Streptococcus suis serotype 2 in Uncooked Pork Meat Products in Khon Kaen, Northeastern Thailand, and their Antimicrobial Profiles

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Abstract— To isolate *Streptococcus suis* serotype 2 from minced pork, pork meat, fresh pig blood, liver and other offal, 320 samples were collected from 8 sub-districts in Khon Kaen province from January to May, 2013. Isolation was done by direct plating technique using selective media with antibiotic supplement. *S. suis* serotype 2 isolates were examined to species level by multiplex PCR. Forty one isolates were identified as *S. suis* serotype 2. Prevalent rate did not depend on areas ($p>0.05$). Overall prevalent rate was moderate at 12.8%. The occurrence in fresh pig blood were highly significant different ($p<0.01$) from other tissue types, and heavily contaminated at 24.6%. Minced pork samples, were least contaminated at 2.7%. This is the first report in fresh pork and pork related samples in northeastern Thailand. Traditional custom of consuming raw pork dishes which included fresh pig blood were normally practiced among Thai popular in the northeastern region. Therefore, control measure must be strengthened. In addition, antimicrobial susceptibilities of the isolates were tested using disk diffusion technique. Inhibitory zone diameters in millimeter for amoxicillin (AML), cephalexin (CL), gentamycin (CN), penicillin G (P), and tetracycline (TE) were measured. It is noted that CN is the most susceptible drug (93.6%) compared with 4 other drugs tested against *S. suis* serotype 2, followed by AML (90.3%), CL (67.7%), P (51.6%), and TE (16.3-35.5%) by in vitro technique. There was significant different between resistance and non-resistance ($p<0.01$) where majority of the isolates were susceptible to the tested drugs.

Index Terms— antimicrobial, multiplex PCR, northeastern Thailand, pork, resistance, *Streptococcus suis* serotype 2

1 Introduction

Streptococcus suis is a gram-positive cocci, its infection in pigs is reported worldwide, from North America to South America, Europe, Asia, Australia, and New Zealand which causes problems including mortality in humans consuming infected products [1]. Healthy pigs can carry multiple serotypes, but serotype 2 is the most important for humans [2], [3], [4]. An outbreak of *S. suis* infection occurred in Sichuan Province, China, during July and August 2005, of which involved 215 cases and 38 deaths [5]. A large series of 151 *S. suis* serotype 2 meningitis cases was found in southern Vietnam over a 10-year period [6]. Human *S. suis* infections are most often reported from countries where pig-rearing is common, this included Thailand where there is a high density of pig farms. In Thailand, infections are sporadic, case fatality rate for adults were 9.5%, primarily in northern Thailand [7], and 16.1% in Phayao Province in 2012 in northern Thailand [4]. This showed an increasing trend of the incidence in northern Thailand. An outbreak of *S. suis* infection including 29 laboratory confirmed cases occurred in the Phu Sang District, Phayao Province, in northern Thailand in April and May, 2007 [8]. Specimens used in most researches were live animals, their viscera, and discharges such as tonsils, livers, and spinal fluids. However, there is little information available regarding the situation in the northeast of Thailand, in terms of prevalence of the infection in products consumed by humans. Therefore, the present study was carried out on the basis of capsular polysaccharides, 35 serotypes of *S. suis* have been identified [9], [10]. Of these, serotype 2 is the most prevalent type in humans infected with this pathogen [2], [11]. Molecular techniques with a *S. suis* serotype 2-specific PCR have been developed, and improved the detection of *S. suis* cases in Asia with the cps2J gene as a target gene [6], [12]. Since this is readily available, and accurate, we applied this approach here. Minced pork, pork meat, fresh pig blood, liver and other offal were chosen as samples since these are commonly consumed. It is well established that strains of *Streptococcus* spp. can become resistant to antimicrobial drugs such as tetracycline, erythromycin, and chloramphenicol [6]. Penicillin resistance has been reported in a single human case, and in some pig isolates [13], [14]. In order to determine the sensitivity of the *S. suis* serotype 2 isolated from samples in Khon Kaen, northeastern Thailand, we also in the present study conducted a test of five drugs i.e. amoxicillin (AML, Oxoid), cephalexin (CL, Oxoid), gentamycin (CN, Oxoid), penicillin G (P, Oxoid), and tetracycline (TE, Oxoid) using disk diffusion technique [15], [16], [17]. In order to avoid possible bias between sites, we selected 8 different locations where products were on sale.

2 Materials and Methods

2.1 Isolations of *Streptococcus* spp.

Streptococcus suis serotype 2 was isolated from minced pork, pork meat, fresh blood, liver and other offal. In total, 320 samples were collected from 8 sub-districts in Khon Kaen province from January to May, 2013. Samples were streaked onto the selective media with antibiotic supplement to suppress other

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bacteria by direct plating technique onto Blood Agar plates. Polymyxin B sulphate (Oxoid, Hampshire, UK), and nalidixic acid sodium salt (Oxoid) were incorporated into the media to inhibit the growth of other bacteria.

2.2 Species identification by multiplex PCR

Streptococcus suis serotype 2 isolates were examined to species level using multiplex PCR. The primer pairs (Thermo Scientific, Lithuania) are used i.e. 16s-195(s) (CAG TAT TTA CCG CAT GGT AGA TAT), 16s-489 (as2) (GTA AGA TAC CGT CAA GTG AGA A), cps2J-s (GTT GAG TCC TTA TAC ACC TGT T), cps2J-as (CAG AAA ATT CAT ATT GTC CAC C), CI6-s (GTT GAG TCC TTA TAC ACC TGT TAC TCA GTG CCG CAG CTA ACG CAT T), and CI6-as (CAG AAA ATT CAT ATT GTC CAC CCG ACT TCA CCC CAA TCA TCT ATC C), respectively.

2.3 PCR master mix for 1 reaction

Comprised of DDW, 3.6 µl; 2 X Phire Plant PCR buffer, 10.0 µl; Primer 16s-195(s), Primer 16s-489 (as2), Primer cps2J-s, Primer cps2J-as, Primer CI6-s, Primer CI6-as, at 1.0 µl each; Phire Hot Start II DNA polymerase, 0.4 µl; and DNA template, 5.0 µl, respectively. Total volume of PCR mixture was 25 µl.

2.4 PCR conditions

It started with denaturation of 98°C, 5 min; and of 98°C, 1 s; 72°C, 20 s; 72°C, 1 min for 40 cycles, then 4°C, ∞, respectively.

2.5 Preparation of the inoculums

Bacterial culture was standardized to McFarland Standard No. 0.5 using sterile 0.9% (w/v) NaCl which was equivalent to 10^8 CFU/ml.

2.6 Antimicrobial susceptibility test

Disc diffusion method was employed in this study followed that of CLSI [15], [16] and EUCAST [17]. The 5 selected antimicrobials were amoxicillin, 10 µg/ml (AML, Oxoid); cephalexin, 30 µg/ml (CL, Oxoid); gentamycin, 10 µg/ml (CN, Oxoid); penicillin G, 10 µg/ml (P, Oxoid), and tetracycline, 30 µg/ml (TE, Oxoid).

3 RESULTS

Results indicated that 41 isolates were identified as *Streptococcus suis* serotype 2 from 320 samples examined. The prevalent rate was 12.8% (Table 1) and the remainders were other Streptococcus spp. of which not indicated as major strain causing illness in most countries.

TABLE 1
OCCURRENCES OF *STREPTOCOCCUS SUIS* SEROTYPE 2 IN PORK MEAT, BLOOD AND OFFAL COLLECTED FROM KHON KAEN PROVINCE DURING JANUARY TO MAY, 2013

Areas (Code)	Total no. samples	Total no. of *Streptococcus suis* serotype 2 founded (%)
Old market	25	3 (12.0)
Aor Jira	16	5 (31.3)
Bang Lumpoo	55	5 (9.1)
Chonabot	15	0 (0.0)
Ban Phai	42	3 (7.1)
Munjakiri	25	3 (12.0)
Nam pong	32	5 (15.6)
Ubonrat	33	5 (15.2)
Somsoong	19	2 (10.5)
Kranuan	36	8 (22.2)
Superstore 1	8	0 (0.0)
Superstore 2	14	2 (14.3)
Total	320	41 (12.8)

Note: p>0.05

There was no significant different of the prevalent rates between the 8 areas (p>0.05). Fresh pig blood samples were heavily contaminated at 24.6%, and significant different were found between each meat type (p< 0.01). In addition, minced pork samples were least contaminated at 2.7% (Table 2). Antimicrobial susceptibility test among 5 drugs were tested using disk diffusion technique. Among 31 isolates available for the drug test, inhibition zone diameters for AML, CL, CN, P, and TE were in the range of 6.0 - 46.0, 6.0 - 47.0, 13.5 - 39.0, 6.0 - 47.0, and 6.0 - 40.0 mm, respectively. There were significant different in inhibitory zone diameters among the 5 tested antibiotics (p<0.01). CN was the most susceptible drug (93.6%) compared with 4 other drugs tested with *S. suis* serotype 2. More information is provided in Table 3.

TABLE 2
PREVALENCE OF *STREPTOCOCCUS SUIS* SEROTYPE 2 BY MEAT TYPES

Meat types	No. of sample collected	Prevalence of *Streptococcus suis* serotype 2
Minced pork meat	75	2 (2.7)
Fresh pork meat	93	10 (10.8)
Fresh pig blood	61	15 (24.6)
Liver and other offal	91	14 (15.4)
Total	320	41 (12.8)

Note: p<0.01

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TABLE 3
ANTIBIOTIC RESISTANCE PROFILES OF *STREPTOCOCCUS SUIS* SEROTYPE 2 CATEGORIZING INTO RESISTANT, INTERMEDIATE, AND SUSCEPTIBLE AMONG THE 5 DRUGS

	Antibiotic resistance profile of *Streptococcus suis* serotype 2 n (%)			References
Drug conc.	Resistance	Intermediate	Susceptible	EUCAST, 2013; CLSI, 2006, 2013
AML 10 µg/ml	2 (6.5)	1 (3.2)	28 (90.3)	*Salmonella* and *E. coli*
CL 30 µg/ml	9 (29.0)	1 (3.2)	21 (67.7)	*Streptococcus pneumonia*
CN 10 µg/ml	0 (0.0)	2 (6.5)	29 (93.6)	*Salmonella* and *E. coli*
P 10 µg/ml	2 (6.5)	11 (35.5)	16 (51.6)	*Salmonella* and *E. coli*
TE 30 µg/ml	5 (16.1)	15 (48.4)	11 (35.5)	For Enterobacteriaaceae
	20 (64.5)	6 (19.4)	5 (16.1)	For most bacteria

Note: conc. = concentration per disc; p<0.01

4 CONCLUSION

The occurrence of *Streptococcus suis* serotype 2 was found in pork and pork related samples especially pig blood in Khon Kaen province, northeastern Thailand. Locals must be educated for the safe consumption, especially pig blood where most people added fresh blood in their meals. Gentamycin may still be the drug of choice as indicated in the in vitro study

5 DISCUSSIONS

Present results showed that the prevalence is intermediate, at total of 12.8%. But varying greatly with the location (from 0.0-31.0%), and the type of sample (minced pork 2.7 to pig blood with 24.6%). These figures are the first for northeastern Thailand, but are similar to a study in Hong Kong where *S. suis* serotype 2 was isolated from 6.1% of raw pork meat from 3 of the 6 wet markets [18] compared to 10.8% from 8 locations (6 wet markets and 2 superstores) in Khon Kaen, northeastern Thailand in the present study. In Vietnam, pork is the most important meat source, with more than 98.0% of households consuming pork, and they prefer to buy fresh pork from wet markets [19]. Mapping of pig density and human *S. suis* cases clearly suggests where S. suis is likely to be present, but, thus far, has not been illucidated. The study in Hong Kong is in accordance with the present study in that, fresh pig blood samples in Khon Kaen, northeastern Thailand were heavily contaminated (24.6%) with *S. suis* serotype 2. In addition, a study in Phayao Province, northern Thailand revealed that a major route of transmission during this outbreak was the consumption of raw pig blood [8]. The first study here in Khon Kaen Province, northeastern Thailand confirmed that fresh raw pig blood may act as a major source for *S. suis* serotype 2 in the human infection. The reason behind this may be attributable to the fact that raw fresh pig blood from individual pig at slaughter are usually mixed together in one container. Therefore, after locally distributed to various wet markets in the area, this practice benefits the wider dissemination or contamination of the uncontaminated fresh blood from other pigs. From the observation, some butchers shared the same bucket of fresh pig blood in the small scale wet market. This unsafe practice needs further monitoring and evaluation by the authority in charge. It is likely that, besides occupational exposure, processing or consuming uncooked or partially cooked pork products is also a risk factor for infection. Local delicacies, such as undercooked pig tonsils, intestines, or uterus and fresh pig blood, may also represent important sources of infection. Hearing loss in *S. suis* meningitis is sensineural, is in the high frequency range [18].

The principles of treatment are the same as those for other causes of bacterial meningitis. For empirical treatment, ceftriaxone with or without vancomycin (depending on the local epidemiology of bacterial meningitis and drug resistance) is a good choice until the diagnosis is laboratory confirmed. The same treatment dose and duration that is used for pneumococcal meningitis is also recommended for *S. suis* meningitis i.e. ceftriaxone (2 g every 12 h for 14 days for adults). This has achieved a high cure rate of 97.0% [20], [21]. Penicillin G (24 million U over 24 h for at least 10 days) has been used successfully for the treatment of *S. suis* meningitis [22]. Nonetheless, 13 years later, it was noted from the present study that penicillin G is now approaching 50.0% resistance by in vitro study. Cephalexin may still be prescribed to *S. suis* serotype 2 cases but with close observation according to the present findings. Nevertheless, gentamycin may still be the drug of choices. Since *S. suis* meningitis is one of the most common causes of adult meningitis in China, northern Thailand, and as well as in Vietnam. Increased awareness of both clinicians and microbiologists is needed to fully appreciate the importance of *S. suis* serotype 2 as a human pathogen. The recommendation for further study is to assess human exposure and outcomes, including treatment options.

In conclusion, this is the first report of the occurrence of *S. suis* serotype 2 in pork and pork related samples especially raw pig blood in Khon Kaen province, northeastern Thailand. Locals must be educated for the safe consumption, especially raw pig blood where most people added fresh in their meals. Gentamycin may still be the drug of choice as indicated in the in vitro study.

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REFERENCES

[1] J.J. Staats, I. Feder, O. Okwumabua and M.M. Chengappa, "*Streptococcus suis*: past and present," *Vet Res Commun*, vol. 21, no. 6, pp. 381-407, Aug. 1997.

[2] Z.R. Lun, Q.P. Wang, X.G. Chen, A.X. Li and X.Q. Zhu, "*Streptococcus suis*: an emerging zoonotic pathogen," *Lancet Infect Dis*, vol. 7, no. 3, pp. 201–209, Mar. 2007.

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[3] C. Suankratay, P. Intalapaporn, P. Nunthapisud, K. Arunyingmongkol and H. Wilde, "*Streptococcus suis* meningitis in Thailand," *Southeast Asian J Trop Med Public Health*, vol. 35, no. 4, pp. 868-876, Dec. 2004.

[4] D. Takeuchi, A. Kerdsin, A. Pienpringam, P. Loetthong, S. Samerchea, P. Luangsuk, et al., "Population--Based Study of *Streptococcus suis* Infection in Humans in Phayao Province in Northern Thailand," *PLoS One*, vol. 7, no. 2, e31265, pp. 1-7, Feb 2012.

[5] H. Yu, H. Jing, Z. Chen, H. Zheng, X. Zhu, H. Wang, et al., "Human *Streptococcus suis* Outbreak, Sichuan, China," *Emerg Infect Dis*, vol. 12, no. 6, pp. 914-920, Jun. 2006.

[6] N.T. Mai, N.T. Hoa, T.V. Nga, et al., "*Streptococcus suis* meningitis in adults in Vietnam" *Clin Infect Dis*, vol. 46, no. 5, pp. 659-667, Mar. 2008.

[7] A. Kerdsin, S. Dejsirilert, P. Puangpatra, S. Sripakdee, K. Chumla, N. Boonkerd, et al., "Genotypic Profile of *Streptococcus suis* serotype 2 and Clinical Features of Infection in Humans, Thailand," *Emerg Infect Dis,* vol. 17, no. 5, pp. 835-842, May. 2011.

[8] N. Khadthasrima, T. Hannwong, P. Thammawitjaya, D. Pingsusean, et al., "Human *Streptococcus suis* outbreak in Phayao Province, Thailand," *OSIR*, http://www.osirjournal.net/upload/files/2_Streptococcus_suis_new.pdf. 2008.

[9] M.M. Gottschalk, M. Segura and J. Xu, "*Streptococcus suis* infections in humans: the Chinese experience, and the situation in North America," *Ani Health Res Rev*, vol.8, no. 1, pp. 29-45, Jun. 2007.

[10] H.F.L. Wertheim, H.D.T. Nghia, W. Taylor and C. Schultsz, "*Streptococcus suis*: An Emerging Human pathogen," *Clin Infect Dis*, vol. 48, no. 5, pp. 617-625, Mar. 2009, 48.

[11] J.E. Hill, M. Gottschalk, R. Brousseau, J. Harel, S.M. Hemmingsen, and S.H. Goh, "Biochemical analysis, cpn60 and 16S rDNA sequence data indicate that *Streptococcus suis* serotypes 32 and 34, isolated from pigs, are *Streptococcus orisratti*," *Vet. Microbiol*, vol. 107, no. 1-2, pp. 63–69, Apr. 2005.

[12] H. Matsuo and S. Sakamoto, "Purulent meningitis caused by *Streptococcus suis* in a pig breeder," *J Infect Dis,* vol. 77, no. 5, pp. 340-342, May 2003.

[13] J.M. Sheerson, B. Chattopadhyay, M.F.G. Murphy and I.W. Fawcett, "Permanent perceptive deafness due to *Streptococcus suis* type-II infection" *J Laryngol Otol*, vol.94, no. 4, pp. 425-427, Apr. 1980.

[14] J. Marie, H. Morvan, F. Berthelot-Herault, et al., "Antimicrobial susceptibility of *Streptococcus suis* isolated from swine in France and from humans in different countries between 1996 and 2000," *J Antimicrob Chemother,* vol. 50, no. 2, pp. 201-209, Apr. 2002.

[15] Clinical Laboratory Standards Institute, "Performance Standards for Antirrcicrobial Disk Susceptibility Tests; Approved Standard - Ninth Edition," CLSI document M2-A9, Wayne, Pennsylvania 19087-1898 USA, 2006.

[16] Clinical and Laboratory Standards Institute, "Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Third Informational Supplement." CLSI AST standards M100-S23, Wayne, Pennsylvania, USA, Jan. 2013.

[17] European Community on Antimicrobial Susceptibility testing (EUCAST), "Zone diameter distributions," EUCAST, http://www.eucast.org/zone_diameter_distributions. 2013.

[18] T.H.M. Nguyen, T.H. Ngo, V.T.N. Tran, D.L. Le, et al., "*Streptococcus suis* Meningitis in Adults in Vietnam," *Clin Infect Dis*, vol. 46, no. 5, pp. 659-667, Mar. 2008.

[19] T.H. Nguyen, T.H. Tran, G. Thwaites, V.C. Ly, et al., "Dexamethasone in Vietnamese adolescents and adults with bacterial meningitis," *N Engl J Med*, vol. 357, no. 24, pp. 2431–2440, Dec.2007.

[20] C. Marois, L. Le Devendec, M. Gottschalk and M. Kobisch, "Detection and molecular typing of *Streptococcus suis* in tonsils from live pigs in France," *Can J Vet Res*, vol. 71, no. 1, pp. 14–22, Jan. 2007.

[21] M. Ip, K.S. Fung, F. Chi, E.S. Cheuk, S.S. Chau, et al., "*Streptococcus suis* in Hong Kong," Diagn Microbiol Infect Dis, vol. 57, no. 1, pp. 15–20, Jan. 2007.

[22] R. Kay, A.F. Cheng and C.Y. Tse, "*Streptococcus suis* infection in Hong Kong," *QJM*, vol. 88, no. 1, pp. 39–47, Jan. 1995.