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High prevalence *of Streptococcus suis* serotype 2 contamination in raw pork and edible organs from retail markets in Phayao, Thailand

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Abstract

Streptococcus suis (*S. suis*) is a Gram-positive bacterium that is causing public health concern worldwide, especially in Northern Thailand. In Phayao province, the cultural preference for consuming raw or undercooked pork has led to a persistent risk of severe human infections. This study aimed to investigate the prevalence and distribution of *S. suis* contamination in retail pork products sole in Phayao province. We reported the prevalence of *S. suis* contamination in raw pork samples, fresh pig blood and edible pork organ samples (livers, lungs, hearts, and pharynx) collected from retail markets in 7 districts in Phayao province, Thailand between April to June 2025. From a total of 200 samples, *S. suis* and *S. suis* serotypes 1, 2, 1/2 and 14 were identified using the multiplex PCR method. The result revealed a high contamination rate, with 114 (57.0 %) of the samples identified as *S. suis*; specifically, 3 % were serotype 2 and 54 % comprised unknown serotypes. Among the *S. suis*-positive samples, serotype 2 was identified at 5.3 % (6/114). Notably, serotypes 1, 1/2, and 14 were absent in this study. Analysis of sample types showed that the highest contamination rate of *S. suis* was found in the pharynx, while the lungs exhibited the highest prevalence of serotype 2. These findings underscore the critical need for increased public awareness regarding the dangers of consuming uncooked pork and highlight the necessity for more stringent food safety policies and continuous monitoring within the pork supply chain to prevent future outbreaks.

Introduction

Streptococcus suis (*S. suis*) is classified as a Gram-positive bacterium that naturally resides in the upper respiratory tract of pigs and is considered a pathogen that can be transmitted from animals to humans, causing diseases such as bloodstream infections, meningitis, arthritis, endocarditis, and hearing loss, which in severe cases can lead to death. Every year, this causes massive losses to the world economy (1-3).

In the northern region, including Phayao Province, there is a tradition of consuming "Larb" and "Loo", which are made from raw pork, raw pig's blood, and uncooked pig organs. This consumption poses a high risk of contracting the *S. suis*, which may be contaminated in pork or pig organs. One of the significant outbreaks

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occurred in the 2007, in Phayao Province, there were a large number of patients due to the consumption of raw pork, which was reported as one of the largest community outbreaks in Thailand (4). Thailand has the second-highest reported cases of *S. suis* infection in the world, accounting for 11% of the total reported cases globally (5), according to data from the Department of Disease Control (2023), it was reported that there were more than 300 cases of *S. suis* infection nationwide. The northern region had the highest number of infections, accounting for 60-70% of all cases, with a mortality rate of approximately 10-12% among those infected. The majority of cases were caused by the consumption of raw or undercooked pork or pork products. Additionally, pig-related occupation is an important risk factor for *S. suis* infection in humans in Asian countries (6). Therefore, monitoring the contamination of *S. suis* in pork sold in high-risk areas is extremely necessary to prevent the spread of the pathogen from animals to humans.

S. suis can be classified into 29 serotypes based on the characteristics of the capsular polysaccharide (CPS) antigen, which plays a crucial role in the pathogen's virulence. The most common serotype in humans is serotype 2, which is the most frequently encountered in infections in human (93.4%) and the second most common serotype 14 (5.2%). Other serotypes include 24 (0.6%), 5 (0.4%), 4 (0.1%), 9 (0.1%), and 31 (0.1%) (3, 7-9). Therefore, identifying serotypes is crucial for assessing the risk of human infection, establishing surveillance guidelines, and effectively controlling the disease. Moreover, there is still a lack of comprehensive information regarding *S. suis* contamination in raw pork, especially in Phayao province, where frequent incidence of infections is observed. Thus, in this study we aimed to assess contamination of *S. suis* in pork and edible pork organ sold in local markets in Phayao province, Thailand. The findings are expected to provide comprehensive data on *S. suis* contamination in raw pork. They may encourage the development of policies that promote food safety to prevent and control this pathogen.

Materials and Methods

Sample collection and preparation

A total of 200 samples consisted of 60 raw pork samples (45 minced pork and 15 pork tenderloin), 34 fresh pig blood, and 106 edible pork organ samples (40 livers, 26 lungs, 29 hearts, and 11 pharynx) that were randomly purchased from different local markets located in Phayao province during April to June 2025. The retail markets in Phayao Province include Chiang Kham, Pong, Mae Chai, Dok Kham Tai, Chun, Phu Kamyao and Mueang Phayao Districts, with 1 to 2 shops per market. All these selected part and edible organ is popular for cooking “Larb”, “Loo” and a kind of Thai curry soup “Gaeng Orm”, Thai traditional dish commonly eaten in Phayao province. The samples were stored in a foam box filled with ice at a temperature of approximately 4 °C and transferred immediately to school of Allied Health Science, University of Phayao, Phayao, Thailand on the same day after purchasing. By following a previous study (1), each sample, consisting of 100 g, was cut into small pieces and then put in a sterile plastic bag and homogenized with 30 – 100 ml of 0.9% saline solution. Then, the samples were mixed using a stomacher machine or blender. The obtained solution was added to 5 ml of Todd-Hewitt broth supplemented with Streptococcus Selective Supplement (Himedia, India) and incubated at 37 ^{o}C for 18-24 hr.

DNA extraction and S. suis detection

According to the manufacturer’s instruction of PureDireX Genomic DNA Isolation Kit (Bio-Helix, Taiwan), bacterial DNA was extracted. The obtained genomic DNA was stored at -20 ^{o}C until used.

S. suis and *S. suis* serotypes 1, 2, 1/2 and 14 were detected using multiplex polymerase chain reaction (PCR). Based on the conserved region of *thrA* gene, all serotypes of *S. suis* were detected using forward and reverse primers thrA and generated a PCR product size of 120 bp as described previously (10). According to a previous study (3), a set of primers was used for *S. suis* serotypes 1, 2, 1/2 and 14 detections, including forward and

reverse primers cps1,14J for serotypes 1 and 14; forward and reverse primers cps2, 1/2J for serotypes 2 and 1/2; and forward and reverse primers for cps2, 14K for serotypes 2 and 14 detection. Based on the *cps* genes for serotype identification, serotype 1 showed PCR product size of 550 bp; serotype 1/2 showed PCR product size of 450 bp; serotype 2 showed two PCR product sizes of 450 bp and 209 and serotype 14 showed two PCR product sizes of 550 bp and 209 (**Table 1**).

The PCR reaction mixture contained 2x Dream Taq® Green PCR Master Mix (Thermo Scientific, USA), 1.25 µM for all *S. suis* specific primers, 0.125 µM for cps1,14J primers, 0.125 µM for cps2,1/2J primers, 0.5 µM for cps2,14K primers. The reaction mixture without a template was used as a negative control. Positive controls used in this study were bacterial DNA of *S. suis* serotype 1/2 (DMST26745) and serotype 2 (DMST18783). The multiplex PCR reaction was carried out at 95°C for 30 min, followed by 35 cycles of 95°C for 30 sec, 61°C for 1 min, 72°C for 1 min, and the final extension step at 72°C for 5 min.

Table 1. Primer sequences of multiplex PCR used in this study

Serotype	Primer	Sequences (5'-3')	Product size (bp)
1 and 14	cps1,14J	F: AATCATGGAATAAAGCGGAGTACAG R: ACAATTGATACGTCAAAATCCTCACC	550
2 and 1/2	cps2,1/2J	F: GATTTGTCGGGAGGGTTACTTG R: TAAATAATATGCCACTGTAGCGTCTC	450
2 and 14	cps2,14K	F: CTTTGTGGTGGCCTGG R: AATGGAAGCGATGGTCAG	209
All *S. suis* specific	thrA	F: GAAAATATGAAGAGCCATGTCG R: GACAACGAACATAACAGAAACTTC	120

The PCR products were analyzed on a 2.0 % agarose gel electrophoresis for 30 min in Tris-Acetate EDTA (TAE) buffer. The PCR products were stained with Novel Juice (Bio-HELIX, Taiwan) and visualized under UV light (Molecular Imager®Gel Doc™ XR System, Bio-Rad Laboratories, USA). The sizes of the PCR products were determined by comparison with a molecular size standard (GeneRuler 100bp DNA ladder, Thermo Fisher Scientific, Waltham, MA, USA).

Statistical Analysis

The prevalence of *S. suis* and *S. suis* serotypes 1, 2, 1/2 and 14 was calculated as percentages. By using GraphPad Prism Software version 10.3.1.509, the Chi-square test and pairwise comparisons with Bonferroni correction were used to determine if significant differences existed in positive samples between raw pork and edible organs throughout local marketplaces, and during the months of sample collection. A *p*-value of less than 0.05 was considered statistically significant.

Results

A total of 200 samples, comprising raw pork (tenderloins and minced pork) and edible organs (hearts, livers, and blood) were randomly collected from retail markets in Phayao province and analyzed for *S. suis* and *S. suis* serotype 1, 2, 1/2 and 14 using multiplex PCR method. *S. suis* was detected in 57.0 % (95% CI: 50.1-63.8) of the samples, with *S. suis* serotype 2 identified in 5.3 % (6 of 114, 95% CI: 2.4-11.0), as shown in **Table 2.** The results of this study revealed the absence of serotypes 1, 1/2, and 14, with 54.0 % (95% CI: 47.1-60.8) of the samples remaining untyped by the method used.

Table 2. Sample types used in this study and *S. suis* serotype detected.

Sample types	Total no. of samples	Total no. of *S. suis* serotypes detected (%)				
		1	1/2	2	14	Unknown
Minced pork	45	0	0	0	0	22 (48.9)
Pork tenderloin	15	0	0	1 (6.7)	0	9 (60.0)
Fresh blood	34	0	0	2 (5.9)	0	19 (55.9)
Liver	40	0	0	0	0	15 (37.5)
Lung	26	0	0	2 (7.7)	0	18 (69.2)
Heart	29	0	0	1 (3.4)	0	16 (55.2)
Pharynx	11	0	0	0	0	9 (81.8)
Total	200	0	0	6 (3.0)	0	108 (54.0)

Regarding the sample type in this study, the highest contamination rate of *S. suis* was detected in pharynx (81.8 %; 9 of 11, 95% CI:52.3-94.9) followed by lung (69.2 %; 18 of 26, 95% CI: 50.0-83.5), pork tenderloin (60.0 %; 9 of 15, 95% CI: 35.7-80.2), fresh blood (55.9 %; 19 of 34, 95% CI:39.5-71.1), heart (55.2 %; 16 of 29, 95% CI: 37.5-71.6), minced pork (48.9%; 22 of 45, 95% CI: 35.0-63.0) and liver (37.5%; 15 of 40, 95% CI: 24.2-53.0), respectively. For *S. suis* serotype analysis, the highest prevalence rate of *S. suis* serotype 2 was found at 7.7% (2 of 26, 95% CI: 2.1-24.1) in lungs followed by pork tenderloin (6.7%; 1 of 15, 95% CI: 1.2-29.8), fresh blood (5.9%; 2 of 34, 95% CI: 1.6-19.1) and heart (3.4%; 1 of 29, 95% CI: 0.6-17.2). The contamination rate of *S. suis* detected in each type of sample had significant statistical differences ($p = 0.018$), as shown in Figure 1.

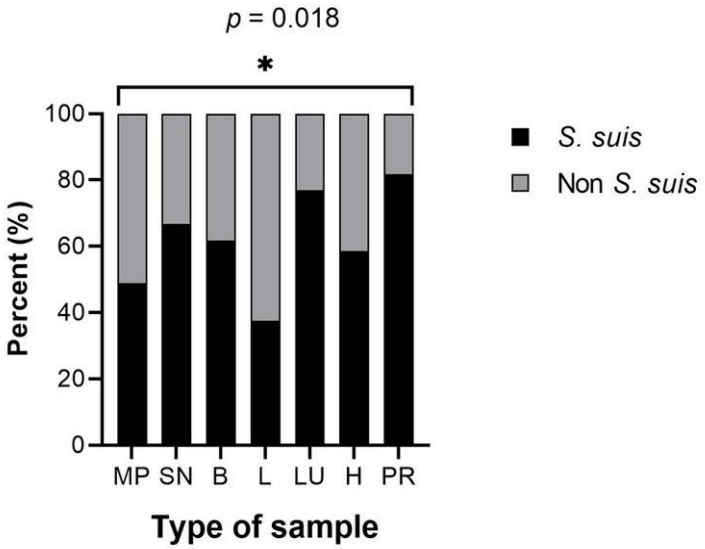

Fig. 1. Prevalence rate of *S. suis* and non *S. suis* detected in this study according to the sample types including minced pork (MP), pork tenderloin (SN), fresh blood (B), liver (L), lung (LU), heart (H), and pharynx (PR). * indicates a statistically significant result ($p < 0.05$).

There are 9 districts in Phayao province, including Chiang Kham, Pong, Mae Chai, Dok Kham Tai, Chun, Phu Kamyao, Phu Sang, Chiang Muan, and Mueang Phayao districts, and only 7 districts were in our study area. Regarding the sample collecting area, the highest prevalence rate of *S. suis* was detected at 80.0%, 95% CI:

37.6-96.4 from sample collected in the retail market at Phu Kamyao district, followed by Mae Chai (73.7%, 95% CI: 51.2-88.2), Chiang Kham (68.8%, 95% CI: 44.4-85.8), Chun (63.6%, 95% CI: 35.4-84.8), Dok Kham Tai (62.5%, 95% CI: 38.6-81.5), Mueang (51.2%, 95% CI: 42.6-59.7), and Pong (50.0%, 95% CI: 18.8-81.2), as shown in **Figure 2**. However, a significant difference between the markets was not found (p = 0.379). *S. suis* serotype 2 was found in fresh pig blood samples from the Dok Kham Tai district, where it was detected at a rate of 12.5%, 95% CI: 3.5-36.0. Whereas 3.1%, 95% CI: 1.2-7.8 of *S. suis* serotype 2 was found in heart, lung, and pork tenderloin samples from the Mueang district. The difference was not statistically significant (p = 0.346).

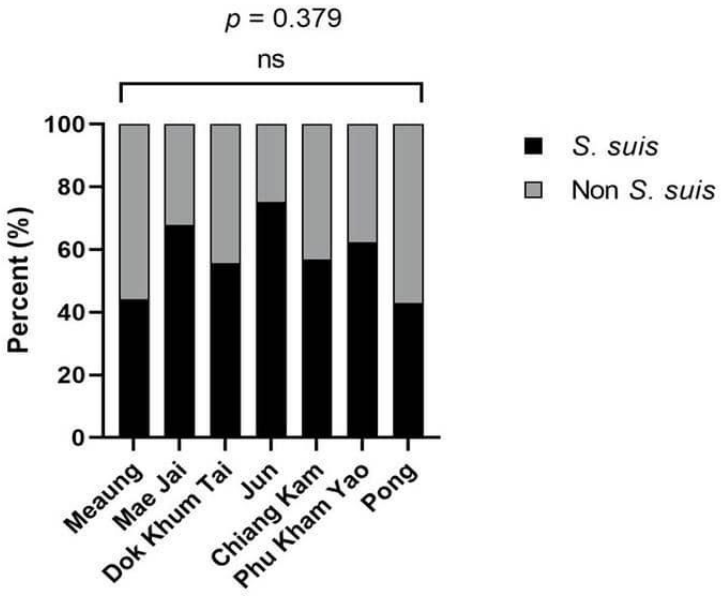

Fig. 2. Prevalence rate of *S. suis* and non *S. suis* detected in this study among 7 districts in Phayao province, the sample collecting area. There was no statistically significant result ($p < 0.05$), indicated as ns.

Discussion

In this study, all samples were collected from retail markets in 7 districts in Phayao province, the northern part of Thailand, including Chiang Kham, Pong, Mae Chai, Dok Kham Tai, Chun, Phu Kamyao and Mueang Phayao Districts. This study demonstrated a higher prevalence of *S. suis* compared to other findings from northeastern Thailand (11-12). Using a similar technique, Rattanadilok na Phuket et al. (12) reported *S. suis* in 12.5% of raw pork and 40.9% of fresh pig blood and visceral organs, respectively. Additionally, 12.8 % of *S. suis* serotype 2 was reported by Noppon et al. (11), with the highest prevalence found in fresh pig blood. A study by Wongnak et al. (13) identified *S. suis* in pork collected from traditional markets across 4 regions in Thailand (northern, northeastern, central and southern) and 4.2% of *S. suis* was found. However, the different techniques such as loop-mediated isothermal amplification assay (LAMP) have been used to detect the prevalence of *S. suis*. Several studies indicated that LAMP could be more sensitive than conventional and multiplex PCR (14-15); thus, it can detect high contamination rates. Using LAMP techniques, a recent study reported by Guntala et al. (1) revealed the prevalence rate at 84.0 % and 34.0 % contamination of *S. suis* and *S. suis* serotype 2, respectively in raw pork and edible pork organs collected from retail market in Chiang Mai province, northern Thailand. Furthermore, the high contamination rate of *S. suis* and *S. suis* serotype 2 was also reported at 85.2% and 17.1%, respectively, in raw pork samples collected in central Thailand (16). The prevalence of *S. suis* and *S. suis*

serotype 2 may vary depending on the locations, research periods, detection technologies, sample collecting strategies, and number of samples analyzed.

In our study, the contamination prevalence rate of *S. suis* and *S. suis* serotype 2 was high in the pharynx and lungs, respectively. The results were consistent with previous studies which have reported *S. suis* and *S. suis* serotype 2 in lungs and heart with high prevalence rate (1, 16). The agreement with earlier studies may reflect the known ecological niche of *S. suis* in pigs, particularly in the upper respiratory tract, including the tonsils and nasal cavities (7). Given this natural localization, detecting *S. suis* in the pharynx is biologically plausible and may be more likely due to direct colonization than to incidental contamination alone. By contrast, the detection of *S. suis* in internal organs such as the lungs and heart may suggest either systemic dissemination in infected or carrier animals or contamination occurring during slaughter and subsequent handling processes. The differences in contamination prevalence among organs may therefore be explained by a combination of biological and processing-related factors. From a biological perspective, *S. suis* can persist in carrier pigs and disseminate via the bloodstream, allowing the organism to be present in multiple tissues (7, 17). This suggested that the *S. suis* can survive in blood and invade in multiple organs and results in cross-contamination between pig organs and meat through post slaughter processes (1, 18). The high prevalence of contamination may be due to poor sanitation and hygiene through slaughtering, meat cutting, meat handling and further in local market. Thus, it is possible that meat and pig organs are major sources of *S. suis* causing human infection, and people who work in close contact with raw pork and pig organs.

This study found a moderate and low prevalence rate of *S. suis* and *S. suis* serotype 2, around 6.0 %, attributed to the sample collecting period from April to June, the summer season and the beginning of rainy season. This is inconsistent with a study in Chiang Mai province which indicated high and stable prevalence of *S. suis* contamination during May to July (1). It is also only partly consistent with epidemiological data from Thailand showing that the incidence of human *S. suis* infection tends to increase during the rainy season, particularly from June to September (19). A previous study showed a high incidence of *S. suis* infection during June to September (20). Similarly, a report from Chiang Mai University Hospital documented a high number of *S. suis* admission cases during both the summer period (April–June) and the rainy season (August–October) (21). In contrast, a study conducted in Phayao province, Thailand, found a high incidence of *S. suis* infection associated with the consumption of raw pork products during the summer months of April to May (22). These differences suggest that the temporal pattern observed in the present study may not be explained by season alone. One possible explanation is that seasonal trends in human infection do not necessarily correspond directly to contamination prevalence in pork and pig organs. During the rainy seasons, pigs may experience more stress during transportation due to hot and muggy conditions, which might raise the risk of illness. Additionally, these circumstances could allow the organism to multiply and become more contagious when exposed to meat (6). In Thailand and other Southeast Asian countries, the infection cases are connected to individuals consuming traditional meals consisting of raw pork, fresh blood, and other related products (19). Moreover, differences among studies may reflect variation in geographic location, sample sources, slaughter and market hygiene, study period, and laboratory methods. Therefore, the lower prevalence observed here may result from a combination of seasonal, methodological, and local environmental factors. One of the current prevention strategies for human *S. suis* infections is largely behavioral and environmental, as no human vaccine is currently available. Good personal hygiene, including frequent handwashing and wearing gloves when handling pigs or raw pork to avoid direct contact, such as thorough cooking of pork and separating raw from cooked products, are also critical. Therefore, this finding suggested that a reduction in human *S. suis* infection cases in Thailand requires effective regulations concerning meat inspection as well as food safety campaigns on hygiene practices in pork processing and preventing the infection.

This study aimed to determine the prevalence of *S. suis*, with a specific focus on serotypes 1, 1/2, 2, and 14. However, a noteworthy limitation is that a definitive serotype could not be assigned to 54% of the collected samples. Moreover, it should be noted that, in this study, positive control used was limited to only DNA extracted from bacteria culture of *S. suis* serotype 1/2 and 2. This is because of the limitation in bacterial transfer between laboratories and the difficulty of cultivation. Some other limitations, including a small sample size and short study period. Therefore, the study period should be extended to encompass all seasons in Thailand to ensure comprehensive temporal data. Moreover, the relatively small number of pharynx samples (n = 11) may have limited the statistical robustness of this group. This was primarily due to the lower retail availability of the pharynx, as it is less commonly consumed than other pig organs. A significant limitation of the current study was its sampling methodology; samples were collected from only one or two retail shops per district, rather than from all available markets. Future study collecting samples from all retail markets in each district could achieve representative coverage of Phayao province, this would enable a more accurate determination of the true contamination rate throughout the province. To prevent disease transmission, the combination of measures should be focused. The continuing *S. suis* surveillance may imply the awareness of consuming raw pork and related products and indicate the successful of food safety campaign in Thailand. In addition, more concrete public health measures should be considered, including mandatory glove use during the handling of raw pork and pig organs, hygiene training or certification programs for pork vendors and meat handlers, and targeted educational campaigns for high-risk groups, particularly those involved in pork processing and those who consume raw pork or fresh blood products. Strengthening meat inspection and food safety monitoring at slaughterhouses and retail markets may also help reduce the risk of contamination and subsequent human infection.

Conclusion

In conclusion, our study revealed the moderate prevalence of *S. suis* and *S. suis* serotype 2 found in raw pork, fresh pig blood, and edible organs used for raw consumption in northern Thai traditional dishes. The finding highlights a critical public health threat. The contamination rate indicates a substantial risk of infection for associated individuals, including pig farm and slaughterhouse workers, vendors, and consumers of uncooked pork products. Therefore, it is imperative to implement safety campaigns to promote awareness of the occupational and dietary risks associated with *S. suis* infection. Continuous public education, emphasizing the dangers of consuming uncooked pork meat and organs, is crucial. Furthermore, regulations ensuring the hygienic handling and sale of pork products must be enforced to prevent pathogen transmission to consumers and workers throughout the production chain and to facilitate disease control within Phayao province.

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Conflict of Interest

The authors declare no conflicts of interest.

Ethical approval

Not applicable.

Artificial Intelligence Statement

The authors declare that we have not used any Artificial Intelligence (AI) tools in the creation of this article.

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