Potentially hazardous *Streptococcus suis* strains latent in asymptomatic pigs in a major swine production area of Thailand

Nattakan Meekhanon,¹ Sarawan Kaewmongkol,¹ Waraphon Phimpraphai,² Masatoshi Okura,³ Makoto Osaki,³ Tsutomu Sekizaki⁴ and Daisuke Takamatsu³,⁵,*

### Abstract

**Purpose.** Carrier pigs have been considered as the major reservoir of *Streptococcus suis* and could be a significant source of human infection. Therefore, we investigated the prevalence and characteristics of latent *S. suis* in asymptomatic pigs in the pig-farming area of central Thailand, and compared the data to those previously reported in other regions.

**Methodology.** We collected samples from 340 asymptomatic pigs. *S. suis* isolates from the samples were confirmed by species-specific PCR (*recN* PCR). The capsular polysaccharide synthesis gene (*cps*) types, virulence-associated gene profiles and sequence types (*STs*) of the isolates were investigated.

**Results/Key findings.** The prevalence of *S. suis* found in this study was 37% (125/340 pigs). The most prevalent genotype was *mrp*/epf*/sly*. Among the 16 *cps*-types identified in 135 isolates, *cps*-type 16 was the most frequent (11%), whereas 44% of the isolates were non-typable. In common with the strains causing human sepsis in Thailand, two *cps*-type 9 isolates and a *cps*-type 24 isolate from slaughtered pigs belonged to ST16 and ST221, respectively. All the isolated *cps*-type 2 strains were confirmed as serotype 2 by co-agglutination tests, and these belonged to ST104, the unique ST commonly found in Thai patients; however, in contrast to the endemic areas, the prevalence of serotype 2 strains was relatively low (2%) and no ST1 isolate was found.

**Conclusion.** Our results showed the population structure differences between *S. suis* in central Thailand and other regions; however, zoonotic *S. suis* is certainly latent in asymptomatic pigs in this intensive swine production area.

### INTRODUCTION

*Streptococcus suis* is a zoonotic pathogen that can cause various diseases including meningitis, arthritis and endocarditis in both swine and humans [1, 2]. *S. suis* is found naturally in the upper respiratory tract, especially in the tonsils and nasal cavities, the genital and alimentary tracts, and the salivary glands of clinically healthy pigs [3–6]. Healthy carrier pigs are important not only regarding the spread of *S. suis* in herds, but also as source of infection for humans [2].

The capsule is among the most important virulence determinants in *S. suis*. According to the antigenicity of the capsular polysaccharide (CP), *S. suis* can be classified into different serotypes. Although many serotypes of *S. suis* have been described to date, most isolates from diseased pigs belong to certain limited serotypes including serotypes 2, 3, 7 and 9 [1, 7, 8]. In humans, the majority of clinical cases are associated with serotype 2 [9, 10]; however, serotypes 4, 5, 9, 14, 16, 21, 24 and 31 have also been reported [11–17]. In addition to the capsule, many other potential virulence factors have been identified, including muramidase-released protein (MRP, encoded by *mrp*) [18], extracellular factor (EF, encoded by *epf*) [19] and suilysin (SLY, encoded by *sly*) [20]. Although the precise role of MRP and EF has not been clearly identified, these
Putative virulence factors are frequently associated with highly virulent serotype 2 strains and thus have commonly served as virulence markers [21, 22].

In the last decade, an increasing number of human S. suis infections has been reported, and more than 500 cases of S. suis infection in humans have been confirmed in Thailand [7, 23]. Most of these patients were infected after consuming raw pork products. According to a previous study by Kerdsin et al. [23], S. suis serotype 2 isolates from the blood and cerebrospinal fluid of patients in Thailand predominantly possessed three virulence-associated genes (mrp, epf and sly) and were mostly classified into sequence types (STs) 1 and 104 by multilocus sequence typing (MLST), suggesting that S. suis strains with these genotypes are especially hazardous for humans in Thailand. Because of the traditional custom of eating raw pork dishes in northern Thailand, outbreaks of S. suis infections have been focused mainly on humans and pigs in northern areas.

Human S. suis infections also sporadically occur in central Thailand, which has the highest density of swine production; however, the prevalence and characteristics of latent S. suis in pigs in this area are almost unknown. Therefore, the objectives of this study were to investigate the prevalence of S. suis on asymptomatic pigs in farms and slaughterhouses in central Thailand, and to compare the characteristics of the isolated S. suis to those previously reported in other regions and neighbouring countries. Here, we show the presence of potentially zoonotic S. suis strains in carrier pigs distributed in the pig-farming area of central Thailand, and differences in the population structures of S. suis between this area and human endemic areas, including northern Thailand.

METHODS

Isolation and identification of S. suis from carrier pigs

Although the carriage rate of S. suis in pigs varies among studies, we assumed that a carriage rate of S. suis in asymptomatic pigs in central Thailand of approximately 30 %, on the basis of several previous studies conducted in Southeast Asia [5, 24, 25]. In this study, we accepted a 5 % margin of error at a confidence level of 95 %. Under these assumptions and conditions, approximately 320 samples are required as an acceptable sample size. Therefore, in this study, we collected samples from 340 asymptomatic pigs (75 nursing sows, 75 suckling piglets, 115 finishing pigs and 75 slaughtered pigs) on five pig farms and two slaughterhouses located in the highest-density area of swine production in Thailand [Nakhon Pathom and Ratchaburi provinces, Fig. S1 (available in the online Supplementary Material)]. All the samples were collected in the period October 2014–July 2015. Since oral cavity secretions, which often contain S. suis, are easily transmitted from pig to pig and from pig to human, saliva was collected from live pigs in this study.

However, due to the limited amount of saliva obtained from piglets and finishing pigs, oral swabs were collected from these pig types rather than saliva. As for the slaughtered pigs, their oral cavity secretions could be rinsed during the slaughtering process. Besides the oral cavity, carrier pigs commonly harbour S. suis in the nasal cavity; therefore, nasal swab samples were collected from the slaughtered pigs, and additionally from finishing pigs, to investigate the presence of potentially hazardous S. suis strains that may come in contact with consumers.

The samples were inoculated onto Columbia blood agar (Oxoid) containing 5 % sheep blood and streptococcal selective supplement (Oxoid). In each sample, up to six streptococcal-like colonies were selected and cultured on Todd–Hewitt (TH) agar (Difco Laboratories, Becton Dickinson). The bacteria were cultured at 37 °C in air plus 5 % CO₂ for 18–24 h. All candidates were then screened by Gram staining and a catalase test.

All suspected isolates were confirmed as S. suis by species-specific PCR for S. suis (recN PCR) [26]. Briefly, the DNA of bacterial cells was extracted using InstaGene Matrix (Bio-Rad) following the manufacturer’s instructions, and recN PCR was performed using Quick Taq HS DyeMix (TOYOBO) according to the manufacturer’s instructions. S. suis strain P1/7 was used as the positive control.

CP synthesis gene (cps)-typing and co-agglutination test with typing sera

The cps-type of all S. suis isolates was identified by two-step multiplex PCR targeting serotype-specific cps genes, following a previous study [27]. Multiplex PCR reactions were carried out with QIAGEN Multiplex Master PCR Mix (Qiagen) according to the manufacturer’s instructions. This typing method can assign S. suis strains to cps-types, which were numbered corresponding to the expected serotypes (e.g. serotype 3 to cps-type 3); however, because serotypes 2 and 1/2 cannot be differentiated solely by PCR, the serotypes of the isolates carrying specific genes for these serotypes were confirmed by a co-agglutination test using anti-serotype 1 and 2 sera as described previously [28, 29].

Virulence-associated gene profiling

Three virulence-associated genes (mrp, epf and sly) were investigated in all S. suis isolates using PCR, with specific primers for each gene following procedures described previously [22]. A multiplex PCR reaction was carried out using QIAGEN Multiplex Master PCR Mix (Qiagen) according to the manufacturer’s instructions, and the presence of variant mrp was further examined by PCR using TaKaRa Ex Taq polymerase (Takara Bio) following the manufacturer’s instructions. S. suis strain P1/7 was used as the positive control for the amplification of mrp, epf and sly genes.

The correlation between isolate sources and the presence of virulence-associated genes was analysed by chi-square test at a 95 % confidence interval.
MLST analysis
MLST was performed by sequencing seven housekeeping genes of *S. suis*, as described elsewhere [30]. Bacterial DNA extraction was performed using the EZNA Bacterial DNA kit (Omega Bio-Tek). The fragments of the seven housekeeping genes were amplified using TaKaRa Ex Taq polymerase (Takara Bio) according to the manufacturer’s instructions. Due to the difficulty in *mutS* amplification, the primers described by Rehm et al. [31] were used in some isolates. Amplified PCR products were purified using a QIAquick PCR purification kit (Qiagen) following the manufacturer’s instructions, and were sequenced using a 3730xl DNA Analyzer (Applied Biosystems). MLST alleles and the resulting STs were determined by comparing the sequences to those in the *S. suis* MLST database (http://ssuis.mlst.net and http://pubmlst.org/ssuis/). Novel alleles and STs were assigned by submission of the respective data to the database. The population structure was analysed using the eBURST program (http://eburst.mlst.net).

RESULTS

Distribution of *S. suis* in asymptomatic pigs in central Thailand

Among the samples of saliva and oral and nasal swabs collected from 340 asymptomatic pigs, *S. suis* was found in 125 samples (37%) from sows (25/75), piglets (16/75), finishing pigs (52/115) and slaughtered pigs (32/75). From the 125 samples, 135 *S. suis* isolates were identified; 28 and 16 isolates were from nursing sows and their suckling piglets, respectively, while 57 and 34 isolates were from finishing and slaughtered pigs, respectively (Table 1).

cps-types of *S. suis* isolates

Using the results of the *cps*-typing assay, 76 of the 135 isolates (56%) were classified into 16 *cps*-types (*cps*-types 2, 3, 8, 9, 10, 11, 15, 16, 17, 18, 19, 21, 24, 28, 29 and 31), and all the *cps*-type 2 isolates were confirmed as serotype 2 by co-agglutination tests; however, 59 of these (44%) could not be classified into any *cps*-type (Fig. 1). The nontypable isolates were mostly isolated from oral swabs of finishing pigs (21/59) and the saliva of sows (16/59). Among the 76 *cps*-typed isolates, *cps*-types 16 (15/76), 8 (10/76), 9 (8/76) and 3 (7/76) were found relatively frequently, while the isolation rate of serotype 2 *S. suis* was low (3/76). In most of the samples from which *S. suis* was isolated, only a single *cps*-type was found from a single sample; however, in the 10 samples from sows, finishing and slaughtered pigs, two different *cps*-types were identified from a single sample.

Among the sow–piglet pairs tested, *S. suis* was recovered from either the sow or piglet in 27 pairs and from both the sow and piglet in only seven pairs. The same *cps*-type of *S. suis* was obtained from just one sow–piglet pair.

Virulence-associated gene profiles (mrp, epf and sly)

Approximately 27% of *S. suis* isolates possessed at least one of these virulence-associated genes. The most prevalent genotype of *S. suis* strains isolated from asymptomatic pigs was *mrp*/epf*/sly* (99/135, 73%), followed by *mrp*/epf*/sly* (15/135, 11%) and *mrp*/epf*/sly* (12/135, 9%). The other profiles identified in this study were *mrp*/epf*/sly*, *mrp*/epf*/sly* and *mrp*/epf*/sly* (Table 2). In three isolates, the *mrp* gene was detected using the primers reported by Silva et al. [22]. However, variant types of the *mrp* gene could not be determined by the variant-PCR for *mrp* due to unusual amplifications (approximately 500 and 700 bp products), suggesting that these have novel variants of *mrp* (*mrp* UT). All isolates classified into *cps*-types 2, 11, 18 and 19 had at least one of the virulence-associated genes (Table S1). However, interestingly, none of our isolates possessed *epf* (Table 2).

Among the 75 *S. suis* isolates obtained from the oral cavity of pigs, only nine (12%) had the virulence-associated gene(s) while 27 of 60 isolates (45%) from the nasal cavity possessed the virulence-associated gene(s) (Table 2). The proportion of *S. suis* isolates possessing the virulence-associated gene(s) was significantly different between the isolates from nasal swabs and saliva/oral swabs (*P*<0.05). Similarly, in the finishing pigs, the proportion of the virulence-associated gene-positive isolates was significantly higher in the nasal cavity (18/26 isolates, 69%) than the oral cavity (5/31 isolates, 16%; *P*<0.05). On the other hand, no significant difference was observed for the presence of the virulence-associated genes between isolates from farms and slaughterhouses (*P*>0.05).

MLST analysis of *S. suis* isolates

Among the 76 *cps*-typed isolates, 31 belonged to *cps*-types 2, 9, 16, 21, 24 and 31, the corresponding serotypes of which have been reported to cause human infections. By MLST analysis, these isolates were assigned to 20 different STs including 16 novel STs (ST715-ST724, ST726-ST727, ST771-ST774) (Tables 3 and S1). Notably, all *serotype 2* isolates from carrier pigs were assigned to ST104, which was the significant ST uniquely isolated from human cases in Thailand. In addition, two *cps*-type 9 isolates from slaughtered pigs were assigned to ST104, which was the significant ST isolated from human cases in Thailand. In addition, two *cps*-type 9 isolates from slaughtered pigs were assigned to ST16, which was an important ST causing infection in a patient in Thailand [15] and predominantly affects pigs in European countries [7,32]. Moreover, as with the *serotype 24-ST221* strain previously reported to cause human infection in northern Thailand [14] and recently reported to cause fatal septic meningitis in children [33], the *cps*-type 24 isolate from a slaughtered pig was also assigned to ST221. On the other hand, although *S. suis* serotype 16-ST106 has been reported to be the cause of a fatal infection case in Vietnam [16], no *cps*-type 16 isolates were classified into ST106 in our study. Most of our *cps*-type 16 isolates were novel STs, and one *cps*-type 16 isolate from a finishing pig was assigned to ST375, which was also found in a field isolate from the tonsil of a carrier pig in Thailand (id 1124; http://pubmlst.org/ssuis/) (Table 3 and S1).
Interestingly, potentially zoonotic types (ST104, ST16 and ST221) were only isolated from nasal swabs.

**DISCUSSION**

*S. suis* is recognized as an emerging zoonotic pathogen throughout the world, and more than 90% of human *S. suis* infection cases have been reported in Asia, especially in Vietnam, Thailand and China [7]. According to the statistical data on swine population in Thailand as of June 2015 (Information and Communication Technology Center, Department of Livestock Development, Ministry of Agriculture and Cooperatives), approximately 6 million finishing pigs were reared in Thailand and more than half of these (3.2 million) were reared in the central area, particularly in Ratchaburi and Nakhon Pathom provinces. As human *S. suis* infection tends to be frequently reported in pig-rearing areas [10], in this study we investigated the prevalence and characteristics of *S. suis* in central Thailand. In this area, the prevalence of *S. suis* in asymptomatic pigs was 37% (125/340) and the carriage rate of serotype 2 strains was 0.9% (3/340). Although the materials and methods used for the isolation, identification and serotyping of *S. suis* have varied somewhat among studies, the carriage rate of *S. suis* serotype 2 in central Thailand is almost comparable to that of healthy fattening pigs in upper northeastern Thailand (0.13%, 1/741 nasal swab samples) [25] but lower than that of healthy slaughterhouse pigs in southern Vietnam (8.3%, 45/542 tonsils) [24]. However, since only a few colonies were investigated for each pig, the prevalence of *S. suis* in this study may be underestimated.

<table>
<thead>
<tr>
<th>Sample source</th>
<th>No. of pigs</th>
<th>No. of pigs carrying <em>S. suis</em></th>
<th>No. of isolated <em>S. suis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sow</td>
<td>75</td>
<td>25 (33%)</td>
<td>28</td>
</tr>
<tr>
<td>Piglet</td>
<td>75</td>
<td>16 (21%)</td>
<td>16</td>
</tr>
<tr>
<td>Finishing pig</td>
<td>115</td>
<td>52 (45%)</td>
<td>57</td>
</tr>
<tr>
<td>Slaughtered pig</td>
<td>75</td>
<td>32 (43%)</td>
<td>34</td>
</tr>
<tr>
<td>Total</td>
<td>340</td>
<td>125 (37%)</td>
<td>135</td>
</tr>
</tbody>
</table>

**Fig. 1.** cps-typing results of 135 *S. suis* isolates from asymptomatic pigs.
In our study, *S. suis* *cps*-type 16 was the most predominant (11%) amongst the isolates latent in carrier pigs, followed by *cps*-types 8 (7%) and 9 (6%), while the prevalence of *S. suis* serotype 2 was only 2%. The results of virulence-associated gene profiling showed that *mrp*/epf*/sly* was the major genotype (73%) found among the *S. suis* population in this study. In addition, none of our isolates possessed epf, and all serotype 2 isolates were *mrp*/epf*/sly+. Interestingly, this is inconsistent with the population structures of *S. suis* in endemic areas in Thailand and neighbouring countries. A study in Chiang Mai province [6], in the endemic area of northern Thailand, demonstrated that *S. suis* serotypes 3 (17.5%) and 2 (12.5%) were the most common serotypes isolated from submaxillary glands of pig carcasses, and the *mrp*/epf*/sly* genotype was highly prevalent (45%). Padungtod et al. [5] also showed that *S. suis* serotype 2 was the most predominant serotype (43%, 12/28) in healthy pigs in Chiang Mai province, and all 28 isolates possessed *mrp* and *sly*. A study of genetic diversity of *S. suis* in Thailand [34] demonstrated that seven of 194 *S. suis* isolates (3.6%) from tonsil

Table 2. Virulence-associated gene profiling results of *S. suis* isolates classified by sample source

| Sample source      | No. of *S. suis* isolates | *mrp*/epf*/sly* | *mrp*/epf*/sly*† | *mrp*/epf*/sly*+ | *mrp*/epf*/sly*+† | *mrp*/epf*/sly*+*** |
|--------------------|---------------------------|----------------|----------------|----------------|----------------|----------------
| Oral cavity        |                           |               |               |               |               |               |
| Sow (saliva)       | 27                        |               |               |               |               |               |
| Piglet (oral swab) | 13                        |               |               |               |               |               |
| Finishing pig (oral swab) | 26                  | 2             | 3             |               |               |               |
| Nasal cavity       |                           |               |               |               |               |               |
| Finishing pig (nasal swab) | 8                   | 6             | 12            |               |               |               |
| Slaughtered pig (nasal swab) | 25                  | 4             |               | 3             | 2             |               |
| Total              | 99                        | 12            | 3             | 15            | 2             | 4             |

†*mrp*†, the *mrp* gene of the isolate was detected by primers reported by Silva et al. [22], but the variant type could not be determined by the *mrp* variant PCR reported in the same publication.

Table 3. *cps*-typing, virulence-associated gene profiling and MLST results of *S. suis* isolates

<table>
<thead>
<tr>
<th><em>cps</em>-type</th>
<th>Virulence-associated gene profile</th>
<th>ST†</th>
<th>No. of isolates from farms</th>
<th>No. of isolates from slaughterhouses</th>
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</thead>
<tbody>
<tr>
<td></td>
<td><em>mrp</em>†</td>
<td>efp</td>
<td>sly</td>
<td>Sow (saliva)</td>
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<tr>
<td>2§</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>104</td>
</tr>
<tr>
<td>9</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>771–774</td>
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<td>*</td>
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<tr>
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<td>−</td>
<td>−</td>
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<tr>
<td>24</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>221</td>
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<tr>
<td>31</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>722, 727</td>
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<td>Other</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>ND</td>
</tr>
<tr>
<td><em>cps</em>-types</td>
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<td>**</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>ND</td>
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<td>Nontypable</td>
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<td>ND</td>
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<tr>
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<td>Total</td>
<td>28</td>
<td>16</td>
<td>31</td>
<td>26</td>
</tr>
</tbody>
</table>

ND, Not done.

†*mrp*†, the *mrp* gene of the isolate was detected by primers reported by Silva et al. [22], but the variant type could not be determined by the *mrp* variant PCR reported in the same publication.

§*cps*-type 2 strains were confirmed as serotype 2 by co-agglutination tests.
homogenate of asymptomatic pigs in Phayao province, in the endemic area of northern Thailand, were serotype 2, and 5 of the 7 serotype 2 isolates were \textit{mpr}+/\textit{epf}+\textit{sly}+; while the remaining two isolates were \textit{mpr}+/\textit{epf}+/\textit{sly} and \textit{mpr}−/\textit{epf}−/\textit{sly}+. In a study of slaughterhouse pigs in southern Vietnam [24], \textit{S. suis} serotype 2 was the most common serotype (14.2 %) isolated from healthy pig tonsils, and 77.8 % of serotype 2 isolates were classified as ST1 and possessed \textit{epf}+/\textit{sly}+ or \textit{epf}+/\textit{sly}+. In China, Wang et al. [35] reported that \textit{S. suis} serotype 2 was the most predominant (17.6 %) in isolates from slaughtered pigs, followed by serotype 2 (13.7 %). That is, the prevalence of \textit{S. suis} serotype 2 strains in carrier pigs in central Thailand was relatively lower than that in human endemic areas, and the virulence-associated gene profiles were different between our serotype 2 isolates and those isolated from the endemic areas. Although sporadic \textit{S. suis} human infections have occurred in central Thailand, the incidence is low compared with northern Thailand. Apart from the difference in consumption behaviour, the lower prevalence of serotype 2 isolates were classified as ST1 and possessed \textit{epf}+/\textit{sly}+ or \textit{epf}+/\textit{sly}+. The most common ST of \textit{S. suis} serotype 2 strains in central Thailand may be one of the significant reasons for the low incidence of \textit{S. suis} human infections in this region. These data additionally emphasize the variation in \textit{S. suis} population structures among carrier pigs in different geographical locations. Of note, in this study, the proportion of isolates possessing the virulence-associated genes was different between those from the nasal and oral cavities; therefore, even in carrier pigs, \textit{S. suis} population structures may vary between different organs, although further study is needed to verify this hypothesis.

Wongsawan et al. [6] reported that 37.5 % of \textit{S. suis} isolates from pig carcasses in Chiang Mai province were non-typable. Likewise, in this study, non-typable \textit{S. suis} isolates were found in 44 % of isolates from carrier pigs. Notably, over 60 % of non-typable isolates were from the saliva of sows and oral swabs of finishing pigs. These non-typable strains may be novel serotypes with unknown \textit{cps} gene clusters. Some isolates showed unusual patterns of the amplified products when using two-step multiplex PCR for \textit{cps}-typing, which were different from those of the serotype reference strains described by Okura et al. [27], and this may imply the presence of novel \textit{cps} gene clusters in these isolates. Alternatively, the failure of \textit{cps}-typing using PCR may result from mutations in the \textit{cps} genes that caused the loss of target genes or primer-template mismatches. Indeed, mutations in the \textit{cps} genes, including deletions, insertions, single-nucleotide substitutions and frame-shift mutations, were frequently found in field \textit{S. suis} isolates from porcine endocarditis, and many of these mutations caused the loss of the capsule in the isolates [29, 36].

The most common ST of \textit{S. suis} serotype 2 strains associated with disease in humans is ST1; however, in Thailand, serotype 2 strains with other STs have also been isolated from patients. An \textit{S. suis} serotype 2-2-ST104 strain was isolated from the blood of a fatal human case in northern Thailand [17]. Moreover, Kerdsin et al. [23] reported that ST104 was the second most common ST of serotype 2 (25.5 %) found in human infections in almost all parts of Thailand. In addition to human infection, a serotype 2-ST104 strain was previously isolated from the blood of a diseased pig in Nakhon Pathom province [34]. Although we could not isolate the ST1 strain from any samples, interestingly, we found \textit{S. suis} serotype 2-2-ST104 strains in nasal swabs of both finishing and slaughtered pigs. As with the human isolates [23], all three \textit{S. suis} serotype 2-2-ST104 strains isolated in this study were the \textit{mpr}+/\textit{epf}+/\textit{sly}+ genotype. Besides serotype 2, \textit{S. suis} serotype 9-ST16 and serotype 24-ST221 strains have been reported to cause sepsis in patients in Thailand [14, 15, 33]. In our study, these two types were found in the isolates from nasal swabs of slaughtered pigs. These findings strongly suggested that healthy carrier pigs distributed throughout the swine industry in Thailand could be an important source of human infection. To the best of our knowledge, this is the first official report of the presence of these potentially hazardous strains in carrier pigs.

The sow is a potential source of infection in the herd, and piglets can become infected from the sow harbouring \textit{S. suis} in her uterus or vagina [37]. In addition, carrier pigs are important in the transmission of \textit{S. suis} in herds via the nasal and oral routes [2]. In this study, we isolated \textit{S. suis} from nursing sows’ saliva and oral swabs of their piglets. Unexpectedly, the same \textit{cps}-type of \textit{S. suis} was found in only one of the pairs of sows and piglets tested in this study. This possibly suggests that \textit{S. suis} could be transferred to piglets not only from their carrier sows but also from other sources, such as other sows or piglets living adjacent to their stall, contaminated fomites/person or from a contaminated environment. Moreover, a recent study by Bonifait et al. [38] suggested the potential transmission of \textit{S. suis} via aerosol in swine confinement buildings; therefore, such horizontal transmission events might have occurred via aerosol. However, since we selected only up to six streptococcal-like colonies in each sample, some \textit{S. suis} isolates might have been overlooked during the colony selection step. Therefore, we cannot rule out the possibility that the number of sow–piglet pairs carrying \textit{S. suis} strains of the same \textit{cps}-type was underestimated. In addition, since \textit{S. suis} could potentially be transmitted from sow to piglet via the uterus or vagina, further investigation of these organs is necessary to evaluate the importance of vertical transmission of \textit{S. suis} on pig farms.

In conclusion, our study showed population structure differences between \textit{S. suis} in central Thailand and human endemic areas in Thailand and neighbouring countries. In addition, we showed the presence of some potentially hazardous \textit{S. suis} strains for humans in asymptomatic pigs distributed in the intensive swine production area of Thailand. Therefore, public awareness should be raised concerning food safety in the overall process of pig production and pork processing to prevent further mass outbreaks of this emerging zoonotic infection.

\textbf{Funding information}

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Conflicts of interest

The authors declare that there are no conflicts of interest.

Ethical statement

The Animal Ethic Committees of Faculty of Veterinary Technology, Kasetsart University approved this study.

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