INCIDENCE AND PRESENCE OF VIRULENCE FACTORS OF *STREPTOCOCCUS SUIS* INFECTION IN SLAUGHTERED PIGS FROM CHIANG MAI, THAILAND

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Abstract. This study was designed to determine the incidence of *Streptococcus suis* infection in slaughtered pigs raised in industrial facility and backyard system in Chiang Mai City, Thailand. A total of 90 tonsils and submaxillary salivary gland/ lymph node samples from slaughtered pigs raised in industrial facility and 122 samples from slaughtered pigs raised in backyard system were collected. Isolation and identification of *S. suis* were conducted using standard bacteriological methods. Farm management and risk factor data were collected by a questionnaire. Serotyping and presence of virulence factor genes, *epf*, *mrp* and *sly*, were determined by multiplex PCR assay. The overall incidence of *S. suis* in this study was 9% (n = 212) and the incidence is significantly higher in districts located at a greater distance south of Chiang Mai City. *S. suis* serotype 2 was present more in healthy pigs (43%) than ill pigs (10%). Every *S. suis* isolate carried *mrp* and *sly* and ill pigs carried *epf* (80%) more than healthy pigs (57%). However, the probability of *S. suis* serotype 2 with epf^+ (0.245) detected in healthy pigs was higher than in ill pigs (0.08) indicating people may have a higher risk of being infected with *S. suis* from healthy than ill pigs.

Key words: *Streptococcus suis*, pig, serotyping, virulence gene

INTRODUCTION

Streptococcus suis is a zoonotic pathogen infecting pigs and humans. It is a gram-positive facultative anaerobic bacterium (Lun *et al*, 2007). All strains of *S. suis* show hemolytic activity when grown on blood agar supplemented with sheep blood (Facklam, 2002). *S. suis* can be categorized into serotypes based on capsular polysaccharides. The most common serotype found in pigs and humans is serotype 2. The putative virulence factors in *S. suis* include muraminidase released protein (MRP), extracellular factor (EF), suilysin (SLY) and thiol-activated hemolysin (Gottschalk and Segura, 2000). MRP and

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EF is present in inner and outer cell wall, respectively. There is a correlation between the presence of SLY, EF and MRP with high virulence of *S. suis* type 2 isolate (Staats *et al*, 1999).

S. suis can be found in the upper respiratory tract of healthy pigs particularly in piglets, which can act as carriers of the disease (MacInnes and Desrosiers, 1999). Disease caused by *S. suis* is usually observed in fattening pigs (pigs younger than 16 weeks old) with fever, lameness, neurological signs, cyanosis, wasting, dyspnea, inappetance, abortion and sudden death (MacInnes and Desrosiers, 1999). Human infection with *S. suis* is most frequently manifested as purulent meningitis followed by hearing loss and other neurological signs (Lun *et al*, 2007), which may be confused with other *Streptococcus* infection (Donsakul *et al*, 2003). Human infection usually is caused by direct contact with carrier pigs or consumption of raw pork contaminated with *S. suis. S. suis* infection in humans has been reported in several countries in Asia, including Japan (Chang *et al*, 2006), Taiwan (Huang *et al*, 2005) and Thailand (Suankratay *et al*, 2004).

S. suis infection in Thailand has been reported as both occupational disease involving butchers and food borne infection from consumption of contaminated raw pork (Leelarasamee *et al*, 1997; Chotmongkol *et al*, 1999; Fongcom *et al*, 2001; Vilaichone *et al*, 2002). Recent outbreaks of *S. suis* in Payao Province in northern Thailand is associated with consumption of raw pork (Khadthasima *et al*, 2007). Alcohol may also be a precipitating cause as most raw pork consumers also drink alcohol with their meals (Khadthasima *et al*, 2007). However, there is no information regarding the incidence of *S. suis* infection in pigs in northern Thailand. Several molecular techniques have been used to study *S. suis* (Chang *et al*, 2006; Baums *et al*, 2007; Marois *et al*, 2007). Molecular profile of the bacteria from different sources may indicate the genetic relatedness and association between the source (pigs) and human cases. This study determined and compared the incidence of *S. suis* infection in fattening pigs raised in industrial facilities and backyards. PCR serotyping and the presence of virulence factor genes (*epf*, *mrp* and *sly*) in *S. suis* isolates were performed.

MATERIALS AND METHODS

Collection of *S. suis* samples

Study districts were selected based on distance from Chiang Mai City (Table 1). Six districts adjacent to Chiang Mai City with distances of less than 50 km and 8 districts farther than 50 km in the south and north/east direction from Chiang Mai City were selected. On the west side of the city is a mountain. Local slaughterhouses in each district were selected by district livestock officers for sample collection.

A total of 90 slaughtered pigs raised in industrial facility and 122 slaughtered pigs raised in backyards were selected. This sample size is satisfactory for comparison of incidence of infection between industrial and backyards if the incidence of infection of pig in industrial group is 4% at 95% confidence, 80% power and expected relative risk of 4. Farm management and risk factor data were collected by a questionnaire from the pig producers.

Approximately 25 g of tonsil and submaxillary lymph node/salivary gland were collected and stored in icebox until delivered to the laboratory. Samples were processed within 12 hours after collection. Identification of *S. suis* was conducted using standard bacteriological method

Table 1
Study districts by zone and direction from Chiang Mai City.

Zone	South	Industry facility, no.	Backyard system, no.	North/East	Industry facility, no.	Backyard system, no.
Near	Doi Lo	17	0	Doi Saket	0	14
(<50 km)	Hang Dong	5	15			
	Mae Ta	13	1			
	San Kampaeng	10	15			
	San Pa Tong	8	0			
Far	Chom Tong	1	10	Chiang Dao	17	0
(>50 km)	Mae Chaem	5	20	Chai Prakan	5	20
	Mae Wang	3	4	Fang	1	1
				Mae Taeng	5	3
				Phrao	0	19

(Wongsawan *et al*, 2006). In brief, tissue samples were aseptically removed and homogenized in sterile normal saline. Approximately 100 µl aliquot of sample suspension was inoculated on 5% sheep blood agar (Merck®, NJ) and incubated at 37ºC for 24 hours. Alpha-hemolytic colonies with mucoid appearance were selected for further biochemical tests. Automated system (API) was used concurrently with regular biochemical tests.

PCR serotyping and determination of virulence factor genes

A total of 38 *S. suis* isolates were used for PCR serotyping and determination for presence of virulence factor genes. Twenty-eight *S. suis* isolates were collected from healthy pigs in 2008 by the Faculty of Veterinary Medicine, Chiang Mai University, and 10 *S. suis* isolates were collected from clinical (ill) pigs in 2006-2007 by Faculty of Veterinary Science, Chulalongkorn University. All *S. suis* isolates were cultured overnight on 5% sheep blood agar (Merck®, Whitehouse station, NJ) at 37ºC. and identified by biochemical tests (API).

Chromosomal DNA was prepared by CTAB precipitation method (Ausubel *et al*, 1999) and stored in TE buffer (pH 8.0) at 4ºC. Multiplex PCR assay was used to detect serotypes and virulence-associated genes as described previously (Silva *et al*, 2006). Primers were designed based on the sequences of the genes for *cps*1J, *cps*2J, *cps*7H and *cps*9H for the PCR serotyping and *epf*, *mrp* and *sly* for the presence of virulence genes (Table 2). PCR amplicons were separated by electrophoresis in 1.0% agarose gel, stained with ethidium bromide and analyzed by a gel documentation system (GelDoc2000, BioRad®, Hercules, CA).

Analysis of risk factors was done using Fisher's exact test and logistic regression analysis adjusting for clustering of sample using a generalized estimating equation (GEE).

RESULTS

Incidence of *S. suis* infection

The overall incidence of *S. suis* in this study was 9% (n = 212). The incidence of

Table 2
Primer sequences and sizes of PCR amplicons.

Gene	Genbank accession number	Primer sequence (5′-3′)	Amplicon size (bp)
*cps*1J	AF155804	TGG CTC TGT AGA TGA TTC TGC T	
		TGA TAC GTC AAA ATC CTC ACC A	637
*cps*2J	AF118389	TTT GTC GGG AGG GTT ACT TG	
		TTT GGA AGC GAT TCA TCT CC	498
*cps*7H	AF164515	AAT GCC CTC CTG GAA TAC AG	
		TCC TGA CAC CAG GAC ACG TA	379
*cps*9H	AF155805	GGG ATG ATT GCT CGA CAG AT	
		CCG AAG TAT CTG GGC TAC TGA	303
epf	X71881	CGC AGA CAA CGA AAG ATT GA	
		AAG AAT GTC TTT GGC GAT GG	744
mrp	X64450	ATT GCT CCA CAA GAG GAT GG	
		TGA GCT TTA CCT GAA GCG GT	188
sly	Z36907	GCT TGA CTT ACG AGC CAC AA	
		CCG CGC AAT ACT GAT AAG C	248

S. suis infection between industrialized pigs (8%) and backyard pigs (10 %) is not significantly different ($p = 0.637$). *S. suis* was found in pigs from Mae Chaem, Mae Ta, Chom Tong, Doi Lo, Mae Taeng and Chai Prakan (Fig 1). The *S. suis* incidence is significantly higher in districts located greater than 50 km south of Chiang Mai City ($p = 0.006$) (Fig 2).

The majority of pig samples were selected from small farms (<400 pigs) (Table 3). Pigs from industry type of production were slaughtered at the slaughterhouse and sold at the market while the majority of backyard pigs were slaughtered at home.

Univariate analysis of risk factors of infection showed that distance and direction from the city, water quality and disinfectant usage are significantly associated with infection (Table 4). Surprisingly, infected pigs were more likely to come from farms that adjusted water quality with chlorine and used disinfectants. However, the final logistic model adjusted for clustering effects of the samples showed that only distance and direction from the city are significantly associated with infection.

PCR serotyping and presence of virulence factor

PCR serotyping showed that most *S. suis* isolates were serotype 2, which was detected more in healthy pigs (43%) than clinical pigs (10%) (Table 5). Detection of virulence factor genes showed every isolate carried *mrp* and *sly*, and 63% (24/38) of the isolates carried *epf*. More clinical pigs (80%) carried *epf* than healthy pigs (57%). However, the probability of *S. suis* serotype 2 with *epf* $^{+}$ detected in healthy pigs was 0.245 and in clinical pigs 0.08.

DISCUSSION

This study shows that the incidence of *S. suis* is not different between industrial and backyard pigs, but the geographic location relative to Chiang Mai City is significant. It is not surprising that no man-

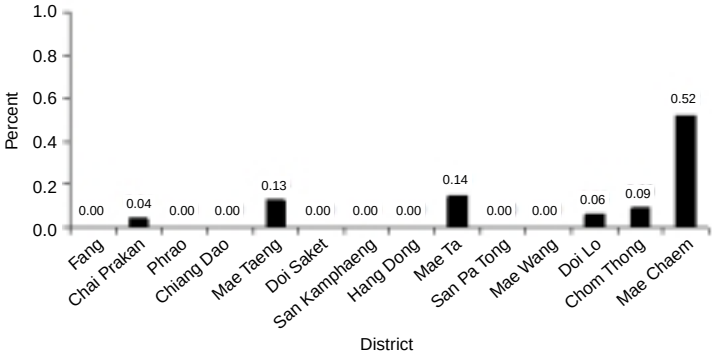

Fig 1– Incidence of *S. suis* by district in Chiang Mai.

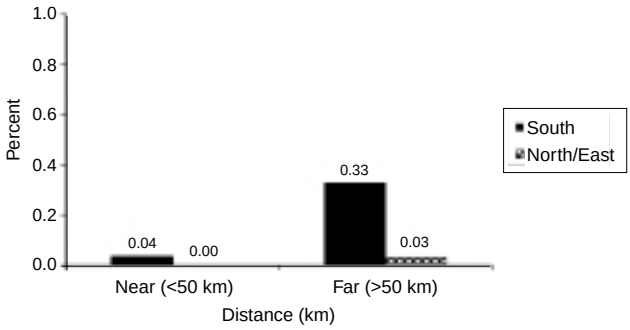

Fig 2–Incidence of *S. suis* by distance and direction from Chiang Mai City.

agement factor is significantly associated with infection in pigs as *S. suis* can survive in the environment and therefore can spread equally well in both industrial and backyard pig raising systems.

There is no apparent reason to explain the effect of distance and direction on *S. suis* infection in pigs. It may have to do with veterinary service. The farther away from the city, the less veterinarian service is provided either by the government or pharmaceutical company which may lead to different production standards, such as stringency level of biosecurity practice. Another possible explana-

Table 3
Number of sample from industry and backyard pig production.

Factor	Level	Industrial facility		Backyard system	
		n	%	*n*	%
Zone:	Near	53	59	45	37
	Far	37	41	77	63
Direction:	South	62	69	65	53
	North/East	28	31	57	47
Farm size:	<400	25	35	100	100
	401 - 3,000	28	40	.	0
	>3,000	17	25	.	0
Death pig destination:	Consuming	0	0	0	0
	Selling	1	1	0	0
	Destroying	77	99	97	100
Live pig destination:	Consuming	0	0	1	1
	Selling	85	100	115	99
Slaughter place:	Home	0	0	19	53
	Slaughterhouse	28	100	17	47
Sold at:	Home	0	0	14	36
	Market	17	100	25	64

Table 4
Univariate analysis of potential risk factors associated with *S. suis* infection in pigs.

Factor	Level	*n*	% infected	*p*-value
Production type	Industry	90	8	0.637
	Backyard	122	10	
Zone	Near	98	3	0.007
	Far	114	14	
Direction	South	127	13	0.006
	North/East	85	2	
Farm size	<400	125	14	0.169
	401 - 3,000	28	0	
	>3,000	17	6	
Watering method	Tray	27	0	0.136
	Tap	167	10	
Adjusting water quality	Yes	78	20	0.000
	No	122	1	
Adding chlorine in water	Yes	64	25	0.000
	No	137	1	
Feed source	Home made	73	5	0.301
	Commercial	127	10	
Adding antimicrobials in feed	Yes	29	7	1.000
	No	172	9	
Floor of the pig house	Ground	115	5	0.112
	Raised	83	12	
Using disinfectant	Yes	127	13	0.000
	No	74	0	
Using antimicrobial for treatment	Yes	40	7	1.000
	No	150	9	

tion is that the bacteria may be localized in these farther districts, where the density of pig production may be higher than those districts closer to the city. Southern area of Chiang Mai City also has a higher density of pig production as the north is mostly mountainous area.

PCR serotyping showed that *S. suis* serotype 2 was detected in healthy pigs much more than clinical pigs. This indicates that consumers may have increased risk of *S. suis* infection from healthy pigs. These results are in agreement with previous study, which detected *S. suis* serotype 2 from patients in northern Thailand (Fongcom *et al*, 2001; Wongsawan *et al*, 2006). More clinical pigs carried *epf* gene than healthy pigs suggesting that *epf* $^{+}$ is correlated with *S. suis* infection similar to previous studies (Vecht *et al*, 1991; Staats *et al*, 1999; Wei *et al*, 2009**)**.

The interesting aspect of *S. suis* infection in humans in northern Thailand is the mode of transmission. In developed countries, only direct contact with pigs was reported as the mode of transmission (Lun *et al*, 2007). However, consumption of raw pork, viscera and blood was reported as

Table 5
Presence of virulence factors in *S. suis* isolates.

Serotype/genotype	Number (%) *S. suis*	
	Healthy pig ($n = 28$)	Clinical pig ($n = 10$)
Cps 1	1	2
epf$^-$*mrp*$^+$*sly*$^+$ *cps* 1	0 (0)	1 (10)
epf$^+$*mrp*$^+$*sly*$^+$ *cps* 1	1 (3)	1 (10)
Cps 2	12	1
epf$^-$*mrp*$^+$*sly*$^+$ *cps* 2	4 (14)	0 (0)
epf$^+$*mrp*$^+$*sly*$^+$ *cps* 2	8 (28)	1 (10)
Cps 7	4	0
epf$^-$*mrp*$^+$*sly*$^+$ *cps* 7	2 (7)	0 (0)
epf$^+$*mrp*$^+$*sly*$^+$ *cps* 7	2 (7)	0 (0)
Cps 9	1	2
epf$^-$*mrp*$^+$*sly*$^+$ *cps* 9	0 (0)	1 (10)
epf$^+$*mrp*$^+$*sly*$^+$ *cps* 9	1 (3)	1 (10)
Non-typeable	10	5
epf$^-$*mrp*$^+$*sly*$^+$	6 (21)	0 (0)
epf$^+$*mrp*$^+$*sly*$^+$	4 (14)	5 (50)

the only mode of transmission in northern Thailand (Khadthasima *et al*, 2007). During this study, an outbreak of *S. suis* in consumers in Chom Tong District was reported (unpublished data) suggesting a correlation between *S. suis* incidence in pigs and humans. The incidence in pigs was also high not only in Chom Tong but also in Mae Chaem and Doi Lo, which are adjacent to Chom Tong. Further molecular epidemiological study is underway to determine the relationship between *S. suis* in pigs and those obtained from human patients.

ACKNOWLEDGEMENTS

The authors would like to express their appreciation to the Research Center for Animal Hygiene and Food Safety, Obihiro University of Agriculture and Veterinary Medicine, Japan; the Veterinary Public Health Center for Asia Pacific, Chiang Mai University, and the Thailand Research Fund for financial support given to the Faculty of Veterinary Medicine, Chiang Mai University (TRF-MRG-WII515S075). Appreciation is extended to Chiang Mai provincial livestock officers for sample collection and logistic support.

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