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Research article

Identification of *Streptococcus suis* carriage in healthy pigs in Chiang Mai, Thailand

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Abstract

Streptococcus suis (*S. suis*) belongs to the critical streptococcal swine pathogens affecting financial losses in pig production globally and being concerned as a zoonotic bacterial that causes a severe invasive disease emerging in pigs and humans. Serotype 2 is considered the major serotype and is the most pathogenic *S. suis* obtained from human cases, and the healthy pigs are known as the major reservoir of *S. suis*. In this cross-sectional study, *S. suis* prevalence was conducted in live pigs from 111 farms across Chiang Mai, Thailand. *S. suis* carriage rate and serotypes were determined from the bacteriological and multiplex PCR method from tonsil swab samples. We found that 18.2% (138/760) of tonsil swab samples and 54.1% (60/111) of pig farms were positive to *S. suis*, and only one (0.72%) from 138 isolates was identified as serotype 9. Meanwhile, all the remains were identified as non-serotype ½/1/2/7/9/14 strains. In addition, there was an independent relation between age-ranged, farm types, and production systems with *S. suis*-positive rates at the farm level. The results indicate that both intensive and smallholder production systems can generally be the source of *S. suis* carriage. Therefore, implementing good husbandry practices and *S. suis*-infection predisposing factors limiting that appropriate for each farm type is essential to minimize the opportunities of *S. suis* outbreak in humans.

Keywords: Chiang Mai province, Healthy pig, Prevalence, *Streptococcus suis*, Zoonosis

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INTRODUCTION

Streptococcus suis (*S. suis*) is considered the foremost streptococcal swine pathogen affecting financial losses in pig production globally. The upper respiratory tracts, especially in the palatine tonsils and the nasal cavities, are major colonization sites of *S. suis* in carrier pigs (Dutkiewicz et al., 2017). Recently, *S. suis* has been concerned as an emerging zoonotic pathogen and the public health threat that causes severe invasive diseases in humans and pigs (Gottschalk and Segura, 2019). *S. suis* infection primarily occurs in people who work in pig to pork processing chains such as pig breeders, abattoir workers, and butchers by percutaneous exposure in North America and European countries (Goyette-Desjardins et al., 2014). On the other hand, in Southeast Asian countries, raw pork consumption is the prominent cause of human *S. suis* infection (Takeuchi et al., 2017). The significantly clinical expression indicated *S. suis* infection is purulent meningitis and arthritis (Gottschalk and Segura, 2019; Rayanakorn et al., 2018). However, multiple systemic pathological conditions have also been reported, such as sudden death due to acute septicemia, endocarditis, polyserositis, and pneumonia. *S. suis* has been admitted as the most frequent bacterial infection leading to meningitis in adults in Vietnam, Thailand, and Hongkong sequentially (Fittipaldi et al., 2012). Since at least one ear permanent deafness and vestibular dysfunction are the important consequently complications after recovery from meningitis in humans (Rayanakorn et al., 2018; Salaneuve et al., 2020) thus, a common name of human streptococcosis from *S. suis* is known as "deafness fever disease" in Thailand.

Among the 29 currently recognized serotypes based on their antigenic structures of the capsular polysaccharide (CPS), serotype 2 is the crucially zoonotic serotype with the most pathogenic and frequently isolated in human cases, followed by serotype 14 and serotype 5, while the distribution of other serotypes is different depends on geographic region (Okura et al., 2016; Segura et al., 2020). Human *S. suis* infection has been sporadically notified worldwide since 1968; nonetheless, there is an enormous human *S. suis* infection in Southeast Asia, especially in Thailand, at 0.487 per 100,000 people. Moreover, 346 million Thai baht, or US$11.3 million, was lost due to the overall health burden from *S. suis* infection in humans (Rayanakorn et al., 2021), whereas more than 2,000 cases of *S. suis* infection in humans with a 6.5% mortality rate have been reported between 2013–2019. Raw pork consumption is a deep-rooted culture in the Northern people and also be a significant source of *S. suis* infection in Thailand. As a result, many severe outbreaks have occurred in Northern Thailand, including Phayao, Lamphun, and Chiang Mai Province, with 73 confirmed cases and six deaths between 2007-2008 (Rayanakorn et al., 2021; Segura et al., 2020). Chiang Mai had the highest incidence rate of human cases in Thailand according to the *S. suis* infection from 2005 to 2014 at 1.55 per 100,000 people, which was 6.5 times greater than other areas of Thailand (Thongsawad, 2016).

Since healthy pigs are known as the major reservoir of *S. suis*, which significantly impacts human health and swine production, in this study, we aimed to investigate the *S. suis*-positive rate and their serotypes in healthy pigs in Chiang Mai regarding their age range and farm types.

MATERIALS AND METHODS

The positive rate of *S. suis* was determined among sows, nursery pigs (3-8 weeks), growing pigs (8-16 weeks), and finishing pigs (16 weeks to slaughter) at 54 backyard and smallholder pigs (1-50 pigs), 40 small-scale (51-500 pigs) and 17 medium-scale (501-5000 pigs) intensive swine farms as described by Thanapongtharm et al. (2016). In addition, clinically healthy pigs without clinical signs related to *S. suis* were randomly selected, if possible, from one pig per pen. Sample collecting was obtained from March to November 2015. The study protocol has been approved on behalf of the Animal Care and Use Committee, the Faculty of Veterinary Medicine, Chiang Mai University, on protocol number S24/2559

Sample collections

The study was determined in pig farms allocated in all 25 districts in Chiang Mai, Northern Thailand (Figure 1). Overall, 760 palatine tonsil swab samples were taken out of 111 farms using an aseptic technique for all specimen collection procedures. First, a tonsillar swab was obtained from each pig using a snare and mouth gag to open the mouth and rub a cotton swab on the tonsil surface ten times. All samples were then placed in a transport tube containing Stuart's transport medium (Oxoid, UK), refrigerated at 4 °C, or kept in a cooler box, delivered to the laboratory, and processed within 24 hours of collection.

Bacterial identification

All samples were incubated onto 5% sheep blood agar (Oxoid, UK) at 37oC for 24-48 hours with 5% CO2. Physical and biochemical characteristics of *S. suis* were identified as previously described (Higgins and Gottschalk, 1990). A typical *S. suis* colony is alpha-hemolytic, small (pin-point in diameter), and mucoidal translucent colony. In addition, a single or double catalase-negative Gram-positive cocci colony was selected for further testing. Finally, *S. suis* tentative phenotypic confirmation was carried out by a set of biochemical tests consisting of a negative for 6.5% NaCl test and Voges-Proskauer (VP) test, and acidity producing in lactose, sucrose, trehalose, salicin, and inulin. In contrast, the acid production in glycerol, sorbitol, and mannitol was absent.

Figure 1 Map of 25 districts of Chiang Mai, Thailand.

S. suis confirmation and capsular gene typing

Genomic DNAs were extracted from *S. suis* suspected isolates using a QIAamp DNA Mini Kit (Qiagen, Germany) per the manufacturer's instructions. A multiplex polymerase chain reaction (PCR) was used for *S. suis* confirmation and the capsular-specific gene detection for serotypes 1 or 14, 2 or 1/2, 7, and 9 as described in Table 1. Multiplex PCR reactions were prepared in a total volume of 25 µL containing 12.5 µL of 2X Qiagen multiplex master mix (Qiagen, Germany), 2.5 µL of 5X Q-solution, 2 µM of each primer, and 2.5 mL of template DNA. All reactions were conducted in a Biorad T100TM thermocycler (Biorad, Hercules, CA, USA) under the conditions as follows: pre-activation step at 95°C for 15 min; 35 cycles of denaturation step at 94°C for 1 min and annealing step at 57°C for 1 min 30 sec; extension step at 72°C for 1 min, and final extension step at 72°C for 10 min. In addition, the amplified products were visualized by electrophoresis of 8 µL of final reaction on a 1.5% agarose gel stained with 0.25g/ml of ethidium bromide and visualized by ultraviolet (UV) transillumination. Positive control of *S. suis* serotype 1, 2, 7, and 9 were included in each amplification, as shown in Figure 2.

Table 1 Primers used for *S. suis* confirmation and capsular type identification.

Primers	Sequence (5'-3')	Gene	Size (bp)
16s-195(s) 16as-489(as2)	CAG TAT TTA CCG CAT GGT AGA TAT GTA AGA TAC CGT CAA GTG AGA A (Marois et al., 2004)	16S rRNA	294
cps1(F) cps1(R)	GGC GGT CTA GCA GAT GCT CG GCG AAC TGT TAG CAA TGA C (Smith Hilde et al., 1999)	type 1 or 14 CPS gene	441
cps2(F) cps2(R)	CAA ACG CAA GGA ATT ACG GTA TC GAG TAT CTA AAG AAT GCC TAT TG (Smith Hilde et al., 1999)	type 2 or ½ CPS gene	675
cps7(F) cps7(R)	GAA TCA ATC CAG TCA GTG TTG G CTA ATT CGA TAC GAA GCT AAA C (Smith et al., 1999)	Type 7 CPS gene	541
cps9(F) cps9(R)	GGC TAC ATA TAA TGG AAG CCC CCG AAG TAT CTG GGC TAC TG (Smith Hilde et al., 1999)	type 9 CPS gene	388

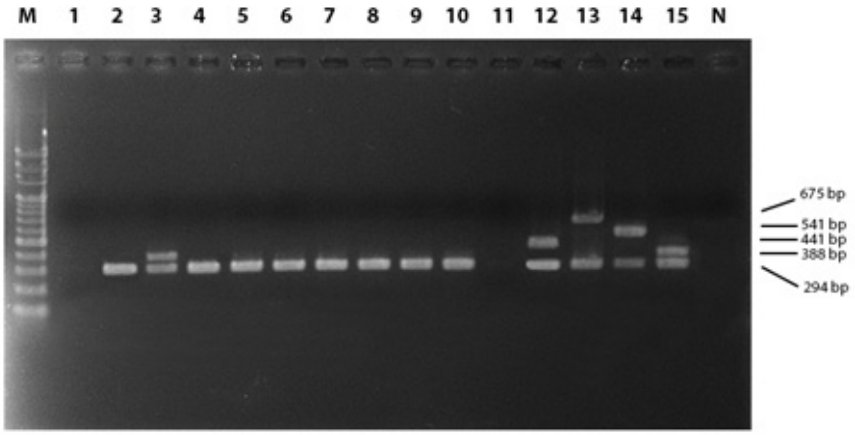

Figure 2 A total of 760 tonsillar samples from healthy pigs were collected for *S. suis* identification and serotyping by bacterial culture and PCR. Ethidium bromide-stained agarose gel electrophoresis of amplified PCR products using the multiplex PCR set of *S. suis* serotypes 1 (and 14), 2 (and ½), 7, 9, and 16S ribosomal RNA. Lane M=100 bp DNA ladder (Invitrogen, USA), lane 1- 11= *S. suis* suspected sample isolates, lane 12 = *S. suis* serotype 1, lane 13=*S. suis* serotype 2, lane 14= *S. suis* serotype 7, lane 15=*S. suis* serotype 9 and lane N= *S. suis* negative control.

Statistical analysis

S. suis identification results were compared by Chi-squared test of independence. Statistical tests were estimated by IBM SPSS Statistics (version 22.0) (IBM, New York, USA), and the differences were indicated statistical significance when the probability (P-value) amongst the tests performed was less than 0.05.

RESULTS

All 111 pig farms in this study consisted of 54 backyard and smallholder pigs, 40 small-scale and 17 medium-scale intensive swine farms. The 760 Palatine tonsil swab samples from pigs in four production stages were collected from the nursery, growing, finishing pigs and sows. The bacterial culture, biochemical tests, and the *S. suis* gene-specific PCR amplification (Figure 2) were used for *S. suis* isolation and identification. A total of 138 tonsil swab samples (18.2%, 95% confidence interval (CI): 15.4 to 20.9) were confirmed to be the *S. suis* positive samples, as listed in Table 2. There was no statistical difference of *S. suis* positive rates along age-ranged; meanwhile, the proportions of *S. suis* positive samples from smallholder pigs and medium-scale intensive farms were significantly higher than small-scale intensive farms at 21.5, 20.6, and 13.9%, respectively (P=0.042). The results of *S. suis* identification in each production age within the production system conditions were considered. The *S. suis* positive proportion was observed and ranged from 10.5 to 72% in all subgroups. Meanwhile, the *S. suis* positive proportion was no significant difference between age-ranged in smallholder farms and small-scale intensive farms; however, a significant distinction of the *S. suis* positive proportion was observed in growing pigs much higher than finishing pigs and sows from medium-scale intensive farms (P<0.001).

Table 2 *S. suis* identification rate from the tonsillar swabs of healthy pigs in Chiang Mai, Thailand, between March to November 2015

Variables	No of samples	%Positive (95%CI)	P-valuea
Age-ranged			0.172
Nursery pigs	164	16.5 (10.8-22.1)	
Growing pigs	264	22.7 (17.7-27.8)	
Finishing pigs	191	17.8 (12.4-23.2)	
Sows	141	12.1 (6.7- 17.4)	
Production systems			0.042*
Smallholder farms	247	21.5 (16.3-26.6)	0.739
Nursery pigs	47	25.5 (13.1-38.0)	
Growing pigs	134	20.1 (13.4-26.9)	
Finishing pigs	46	23.9 (11.6-36.2)	
Sows	20	15.0 (0-30.6)	
Small-scale intensive farms	309	13.9 (10.1-17.8)	0.931
Nursery pigs	117	12.8 (6.8-18.9)	
Growing pigs	105	14.3 (7.6-21.0)	
Finishing pigs	71	14.1 (6.0-22.2)	
Sows	16	18.8 (0-37.9)	
Medium-scale intensive farms	204	20.6 (15.04-26.1)	<0.001*
Nursery pigs	-	-	
Growing pigs	25	72.0 (54.4-89.6)	
Finishing pigs	74	17.6 (8.9-26.2)	
Sows	105	10.5 (4.6-16.3)	
Total samples	760	18.2 (15.4-20.9)	

[a]P-value across groups and subgroups was determined using the Chi-square-test; the P-value in the line for age-ranged and production systems are for the variation across their groups; the P-value in the line of smallholder farms, small-scale intensive farms, medium-scale intensive farms are for the variation across their subgroups. An asterisk (*) indicates a statistically significant difference between groups or subgroups (P<0.05).

The farm-level positive rate of *S. suis* is shown in Table 3. Overall, *S. suis* was obtained at 54.1% (95% CI: 44.8 to 63.3) of 111 farms located in 23 districts in Chiang Mai and were absent in samples from Muang Chiang Mai and Fang districts. The identification rates of *S. suis* at the farm level were observed in the fattening farm system at 61.9% and the farrow-to-finishing farm system at 49.2%. Likewise, the *S. suis* positive ratio of smallholder, small-scale, and medium-scale intensive farms was observed at 51.9, 52.5, and 64.7%, respectively. There was no significant difference in the farm-level identification rate considering the farm sizes (P=0.631) and pig production systems (P=0.195).

Table 3 *S. suis* identification rate at farm level considering the farm type and farm production system

District	% *S. suis* positive farms (positive farms/total farms)					
	Total farms	Farm types			Production systems	
		Smallholders	Small-scale intensive farms	Medium-scale intensive farms	Fattening farm	Farrow-to-finish system
Chai Prakan	40.0 (2/5)	25.0 (1/4)	-	100 (1/1)	0 (0/1)	50.0 (2/4)
Chiang Dao	50.0(2/4)	-	0 (0/1)	66.7 (2/3)	66.7 (2/3)	0 (0/1)
Chom Thong	40.0 (2/5)	40.0 (2/5)	-	-	0 (0/1)	50.0 (2/4)
Doi Lo	66.7 (2/3)	-	66.7 (2/3)	-	100 (2/2)	0 (0/1)
Doi Saket	100 (3/3)	-	100 (3/3)	-	-	100 (3/3)
Doi Tao	60.0 (3/5)	60. (3/5)	-	-	66.7 (2/3)	50.0 (1/2)
Fang	0 (0/2)	0 (0/2)	-	-	-	0 (0/2)
Galayani Vadhana	25.0 (1/4)	25.0 (1/4)	-	-	0 (0/1)	33.3 (1/3)
Hang Dong	100 (3/3)	-	100 (3/3)	-	-	100 (3/3)
Hot	80.0 (4/5)	80. (4/5)	-	-	80.0 (4/5)	-
Mae Ai	33.3 (1/3)	33.3 (1/3)	-	-	50.0 (1/2)	0 (0/1)
Mae Chaem	60.0 (3/5)	60.0 (3/5)	-	-	100 (2/2)	33.3 (1/3)
Mae On	75.0 (6/8)	-	66.7 (2/3)	80.0 (4/5)	80.0 (4/5)	66.7 (2/3)
Mae Rim	100 (3/3)	100 (3/3)	-		100 (1/1)	100 (2/2)
Mae Tang	20.0 (2/10)	0 (0/3)	0 (0/1)	33.3 (2/6)	25.0 (2/8)	0 (0/2)
Mae Wang	75.0 (3/4)	-	75.0 (3/4)	-	-	75.0 (3/4)
Muang	0 (0/2)	0 (0/1)	0 (0/1)	-	-	0 (0/2)
Omkoi	75.0 (3/4)	75.0 (3/4)	-	-	100 (1/1)	66.7 (2/3)
Phrao	75.0 (3/4)	75.0 (3/4)	-	-	-	75.0 (3/4)
Samoeng	45.5 (5/11)	66.7 (2/3)	37.5 (3/8)	-	50.0 (2/4)	42.9 (3/7)
San Kamphaeng	16.7 (1/6)	-	16.7 (1/6)	-	-	16.7 (1/6)
San Pa Tong	66.7 (2/3)	-	66.7 (2/3)	-	-	66.7 (2/3)
San Sai	66.7 (2/3)	-	0 (0/1)	100 (2/2)	100 (1/1)	50.0 (1/2)
Saraphi	66.7 (2/3)	-	66.7 (2/3)	-	-	66.7 (2/3)
Wiang Haeng	66.7 (2/3)	66.7 (2/3)	-	-	100 (2/2)	0 (0/1)
Total (95% CI)	54.1 (60/111) (44.8-63.3)	51.9 (28/54) (38.5-65.2)	52.5 (21/40) (37.0-68.0)	64.7 (11/17) (42.0-87.4)	61.9 (26/42) (47.2-76.6)	49.2 (34/69) (37.5-61.1)
P-value[a]			P=0.631		P=0.195	

[a]P-value across the groups was determined using the Chi-square test.

Multiplex PCR was done for *S. suis*-specific gene detection and capsular gene identification for serotype 1 (and 14), serotype 2 (and ½), serotype 7, and serotype 9. Of 138 *S. suis* obtained isolates, only one (0.72%) isolate was *S. suis* serotype 9, while the rest were undetected with all above PCR capsular-specific gene typing and categorized as non-serotype ½/1/2/7/9/14 strains.

DISCUSSION

Generally, the tonsils are an essential l ymphoid organ in the innate and adaptive immune system and are entrance paths and reservoirs for various microorganisms. Numerous pathogenic and commensal bacteria in swine are often colonized at the palatine tonsil (Opriessnig et al., 2011; Pena Cortes et al., 2018). In addition, the Streptococcaceae family has been one of the most common and mainly identified from the tonsil microbiota (Niazy et al., 2022; Pena Cortes et al., 2018). Hence, tonsils sampling is often performed to determine live pigs' viral and bacterial infectious status, including *S. suis* (Suzuki and Fuchimoto, 2019).

Tonsil swab sample collection, which had more sensitivity, simplicity, and safety than tonsil biopsy sampling in live pigs (Marois et al., 2007), was used in this study. One hundred thirty-eight *S. suis* strains were carried out from 760 tonsil swab samples using microbiological techniques and multiplex PCR. In this study, the positive rate of *S. suis* isolated from healthy live pigs' tonsil swab samples was 18.2% (95% CI: 15.4 to 20.9), which higher than the previous studies in the same area at 4.8% (Thongsawad, 2016) and 16.8% (Kongkaew et al., 2012). Meanwhile, the *S. suis* prevalence in tonsil samples from pig carcasses at the slaughterhouse in Chiang Mai were previously documented at 9% (Padungtod et al., 2010) and 28.1% (Lakkitjaroen et al., 2011). On the other hand, the *S. suis*-positive rate in healthy pigs was remarkably high in other areas of the Northern, Lampang and Phayao, at 64.8% and 61.4%, respectively (Pathanasophon et al., 2013). The positive rate of *S. suis* obtained from lived pigs and slaughter pigs varied from 4.0 to 37.0% in multiple studies from Central, Eastern, and Western Thailand (Meekhanon et al., 2017; Pathanasophon et al., 2009). The difference in *S. suis* prevalence depends on the geographical area and climate diversity; in addition, the difference of selective media for bacterial isolation and the sampling method, such as tonsil swabs, tonsil biopsy, saliva samples, and oral swabs, also influenced the variation of *S. suis* prevalence (Kerdsin et al., 2020; Nguyen et al., 2021).

To date, from a total of 29 serotypes of *S. suis*, serotype 2 is remarkably isolated from human cases, followed by serotypes 14 at 74.7% and 2%, respectively (Goyette-Desjardins et al., 2014). Nevertheless, Serotype 4, 5, 9, 16, 21, 24, and 31 have also been rarely reported as the disease-causing in humans, while serotype ½, 2, 7, 9, and 14 are the most frequent serotypes recovered from diseased pigs (Dutkiewicz et al., 2017; Hatrongjit et al., 2015; Kerdsin et al., 2017; Wisselink et al., 2002). Hence, Serotype 2 is vigorously considered a majority and the most virulent strain causing disease in humans and pigs. However, the positive rate of *S. suis* and their serotypes in healthy live pigs and slaughter pigs are variable. The specific PCR primer sets for the most frequent serotype in diseased pigs (serotype 1 (and 14), 2 (and ½), 7, and 9) were used in this study. Therefore, samples that showed negative results to

all the primer sets were categorized as non-serotype ½/1/2/7/9/14 strains in this study and were strongly predominant with 99.28% (n=138), while serotype 2 was undetected. Only one typable strain in this study was obtained from nursery pigs on a small-scale intensive farm and was identified as serotype 9, which frequently causes disease in pigs and can also be infected in humans. In various countries, including Thailand, *S. suis* serotypes are distributed in healthy pigs' tonsil samples. Previous findings documented a high proportion of serotype 2 in the *S. suis* population in healthy pigs from farms at 23.3% in Chiang Mai and from pig carcasses in slaughterhouses at 19.1% in Phayao and 42.8% in Chiang Mai (Kerdsin et al., 2020; Kongkaew et al., 2012; Padungtod et al., 2010). This high fraction of *S. suis* serotype 2 might be consistent with the incidence of human *S. suis* infection cases that frequently occurred in Chiang Mai and Phayao (Takeuchi et al., 2012; Wongkumma et al., 2014). However, lower identification rates of serotype 2 obtained from healthy pigs in Northern Thailand are also observed at 3.8-5.9% (Pathanasophon et al., 2013; Thongkamkoon et al., 2017). Moreover, Thongkamkoon et al. (2017) reported that serotype 23 was the most frequently observed in healthy pigs at 10.2%, followed by serotype 9 equally with serotype 7 at 8.2% and serotype 2 at 5.6%. Otherwise, the prevalence of *S. suis* serotype 16 (11%), serotype 8 (7%), serotype 9 (6%), and serotype 3 (5%) were showed in relatively frequent in Central Thailand, while the positive rate of serotype 2 is lower at 0.9% (Meekhanon et al., 2017). These *S. suis* serotype diversity would depend on geographical areas and the competitive factor between non-pathogenic serotype and virulent serotype on the target site in tonsils. Moreover, the beneficial and prosperous commensal bacteria in the microbiota community may impact the presence of *S. suis* virulent strains and other respiratory pathogens. Besides, many factors from farm husbandry are influential factors of tonsil microbiota diversity, such as ventilation, pig density, and flow system, herd status, feed quality, in-feed medication as well as the individual factors, for example, breeds and age (Correa-Fiz et al., 2016; Niazy et al., 2022). Generally, a majority population of non-typeable *S. suis* isolated from healthy pigs is frequently observed in both co-agglutination test with typing-sera and capsular gene-specific PCR assay (Arndt et al., 2018; Meekhanon et al., 2017; Thongkamkoon et al., 2017) probably as a consequence of the inability of CPS producing, cps loci losing and cps gene mutation (Gottschalk and Segura, 2019; Zheng et al., 2017). Nonetheless, this study's capsular-gene-specific PCR assay was not covered for all capsular types. Hence, further studies to ascertain the distribution of the *S. suis* serotype in population by the complete set of capsular-gene typing, serotyping by co-agglutination test or genome sequencing, and the virulence and antimicrobial resistance data of these *S. suis* strains would be applicable. The non-serotype ½/1/2/7/9/14 strains in this study were not pathogenic and would be classified as a commensal *S. suis*, which was widely considered harmless microbes. However, several studies reported that commensal bacteria such as *Escherichia coli* (*E. coli*), *Enterococcus* spp., and *S. suis* could display the high potential to be the reservoir of transferable antimicrobial resistance (AMR) genes and are recognized as severe public health traits (Djordjevic et al., 2013; Hadjirin et al., 2021; Thanh Duy et al., 2020). Hence, besides the pathogenicity of *S. suis*, the AMR issue is essential to be concerned, and further study of the AMR determinants in the commensal *S. suis* population is needed.

A resemblance of *S. suis*-carriage rate in smallholder pigs and intensive pig farms was observed in this study. The positive rate of *S. suis* per tonsillar swab samples from smallholder pigs (21.5%) is close to the medium-scale intensive farms (20.6%) and both significantly higher than small-scale intensive farms (13.9%). Our *S. suis*-positive ratio in smallholder pigs is higher than the previous studies in Chiang Mai area reported by Thongsawad (2016) at 4.8 % and Padungtod et al. (2010) at 10%. However, there was no statistical difference in *S. suis*-positive rate between the production stage in smallholder pigs, probably due to the continuous flow production system and multi-age groups on one-site of the smallholder production system. A significant proportion of pigs in the swine industrial production in Thailand were raised by the smallholders; approximately 435,000 pigs were raised in Chiang Mai, with the highest density of smallholders' farms in Thailand (DLD, 2015; Thanapongtharm et al., 2016). Generally, most smallholder farmers usually have a small budget for husbandry practices improvement; consequently, disease prevention and control practices are relatively poor. Therefore, smallholder pigs can be an important source of many contagious diseases spreading in pigs and humans (Beltran-Alcrudo et al., 2019; Thanapongtharm et al., 2016). The heaviest *S. suis* outbreak in humans was appeared in China during the year 2005 and originated from smallholder pigs related to backyard rearing and slaughtering practices (Du et al., 2017; Yu et al., 2006). Furthermore, *S. suis* be capable of inter-species transmission between pigs and other animals such as chickens, cattle, and goats (Nhung et al., 2020; Okwumabua et al., 2017; Yu et al., 2006), while multi-species of animals were often reared together in one backyard and smallholder farm without appropriate biosecurity and biocontainment. Therefore, the opportunities of inter-species transmission and *S. suis*-harboring transferable from the backyard and smallholder animals to humans should be concerned.

Overall, there was no difference in the *S. suis*-positive rate between the production age-ranged consisting of the nursery, growing, finishing pigs, and sows. Inconsistently, Kongkaew et al. (2012) reported that the positive rate of *S. suis* from live pigs in each production stage was highest in sows, while *S. suis*-carriage rate was reported in piglets and growing to finishing pigs in Central Thailand as 21% and up to 45%, respectively (Meekhanon et al., 2017). In Spain, the proportion of *S. suis* isolated in the tonsillar samples of live pigs in the nursery, growing, finishing pigs, and sows as 34, 38.3, 38.3, and 19.9%, respectively (Luque et al., 2010). Generally, symptomatic *S. suis* infection is usually found in piglets up to 10 weeks; nonetheless, non-invasive *S. suis* colonization can be found in all age ranges of pigs. (Gottschalk and Segura, 2019). These data suggested that age may not be directly related to *S. suis* carriage rate in healthy pigs on a single farm. However, several predisposing factors such as overpopulation, inadequate air circulating, overmuch inconstancy temperature, and other stress-induced factors are associated with pathogenic *S. suis* infection in herds as the disease-triggering factors (Gottschalk and Segura, 2019).

From a herd status perspective, the healthy *S. suis* -carriage pigs were presented on 54% of pig farms with no significant difference between smallholder pigs, small-scale and medium-scale intensive farms, and the production system. Following the UK and China study, age and farm type, consisting of intensive-farm and small-farm subgroups, were unrelated to the *S. suis* carriage rate at farm-level. Meanwhile, the higher air temperature

increased the *S. suis* carriage rate and the prevalence of human disease-causing *S. suis* (Zou et al., 2018). *S. suis* infection rate and virulence vary depending on factors such as pathogenic attributes of strains, host factors, environmental factors, management factors, and co-infection with other pathogens (Gottschalk and Segura, 2019). Given that healthy pigs can carry *S. suis*, it would be essential to focus on good husbandry practices and predisposing *S. suis*-infection factors control in both intensive and smallholder production systems to minimize the opportunities to be an important source of transferable pathogenic *S. suis* to humans. Besides, raw pork and pig blood consumption are crucial factors leading to the *S. suis* infection incidence in the Northern people of Thailand; hence, an effective public health education campaign is needed to improve these cultural food habits.

CONCLUSION

For two decades, conscious knowledge of *S. suis* as an invasive zoonotic pathogen and public health threat has risen. Healthy pigs are addressed as the *S. suis* colonization and can be a potentially *S. suis*-carriage transmittable to humans. Overall, the *S. suis*-positive rate at 18.2% in healthy live pigs in Chiang Mai province was determined in this study and can be found *S. suis* in all age-range pigs both in smallholder and intensive production systems. Accordingly, proper practices and regulations should be standardized and carried out based on the characteristics of each farm type to limit the *S. suis* infection and keep disintegrated within the links along the potential human-animal-environment infection chain. Nonetheless, public health education campaigns for the awareness of human *S. suis* infection in Northern Thailand are challenging but essential for *S. suis* outbreak control, and further study of the AMR determinants in the commensal *S. suis* population is needed from the public health perspective.

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AUTHOR CONTRIBUTIONS

Conceptualization and methodology designation: NK, PP
Sample collection: NK, PT, PT
Performed the laboratory analysis: NK
Analyzed the data: NK, PP
Contributed reagents/materials/analysis tools: NK, OB
Wrote the paper (review and editing): NK, PT, PT, TS, TB, SR, PP

CONFLICT OF INTEREST

All authors have no conflicts of interest to declare.

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