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Prevalence and Antimicrobial Susceptibility of *Streptococcus suis* Isolated from Slaughter Pigs in Northern Thailand

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ABSTRACT

Streptococcus suis is an important zoonotic agent globally, including Thailand, especially in the northern part, where periodic outbreaks have occurred recently. The aims of this study were to determine the prevalence and antimicrobial susceptibility of *S. suis* isolated from slaughter pigs in northern Thailand. A total of 125 salivary glands and 116 tonsillar swabs were collected from 190 slaughter pigs in six slaughter houses during the summer of 2008. In the winter of 2009, 117 salivary glands and 115 tonsillar swabs were collected from 193 slaughter pigs at the same processing plants. Samples were cultured out on Columbia blood agar and the suspected colonies were confirmed by conventional biochemical tests. The prevalence of *S. suis* isolated from healthy slaughter pigs in northern Thailand sampled in summer and winter was 20% (38/190) and 7.3% (14/193), respectively. Antimicrobial susceptibility testing indicated that the most effective antimicrobial drugs against *S. suis* were ceftiofur (96%), ampicillin (73%) and amoxicillin (69%), respectively.
Keywords: *Streptococcus suis*, slaughter pigs, prevalence, antimicrobial drug, northern Thailand

INTRODUCTION

Streptococcus suis, a Gram-positive facultative anaerobic bacterium, is known as an important zoonotic agent in northern Thailand, where periodic outbreaks have occurred recently. This microorganism can be isolated from the upper respiratory tract, especially the tonsil and nasal cavity, and from the genital organs and the gastrointestinal tract of healthy pigs (Gottschalk and Segura, 2000). Wisselink *et al*. (1999) reported that carrier rates within herds varied from 0 to 100% and there was no relationship between the carrier rate and disease level. Moreover, healthy carrier pigs represented potential sources of infection to herds or to human beings (Breton *et al*., 1986).

Aside from outbreaks of *S. suis* infection causing heavy economic losses in pig production, there is the additional risk of severe illness to humans. Disease transmission to humans might occur through wounds on skin, via mucous membrane or by ingestion (WHO, 2005). The clinical signs in human infection are meningitis,

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septicemia, septic shock, arthritis, endocarditis, pneumonia and peritonitis (Lun *et al*., 2007). According to the capsular polysaccharides, 33 serotypes of *S. suis* have been identified. Moreover, serotype 2 was associated most frequently with infected pigs and patients (Gottschalk, 2004).

Due to the antigenic variation, there are currently no effective vaccines against *S. suis* available for the swine industry (Zhang *et al*., 2008). Therefore, antimicrobial drugs have been applied intensively for therapeutic and preventive purposes. In addition, previous studies demonstrated that *S. suis* strains isolated from infected or healthy pigs were resistant often to commonly used antimicrobial agents (Vela *et al*., 2005; Wisselink *et al*., 2006; Zhang *et al*., 2008).

The prevalence and antimicrobial susceptibility patterns of *S. suis* recovered from healthy pigs are important factors for controlling the outbreak of *S. suis*. However, this information has been limited in endemic areas, such as the northern part of Thailand. The objectives of this study were to determine the prevalence and antimicrobial susceptibility of *S. suis* isolated from slaughter pigs in the northern part of Thailand.

MATERIALS AND METHODS

Specimens

The first sampling was performed in March 2008, with a total of 125 salivary glands and 116 tonsillar swabs collected from 190 pigs in six slaughter houses in the five northern Thai provinces of Phitsanulok, Sukhothai, Lamphun, Chiang Mai and Chiang Rai. The second sampling was done in January 2009, with 117 salivary glands and 115 tonsillar swabs collected from 193 pigs at the same slaughterhouses. The former and the latter sampling times were assumed to represent the summer and winter season, respectively. The swabbing of palatine tonsils used the methods described by Breton *et al*. (1986). An aseptic technique was used for all specimen collection procedures. The swabs were kept in Stuart transport medium (Britania, Argentina). All specimens were transported to the laboratory at 4-8°C within 24-48 h of collection.

Bacterial isolates

The specimens were inoculated onto Columbia agar plates (Oxoid, UK), supplemented with 5% sheep blood and incubated at 37°C for 24-48 h. Two to three colonies, which did not exceed 1 mm in diameter and exhibited alpha hemolysis, were picked from each plate to subculture on Columbia blood agar plates and were incubated in the same manner. Suspected colonies, which were Gram-positive cocci and negative for the catalase test, were selected for further screening with 11 different conventional biochemical tests (Han *et al*., 2001). Isolates were identified as *S. suis* species when they were negative for the arginine dihydrolase and Voges-Proskauer tests. Isolates were able to produce acid from trehalose, lactose, sucrose, salicin and inulin, but none of them could produce acid from glycerol, sorbitol and mannitol. They were also unable to grow in 6.5% NaCl solution.

Antimicrobial susceptibility test

Disk diffusion susceptibility was tested, according to the Clinical and Laboratory Standards Institute (CLSI, 2008) guidelines. The procedure was carried out on Mueller Hinton agar (Becton Dickinson, USA) supplemented with 5% sheep blood. The antimicrobial agents applied in this trial were amoxicillin (25μg/disk), penicillin G (10 unit/disk), ampicillin (10μg/disk), ceftiofur (30μg/disk), lincomycin (2μg/disk), tetracycline (30μg/disk), enrofloxacin (5μg/disk) and trimethoprim-sulfamethoxazole 1:19 (25μg/disk) (Oxoid, UK). The inhibition zone diameters were measured and classified (Table 1).

RESULTS

A total of 241 specimens from the summer sampling were analyzed. Thirty-eight isolates (15.8%) were positive for *S. suis*. The numbers of *S. suis* isolated from tonsillar swabs and salivary glands were 29 (25%) and 9 (7.2%), respectively. In the winter sampling, 14 isolates (6.0%) were recovered from 232 specimens. There were 11 (9.6%) isolates from tonsillar swabs, while the number of isolates from salivary glands was 3 (2.6%). The numbers and percentages of isolates classified by province are shown in Table 2. The prevalence of *S. suis* isolated from healthy slaughter pigs in the northern part of Thailand in summer and winter was 20% (38/190) and 7.3% (14/193), respectively.

An antimicrobial susceptibility test was

Table 1 Interpretation of inhibition zone size.*

Antibiotic disk (μg/disc)	Zone diameter (mm.)		
	Resistant	Intermediated	Susceptible
Amoxicillin (25μg/disk)	≤ 18	19 - 25	≥ 26
Penicillin G (10 unit/disk)	≤ 19	20 - 27	≥ 28
Ampicillin (10μg/disk)	≤ 18	19 - 25	≥ 26
Ceftiofur (30μg/disk)	≤ 17	18 - 20	≥ 21
Lincomycin (2μg/disk)	≤ 15	16 - 18	≥ 19
Tetracycline (30μg/disk)	≤ 18	19 - 22	≥ 23
Enrofloxacin (5μg/disk)	≤ 16	17 - 22	≥ 23
Trimethoprim-sulfamethoxazole (25μg/disk)	≤ 16	16 - 18	≥ 19

* = modified from CLSI (2008).

Table 2 Numbers and percentages of *S. suis* isolates.

Province	Specimen	Numbers of specimens		Numbers of isolates		Percentage of isolated Specimens (%)	
		Summer	Winter	Summer	Winter	Summer	Winter
Phitsanulok	Gland	30	30	2	2	6.7	6.7
	Swab	15	30	0	3	0	10
	Total	**45**	**60**	**2**	**5**	**4.4**	**8.3**
Sukhothai	Gland	16	21	1	0	6.3	0
	Swab	15	21	8	3	53.3	14.3
	Total	**31**	**42**	**9**	**3**	**29**	**7.1**
Lamphun	Gland	4	4	0	0	0	0
	Swab	5	6	1	1	20	16.7
	Total	**9**	**10**	**1**	**1**	**11.1**	**10**
Chiang Mai	Gland	40	42	4	1	10	2.4
	Swab	32	40	9	0	28.1	0
	Total	**72**	**82**	**13**	**1**	**18.1**	**1.2**
Chiang Rai	Gland	35	20	2	0	5.7	0
	Swab	49	24	11	4	22.5	16.7
	Total	**84**	**44**	**13**	**4**	**15.5**	**9.1**

carried out on a total of 52 isolates. The most effective antimicrobial drugs against *S. suis* were ceftiofur, ampicillin and amoxicillin, with 96%, 73% and 69%, respectively, of the isolated strains being susceptible (Table 3). In addition, none of the isolates was susceptible to lincomycin, and 96% were resistant to tetracycline (Table 3).

DISCUSSION

The number of isolates from summer sampling was more than the number of isolates from winter sampling in almost all provinces. Similarly, Ma *et al.* (2008) found that in Hong Kong, more *S. suis* infected human cases occurred in summer, with the severe outbreaks in China reported also during summer. It is possible that pigs might become stressed during summer and the *S. suis* pathogen could survive and grow well under this condition. This suggests that the high carrier rate in pigs during summer leads to an increase in the chance of human infection.

Among the 33 capsular serotypes that have been described, serotype 2 is frequently recovered from infected animals and it is also correlated with human diseases (Higgins and Gottschalk, 2001; Greeff *et al.*, 2002; Madsen *et al.* 2002; Marois *et al.*, 2004; Wongsawan *et al.*, 2006). However, the identification methods used in the present study (the morphology and biochemical tests) were unable to identify the strains of the isolates. Therefore, capsular serotyping and a molecular technique, such as polymerase chain reaction (PCR), would be useful for further study to determine the disease potential of the isolated strains.

The results of the antimicrobial susceptibility test indicated that almost all isolates were susceptible to ceftiofur, which is a third-generation cepharosporin. Aarestrup *et al.* (1998a), Ruzauskas *et al.* (2006) and Wisselink *et al.* (2006) indicated that treatment of *S. suis* infection with ceftiofur was effective still. On the other hand, *S. suis* showed the highest resistance to lincomycin (100%) and tetracycline (96%). These results were consistent with many other studies that described *S. suis* as being resistant to these antimicrobial agents (Martel *et al.*, 2001; Burch, 2005; Ruzauskas *et al.*, 2006). The high rates of resistance to macrolides/lincosamides and to tetracycline might be explained by the intensive use of tylosin as a growth promoter and tetracycline as a therapeutic in pig production (Aarestrup *et al.*, 1998b).

In conclusion, the prevalence of *S. suis* isolated from slaughter pigs in the northern part of Thailand was 13.6% (52 / 383), which suggests that healthy carrier pigs might spread *S. suis* on the slaughter line. Moreover, pig carcasses may be contaminated with *S. suis*, which is a potential hazard for slaughterhouse workers, butchers and consumers. The study by Ip *et al.* (2007) reported that *S. suis* was isolated from 6.1% of the meat pork from 3 of 6 markets in Hong Kong. This result

Table 3 Antimicrobial susceptibility test of *S. suis* isolates.

Antimicrobial agent	Resistant strains	Intermediate strains	Susceptible strains
Amoxicillin	13 (25%)	3 (5.8%)	36 (69%)
Penicillin G	14 (27%)	6 (12%)	32 (62%)
Ampicillin	12 (23%)	2 (3.9%)	38 (73%)
Ceftiofur	0 (0%)	2 (3.9%)	50 (96%)
Lincomycin	52 (100%)	0 (0%)	0 (0%)
Tetracycline	50 (96%)	0 (0%)	2 (3.9%)
Enrofloxacin	11 (21%)	10 (19%)	31 (60%)
Trimethoprim-sulfamethoxazole	19 (37%)	9 (17%)	24 (46%)

confirmed that *S. suis* is a potential biological hazard for both consumers and workers in slaughterhouses.

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