pigs in Northern region of Thailand)

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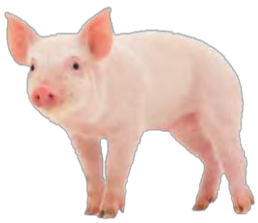

ปีงบประมาณ 2560

กิตติกรรมประกาศ

โครงการวิจัยนี้ได้รับทุนอุดหนุนการวิจัยจากเงินงบประมาณแผ่นดินประจำปีงบประมาณ 2560 คณะผู้วิจัยขอขอบคุณผู้วิจัยขอขอบคุณนิสิตปริญญาโท ผู้ช่วยวิจัยและเจ้าหน้าที่ภาควิชาสัตวแพทยสาธารณสุข คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัยทุกท่าน ที่ให้ความช่วยเหลือในห้องปฏิบัติการและจัดทำรายงานการวิจัย

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บทคัดย่อ

ชื่อโครงการวิจัย สถานการณ์การแพร่กระจายและการดื้อยาต้านจุลชีพของ *Streptococcus suis* ที่แยกได้จากสุกรในเขตจังหวัดภาคเหนือของประเทศ

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การศึกษาครั้งนี้มีวัตถุประสงค์การวิจัยเพื่อศึกษาสถานการณ์ที่แท้จริงของ *S. suis* ในเขตภาคเหนือประเทศไทย เก็บตัวอย่าง nasal swab จำนวน 768 ตัวอย่างจากสุกรในโรงฆ่า โดยเก็บตัวอย่างจำนวน 4 ครั้งในช่วงเดือนเมษายน 2018 ถึงตุลาคม 2018 สถานที่เป้าหมายในการเก็บตัวอย่างคือ โรฆ่าสัตว์เทศบาลประจำอำเภอใน 5 จังหวัดภาคเหนือคือ โรงฆ่าสัตว์ในจังหวัดเชียงใหม่ (n=130) เชียงราย (n=180) น่าน (n=190) พะเยา (n=202) และแม่ฮ่องสอน (n=66) ตัวอย่างทั้งหมดนำมาแยกเชื้อและตรวจ serotypes จากผลทดสอบทางชีวเคมีพบว่า ใน 5 จังหวัดมีความชุกของ *S. suis* 6.8%-15% พบสูงสุดที่เชียงราย (15%) ตามด้วยพะเยา (11.4%) แม่ฮ่องสอน (10.6%) เชียงใหม่ (9.2%) และน่าน (6.8%) จากการปรากฏของยีน 16SrRNA หรือ *gdh* ใน 5 จังหวัดมีความชุกของ *S. suis* 3.0%-9.4% ไม่พบว่าเชื้อจากเชียงใหม่ให้ผลบวกต่อ 16SrRNA หรือ *gdh* ความชุกของ *S. suis* สูงสุดที่เชียงราย (9.4%) และแม่ฮ่องสอน (9.1%) ตามด้วยน่าน (6.3%) และพะเยา (3%) จากเชื้อจำนวน 59 isolates ที่ยืนยันด้วยการเพิ่มจำนวน 16SrRNA หรือ *gdh* พบ serotype 8 มากที่สุด ตามด้วย serotype 10 (3.4%) serotype 2 (1.7%) และ 9 (1.7%) โดยพบ serotype 20 จำนวน 1 isolate จากตัวอย่างในจังหวัดพะเยา ตัวอย่างที่ให้ผลบวกส่วนใหญ่มี *S. suis* เพียง serotype เดียว แต่มี 3 ตัวอย่างจากเชียงราย (2) และพะเยา (1) ที่มีมากกว่า 1 serotype เชื้อทั้งหมดมียีน *arcA* และไม่พบเชื้อที่มียีน *epf* พบยีน *sly mrp* และ *hyl* ในอัตรา 91.5% ตามด้วย 69.5% และ 37.3% ตามลำดับ ได้รูปแบบ virulence gene จำนวน 15 รูปแบบ โดยที่พบมากที่สุดคือ *mrp, sly*, *arcA, hyl* (33.9%) ตามด้วย *mrp, sly*, *arcA* (28.8%) และ *sly*, *arcA* (25.4%) การศึกษาครั้งนี้ยืนยันบทบาทสำคัญของสุกรที่ไม่แสดงอาการในการเป็นพาหะของ *S. suis* และความจำเป็นในการตรวจติดตามเชื้อนี้ในสุกรที่มีสุขภาพดี

Abstract

Title Situation and antimicrobial resistance of *Streptococcus suis* isolated from pigs in Northern region of Thailand

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This study aims to study prevalence of *S. suis* in pigs and pork in Northern Thailand. A total of 768 nasal swab samples (n=768) were obtained from pigs at slaughterhouses. The cross-sectional sampling was performed 4 times during April 2018 to October 2018. The targeted sampling sites included the municipal slaughterhouse in each district in five provinces in Northern Thailand including Chiang Mai (n=130), Chiang Rai (n=180), Nan (n=190), Phayao (n=202) and Mae Hong Son (n=66). Based on biochemical test results, the prevalence of S. suis in five provinces varied from 6.8%-15%. The highest prevalence was observed in Chiang Rai, followed by Pha Yao (11.4%), Mae Hong Son (10.6%), Chiang Mai (9.2%) and Nan (6.8%). Based on the presence of 16SrRNA or gdh, the prevalence of *S. suis* in five provinces varied from 3.0%-9.4%. None of the isolates collected in Chiang Mai were positive to 16SrRNA or *gdh*. The prevalence of *S. suis* confirmed by these genes was highest Chiang Rai (9.4%) and Mae Hong Son (9.1%), followed by Nan and Pha Yao. Of all 59 isolates confirmed to be *S. suis* by PCR amplification of either 16S rRNA or *gdh,* serotype 8 was most commonly identified, followed serotype 10 (3.4%) and serotype 2 (1.7%) and 9 (1.7%). The only one *S. suis* serotype 2 found was originated from the sample collected from Phayao. Most of positive samples carried only one *S. suis* serotype, but 3 samples from Chiang Rai (2) and Phayao (1) carried more than one *S. suis* serotypes. All the isolates carried *arcA*, but none were positive to *epf.* The *sly* gene was very common (91.5%), followed by *mrp* (69.5%) and *hyl* (37.3%). Fifteen virulence gene patterns were obtained. The most commonly identified pattern was *mrp, sly*, *arcA, hyl* (33.9%), followed by *mrp, sly*, *arcA* (28.8%) and *sly*, *arcA* (25.4%). This study confirms the important role of subclinical carrier pigs of *S. suis* and emphasize the need for detection of *S. suis* in clinically healthy pigs.

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1. Introduction

Streptococcus suis is a zoonotic bacterium that causes severe infections in both human and pigs. The pathogen easily colonizes in pigs and is a major pathogen in pig industry worldwide and can be isolated from new borne to weaning pigs. In the past, *S. suis* was most commonly a opportunistic pathogen involved in secondary infection in pigs. It usually caused clinical disease in pigs with immunocompromise. However, *S. suis* is being isolated in the absence of viruses or agents that would suppress the immunity. Therefore, this pathogen is now considered a primary pathogen that can cause clinical disease in normal or healthy pigs.
Infection with *S. suis* has created a negative impact on pig production. The pathogen typically affects organs or tissues such as brains, tonsils, lungs, or joints of diseased pigs and the common symptoms in infected pigs include fever, septicemia, endocarditis, arthritis, pneumonia and meningoencephalitis. *S. suis* can adapt well to its environment and can exist in clinically healthy pigs without causing illnesses. Therefore, pigs serve as a symptomatic carrier of the pathogen.

S. suis is an emerging zoonotic agent that can be transmitted to human mainly via direct contact (e.g. wound, skin cut) and ingestion of contaminated food, in particular uncooked pork and blood. The pathogen is responsible for septicemia with or without septic shock, meningitis and other less common infections in human. The people at risk of *S. suis* infection include pig farmer, worker in pig farm, worker in pig slaughterhouse, butcher and veterinarians. From a report in Vietnam, it was demonstrated that pigs in slaughterhouse are a major reservoir for *S. suis* serotype 2 that was confirmed to be in complex 1 and in the same clonal with those causing infections in China in 1998 and 2005 (1). In the same country, the *S. suis* strains from patients in 1997-2008 exhibited resistantance to multiple drugs with high resistance rates (2).

The first human case of *S. suis* infection in humans was in Denmark in 1968 (3). A large outbreak occurred in China in 2005, resulting in 215 cases, 102 patients with encephalitis, 48 patients with septicemia and 28 deaths (4). Since then, intensive outbreaks of human *S. suis*

infection have repeatedly reported and have raised a particular public health concern worldwide regarding the pathogen as an emerging zoonotic pathogen. Currently, *S. suis* is considered one of the most important "emerging zoonotic pathogens" in Asia and it is also a world-leading cause of enphalocephalitis and septicemia in humans (1).

In Thailand, infection with *S. suis* has been isolated from pigs in all regions. Patients are mostly in Northern provinces (e.g. Lamphun, Chiangmai, Phayao, Phitsanulok, Kamphaeng Phet and Phichit), some provinces in Northeastern and upper center regions. It has been shown that an important risk factor is consuming of raw pork and uncooked pig's blood (5). Infection with *S. suis* is frequently identified in Hill tribe, who usually slaughter pigs for family consumption and traditional celebration. Number of illness and death due to *S. suis* infection has been increasingly reported (6). In 2012, Bureau of Epidemiology, Thailand reported 186 *S. suis* cases and 18 death in the country. The *S. suis*-infected patients were most commonly found in Northern Thailand (5) and the highest morbidity rate was also in the region. Later in 2013, there were 189 *S. suis* infected cases and 22 deaths (6). Patients infected with *S. suis* were between 23-72 years of age with history of exposure to raw pork and raw blood (7). Later, it reported that the isolates from patients during 1998-2008 carried the similar virulence factors and serotypes ST1, 27 and 87 were most common and genetically related (8). In 2007, there was an outbreak in Pha Yao with 29 patients and 3 deaths. Infection was suspected in 21 persons and nine patients consumed raw blood and raw pork (9).

Up to date, there are 35 *S. suis* serotypes described and serotypes 1/2, 1, 2, 7, 9 and14 have been reported to be associated with diseases. Among these, *S. suis* serotype 2 is most commonly isolated with diseased pigs and humans and most frequently reported worldwide. It has caused more damages to economy and public health than other serotypes (10-12). The type 4 is an important zoonotic agent causing infection with high mortality (13). Infection with *S. suis* is a fatal disease. The disease is usually associated with hearing loss and vestibular impairment**.** The majority of those who survive from meningitis face the permanent hearing loss or deafness of both ears and some suffer from paralysis.

Up to date, there is no monitoring or surveillance program existing in Thailand and as a result, the real prevalence and incidence of *S. suis* infection remains largely unclear. It is expected that the real *S. suis* prevalence is much higher than reported. Importantly, *S. suis* vaccine is still not available for human use. Therefore, antibiotic therapeutic is the important treatment choice for *S. suis* infection. However, the *S. suis* strains resistant to antibiotics have increasingly emerged worldwide, resulting in ineffective treatment of *S. suis* infection and increasing high mortality in humans and pigs (14-16). Currently, knowledge on antimicrobial resistance phenotype of the *S. suis* strains is still limited and the genotype is much less. Such data is required for development of control and prevention strategic plan for S. *suis*, as well as development of vaccine.

This project consists of two phases in two continuous years:

Year 1 Situation and antimicrobial resistance of *Streptococcus suis* isolated from pigs in Northern region of Thailand: Prevalence of *S. suis*

Year 2 Situation and antimicrobial resistance of *Streptococcus suis* isolated from pigs in Northern region of Thailand: Antimicrobial resistance of *S. suis*

The current repost is for the Year 1 project. which is ”Situation and antimicrobial resistance of *Streptococcus suis* isolated from pigs in Northern region of Thailand: Prevalence of *S. suis”*

2. Characteristics of *Streptococcus suis*

S. suis is a Gram-positive facultative anaerobe. This bacterium is coccoid or ovoid, and occurs as single cells, in pairs, or in short chains. The natural habitat of *S suis* is the upper respiratory tract, particularly tonsils and nasal cavities and therefore, is usually transmitted nasally or orally. It can colonize genital and alimentary tracts of pigs. *S suis* serotype 2 colonises the palatine tonsils of clinically ill and apparently healthy pigs (17). Carriers of *S suis* are infectious to other pigs and very important in dissemination in herds. *S suis* serotype 2 is very good in adaptation and tolerates to various environmental conditions. The bacterium can survive in 60°C for 10 min, 50°C for 2 h, and 10°C carcasses for 6 weeks (18). This bacterium can at 0°C for 1 month in dust and for over 3 months in faeces. At 25°C, it can survive for 24 h in dust and for 8 days in faeces. However, *S suis* serotype 2 can be killed easily with 5% bleach.

Based on the capsular polysaccharides, there are 35 serotypes that have been identified including types 1–34 and ½ (19-23), with the exception serotypes 32 and 34 that have been proven to be *S. orisratti* (24). Of all identified serotypes, serotype 2 is the most commonly reported serotype worldwide and most frequently associated with diseases in pigs and *S suis* infections in humans (25, 26). Almost all human *S suis* infections are attributed to serotype 2, except two cases caused by serotype 1 and one case of septicaemia caused by serotype 14 (27, 28).

The genome of *S suis* has been completely sequenced and it contains 20,074,917 bp with a 41·3% G+C content (29). Approximately 20–30% of the genes are still unknown. And the current information reveals that many genes play a part in the pathogenesis of *S suis* infection, including polysaccharide production, capsular transport, iron-restriction factors, suilysin, virulence-associated proteins, various enzymes, arginine deiminase system, and IgG binding proteins.

The virulence of *S. suis* differs from serotypes to another and is different between different strains of the same serotype (30). Most studies on the *S. suis* virulence have been done with

serotype 2. *S suis* is generally susceptible to antibiotics, including penicillin, ceftriaxone, cephalosporin, ampicillin, and amoxicillin (31, 32).

3. Objectives

The objectives of the year 1 and year 2 research project sare:

2.1. to study prevalence of *S. suis* in pigs and pork in Northern Thailand

2.2. to investigate antimicrobial resistance phenotype of *S. suis* in pigs and pork in Northern Thailand

2.3. to examine genetics underlying antimicrobial resistance of *S. suis* in pigs and pork in Northern Thailand

2.4. to examine genes encoding virulence factors of *S. suis* in pigs and pork in Northern Thailand

4. Materials and Methods

This study consists of phases as follows:

Phase 1 Sampling location and sample collection

Phase 2 Bacterial isolation

Phase 3 Bacterial confirmation

4.1. Sampling location and sample collection

A total of 768 nasal swab samples (n=768) were obtained from pigs at slaughterhouses. The cross-sectional sampling was performed 4 times during April 2018 to October 2018. The targeted sampling sites included the municipal slaughterhouse in each district in five provinces in Northern Thailand including Chiang Mai, Chiang Rai, Nan, Phayao and Mae Hong Son (Figure 1). The sampling areas were chosen based on the previous report of *S. suis* cases, slaughterhouse owner aggreement and distance not farther than 15-20 km from public delivery service to ensure that the samples would arrive at the laboratory within 24 hours after collection .The slaughterhouses were of large production facilities with daily throughput of 80 or more pigs or small-scale plant with daily throughput of 50 or lesser animals and one large production facility with daily throughput of 200 or more heads were chosen .The nasal swab samples were collected from pigs after stunning and bleeding and stored in Stuart transport medium (Modified) (Oxiod, Hamshire, England) or transport medium (See Annex) prepared in our laboratory. All collected samples were transported to the laboratory within 2 h of collection. Due to logistical constraints, random sampling was not possible.

The sampling area are provinces along the border between Thailand and Lao PDR, Cambodia and Myanmar (Figure 1). Number of samples and the provinces are shown in Table 1 and Figure 1. The proportion of samples collected is shown in figure 2

Table 1 Sampling location**,** number of samples obtained from each province (n=768)

Province	District	No of samples
Chiang Mai	San Pa Tong	40
	Mueang	90
	Total	**130**
Chiang Rai	Mueang	80
	Thoeng	50
	Wiang Pa Pao	50
	Total	**180**
Nan	Wiang Sa	40
	Mueang	130
	Poa	20
	Total	**190**
Pha Yao	Mae Chai	10
	Chiang Kham	45
	Mueang	48
	Pong	20
	Phu Sang	42
	Chiang Muan	10
	Phu Kamyao	7
	Dok Khamtai	10
	Total	**202**
Mae Hong Son	Mueang	
	Total	**66**
Grand total		**768**

Figure 1 Map of sampling area and sample number (n=768). The provinces where the sample collection was performed, are indicated. The number of samples collected in each province are shown in corresponding callouts.

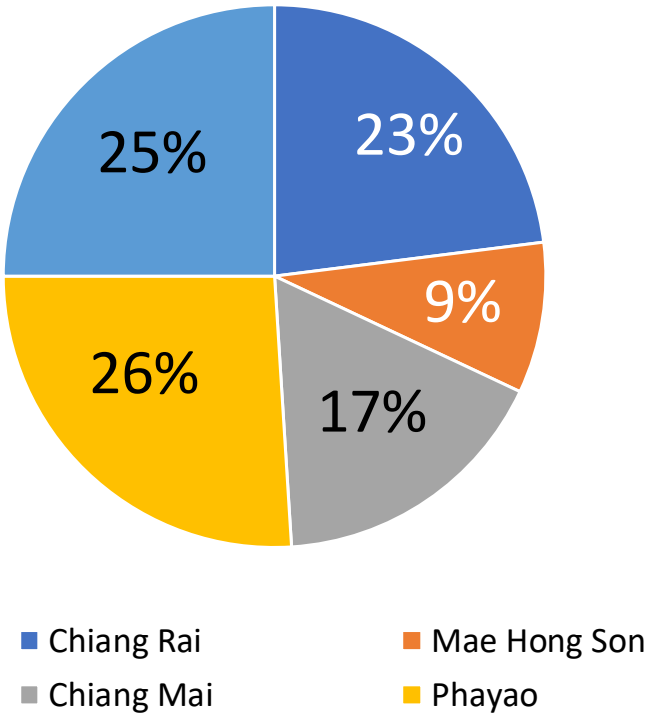

Figure 2 Proportion of sample number collected in 5 provinces in Northern Thailand (n=768)

4.2. Isolation and confirmation of *Streptococcus suis*

Steps for isolation and identification of *S. suis* is shown in Figure 3.

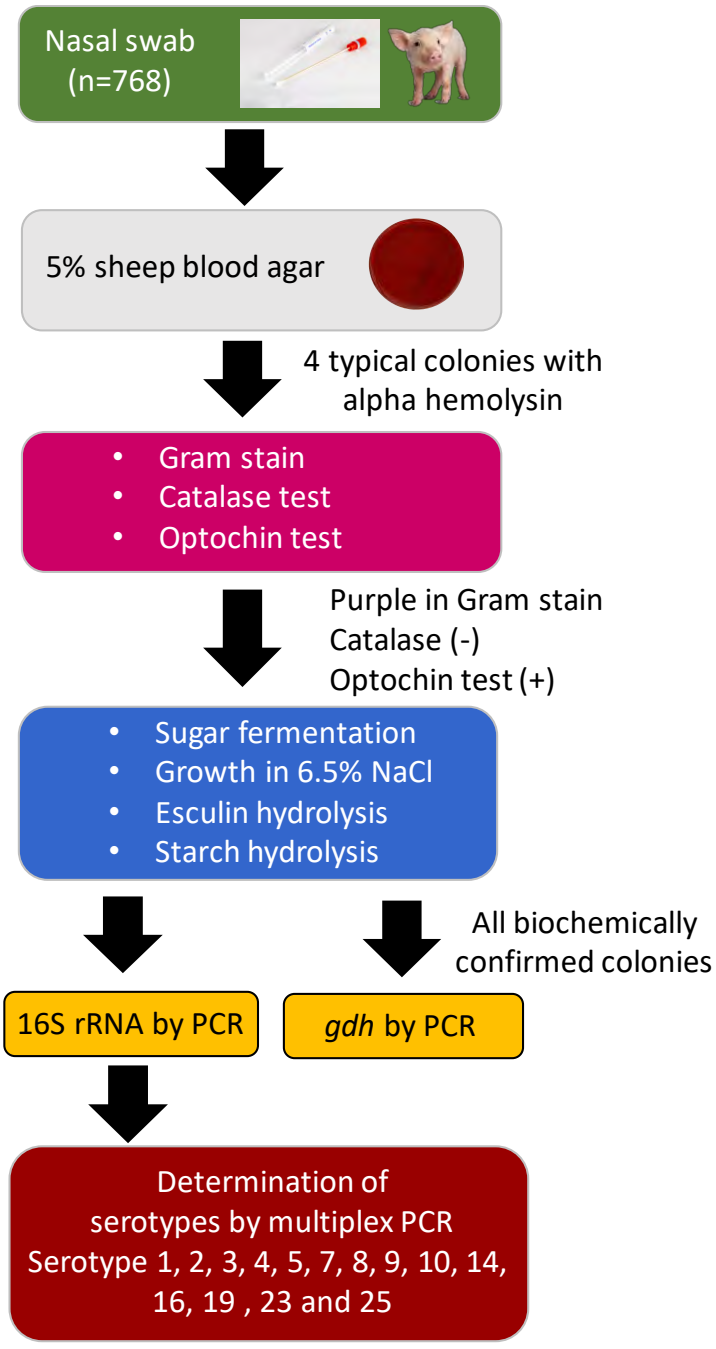

Figure 3 Diagram of isolation and identification of *S. suis*

4.2.1. Isolation of *Streptococcus suis*

Isolation of *S. suis* was performed as previously described (33) . Briefly, nasal swab was streaked on 5% sheep blood agar containing 20 µg/ml polymixin B (colistin) (Sigma-Aldrich, St. Louis, MO, USA) and 15 µg/ml nalidixic acid (Sigma-Aldrich). The antibiotics were added to inhibit growth of Gram-negative bacteria and allow better growth and distinct colonies of *S. suis*. The plates were incubated at 37°C for 24 hour in atmosphere with 5% CO_2. Four typical colonies of *S. suis* (gray small colonies with alpha-hemolysis) from each positive sample were purified and species-identified by the following tests:

1. Gram staining

Typical colonies of *S. suis* were Gram stained. *S. suis* is a gram-positive coccus, stained purple and frequently seen in pairs but can also be single or in short chains.

2. Catalase test

A well-isolated typical colonies of *S. suis* were picked by using inoculating loop and placed onto a glass slide. It is important not to pick any agar, especially agar containing red blood cells because carryover of red blood cells into the test may result in a false-positive reaction. A drop of 3% H_2O_2 was placed onto the bacteria on the glass slide by using a dropper or Pasteur pipette. The positive reaction is the immediate bubble formation. *S. suis* is catalase-negative.

3. Optochin susceptibility test

Typical colonies of *S. suis* on 5% sheep blood agar were collected and 2-3 colonies of a pure culture to be tested were streaked on 5% sheep blood agar. Optochin (ethylhydrocupreine hydrochloride) disk (5 µg/disk) (Oxoid, Hampshine, England) was placed on the streaked area by using sterile forceps. The inoculated plates were Incubated at 35-37°C with ~5% CO_2 for 18 to 24 hour. The zone of inhibition around the optochin disk and was measured in millimeters. For a 6 mm, 5 µg disk, a zone of inhibition of 14 mm or greater indicates sensitivity. A zone of inhibition of 14 mm or lesser indicates resistance. Optochin sensitivity allows for the

presumptive identification of alpha-hemolytic streptococci and *S. suis* is optochin-resistant.

4.2.2. Biochemical tests

The colonies that are purple in Gram stain, catalase negative and optochin-resistant were suspected to be *S. suis*. The suspected colonies were further tested to identify the species as follows (20, 22):

1. Sugar fermentation

The suspected colonies were resuspended in 1 ml of 0.9% NaCl solution. Two µl of the suspension was added into 96-well plate containing 100 µl of different sugar, including arabinose, inulin, lactose, mannitol, raffinose, salicin, sorbitol, sucrose, trehalose. In concentration of sugar used was 2%. The plates were incubated at 37 °C, 18-24 hour. The color change from pink to yellow indicate the positive results.

2. Growth in 6.5% NaCl

The suspected colonies were resuspended in 1 ml of 6.5% NaCl solution and incubated at 37 °C, 24 hour. Turbidity of the culture indicates growth and the positive result.

3. Esculin hydrolysis

Streak the colony on bile esculin slant and incubate at 37 °C, 24 hour. Growth of the bacteria indicates bile salt tolerance and the ability to use esculin. The color change to be black or dark brown indicates the positive result.

4. Starch hydrolysis

The colony was streaked on starch agar and incubate at 37 °C for 48 hour. The grown colovies were tested with iodine solution. If the bacteria can produce amylase enzyme and use starch, there will be the presence of clear zone around the colonies, indicating positive result.

The expected results from biochemical tests for different *S. suis* serotypes are as follows (Table 2):

Table 2 Biochemical characteristics of different *S. suis* serotypes

Biochemical test	Expected result
1. Arabinose	-
2. Inulin	+/-
3. Lactose,	+
4. Mannitol	-
5. Raffinose	+/-
6. Salicin	+
7. Sorbitol	-
8. Sucrose	+
9. Trehalose	+
10. Bile esculine	+/-
11. Growth in 6.5% NaCl	-
12. Starch hydrolysis	+

4.2.3. Confirmation of *S. suis*

Single colonies of *S. suis* confirmed by biochemical test were collected and genomic DNA was prepared from each colony by using Gentra® Puregene® (Qiagen, Hilden, Germany). Each single colony was grown in 2 ml Todd Hewitt broth at 37 °C for 24 hour. The DNA was served as PCR templates for detection of 16S rRNA and glutamate dehydrogenase (*gdh*) that are specific to *S. suis* (34-36). All primers used in this study are listed in Table 3.

4.2.4. Identification of serotype by multiplex PCR

The confirmed *S. suis* colonies were subjected to determination of serotypes by multiplex PCR. The serotype testing based on *cps* genes was performed and included serotype 1, 2, 3, 4, 5, 7, 8, 9, 10, 14, 16, 19, 23 and 25 (34, 37). The template DNA was prepared by Gentra® Puregene® (Qiagen). The PCR reaction was performed using GeNei™ mastermix (Merck, Munich, Germany) and PCR condition was as follows:

1. Predenaturation at 95 °C, 5 min
2. Denaturation at 95 °C, 20 sec
3. Annealing at 58°C, 30 sec
4. extension at 72 °C, 40 sec

Repeat 2-4 for 30 cycles

4.2.5. Nucleotide sequencing analysis

The PCR amplicons were gel purified using Nucleospin® Gel (Mccherey-Nagel, Düren, Germany) and submitted for sequencing PCR primers at First Base Laboratories (Selangor Darul Ehsan, Malaysia). The obtained DNA sequence was BLAST compared with GenBank database (http://www.ncbi.nlm.nih.gov/BLAST).

4.3. Screening for virulence determinants

Virulence determinants including *mrp, epf, sly*, *arcA* and *hyl* (38, 39) were screened by either conventional or multiplex PCR. The PCR reaction was performed using GeNei™ mastermix (Merck, Munich, Germany) and PCR conditions are described below:

mrp, epf and *sly* (38)

1. Predenaturation at 94 °C, 5 min
2. Denaturation at 94 °C, 1 min
3. Annealing at 58°C, 1 min
4. extension at 72 °C, 90 sec.

Repeat 2-4 for 30 cycles

arcA and *hyl* (39)	1. Predenaturation at 94 °C, 5 min
	2. Denaturation at 94 °C, 30 sec
	3. Annealing at 54°C, 1 min
	4. Extension at 72 °C, 1 min..
	Repeat 2-4 for 30 cycles

Table 3 Primers used in this study

Gene	Primer sequences (5'-3')	Amplicon (bp)	Reference
16S rRNA	F: CAGTATTTACCGCATGGTAGATAT R: GTAAGATACCGTCAAGTGAGAA	294	(35)
Serotypes			
1 and 14	F: AATCATGGAATAAAGCGGAGTACAG R: ACAATTGATACGTCAAAATCCTCACC	550	(34)
2 and 1/2	F: GATTTGTCGGGAGGGTTACTTG R: TAAATAATATGCCACTGTAGCGTCTC	450	(34)
3	F: TGGGAGAAGGCAGAAAGTACGAGA R: ACCCCCAGAAGAGCCGAAGGA	1273	(34)
4	F: ACTTGGAGTTGTCGGAGTAGTGCT R: ACCGCGATGGATAGGCCGAC	783	(34)
5	F: TGATGGCGGAGTTTGGGTCGC R: CGTAACAACCGCCCCAGCCG	166	(34)
7	F: GATGATTTATGGCACCCGAGTAAGC R: AGTCACAATTGCTGGTCCTGACACC	150	(34)
8	F: ATGGGCGTTGGCGGGAGTTT R: TTACGGCCCCCATCACGCTG	320	(34)
9	F: GGCTACATATAATGGAAGCCC R: CCGAAGTATCTGGGCTACTG	300	(37)
10	F: TCGCTCTGCGTTCGTCGAGT R: GCCCACCCGCCACGAGAAAG	1756	(34)
16	F: TGGAGGAGCATCTACAGCTCGGAAT R: TTTGTTTGCTGGAATCTCAGGCACC	202	(34)
19	F: AGCAGGGTTGCGTATGGCGG R: ACAAGCACCAGCAAAGACCGCA	1024	(34)
23	F: GCGGGCATATGCAGTGGGCA R: ACCGAATGCCACATCGGGTG	825	(34)
25	F: GGAGGAGCTGCGGGCTCATA R: GGAGGAGCTGCGGGCTCATA	1211	(34)
Virulence determinants			
gdh	F: TTCTGCAGCGTATTCTGTCAAACG R: TGTTCCATGGACAGATAAAGATGG	695	(34)
mrp	F: ATTGCTCCACAAGAGGATGG R: TGAGCTTTACCTGAAGCGGT	188	(38)
epf	F: ATCTACTGGGTATCCTTCTGC R: CTATCTGGATCTGTGATTGGA	626	(39)
sly	F: GCTTGACTTACGAGCCACAA R: CCGCGCAATACTGATAAGC	248	(38)
arcA	F: TGATATGGTTGCTGCTGGTC R: GGACTCGAGGATAGCATTGG	118	(38)
hyl	F: CTCAGATGAAAGCCTTTCTA R: TTTGTCCTTGGTCGTTGTC	1290	(40)

5. Results

5.1. Prevalence of *S. suis*

A total of 768 nasal swab were obtained from pigs in slaughterhouses in five provinces in Northern Thailand (Table 3). Based on biochemical test results, the prevalence of *S. suis* in five provinces varied from 6.8%-15%. The highest prevalence was observed in Chiang Rai, followed by Pha Yao, Mae Hong Son, Chiang Mia and Nan. Based on the determination of 16SrRNA or *gdh*, the prevalence of *S. suis* in five provinces varied from 3.0%-9.4%. None of the isolates collected in Chiang Mai were positive to 16SrRNA or *gdh*. The prevalence of *S. suis* confirmed by these genes was highest Chiang Rai (9.4%) and Mae Hong Son (9.1%), followed by Nan and Pha Yao. The prevalence of *S. suis* by biochemical test (10.3%) is significantly different from that confirmed by determination of 16S rRNA (5.3%) ($p<0.05$).

The *gdh* gene was additionally screened in all the isolates. It is evident that not all the 16S rRNA-positive isolates carried *gdh* (Table 4). Eighty-eight percent of the 16S rRNA-positive isolates (n=39) were found to carry *gdh*. However, the number of 16S rRNA-positive isolates and that of *gdh*-positive isolates are not significantly different.

Based on biochemical test results, 120 isolates of *S. suis* (15.6%) were obtained and all the isolates were subjected to determination of 16S rRNA. Among these, 59 isolates (7.7%) were confirmed to be *S. suis* by PCR amplification of 16S rRNA. The isolates were originated from samples in different provinces as shown in Table 4-5.

The PCR amplicons for 16S rRNA, *gdh* and *cps* for serotyping are shown in figure 4.

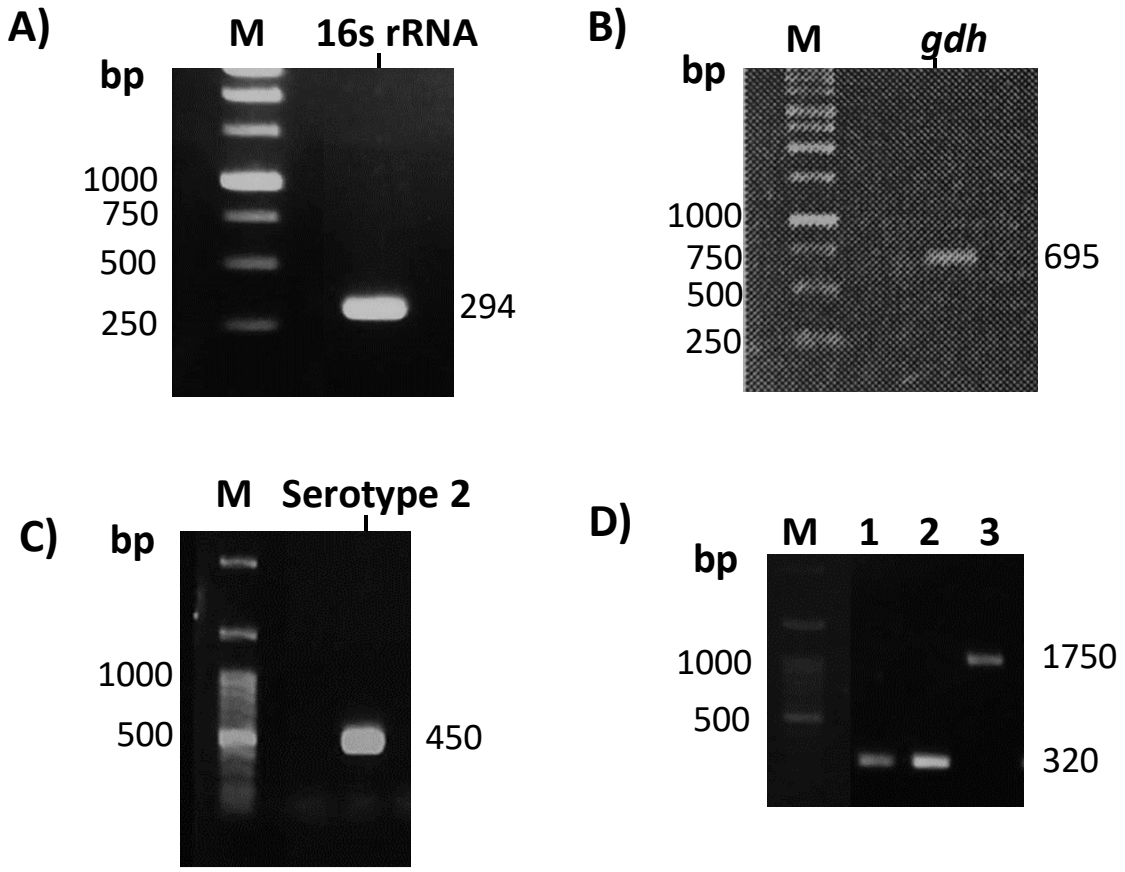

Figure 4 PCR amplicons for A) 16S rRNA, B) *gdh* and C) and D) *cps* for serotyping of *S. suis*

M, Molecular weight marker; 1-2, serotype 8; 3, serotype 10

Table 4 Identification and confirmation of *S. suis* isolated from nasal swab of pigs in slaughterhouses in Northern Province, Thailand

Province	Total no. of samples (n)	Positive by biochemical test		Positive by 16S rRNA or *gdh*	
		No of samples (%)	No of isolates collected	No of samples (%)	No of isolates collected
Chiang Mai	130	12 (9.2)	16 (12.3)	0	0
Chiang Rai	180	27 (15.0)	49 (32.7)	17 (9.4)	27 (45.8)
Nan	190	13 (6.8)	14 (7.4)	12 (6.3)	13 (22)
Pha Yao	202	23 (11.4)	29 (14.4)	6 (3.0)	8 (13.6)
Mae Hong Son	66	7 (10.6)	12 (18.2)	6 (9.1)	11 (18.6)
Total	**768**	82 (10.7)	**120 (15.6)**	41 (5.3)	**59 (100)**

Table 5 Summary of *S. suis* and their serotypes isolated from pigs in slaughterhouses in Northern Province, Thailand

Province	16S rRNA or *gdh* positive (%)	16S rRNA positive (%)	*gdh* positive (%)	Serotypes (no, %)				
				2	8	9	10	Unidentified[a]
Chiang Rai	27 (45.8)	10(16.9)	26 (44.1)	0	3 (5.1)	1(1.7)	2(3.4)	21(35.6)
Nan	13 (22)	13(22.0)	8 (13.6)	0	11 (18.6)	0	0	2(3.4)
Pha Yao	8 (13.6)	8(13.6)	8 (13.6)	1 (1.7)	5 (8.5)	0	0	2(3.4)
Mae Hong Son	11 (18.6)	8(13.6)	10 (16.9)	0	11 (18.6)	0	0	0
Total	59 (100)	39(66.1)	52 (88.1)	1 (1.7)	30 (50.8)	1(1.7)	2 (3.4)	25 (42.4)
			Total	34 (57.6)				

[a]Not positive to 14 serotypes tested in this study

5.2. Serotypes of *S. suis*

Of the 14 serotypes tested, four serotypes (i.e. 2,8, 9 and 10) were identified and the others were designated as “unidentified” (Table 5-7 and figure 5).

Of all the isolates collected, serotype 8 was most commonly identified in all provinces, followed serotype 10 (3.4%), serotype 2 (1.7%) and 9 (1.7%). All the isolates from Mae Hong Son were serotype 8. Most isolates from Nan (11/12) were serotype 8 and the others are classified as “unidentified”.

Only one *S. suis* serotype 2 was found and it was originated from the sample collected from Phayao. Most of positive samples carried only one *S. suis* serotype, but 3 samples from Chiang Rai (2) and Phayao (1) carried more than one *S. suis* serotypes. Both Chiang Rai isolates carried serotype 10 and unidentified serotype. The isolate from Phayao harboured serotype 2 and 8.

Table 6 Summary of positive samples and serotype of *S. suis* isolated from pigs in slaughterhouses in Northern Province, Thailand (n=768)

Province	No. of positive samples	No. of isolates collected)	Serotypes (No. of isolates, %)				
			2	8	9	10	Unidentified[a]
Chiang Rai	17	27	0	3 (11.1)	1 (3.7)	1 (3.7)	21 (77.8)
Nan	12	13	0	11 (84.6)	0	0	2 (15.4)
Phayao	6	8	1 (12.5)	5 (62.5)	0	0	2 (25.0)
Mae Hong Son	6	11	0	11 (100.0)	0	0	0
Total	**41**	**59**	**1 (1.7)**	**30 (50.8)**	**1 (1.7)**	**2 (3.4)**	**25 (42.4)**

[a]UD, not positive to 14 serotypes tested in this study

Table 7 Sources and characteristics of the *S. suis* isolates confirmed by 16SrRNA or *gdh* (n=59)

Provinces	Sample no	No of isolates collected	Code	Positive to *gdh*	Positive to 16s rRNA	Serotype
Nan (n=13)	1	1	42.1	-	+	8
	2	1	45.4	+	+	UD
	3	1	65.3	-	+	8
	4	1	85.1	+	+	8
	5	1	86.2	+	+	8
	6	2	90.1	+	+	8
			90.3	+	+	8
	7	1	91.3	+	+	8
	8	1	100.2	-	+	8
	9	1	118.1	+	+	8
	10	1	139.3	-	+	8
	11	1	150.2	+	+	UD
	12	1	175.4	-	+	8
Mae Hong Son (n=11)	1	1	197.1	+	+	8
			197.4	+	+	8
	2	2	211.3	+	+	8
			211.4	+	+	8
	3	1	213.2	+	+	8
	4	1	219.2	+	+	8
	5	1	251.4	-	+	8
	6	6	253.1	+	+	8
			253.2	+	-	8
			253.3	+	-	8
			253.4	+	-	8
Chiang Rai (n=27)	1	3	628.1	+	-	UD
			628.3	+	-	UD
			628.4	+	-	10
	2	1	629.2	+	-	UD
	3	1	630.1	+	-	UD
	4	1	635.1	+	-	8
	5	3	637.1	+	+	UD
			637.2	+	+	UD
			637.4	+	+	UD
	6	4	642.1	+	+	UD
			642.2	+	-	UD
			642.3	+	-	UD
			642.4	+	-	D
	7	1	656.1	+	+	UD
	8	2	661.1	+	-	UD
			661.4	+	-	UD
	9	1	670.3	+	-	UD
	10	1	672.4	+	+	8
	11	1	680.1	+	-	UD
	12	2	710.1	+	-	UD
			710.3	+	-	UD
	13	2	725.1	+	-	UD

			725.2	+	+	10
	14	1	754.2	+	+	9
	15	1	775.1	+	+	8
	16	1	700.1	+	-	UD
	17	1	751.4	-	+	UD
Phayao (n=8)	1	1	507.1	+	+	8
	2	1	515.3	+	+	8
	3	1	528.3	+	+	UD
	4	2	565.1	+	+	8
			565.4	+	+	8
	5	1	597.4	+	+	UD
	6	2	607.2	+	+	8
			607.3	+	+	2

UD unidentified serotype, not positive to 14 serotypes tested in this study
The highlighted box indicates a single sample positive to two *S. suis* serotypes.

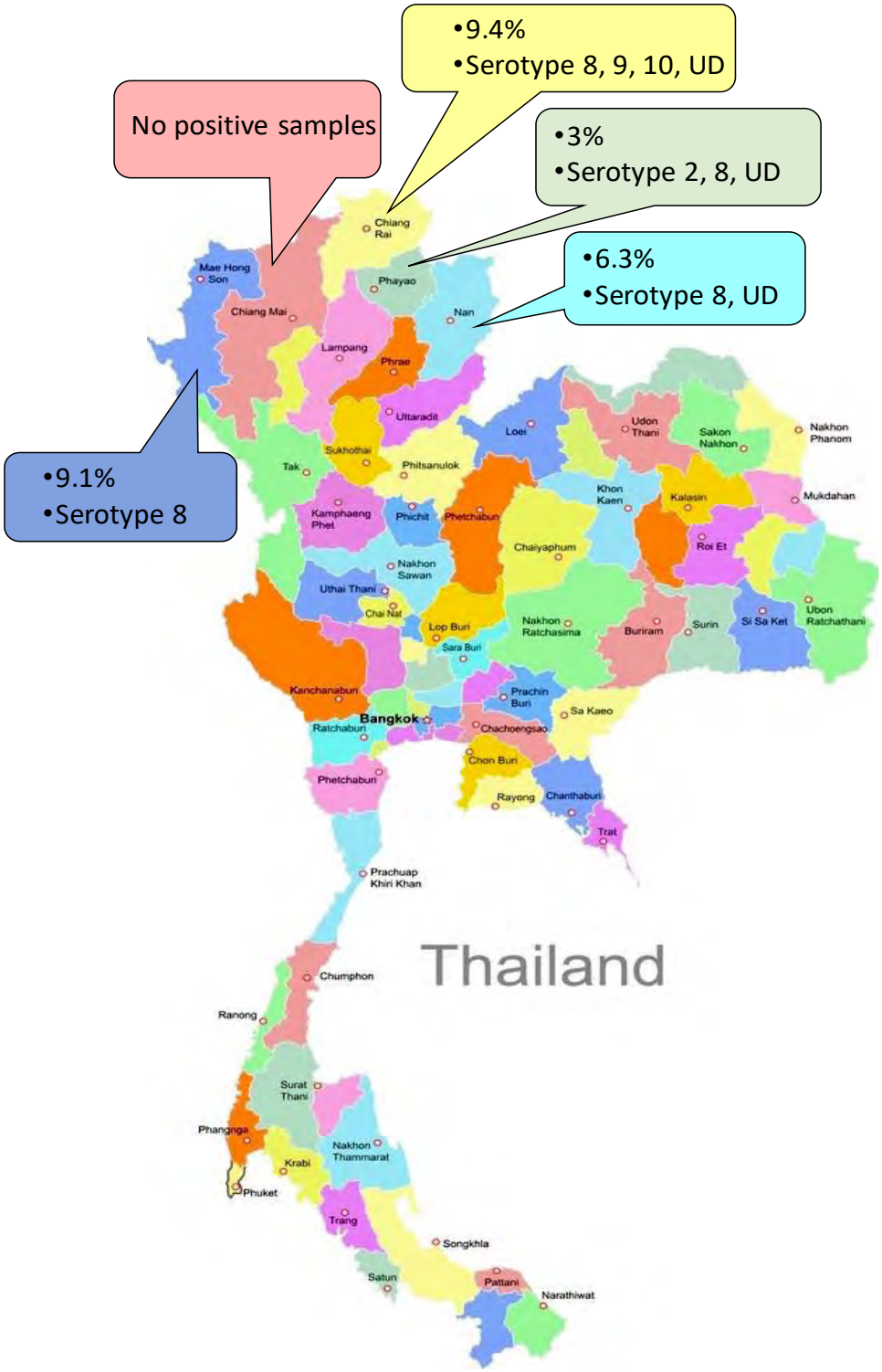

Figure 5 Prevalence and serotypes of *S. suis* in Northern Thailand. The prevalence (%) and serotypes identified in each province is shown in corresponding callouts.

UD, unidentified serotype

5.3. Virulence determinants of *S. suis*

Five virulence determinants commonly found in *S. suis* including *mrp, epf, sly*, *arcA* and *hyl* were chosen for detection in this study. All the isolates carried *arcA*, but none were positive to *epf.* The *sly* gene was very common (91.5%), followed by *mrp* (69.5%) and *hyl* (37.3%) (Table 8 and figure 6).

Based on the presence of virulence genes test, 15 virulence gene patterns were obtained. The most commonly identified pattern was *mrp, sly*, *arcA, hyl* (33.9%), followed by *mrp, sly*, *arcA* (28.8%) and *sly*, *arcA* (25.4%) (Table 9 and 10).

Table 8 Percentage of virulence determinants in of *S. suis* and their serotypes isolated from nasal swab of pigs in slaughterhouses in Northern Provinces, Thailand (n=59)

Virulence gene	Function/Protein	Percentage
mrp	muramidase-released protein	69.5%
epf	extracellular protein factor	0
sly	suilysin	91.5%
arcA	arginine deiminase	100%
hyl	hyaluronidase	37.3%

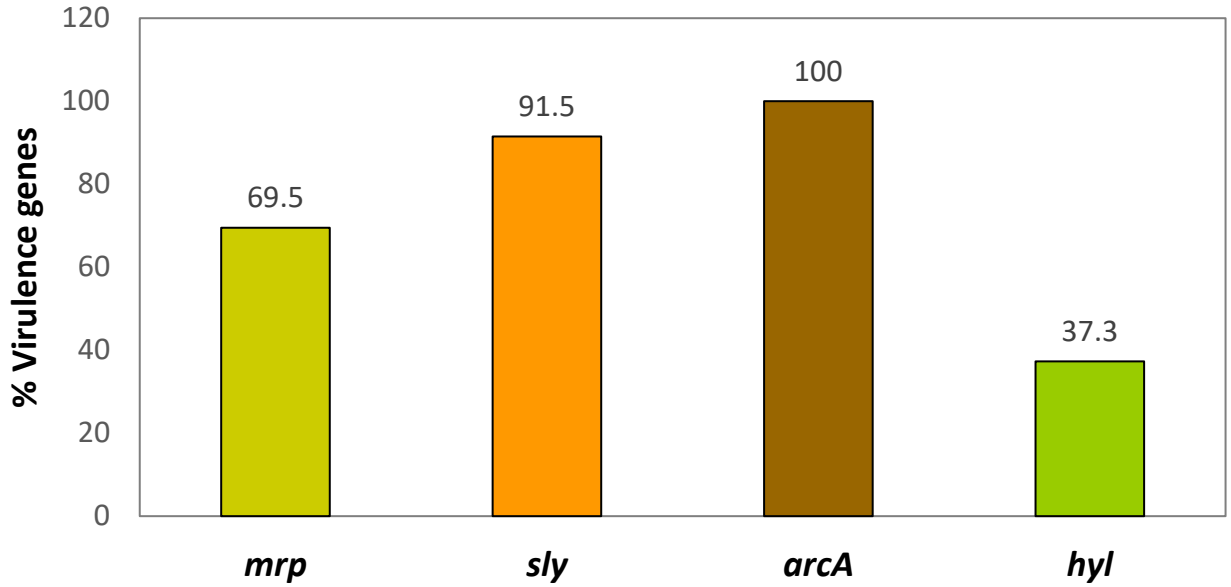

Figure 6 Percentage of virulence determinants in of *S. suis* and their serotypes ที่ isolated from nasal swab of pigs in slaughterhouses in Northern Provinces, Thailand (n=59)

Table 9 Virulence gene patterns in *S. suis* Percentage of virulence determinants in of *S. suis* and their serotypes ที่ isolated from nasal swab of pigs in slaughterhouses in Northern Provinces, Thailand (n=59)

Virulence gene pattern	No of isolates (%)
mrp, sly, arcA, hyl	20 (33.9)
mrp, sly, arcA	17 (28.8)
mrp, sly, hyl	0
mrp, arcA, hyl	0
sly, arcA, hyl	2 (3.4)
mrp, sly	0
mrp, arcA	4 (6.8)
mrp, hyl	0
sly, arcA	15 (25.4)
sly, hyl	0
arcA, hyl	0
mrp	0
sly	0
arcA	1 (1.7)
hyl	0
Total	**59**

Table 10 Virulence genes in *S. suis* of different serotypes (n=59)

Province	Serotypes (n)	No of isolates	Virulence gene patterns (%)			
			mrp	***sly***	***arcA***	***hly***
Chiang Rai (27)	8 (3)	2	+	+	+	+
		1	-	+	+	+
	9 (1)	1	+	+	+	-
	10 (2)	1	+	+	+	+
		1	+	+	+	-
	Unidentified[a] (21)	7	+	+	+	+
		4	+	+	+	-
		3	+	-	+	-
		6	-	+	+	-
		1	-	-	+	-
Nan (13)	8 (11)	3	+	+	+	+
		6	+	+	+	-
		2	-	+	+	-
	Unidentified[a](2)	1	+	+	+	+
		1	-	+	+	-
Phayao (8)	2 (1)	1	+	+	+	+
	8 (5)	1	+	+	+	+
		2	+	+	+	-
		1	-	+	+	+
		2	-	+	+	-
	unidentified[a] (2)	1	+	-	+	-
		1	-	+	+	-
Mae Hong Son (11)	8 (11)	4	+	+	+	+
		3	+	+	+	-
		4	-	+	+	-
	Total	**59**	**41 (69.5)**	**54 (91.5)**	**59 (100)**	**22 (37.3)**

[a] not positive to any serotypes tested in this study

6. Discussion

Based on the presence of either 16s rRNA or *gdh*, 5% of the slaughtered pigs in Northern Thailand were positive to *S. suis*. This is in an agreement with a previous study in healthy fattening pigs in Northern and Northeastern Thailand where 5.7% positive samples were reported (33). The samples were originated from pigs in slaughterhouse and are clinically healthy. *S. suis* can colonize healthy pigs without clinical symptoms and can be isolated from tissues of healthy pigs, such as bone and tail, sold in retail markets. Therefore, the results highlight an important role of pigs as carriers of *S. suis* that may enter food chain and potentially pose risk to pig farmers, butchers or slaughterhouse employees. Therefore, the role of healthy pigs in the maintenance of the *S. suis* strains supports that the animals are responsible for outbreaks of clinical diseases. However, it has been hypothesized that *S. suis* in those tissues may be due to cross-contamination during slaughter and post-slaughter processing. It was previously demonstrated that *S. suis* from patients and pigs in Lamphun, Thailand were in the same pulsotype or genetically related (41). In addition, a genetic analysis revealed that pigs at different stage of production carried the of S. *suis* isolates with similar phenotypic and genetic characteristics. However, this was not pursued in this study and further investigations to explore sources of *S. suis* contamination and genetic relatedness to human and retail pork are suggested.

Despite a deadly disease, determination of *S. suis* is still a problematic issue. There is still a lack of absolute diagnostic techniques with high specificity and sensitivity for the bacteria. Bacterial culture techniques are commonly used to detect and isolate *S. suis* in clinical samples. It is usually followed for confirmation with biochemical testing. However, these methods are usually ineffective in environmental samples with frequent contaminations with a variety of environmental microorganisms e.g. raw pork sold at retail markets where contamination could occur during slaughter, post-slaughter processing, distribution, storage and display. Such high concentration of background microflora can result in the overcrowding of culture plates even in selective culture media and cause difficulty in identification of the *S. suis* strains. Plating of diluted cultures is not a solution for this issue as it inevitably leads to

the loss of the *S. suis* isolates because the concentration of *S. suis* in slaughtered pork or meat is usually low.

Biochemical tests are commonly used to screen the isolates in health pigs (Ref). The conventional diagnostic test for *S. suis*, comprising 11 biochemical tests was developed (20) and now commercially available. Another method is the combination of *S. suis* identification by biochemical test and confirmation by agglutination tests for common serotypes (i.e. 1, 1/2, 2, 5, 7, 8, 9, 14 and 16). However, the latter is quite costly for routine laboratory diagnosis and may not be practical for every country. In recent years, more rapid and specific simplex or multiplex conventional PCR assays have been developed based on different conserved DNA sequences of *S. suis* including the species-specific 16S rRNA genes (35), the glutamate dehydrogenase gene (*gdh*) of serotype 2 (36) and the capsular polysaccharide biosynthesis gene (*cps*) of S. suis serotypes 2 (and 1/2), 1 (and 14), 7, and 9 (35, 36, 42-44). Some studies used the presence of 16S rRNA and *gdh* alone as a sole confirmation method. Screening and sequencing of 16s rRNA is a goal standard for determination of *S. suis* and its sequence variations can describe genetic relationship by construction of a phylogentic tree (45). It was suggested that the *gdh* gene was a good target for detection of *S. suis* in clinical cases (36). As PCR was proved as a reliable species-specific molecular diagnostic tool, detection of *gdh* together with genes encoding *S. suis* capsular biosynthesis (*cps*) (as used in this study) was shown as the accurate identification of *S. suis* isolates and a serotype-specific method for the detection of strains of serotypes 1/2, 1, 2, 7, and 9 (46). It was shown that the sensitivity of biochemical test, detection of 16S rRNA and detection of *gdh* was similar (33). However, detection of *qdh* could yield 31% false negative results, while conventional biochemical test generated 2.3% false positive results (33). PCR technique has gained attention due to its high specificity, less expensive and better reproducibility. In developing countries, the conventional biochemical method is frequently used to screen for *S. suis* in large numbers of samples from healthy pigs. Still, PCR methods is used determination of *S. suis* serotypes due to its versatility, better reproducibility, easy maintenance and less price.

The standard method based on capsular typing or detection of *cps* genes remains to detect virulent strains in clinical samples from diseased pigs and humans In this study, we chose to perform biochemical testing as presumptive test and screening of 16srRNA and *gdh* as

confirmation methods. These three confirmation methods (including biochemical test, screening of 16s rRNA and detection of *gdh*) were performed in the isolates obtained from conventional isolation. The isolates that were positive to either 16srRNA or *gdh* were defined as *S. suis* to avoid the missing isolates. However, the results showed discrepancy between biochemical tests and determination of 16S rRNA/*gdh*. Based on the biochemical test results, the prevalence of *S. suis* was found to be 10.7%, which is twice of that reported by the presence of 16s rRNA and *gdh* (5.3%). Some (12%) of the 16S rRNA-positive isolates were positive to *gdh*. It suggests that biochemical tests should serve as presumptive identification methods and additional confirmation tests are required. It is critical to use appropriate-standard methods to avoid over estimation of the *S. suis* prevalence.

Of all the *S. suis* isolates in the present study, most were serotype 8 (50.8%), while many of them were in unidentified serotypes (42.4%). This serotype 8 was identified in pigs in the US and Australia (REF 34-35). Up to date, serotype 8 has not been reported in humans in Thailand. Only one serotype 2 (1.7%) was found, in agreement with a previous study (33). In general, serotype 2 strains are highly prevalent among the isolates from diseased pigs worldwide (47-49). This is in consistent with the result of this study where only one serotype 2 isolate was detected. This could be because the samples in the present study were originated from clinically healthy pigs.

The serotype 9 was previously found in pig origins in Italy (50) and in clinically healthy and ill pigs in Spain (51). The serotype 9 has become a highly prevalent or even the most prevalent serotype found among clinical isolates from diseased pigs and humans in many European countries (26, 38, 52-55). However, only one serotype 9 was identified in this study. This could be explained by the origin (clinically healthy pigs) of the samples. The first human case infected with serotype 9 was reported in 2013 and the serotype has been considered as emerging serotypes in Thailand since then (48). The serotype 10 was formerly reported in pigs in Australia (56) and in this strain collection, only 2 isolates (3.4%) were positive this serotype.

It is of interest to observe that most serotype 8 observed in the resent study were originated from the samples collected in Nan, Mae Hong Son and Phayao. In contrast, serotype 2, 9 and

10 were found in Chiang Rai only. In addition, most of the *S. suis* isolates from Chiang Rai were classified into unidentified group. The results support that the prevalence of specific serotypes differs between regions, and can change in time.

In Thailand and other Asian countries, the number of patients infected with 2, 14 and 16 has been increasingly emerged (57). In particular, serotypes 2, 5, 14 have been recently reported in Thailand (58){Kerdsin, 2011 #4481. With the exception of serotype 2, these serotypes were not detected among the isolates in the present study. Regardless of the serotypes identified, the results confirm the role of clinically healthy pigs as carriers for *S. suis* in Northern Thailand.

Previous studies showed that multiple *S. suis* serotypes can be identified in tonsils of diseased pigs and pigs can be infected with multiple serotypes of *S. suis* {Wisselink, 2002 #4421}(59, 60). This agrees with the observations in this study, of which three samples from Chiang Rai (n=2) and Phayao (n=1) carried *S. suis* of two different serotypes. These findings may partly contribute to the difficulty in disease control by vaccines. Currently, autogenous bacterins are used as vaccines for control of the disease. However, it has been hypothesized that a bacterin vaccine prepared from one serotype may suppress clinical disease caused by that particular serotype. In this case, these vaccines confer protection only against challenge with strains of a homologous serotype (61)). As a result, they may not affect new outbreaks caused by *S. suis* strains belonging to other serotypes. This highlights the need for identification of specific *S. suis* strains, not only those involved in diseased animals but also those involved in the carrier state. This is to provide data for development of adequate control measures and vaccine production. It also highlights the need for the methods that can detect several *S. suis* serotype simultaneously and multiplex PCR assays can contribute to such requirement.

Virulence-associated phenotypes of *S. Suis* were detected in this study. As a worldwide-important pathogen, difference of virulence among strains of serotype 2 is well documented. Such research on *S. suis* serotype 2 has mainly concentrated on *cps* encoding polysaccharide capsule potential virulence factors and other pathogenic mechanisms. Of all virulence genes tested in this study, *mrp*, *epf*, and *sly* were considered as the most relevant factors to the pathogenesis of *S.suis* (38, 62-64). The *arcA* gene encoding arginine deiminase was previously

found in all pathogenic serotype 2 obtained from Thai isolates (39, 65). This is consistent with this study where all the isolates carried *acrA*. It was suggested that ArcA in serotype 2 may have a presumptive role in the bacterial pathogenicity and could be one of the virulence-associated factors. It is important to observe that many *S. suis* isolates from clinically healthy pigs harbored the *mrp* and *sly* genes, even though none were positive to *epf*. Up to date, study of virulence factors in the isolates from clinically healthy pigs is still limited. Currently, virulence difference between the isolates from clinically healthy pigs and those from diseased pigs may focus on the frequency of the *mrp*, *epf*, and *sly* genes. It is crucial that genotyping based on other virulence factors should be performed and comparison with the isolates from diseased should be done.

7. Conclusions and suggestions

In summary, the objectives of this project were accomplised. This study revealed that healthy pigs can serve as reservoirs for *S. suis* that can constitute a threat to public health. It can be concluded that:

1. The slaughtered pigs in Chiang Mai, Chiang Rai, Nan, Phayao and Mae Hong Son of Thailand served as carriers of *S. suis*. This confirms the important role of subclinical carrier pigs as the source of infection for young sensitive pigs and emphasize the need for detection of *S. suis* in clinically healthy pigs. This can lead to a better understanding of the epidemiology of *S. suis* infections and facilitate in the development of effective control measures.
2. The serotypes 8 is most commonly found among healthy pigs slaughtered in Chiang Mai, Chiang Rai, Nan, Phayao and Mae Hong Son of Thailand. However, serotypes cannot be identified in a large portion of the isolates.
3. *S. suis* serotype 2, 9 and 10 were found at very low rate in clinically healthy pigs for slaughtering in the region.
4. Pigs can be carriers of multiple *S. suis* serotypes.
5. Biochemical test is not an accurate method for *S. suis* identification. Molecular techniques are essential for confirmation of the *S. suis* strains.

Attempts to control and prevent spreading of *S. suis* is halted by lack of data of epidemiology, no available vaccine for humans, and no effective-diagnostic tool. Therefore, the following suggestions are made:

1) The study highlights the need for national monitoring and surveillance program for *S. suis*. The program should not limit to pigs but should cover pork and related food.
2) National control and prevention strategic plan for *S. suis* in pigs, pork and related food should be established. Such epidemiological data will help to understand the root cause, provide information to guide the interventions and evaluate the success of the interventions.

3) It is important to raise public awareness on possible infection with *S. suis* from consumption of uncooked pork and blood.
4) The control and prevention strategies of *S. suis* in pigs should be developed and implemented.
    4.1 **Biosecurity:** This is to prevent other pathogens that impair the immunity.
    4.2 **Ventilation in housing:** Barns with poor or improper ventilation can cause increased clinical signs of *S. suis* and pigs tend to have more clinical disease.
    4.3 **Vaccination: Autogenous vaccines:** Autogenous vaccine is an option to when *S. suis* is present in healthy animals. However, the benefits of these vaccines is not as certain.
    4.4 **Responsible antibiotic use:** Antimicrobials should be prudently used to minimize emergence of antimicrobial resistance.
    4.5 Treatment Regimen: If clinical *S. suis* is present, antibiotic treatment is needed. Choosing of antibiotics should depend on the antimicrobial susceptibility test result.
5) Several factors halt the control *S. suis* related disease including lack of knowledge about the disease caused by *S. suis* and lack of the sensitive diagnostics and effective vaccines. Therefore, future investigations are suggested as follows:
    5.1 Development of rapid and accurate diagnostic methods with reasonable price for multiple strains in large number of samples, particularly in clinically healthy pigs.
    5.2 Monitoring *S. suis* from farm to fork.
    5.3 Monitoring antimicrobial resistance phenotypes, genotypes, and genetic patterns in *S. suis* from farm to fork.
    5.4 Study of association between sequence types and virulence markers in *S. suis*.
    5.5 Study of association between pig and human *S. suis* sequence types.
    5.6 Establishment of the *S. suis* database to set up a complete and reliable serotype identification and to facilitate the sharing of knowledge.
    5.7 Comparison of phenotype, genetic characteristics, virulence factors and antimicrobial resistance between *S. suis* from clinically healthy and diseased pigs.

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9. Appendix

9.1. Culture media and Chemicals

1. Transport media

Sodium Thioglycolate	1.5	g
Disodium Phosphate	1.1	g
NaCl	5	g
Agar	5	g
Distilled water	1	L

Preparation: Dissolve the components in the water by heating, with frequent agitation. Sterilize for 15 min in the autoclave set at 121 °C.

2. Sheep Blood Agar

Columbia Blood agar base	39	g
Distilled water	1	L

Preparation: Dissolve the Columbia Blood agar base in the water by heating, with frequent agitation. Sterilize for 15 min in the autoclave set at 121 °C. Cool to 45-50°C and aseptically 5% Sheep blood, 15 µg/ml polymixin B and 15 µg/ml nalidixic acid. Mix well and pour into sterile Petri plates.

3. Gram Staining

Gram stain

Crystal violet solution	500	ml
Iodine solution	500	ml
Decolorizer Solution I	500	ml

Decolorizer Solution II	500	ml
Safranin Solution	500	ml

Gram Staining Protocol: Heat-fixed smear of cells for 1 minute with crystal violet staining reagent. Wash slide in a gentle and indirect stream of tap water. Flood slide with iodine solution. Flood slide with decolorizing agent. Flood slide with safranin, wait 1 minute. Wash slide in a gentile and indirect stream of tap water and then blot dry with absorbent paper. Observe the results of the staining procedure using microscope.

4. Catalase test

3% H_2O_2 (Hydrogen peroxide)

Glass slide

Catalase test: Transfer a small amount of bacterial colony to a surface of clean, dry glass slide. Place a drop of 3% H_2O_2 on to the slide and mix. A positive result is the rapid evolution of oxygen (within 5-10 sec.) as evidenced by bubbling.

5. Optochin Sensitivity Test

Optochin 6 mm disks	5 µg/disk

Optochin Sensitivity Test: Using an inoculating loop, streak two or three suspect colonies on 5% sheep blood agar plate. Place optochin disk within the streaked area of the plate. Incubate at 35-37°C with ~5% CO_2 (or in a candle-jar) for 18 to 24 hours. Measure the zone of inhibition.

6. Phenol red base broth (double strength)

Phenol red base broth	30	g
Distilled water	1	L

Preparation: Dissolve the Phenol red base broth in the water. Sterilize for 15 min in the autoclave set at 121 °C.

7. Sugar fermentation

Arabinose	1	g
Inulin	1	g
Lactose	1	g
Mannitol	1	g
Raffinose	1	g
Salicin	1	g
Sorbitol	1	g
Sucrose	1	g
Trehalose	1	g
Distilled water	50	ml

Preparation: Dissolve the sugar in the Distilled water. Sterilize by filtration through a syring filter with a pore size of 0.45 µm and mix with Phenol red base broth 1:1.

8. 6.5% NACl

NaCl	6.5	g
Distilled water	100	ml

Preparation: Dissolve the NaCl in the water. Sterilize for 15 min in the autoclave set at 121 °C.

9. Bile Esculin Agar

Peptone	14	g
Bile salts	15	g
Ferric citrate	0.1	g

Esculin	1	g
Agar	14	g
Distilled water	1	L

Preparation: Dissolve the components in the water by heating, with frequent agitation. Sterilize for 15 min in the autoclave set at 121 °C.

10. Starch agar

Soluble starch	10	g
Beef/Yeast extract	3	g
Agar	12	g

Preparation: Dissolve the components in the water by heating, with frequent agitation. Sterilize for 15 min in the autoclave set at 121 °C.

9.2. Sources of chemicals and media

Table A1 Sources of chemicals and media

Item	Source
Agar	Difco, USA
Arabinose	Sigma-Aldrich, USA
Bile esculin	Oxoid, England
Columbia Blood Agar Base	Oxoid, England
Inulin	Sigma-Aldrich, USA
Lactose	Sigma-Aldrich, USA
Mannitol	Sigma-Aldrich, USA
Phenol red base broth	Difco, USA
Raffinose	Sigma-Aldrich, USA
Salicin	Sigma-Aldrich, USA
Soluble Strach	Difco, USA
Sorbitol	Sigma-Aldrich, USA
Sucrose	Sigma-Aldrich, USA
Trehalose	Sigma-Aldrich, USA
Antimicrobial agents	
Ampicillin	Sigma-Aldrich, USA
Cetriofur	Sigma-Aldrich, USA
Chloramphenicol	Sigma-Aldrich, USA
Chlortetracycline	Sigma-Aldrich, USA
Clindamycin	Sigma-Aldrich, USA
Enrofloxacin	Sigma-Aldrich, USA
Erythromycin	Sigma-Aldrich, USA
Gentamicin	Sigma-Aldrich, USA
Florfenicol	Sigma-Aldrich, USA
Nalidixic acid	Sigma-Aldrich, USA
Neomycin	Sigma-Aldrich, USA
Polymicin B	Sigma-Aldrich, USA
Sulfamethoxazole	Sigma-Aldrich, USA
Tettracycline	Sigma-Aldrich, USA
Tiamulin	Sigma-Aldrich, USA
Tilmicosin	Sigma-Aldrich, USA
Trimethroprim	Sigma-Aldrich, USA
Tylosin	Sigma-Aldrich, USA
Oxytetracycline	Sigma-Aldrich, USA
Optochin disk	Oxoid, England
Chemicals	
Disodium Phosphate	Merck, Germany
Gram stain	Merck, Germany
NaCl	BAKER ANALYZED® A.C.S> Reagent, USA

Sodium Thioglycilate	Himedia, India
Genomic DNA	
Todd Hewitt broth	Difco, USA
Gentra®Puregene®	Qiagen, Germany
Conventional and multiplex PCE	
GeNei™mastermix	Merck, Germany

9.3. Additional data

Table A2. Summary of phenotypic and genotypic profiles of *S. suis* isolated from slaughtered pigs in Northern Thailand (n=59)

Province	Sample No.	Catalase	Biochemical tests									B	Na	Optochin	Starch	Species-specific genes		Serotypes			
			A	I	L	Ml	R	Sa	So	Su	T					*gdh*	16S rRNA	2	8	9	10
Nan (n=13)	42.1	-	-	-	+	-	-	+	-	+	+	-	-	R	+	-	+		+		
	45.4	-	-	+	+	-	-	+	-	+	+	-	-	R	+	+	+				
	65.3	-	-	-	+	-	-	+	-	w+	+	+	-	R	+	-	+		+		
	85.1	-	-	-	+	-	-	+	-	+	+	+	-	R	+	+	+		+		
	86.2	-	-	+	+	-	+	+	-	+	+	-	-	R	+	+	+		+		
	90.1	-	-	+	+	-	+	+	-	+	+	-	-	R	+	+	+		+		
	90.3	-	-	+	+	-	+	+	-	+	+	-	-	R	+	+	+		+		
	91.3	-	-	+	+	-	+	+	-	+	+	-	-	R	+	+	+		+		
	100.2	-	-	-	+	-	-	+	-	+	+	-	-	R	+	-	+		+		
	118.1	-	-	-	+	-	-	+	-	+	+	-	-	R	+	+	+		+		
	139.3	-	-	-	+	-	-	+	-	+	+	-	-	R	+	-	+		+		
	150.2	-	-	-	+	-	-	+	-	+	w+	-	-	R	+	+	+				
	175.4	-	-	-	+	-	-	+	-	+	+	-	-	R	+	-	+		+		
Mae Hong Son (n=11)	197.1	-	-	-	+	-	w+	+	-	+	+	-	-	R	+	+	+		+		
	197.4	-	-	-	+	-	-	+	-	+	+	-	-	R	+	+	+		+		
	211.3	-	-	-	+	-	-	w+	-	+	+	-	-	R	+	+	+		+		
	211.4	-	-	-	+	-	-	w+	-	+	+	-	-	R	+	+	+		+		
	213.2	-	-	-	w+	-	-	w+	-	+	+	-	-	R	+	+	+		+		

	219.2	-	-	-	w+	-	-	+	-	+	+	-	-	R	+	+	+		+		
	251.4	-	-	+	+	-	+	+	-	+	+	-	-	R	+	-	+		+		
	253.1	-	-	w+	+	-	-	w+	-	+	+	-	-	R	+	+	+		+		
	253.2	-	-	w+	+	-	-	+	-	+	+	-	-	R	+	+	-		+		
	253.3	-	-	w+	+	-	-	w+	-	+	+	-	-	R	+	+	-		+		
	253.4	-	-	-	w+	-	-	w+	-	+	+	-	-	R	+	+	-		+		
Chiang Rai (n=27)	628.1	-	-	-	+	-	w+	+	-	+	+	-	-	R	+	+	-				
	628.3	-	-	-	+	-	w+	+	-	+	+	-	-	R	+	+	-				
	628.4	-	-	+	+	-	+	+	-	+	+	-	-	R	+	+	-				+
	629.2	-	-	-	+	-	w+	+	-	+	+	-	-	R	+	+	-				
	630.1	-	-	-	+	-	w+	+	-	+	+	-	-	R	+	+	-				
	635.1	-	-	w+	+	-	-	+	-	+	+	-	-	R	+	+	-		+		
	637.1	-	-	w+	+	-	w+	+	-	+	+	-	-	R	+	+	+				
	637.2	-	-	+	+	-	w+	+	-	+	+	-	-	R	+	+	+				
	637.4	-	-	+	+	-	w+	+	-	+	+	-	-	R	+	+	+				
	642.1	-	-	-	+	-	+	+	-	+	+	-	-	R	+	+	+				
	642.2	-	-	-	+	-	w+	+	-	+	+	-	-	R	+	+	-				
	642.3	-	-	-	+	-	+	+	-	+	+	-	-	R	+	+	-				
	642.4	-	-	-	+	-	w+	+	-	+	+	-	-	R	+	+	-				
	656.1	-	-	-	+	-	w+	+	-	+	w+	-	-	R	+	+	+				
	661.1	-	-	-	+	-	-	+	-	+	+	-	-	R	+	+	-				
	661.4	-	-	-	+	-	w+	+	-	+	+	-	-	R	+	+	-				
	670.3	-	-	-	+	-	w+	+	-	+	+	-	-	R	+	+	-				
	672.4	-	-	w+	+	-	w+	+	-	+	+	-	-	R	+	+	+		+		
	680.1	-	-	w+	+	-	-	+	-	+	+	-	-	R	+	+	-				
	710.1	-	-	-	+	-	w+	+	-	+	w+	-	-	R	+	+	-				

	710.3	-	-	-	+	-	w+	+	-	+	w+	-	-	R	+	+	-				
	725.1	-	-	-	+	-	w+	+	-	+	+	-	-	R	+	+	-				
	725.2	-	-	-	+	-	w+	+	-	+	+	-	-	R	+	+	+				+
	754.2	-	-	-	+	-	w+	+	-	+	+	-	-	R	+	+	+			+	
	775.1	-	-	-	w+	-	w+	+	-	w+	w+	-	-	R	+	+	+		+		
	700.1	-	-	-	+	-	w+	+	-	+	+	-	-	R	+	+	-				
	751.4	-	-	-	+	-	w+	+	-	+	+	+	-	R	+	-	+				
Chiang Mai (n=0)																					
Phayao (n=8)	507.1	-	-	-	w+	-	-	w+	-	+	+	+	-	R	+	+	+		+		
	515.3	-	-	-	+	-	-	+	-	+	+	+	-	R	+	+	+		+		
	528.3	-	-	-	w+	-	-	+	-	+	+	+	-	R	+	+	+				
	565.1	-	-	+	+	-	w+	+	-	+	+	-	-	R	+	+	+		+		
	565.4	-	-	w+	+	-	w+	+	-	+	+	-	-	R	+	+	+		+		
	597.4	-	-	+	+	-	-	+	-	+	+	+	-	R	+	+	+				
	607.2	-	-	-	+	-	-	+	-	+	+	-	-	R	+	+	+		+		
	607.3	-	-	+	+	-	w+	+	-	+	+	+	-	R	+	+	+	+			

W+, weak positive

[a] Gray box indicates not positive to any serotypes tested and defined as unidentified serotypes.

Abbreviations: A, arabinose; I, inulin; L, lactose; M, mannitol; R, raffinose; Sa, salicin; So, sorbitol; Su, sucrose; T, trehalose; B, bile aesculin; Na, 6.5% NaCl, O, Optochin

Table A3. Serotypes and virulence genes in *S. suis* isolated from slaughtered pigs in Northern Thailand (n=59)

Province	Samples	Serotypes				Virulence genes				
		2	8	9	10	*efp*	*mrp*	*sly*	*arcA*	*hly*
Nan (n=13)	42.1		+			-	+	+	+	-
	45.4					-	+	+	+	+
	65.3		+			-	+	+	+	-
	85.1		+			-	+	+	+	-
	86.2		+			-	+	+	+	-
	90.1		+			-	+	+	+	+
	90.3		+			-	+	+	+	+
	91.3		+			-	+	+	+	+
	100.2		+			-	-	+	+	-
	118.1		+			-	+	+	+	-
	139.3		+			-	+	+	+	-
	150.2					-	-	+	+	-
	175.4		+			-	-	+	+	-
Mae Hong Son (n=11)	197.1		+			-	+	+	+	+
	197.4		+			-	+	+	+	+
	211.3		+			-	-	+	+	-
	211.4		+			-	+	+	+	-
	213.2		+			-	+	+	+	-
	219.2		+			-	+	+	+	+
	251.4		+			-	+	+	+	+
	253.1		+			-	+	+	+	-
	253.2		+			-	-	+	+	-
	253.3		+			-	-	+	+	-
	253.4		+			-	-	+	+	-
Chiang Rai (n=27)	628.1					-	+	+	+	+
	628.3					-	+	+	+	+
	628.4				+	-	+	+	+	+
	629.2					-	+	-	+	-
	630.1					-	+	-	+	-
	635.1		+			-	+	+	+	+
	637.1					-	+	+	+	+
	637.2					-	+	+	+	+
	637.4					-	+	+	+	+
	642.1					-	+	+	+	-
	642.2					-	+	+	+	-
	642.3					-	+	-	+	-
	642.4					-	+	+	+	-
	656.1					-	-	+	+	-

	661.1					-	-	+	+	-
	661.4					-	-	-	+	-
	670.3					-	-	+	+	-
	672.4		+			-	-	+	+	+
	680.1					-	+	+	+	+
	710.1					-	-	+	+	-
	710.3					-	+	+	+	-
	725.1					-	-	+	+	-
	725.2				+	-	+	+	+	-
	754.2			+		-	+	+	+	-
	775.1		+			-	+	+	+	+
	700.1					-	-	+	+	-
	751.4					-	+	+	+	+
Chiang Mai (n=0)										
Phayao (n=8)	507.1		+			-	-	+	+	-
	515.3		+			-	+	+	+	+
	528.3					-	+	-	+	-
	565.1		+			-	+	+	+	-
	565.4		+			-	+	+	+	-
	597.4					-	-	+	+	-
	607.2		+			-	-	+	+	+
	607.3	+				-	+	+	+	+

10.4. List of photo

Figure A1 Nasal swab from slaughtered pigs

Figure A2 Pigs at the rest area before slaughtering

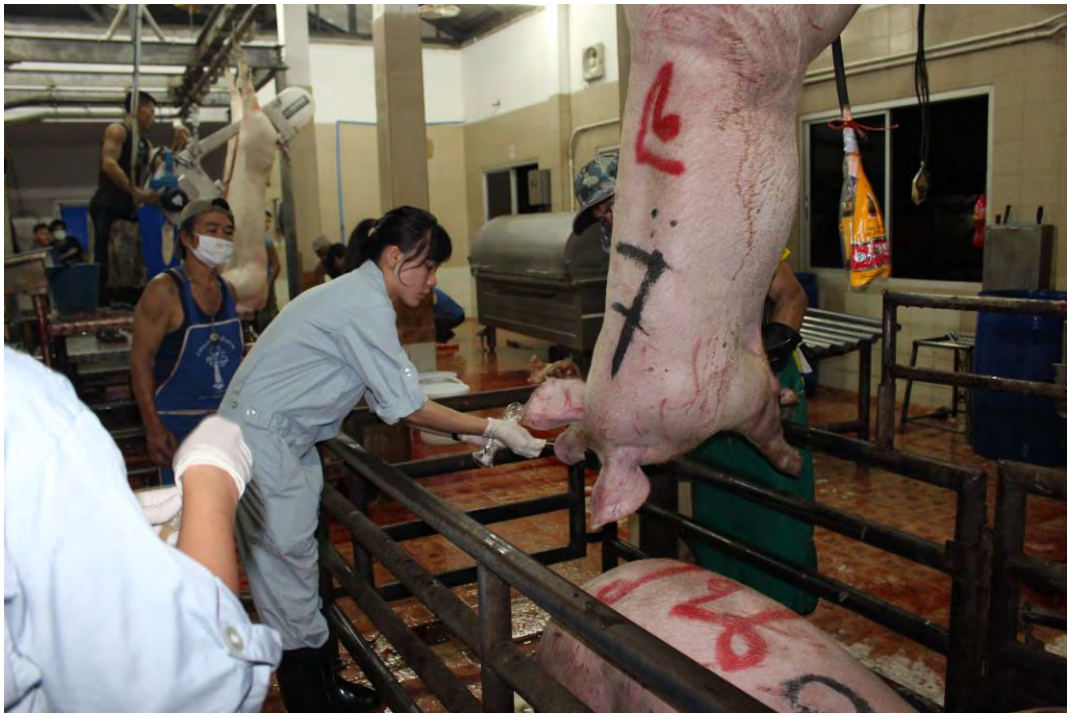

Figure A3 Nasal swab from slaughtered pigs

Figure A4 Nasal swab from slaughtered pigs

10. Curriculum vitae

1. หัวหน้าโครงการวิจัย

ชื่อ (ภาษาไทย) รองศาสตราจารย์ สพ.ญ.ดร. รุ่งทิพย์ ชวนชื่น

(ภาษาอังกฤษ) Assoc. Prof. Dr. Rungtip Chuanchuen

เลขหมายบัตรประจำตัวประชาชน 3749800061680

ตำแหน่งทางวิชาการ

-หัวหน้าภาควิชาสัตวแพทยสาธารณสุข

-หัวหน้าหน่วยปฏิบัติการความปลอดภัยอาหารทางจุลชีววิทยาและเชื้อดื้อยา
-ผู้อำนวยการศูนย์ติดตามการดื้อยาของเชื้อโรคอาหารเป็นพิษ ภายใต้ความร่วมมือของ) องค์การอนามัยโลก; Global Foodborne Infections Network: South-East Asia and Western Pacific Region
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ประวัติการศึกษา

ปริญญา	สาขาวิชา	มหาวิทยาลัย	ปี พ.ศ. ที่ได้รับ
Ph.D.	Microbiology (Bacterial genetics)	Colorado State University	2547
M.S.	Animal sciences (Food safety)	Colorado State University	2542
DVM.	สัตวแพทยศาสตร์	จุฬาลงกรณ์มหาวิทยาลัย	2536

สาขาวิชาการที่มีความชำนาญพิเศษ

1. Antimicrobial resistance of bacteria
2. Foodborne diseases
3. Bacterial zoonotic diseases

Current research topics:

1. Antimicrobial resistance and virulence factors in foodborne pathogens, particularly *Salmonella enterica* and *Escherichia coli.*

2. Antimicrobial resistance and virulence factors in commensal bacteria (particularly *E. coli* and *Enterococcus*).
3. Multidrug-resistance mechanisms (in particular Multidrug Efflux Systems) in *Pseudomonas aeruginosa Acinetobacter baumaii* and other bacteria associated with nosocomial infections.
4. Antimicrobial resistance in bacterial pathogens in aquaculture (eg. *Vibrio* spp and *Aeromonas hydrophila*).
5. Cross resistance between antibiotics and biocides in bacteria of veterinary public health importance.
6. Molecular epidemiology and characteristics of foodborne pathogens associated with animals in Southeast Asia

Publications:

1. Trongjit, S., Angkititrakul, S., R. E. Tuttle, Padungtod, P, and **R. Chuanchuen**. 2016. Pevalence and antimicrobial resistance of *Salmonella enterica* isolated from broiler chickens, pigs and meat products in the Thailand-Cambodia border provinces. Food Microbiol. **(submitted)**
2. Trongjit, S., Angkititrakul, and **R. Chuanchuen.** 2016. Presence and phenotypic-genotypic antimicrobial resistance of *Escherichia coli* in broilers, pigs and meat products in the Thailand and Cambodia border provinces. Food Res. Inter. **(submitted)**
3. Srisanga, S., Angkititrakul. S, Sringam, P., Phuong T. Le Ho[1,4], An T. T. Vo and **R. Chuanchuen**. 2016. Phenotypic and genotypic antimicrobial resistance and virulence genes of *Salmonella enterica* isolated from pet dogs and cats. Res Vet Sci. **(submitted)**
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19. M. Lukkana, J. Wongtavachai, and **R. Chuanchuen**. Characterization of class 1 integrons *Aeromonas hydrophila* isolated from tilapia in Thailand. August 23-26, 2010. The 13th Association of institutions for tropical veterinary medicine conference 2010 (AITVM). Sofitel Centara Grand Hotel, Bangkok, Thailand.
20. K. Poonsuk, S. Pagdepanichkit, **R. Chuanchuen**. Contribution of the multidrug efflux system MexXY-OprM in aminoglycoside resistance in *Pseudomos aeruginosa* clinical isolates from dogs and cats. International Conference on Veterinary Science IICAB, AHIS, FAO Joint Symposiums. February 29-March 2, 2012.

21. K. K. Lay, C. Koowatananukul, **R. Chuanchuen**. Antibiotic resistance and virulence genes of *Eschericia coli* isolated from swine. International Conference on Veterinary Science IICAB, AHIS, FAO Joint Symposiums. February 29-March 2, 2012.
22. S. Pagdepanichkit, Chanwit Tribuddharat and **R. Chuanchuen.** 2014. Antimicrobial-resistance profile and RND efflux pump-expression of the *Acinetobacter baumannii* clinical isolates. The 3rd International Conference on Responsible Use of Antibiotics in Animals, 29 September – 1 October 2014, Royal Tropical Institute, Amsterdam, the Netherlands.
23. N. Sinwat, S. Angkittitrakul and **R. Chuanchuen.** 2014. Widespread districution of antimicrobial-resistance of *Salmonella enterica* isolated from retail pork and humans in Northeastern Thailand. The 3rd International Conference on Responsible Use of Antibiotics in Animals, 29 September – 1 October 2014, Royal Tropical Institute, Amsterdam, the Netherlands.
24. K. Poonsuk and **R. Chuanchuen**. 2014.The MexEF-OprN multidrug efflux systems is predominant in *Pseudomonas aeruginosa* isolates from dogs and cats.The 18th Federation of Asian Veterinary Association Congress (FAVA 2014), Marina Bay Sands, Singapore. November 28-30, 2557.
25. Tuanyok, A, Poonsuk, K, Hoang, TT, Wuthiekanan, V, Limmathurotsakul, D, **Chuanchuen, R**, Bollig, M, Allender, CJ, Keim, P, Nerurkar, V and Yanagihara, R. The Study of Ceftaxidime Resistance Mechanisms in *Burkholderia pseudomallei*. JABSOM Biomedical Sciences and Health Disparities Symposium, John A. Burns School of Medicine, University of Hawaii, Honolulu, Hawaii, March 3-4, 2014.
26. Thu, W.P., S. Angkititrakul and **Chuanchuen, R.** Occurrence of Virulence Genes in *Enterococcus faecium* and *Entercoccus faecalis* Isolated from Pigs and Pig Products in Thailand and Laos PDR Border Area. The 15th Chulalongkorn University Veterinary Conference (CUVC). Chaloem Rajakumari Building (Chamchuri 10), Chulalongkorn University, Bangkok, Thailand. April 20-22, 2016. Thai J Vet Med. Suppl. 2016. 46:387.
27. H.E. Torio, Puangseree, J, Pungpian, C, Srisanga, S. and **Chuanchuen, R**. Phenotypic and Molecular Characterization of Extended-β-lactamase-Producing *Escherichia coli* from Pigs in Selected Provinces in Thailand. The 15th Chulalongkorn University Veterinary Conference (CUVC). Chaloem Rajakumari Building (Chamchuri 10), Chulalongkorn University, Bangkok, Thailand. April 20-22, 2016. Thai J Vet Med. Suppl. 2016. 46:407.

28. R. Prathan, Sinwat, .N, Ankititrakul, S. and **Chuanchuen, R.** Genetic Relatedness of *Salmonella enterica* Isolated from Pig Production and Humans in Thailand-Loas Broder Provinces. The 15th Chulalongkorn University Veterinary Conference (CUVC). Chaloem Rajakumari Building (Chamchuri 10), Chulalongkorn University, Bangkok, Thailand. April 20-22, 2016. Thai J Vet Med. Suppl. 2016. 46:439.

Invited speaker:

1. **Chuanchuen, R.** Food safety II. The first International Training Course on Aquatic Animal Diseases & Diagnostic Techniques. 7 April 2553
2. **Chuanchuen, R.** Molecular Technique in VPH I. The first International Training Course on Aquatic Animal Diseases & Diagnostic Techniques. 7 April 2553
3. **Chuanchuen, R.** Disinfectants. The Regional Marketing Meeting arranged by Novatis Animal Health inc, Switzerland. Harbour Grand Hotel,North Point,Hong Kong. May 17-18, 2010
4. **Chuanchuen, R.** Disinfectants. The Regional Marketing Meeting arranged by Novatis Animal Health inc, Switzerland. Ramada Park Hotel, Geneva, Switzerland. June 3-4, 2010.
5. **Chuanchuen, R.** Disinfectants in aquaculture. September 15, 2010.Veterinary Medical Aquatic Animal Research Center (VMRC). Chulalongkorn university, Bangkok.
6. **Chuanchuen, R.** Emerging antimicrobial resistance bacteria (Cross resistance, current issue). International Training Program in Tropical Emerging and Reemerging Diseases in Animals: Surveillance and Diagnosis. Faculty of Veterinary Science, Chulalongkorn University. 22 Nov 2012
7. **Chuanchuen, R.** Emerging antimicrobial resistance bacteria (MDR salmonella, E. coli, Campylobacter).

   International Training Program in Tropical Emerging and Reemerging Diseases in Animals: Surveillance and Diagnosis. Faculty of Veterinary Science, Chulalongkorn University. 22 Nov 2012
8. **Chuanchuen, R.** Multidrug resistance in bacteria of veterinary public heath importance. The 11th Chulalongkorn University Veterinary Annual Conference, Faculty of Veterinary Science, Chulalongkorn university, Bangkok. May 18, 2012.

9. **Chuanchuen, R.** Cross-resistance between antibiotics and biocides. The 12th Chulalongkorn University Veterinary Annual Conference, Faculty of Veterinary Science, Chulalongkorn university, Bangkok. April 19, 2013.
10. **Chuanchuen, R.** Antimicrobial resistance and food of animal origins in Thailand. The KOSFA Annual Meeting organized by Korean Society of Food from Animal Resources. **Kwa Hak Gi Sul Hoe Kwan.** May 23-24, 2013. Seoul, Korea.
11. **Chuanchuen, R.** Trends of Antimicrobial Resistance in Broilers: Updates & Global Responses. The annual conference Betagro North Park, Bangkok, Thailand. June 20, 2013.
12. Chuanchuen, R. **How do bacteria become resistant? The annual seminar organized by Huvepharma (Thailand). Centra Government Complex Hotel & Convention Centre Chaeng Watthana, Bangkok, Thailand. July 29, 2013. Bureau of Quality Control of Livestock Products, DLD, Pathumtani**
13. **Chuanchuen, R.** Dynamics, Transfer & Distribution of AMR. Workshop "Antimicrobial-resistant bacteria: A problem of Veterinary Public Health importance" August 6, 2013. Bureau of Quality Control of Livestock Products, DLD, Pathumtani
14. **Chuanchuen, R.** Responsible and prudent use of antimicrobial agents in food animals. International Seminar "Strides on Antimicrobial Resistance in Thai Livestock". August 29, 2013. Bitec Bangna, Bangkok.
15. **Chuanchuen, R**. Antimicrobial susceptibility test. Workshop "Antimicrobial-resistant bacteria: A problem of Veterinary Public Health importance" August 7, 2013. Bureau of Quality Control of Livestock Products, DLD, Pathumtani
16. **Chuanchuen, R.** Antimicrobial resistance: Dynamics, selection & transfer. International training on "Antimicrobial Resistance in Foodborne Pathogens for ASEAN Universities" August 27, 2013.
17. **Chuanchuen, R**. Antimicrobial Resistance: Global Responses. International training on "Antimicrobial Resistance in Foodborne Pathogens for ASEAN Universities" August 27, 2013.
18. **Chuanchuen, R.** Get It Started! Standardization and harmonization on antimicrobial resistance monitoring in Thai livestock. International Seminar "What's new & next for antimicrobial resistance in Thai livestock?" Faculty of Veterinary Science, Chulalongkorn University, Bangkok. November 7, 2013.

19. **Chuanchuen, R**. Disinfectants: Action & Resistance. International Seminar "What's new & next for antimicrobial resistance in Thai livestock?" Faculty of Veterinary Science, Chulalongkorn University, Bangkok. November 7, 2013.
20. Chuanchuen, R. **Brush up & Update on disinfectants. The conference on Hazardous Substances Act organized by Division of Animal Food and Veterinary Products Control (กองควบคุมอาหารและยาสัตว์), DLD at Maruay Garden Hotel, Bangkok. December 17, 2013.**
21. **Chuanchuen, R.** Update on Antimicrobial Resistance in Thai Livestock. Faculty of Allied Health Sciences, Chulalongkorn University. April 21, 2014.
22. **Chuanchuen, R.** Antimicrobial resistance monitoring in Thai livestock. The seminar on "Driven on the standard and certification related to livestock (การประชุมสัมมนาการขับเคลื่อนงานด้านมาตรฐานปศุสัตว์) organized by Bureau of Livestock Standards and Certification, DLD. Moutain Beach Hotel, Chonburi. May 1, 2014
23. **Chuanchuen, R.** Best use of disinfectants in animal production. Novatis Animal Heath Pigs & Poultry Management Symposium The Empress Hotel, ChiangMai, Thailand. May 20, 2014
24. **Chuanchuen, R.** Antimicrobial resistance & regulation of antimicrobial use in Thailand.Workshop "Antimicrobial Use in Livestock & Antimicrobial Resistance in Foodborne Pathogens". Ibis Bangkok Riverside, Bangkok. July 7, 2014.
25. **Chuanchuen, R**. Standardized and harmonized antibiotic susceptibility testing and data interpretation. The International workshop " Strategic Action Plan on Control, Prevention and Containment of Antimicrobial Resistance associated with Food Animals in ASEAN countries", Faculty of Veterinary Science, Chulalongkorn University, 2 September 2014 (2-5 Sep.)
26. **Chuanchuen, R**. Guideline on AMR monitoring in food animals. The International workshop " Strategic Action Plan on Control, Prevention and Containment of Antimicrobial Resistance associated with Food Animals in ASEAN countries", Faculty of Veterinary Science, Chulalongkorn University, 3 September 2014 (2-5 Sep.)
27. **Chuanchuen, R**. Responsible antibiotic use guidelines. The International workshop " Strategic Action Plan on Control, Prevention and Containment of Antimicrobial Resistance associated with Food Animals in ASEAN countries", Faculty of Veterinary Science, Chulalongkorn University, Bangkok. 3 September 2014 (2-5 Sep.)

28. **Chuanchuen, R**. Guideline for antimicrobial resistance monitoring. FAO-APHCA-DLD ASEAN Regional Training Workshop on "Antimicrobial Susceptibility Testing (AST) of Bacteria Isolated from Farm Animals" Faculty of Veterinary Science, Chulalongkorn University, Bangkok. 7 October 2014 (7-8 Oct.).
29. **Chuanchuen, R**. AST of Fastidious microorganisms. FAO-APHCA-DLD ASEAN Regional Training Workshop on "Antimicrobial Susceptibility Testing (AST) of Bacteria Isolated from Farm Animals" Faculty of Veterinary Science, Chulalongkorn University, Bangkok. 8 October 2014 (7-8 Oct.).
30. **Chuanchuen, R**. Overview of antimicrobial resistance, prevalence and intervention in ASEAN countries. The 3rd International Conference on Responsible Use of Antibiotics in Animals. Royal Tropical Institute, Amsterdam, the Netherlands. 30 September 2014 (29 Sep– 1 Oct 2014)
31. **Chuanchuen, R**. Overview of AMR associated with livestock in ASEAN (20 Oct.)
    i. AMR in bacteria (Dynamics, transfer & cross-resistance) (23 Oct)
    ii. AMR in bacteria (MDR foodborne pathogens) (23 Oct)
    iii. Standardization and harmonization on AMR monitoring (29 Oct)
    iv. Standard antimicrobial susceptibility test (29 Oct)

    The international workshop "Antimicrobial Resistance and Foodborne Diseases Associated with Livestock: Mechanisms, Diagnosis and Control" Faculty of Veterinary Science, Chulalongkorn University, Bangkok. 20 October-7 November 2014.
32. **Chuanchuen, R.** Addressing Antimicrobial Resistance in Zoonotic Bacteria from Livestock in East, South and Southeast Asia IBCs Asia Animal Health Conference organized by IBC Asia(S) Pte Ltd., Grand Hyatt Shanghai, Shanghai, China. 3 November 2014 (3-6 Nov)
33. **Chuanchuen, R.** Ways to tackle AMR associated with ASEAN livestock Food Safety Strategy: Antimicrobial Resistance in ASEAN Livestock (AMRAL) FAVA VIV 13 March 2015 room MR202 Bitec Bangna
34. **Chuanchuen, R.** Addressing antimicrobial resistance prevalence and interventions in ASEAN countries. The 6th Pan Commonwealth Veterinary Conference & 27th Veterinary Association on Malaysia Congress (PCVC 6 & 27 VAM). The Royale Chulan Hotel, Kuala Lumpur, Malaysia 25 March 2015

35. **Chuanchuen, R.** Efficacy of disinfectants. The workshop on "More on Disinfectant Efficacy Test: PC, MIC, D-value". The 14th Chulalongkorn University Veterinary Conference. Royal Paragon Hall, Siam Paragon, Bangkok, Thailand. 22 April 2015.
36. **Chuanchuen, R.** Evaluation of disinfectant efficacy. The workshop on "More on Disinfectant Efficacy Test: PC, MIC, D-value". The 14th Chulalongkorn University Veterinary Conference. Royal Paragon Hall, Siam Paragon, Bangkok, Thailand. 22 April 2015.
37. **Chuanchuen, R.** Implementation of strategies to control antimicrobial resistance in South East Asian Countries. In: The 4th Food Safety and Zoonoses Symposium for Asia Pacific. By Veterinary Public Health Centre for Asia Pacific (VPHCAP). Holidays Inn Chiang Mai. 3-5 (4) August 2015.
38. **Chuanchuen, R.** AMR in veterinary medicine – Current situation across Asia. In Pre-congress workshop in AMR – Globlal Impact and Management Strategies associated with The Third International Congress on Pathogens at the Human Animal Interface (ICOPHAI 2015), Holiday Inn, Chiang Mai, Thailand. 5August 2015
39. **Chuanchuen, R.** Non-antibiotic selection pressure for AMR: An emerging challenge In Pre-congress workshop in AMR – Globlal Impact and Management Strategies associated with The Third International Congress on Pathogens at the Human Animal Interface (ICOPHAI 2015), Holiday Inn, Chiang Mai, Thailand. 5August 2015.
40. **Chuanchuen, R.** Guideline for monitoring and surveillance of antimicrobial resistance in bacteria assocated with food animals. Training course on "Control, prevention and strategic plan on AMR in animals" 3-5 February 2016, Asia Bangkok Hotel, Bangkok.
41. **Chuanchuen, R.** ESBL-producing bacteria: Significance and laboratory detection. Training course on "Control, prevention and strategic plan on AMR in animals" 3-5 February 2016, Asia Bangkok Hotel, Bangkok.
42. **Chuanchuen, R.** AMR in food animal production and small animal sectors: What are the links? The 15th Chulalongkorn University Veterinary Conference (CUVC). Chaloem Rajakumari Building (Chamchuri 10), Chulalongkorn University, Bangkok, Thailand. April 21, 2016.
43. **Chuanchuen, R.** AMR genotypes as a tool for AMR monitoring. The 15th Chulalongkorn University Veterinary Conference (CUVC). Chaloem Rajakumari Building (Chamchuri 10), Chulalongkorn University, Bangkok, Thailand. April 21, 2016.

Book chapters, expository and review articles:

1. **Chuanchuen, R.** and K. Saitanu. 1996. Milk Quality. In: Research of Dairy Cow. p 245-255.
2. **Chuanchuen, R**. and H.P. Schweizer. 2004. Multidrug Efflux Systems: A mechanism of resistance to multidrug in bacteria. Thai J. Vet. Med. 34 (4):2004.
3. **Chuanchuen, R.** and A. Amonsin. 2007. Determination of milk quality. Point Graphic, Bangkok. p. 158.
4. **Chuanchuen, R.** 2009. Biocides: antiseptics and disinfectants. Treernnasarn, Bangkok. p.134.
5. **Chuanchuen, R.** 2011. Emerging drug-resistant superbugs. Thai J. Vet. Med. 41(1):7-11.
6. Archawakulathep et al. 2014. Perspectives on Antimicrobial Resistance in Livestock and Livestock Products in ASEAN Countries .The Thai Journal of Veterinary medicine. 44(1): 5-13.
7. **Chuanchuen, R**., N. Pariyotorn, K. Siriwattanachai, S. Pagdepanichkit, S. Srisanga, W. Wannaprasat, W. Phyo Thu, S. Simjee and J. Otte. 2014. Review of the Literature on Antimicrobial Resistance in Zoonotic Bacteria from Livestock in East, South and Southeast Asia. pp 81.
   http://cdn.aphca.org/dmdocuments/REP_AMR_141022_c.pdf

Awards:

2014 Best Teaching award, Chulalongkorn University

2013 NOVARTIS Best Research Award, 2013 Faculty of Veterinary Science, Chulalongkorn University

2011 RF-CHE-SCOPUS 2011 Young Researcher Award, Life Sciences & Agricultural Sciences

2001-2003 The Dr. Virgil and Mitzy Yount Postgraduate Veterinary Medical Award, College of Veterinary Medicine and Biomedical Sciences, CSU

2001 and 2003 Student Traineeship, Cystic Fibrosis Foundation, MD, USA

2000 "Multidrug Efflux Pumps and Triclosan Resistance in *Pseudomonas aeruginosa*" presented in The 100th ASM meeting, LA, USA was chosen to present in the press conference

Research Project:

	Research project	Role	Period	Funding
1.	Functional characterization of MexXY and OpmG in aminoglycoside efflux in *Pseudomonas aeruginosa*	PI	Aug 2005 –Jul 2007	TRF
2.	Monitoring of biocide and antibiotic resistanceof *Salmonella* spp . and *Escherichia coli* isolated in Thailand	PI	Oct 2005 –Sep2006	NRCT 2549
3.	Preparation of specific-polyclonal antibodies against protein of multidrug efflux systems in *Pseudomonas aeruginosa*	PI	Aug 2006 –Jul 2007	Rachadapisaksompoch grant
4.	Quality, antibiotic resistance and the resistance transfer in probiotic bacteria from commercial-probiotic products for aquatic animals in Thailand	PI	Oct 2006 -Sep 2007	NRCT 2550
5.	Detection and molecular characterization of antimicrobial resistance genes in *Salmonella enterica* isolated from broilers and swine in Thailand	PI	Apr2007 - Mar 2008	Rachadapisaksompoch grant
6.	Antimicrobial resistance in zoonotic bacteria	Co-PI	Oct 2007 -Sep 2008	NRCT 2551
7.	The study of antibiotic contamination in table eggs and antimicrobial resistance of contaminated bacteria in layer farms.	Co-PI	Oct 2007 -Sep 2008	NRCT 2551
8.	Cross-resistance between halquinol and antibiotics	Co-PI	Dec2 007-Nov2 008	Novartis (Thailand) Ltd.
9.	The study of antibiotic contamination in table eggs and antimicrobial resistance of contaminated bacteria in layer farms.	Co-PI	Oct 2008-Sep 2009	NRCT 2552
10.	Molecular characterization of *Salmonella enterica*: virulence factors, antibiotic resistance and genetic relatedness	PI	May 2008- Jun 2010	TRF
11.	Characterization of integrating conjugative elements (ICEs) in *Vibrio*	PI	Feb-Sep 2009	Faculty of Veterinary Science,

	species isolated from cultivated marine shrimps in Thailand			Chulalongkorn University
12.	Characterization of integrons in *Pseudomonas aeruginosa* and *Acinetobactor baumannii*	PI	Jul 2009-Aug 2010	Rachadapisaksompoch grant
13.	Study of Multidrug Efflux Pumps in *Pseudomomas aeruginosa* Isolated from Patients and Animals: Role in Antibiotic Resistance and Development of Detection Method for the Differential Diagnosis of Efflux-Mediated Resistance	PI	Jun 2010-May 2013	TRF
14.	Role of multidrug efflux pumps in antimicrobial resistance and efficacy of efflux pump inhibitors in *Acinetobactor baumannii*	PI	2012-2013	Rachadapisaksompoch grant
15.	Guideline for judicious use of antibiotics in broiler farm	PI	2556-2555	ACSF
16.	Molecular Characterization of Antimicrobial Resistance, Virulence factors and Genetic Relatedness of Zoonotic *Salmonella* isolates from Thailand-Laos border area	PI	2013-2016	TRF
17.	Determination of Efficacy for Disinfectants for Veterinary Uses	PI	Jul-Oct 2013	Novartis (Thailand) Ltd.
18.	A review of antimcrobial resistance in bacterial micro-organisms isolated from livestock and livestock products in the Asia-Pacific region	PI	Apr-July 2013	FAO Regional Office for Asia and the Pacific
19.	A literature review of microbial contamination of animal food products in the Asia-Pacific region	PI	Jan-May 2014	FAO Regional Office for Asia and the Pacific
20.	Foodborne pathogens and antimicrobial resistance monitoring in livestock and livestock products in the areas along Thailand-Cambodia border	PI	2014-2015	Chulalongkorn University-World-Class University Grant (WCU) Health Cluster
21.	Effects of flavophospholypol (Flavomycin®) on antimicrobial	PI	31 Aug-31 May 2017	Huvepharma

	resistance and antimicrobial activity of the clinically-important antibiotics in *Escherichia coli*			
22.	Effects of flavomycin on antimicrobial resistance of commensal *Escherchia coli* isolated from swine	PI	1 Aug-31 Jul 2017	Huvepharma
23.	Monitoring of resistance to extended-spectrum β-lactam sand colistin in foodborne bacteria associated with food animals in Southeast Asia	PI	Contact in preparation	Chulalongkorn University-World-Class University Grant (WCU) Health Cluster
24.	Situation and antimicrobial resistance of *Streptococcus suis* isolated from pigs in Northern region of Thailand	PI	Contact in preparation	NRCT 2559

2. ผู้ร่วมวิจัย

ชื่อ (ภาษาไทย) รองศาสตราจารย์ น.สพ.ดร. สรรเพชญ อังกิติตระกูล

(ภาษาอังกฤษ) Assoc. Prof. Dr. Mr. Sunpetch Angkititrakul

ตำแหน่ง รองศาสตราจารย์ ระดับ 8

สถานที่ทำงาน ภาควิชาสัตวแพทย์สาธารณสุข คณะสัตวแพทยศาสตร์ มหาวิทยาลัยขอนแก่น 40002
โทรศัพท์ 043-364493 E-mail: sunpetch@kku.ac.th

การศึกษา

ปีที่จบ	คุณวุฒิ	สถาบัน
2532	ปริญญาตรี สัตวแพทยศาสตรบัณฑิต	จุฬาลงกรณ์มหาวิทยาลัย
2541	ปริญญาตรี บริหารธุรกิจบัณฑิต	มหาวิทยาลัยสุโขทัยธรรมาธิราช
2541	ปริญญาโท วิทยาศาสตรมหาบัณฑิต (สัตวแพทย์สาธารณสุข)	จุฬาลงกรณ์มหาวิทยาลัย
2550	ปริญญาเอก สาธารณสุขศาสตรดุษฎีบัณฑิต	มหาวิทยาลัยขอนแก่น

สาขาวิชาการที่มีความชำนาญพิเศษ

- การตรวจคุณภาพเนื้อสัตว์ และผลิตภัณฑ์จากสัตว์
- วิทยาการทางระบาดวิทยา
- โรคติดต่อระหว่างสัตว์และคน

ผลงานวิจัยที่ตีพิมพ์:

1.**สรรเพชญ อังกิติตระกูล** อรุณี บุตรตาสี เดชา สิทธิกล และชัยวัฒน์ พูลศรีกาญจน์. 2554. การดื้อต่อสารต้านจุลชีพของเชื้อซัลโมเนลลาที่แยกได้จากซากสุกร และพนักงานฆ่าสัตว์ในโรงฆ่าสัตว์เขตจังหวัดขอนแก่น. สัตวแพทย์มหานครสาร. ปีที่ 6 ฉบับที่ 1 (มกราคม-มิถุนายน). หน้า 11-19.

2.เดชา สิทธิกล และ**สรรเพชญ อังกิติตระกูล.** 2554. ความชุกของเชื้อซัลโมเนลลาที่แยกได้จากสุกร ซากสุกร น้ำใช้ และพนักงานฆ่าสัตว์ในโรงฆ่าสัตว์ในเขตจังหวัดขอนแก่น. วารสารสัตวแพทยศาสตร์ มข. ปีที่ 21 ฉบับที่ 1 (มกราคม – มิถุนายน) หน้า 33 – 40.

3.ธีรพงศ์ ใจซื่อ และ**สรรเพชญ อังกิติตระกูล.** 2554. การปนเปื้อนเชื้อ Staphylococcus aureus ในเนื้อสุกร และฝ่ามือของคนขายในตลาดสดเขตเทศบาลนครขอนแก่น. วารสารสัตวแพทยศาสตร์ มข. ปีที่ 21 ฉบับที่ 2 (กรกฎาคม - ธันวาคม). หน้า 147-153.

4.Nithiphonh Somsanith บงกช นพผล **สรรเพชญ อังกิติตระกูล** วราภรณ์ ศุกลพงศ์ และขวัญเกศ กนิษฐา นนท์. 2555. ความชุกของเชื้อซัลโมเนลลาที่แยกได้จากสุกรในโรงฆ่าสัตว์หนองด้วง นครหลวงเวียงจันทร์ ประเทศสาธารณรัฐประชาธิปไตยประชาชนลาว. สัตวแพทยศาสตร์ มข. ปีที่ 22 ฉบับที่ 2 (เดือนกรกฎาคม – ธันวาคม) หน้า 167 – 173.

5.ชัยวัฒน์ พูลศรีกาญจน์ และ**สรรเพชญ อังกิติตระกูล.** 2556. การแทรกซึมของเชื้อซัลโมเนลลา เอนเทอริติดิส และการสูญเสียน้ำหนักของไข่ไก่ขณะเก็บรักษา. วารสารกรมวิทยาศาสตร์การแพทย์. 55 (1): 1-7.

6.**Angkititrakul S.**, Chomvarin C., Chaita T., Kanistanon K. and Waethewutajarn S. 2005. Epidemiology of antimicrobial resistance in *Salmonella* isolated from pork, chicken meat and humans in Thailand. Southeast Asian J Trop Med Public Health. Nov; 36 (6): 1510-5.

7.Nutravong T.C., **Angkititrakul S.**, Panomai N., Tharavichitkul P. and Chimanee P. 2010. Epidemiology Study of Serotyping and Genotyping of *Streptococcus suis* in Thailand. Journal of Science (Medical and Pharmacy Issue). 27 (61): 8. 321-324.

8.Arunee Pulpakdee, **Sunpetch Angkititrakul**, Fanan Suksawat, Olivier Sparagano and Kwankate Kanistanon. 2012. Epidemiology and Antimicrobial Resistance of *Salmonella* spp. Isolated from Dogs and Cats in Northeastern Thailand. Journal of Animal and Veterinary Advances. 11 (5): 618-621

9.**Sunpetch Angkititrakul** Arunee Polpakdee and Rungtip Chuanchuen. 2013. Prevalence of *Salmonella enterica*, *Escherichia coli* and *Staphylococcus aureus* in Raw Meat in Thai Self-Service style Restaurant in Khon Kaen Municipality. Thai Journal Veterinary Medicine. 43 (2): 265 – 268.