STREPTOCOCCUS SUIS INFECTION: A PROSPECTIVE STUDY IN NORTHERN THAILAND

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Abstract. A prospective study of all cases of *Streptococcus suis* blood or CSF culture positive admitted to Lamphun Provincial Hospital in northern Thailand was carried out. Fifty-three cases of *S. suis* were identified, which comprised 70% of all viridans streptococci cases. The majority of cases (88.6%) were contracted orally and 83.0% had an underlying disease present. Five clinical syndromes were identified: acute meningitis (37.2%), septicemia (27.9%), toxic shock syndrome (TSS) (23.3%), subacute bacterial endocarditis(SBE) (9.3%) and spondylitis (2.3%). The patients with TSS had a lower mean age than those without TSS. *S. suis* IgG and IgM antibody levels in the TSS group were lower than those without TSS which is important when considering the clinical syndrome and severity of the infection.

INTRODUCTION

Before the large outbreak in Sichaun Province, China in 2005, *Streptococcus suis* infection was considered to be an uncommon zoonotic or occupational disease in humans, with cases reported from many countries, including Thailand (Vilaichone *et al*, 2002; Wangkaew *et al*, 2006). Acute meningitis with hearing loss and vestibular dysfunction is the most common clinical manifestation, followed by septicemia with or without subacute bacterial endocarditis(SBE), arthritis, spondylitis and endopthalmitis. The mortality rate due to *S. suis* meningitis has been reported to be 7-12% (Kay *et al*, 1995; Arends and Zanen, 1998). The overall case fatality rate due to *S. suis* infection has been reported to be 13% in Europe and 20% in Asia (Huang *et al*, 2003). The mortality rate is higher with *S. suis* septicemia cases, especially in those with toxic shock syndrome (TSS) (Tang *et al*, 2006) which was first reported in Thailand in 1997 (Leelarasamee *et al*, 1997). TSS caused by *S. suis* was reported to be a major cause of death in the outbreak in China (Tang *et al*, 2006). In Lamphun Provincial Hospital, Thailand, in the year 2000, 10 patients died within 48 hours of admission due to TSS caused by *S. suis* resulting in sepsis and multiorgan failure. All the patients gave a history of eating raw pork or uncooked pig blood 2-4 days prior to the onset of illness, indicating an oral route of infection (Fongcom *et al*, 2001). We carried out a prospective study of *S. suis* infected patients presenting to Lamphun Provincial Hospital, northern Thailand. We evaluated the epidemiology, clinical manifestations and outcomes of those cases in order to obtain information to help prevent and early detect *S. suis* infection.

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MATERIALS AND METHODS

We carried out a prospective study of *S. suis* cases culture positive on blood or CSF for viridans streptococci admitted to Lamphun Provincial Hospital, northern Thailand from April 2001 to April 2002 and July 2005 to July 2007. We also retrospectively reviewed the 10 cases of *S. suis* infection described in the Introduction section. The epidemiology and medical records of each patient were reviewed. All blood and CSF specimens positive for viridans streptococci were identified as to species using biochemical reactions and confirmed using PCR of the16S rRNA, capsular type 2 (cps-2J) genes and DNA sequencing (Okwumabua *et al*, 1995). Sera from 19 subjects were tested for *S. suis* specific antigens with quantitative IgG and IgM antibody levels by ELISA technique. The antigens used for coating the 96-well plate were heated killed whole cells from 4 different isolates of *S. suis* type 2. Sera from the patients were added followed by the addition of goat antihuman IgG or IgM. O-phenylenediamine(OPD) substrate was added, then the optical density was measured at 492 nm. The titer was calculated from the mean optical density (OD) of the blank plus 3 standard deviations (SD) (Trott *et al*, 2008).

Statistical analysis

The rate of infection and demographic data were analyzed with descriptive statistics. The differential risk factor between patients who were critically ill (those with TSS) and patients who had other clinical syndromes (non-TSS) were analyzed using a statistical exact probability test for qualitative data and two sample *t*-tests for normal distribution of quantitative data plus a Mann-Whitney *U* test for abnormal distribution of quantitative data. TSS was defined as patients who had signs of toxicity and a rapidly progressive clinical course within the first 48 hours of illness, including hypotension, multiorgan failure and a characteristic branching rash. The definition of streptococcus TSS is the 1996 case definition criteria for severe TSS caused by *Streptococcus pyogenes* (http://www.cdc.gov/epo/dphsi/print/streptococcalcurrent.htm).

RESULTS

Fifty-three patients were included in this study, 10 cases were from the outbreak described above which took place in 2000. Nineteen cases were from the first period (April 2001 - April 2002) and 24 cases from the second period (July 2005 - July 2007) of the prospective study. During the first period, 28 cases were positive on blood or CSF cultures for viridans streptococci, 19 of these cases (67.9%) were shown to be *S. suis* after confirmation tests. During the second period, 33 cases were positive on blood or CSF cultures for viridans streptococci, 24 of these cases were shown to be *S. suis* (72.7%). The majority of cases (92.5%) were men; the mean case age was 50 years. The majority of cases were laborers and farmers. Eighty-three percent had underlying diseases, the most common being alcohol abuse followed by diabetes, valvular heart disease and others. Three patients contacted the disease from raw pork while cooking. Most patients (88.7%) had a history of raw pork or pig blood consumption before their illness, suggesting an oral route of infection. The mean incubation period was 4.3 days.

The clinical presentations and outcomes due to *S. suis* infection are shown in Table 1 (excluding the 10 cases in 2000 because the outbreak situation is an uncommon feature). Acute meningitis was the most common clinical manifestation, followed by septicemia without meningitis, TSS, SBE and spondylitis. The highest mortality rate occurred in patients with TSS (80%) followed by SBE (50%). Permanent deafness occurred

Table 1
Clinical presentations and outcomes in studied patients (*N*=43).

Clinical syndrome	Case	Proportion (%)	Clinical outcome			
			Recovery	Deafness	Disability	Death
TSS	10	23.26	2 (20.0%)	0	0	8 (80.0%)
Meningitis	16	37.21	7 (43.8%)	8 (50.0%)	1 (6.2%)	0
Septicemia	12	27.91	10 (83.3%)	0	0	2 (16.7%)
SBE	4	9.30	0	0	2(50.0%)	2 (50.0%)
Spondylitis	1	2.33	1 (100%)	0	0	0
Total	43	100	46.5%	18.6%	7.0%	27.9%

Table 2
Comparison of clinical signs and symptoms between TSS and non-TSS patients.

	Case (%)		*p*-value
	TSS (*n*=20)	Non-TSS (*n*=33)	
Myalgia	16 (80.0)	5 (15.2)	< 0.001
Diarrhea	15 (75.0)	7 (21.1)	< 0.001
Dyspnea	18 (90.0)	1 (3.0)	< 0.001
Skin lesion	19 (95.0)	2 (6.1)	< 0.001
Vertigo	1 (5.0)	10 (30.3)	< 0.037
Neck stiffness	0	16 (48.5)	< 0.001
UA abnormal	11 (91.7)	13 (54.2)	< 0.031
Sub-temp (<37°C)	13 (65.0)	4 (12.1)	< 0.001
Hypotension	11 (55.0)	1 (3.0)	< 0.001
Leukopenia	8 (44.4)	1 (3.2)	< 0.001
Leukocytosis	7 (38.9)	26 (83.9)	< 0.001
Platelet <100,000	14 (77.0)	9 (29.0)	< 0.001
Death	18 (90.0)	4 (12.1)	< 0.001

in half the meningitis cases.

Tables 2 and 3 compare different factors between the patients with TSS and those without TSS, according to their differential severity. Patients with TSS had a lower mean age, shorter incubation period and shorter length of stay in the hospital than non-TSS patients since nearly all died within 24-72 hours of admission. Predominating symptoms were severe myalgia, skin lesions, dyspnea and diarrhea. More than half the cases presented with hypotension and hypothermia on admission. The conspicuous laboratory finding in the TSS group was a very low blood glucose level in almost all cases. Rising levels of serum creatinine, SGOT, SGPT and bilirubin were common in the TSS group. Total serum protein in the TSS group was lower than in the non-TSS; metabolic acidosis was commonly found in the TSS group.

Sera from19 patients showed that IgG

Table 3
Comparison of some variables between TSS and non-TSS patients.

Clinical data	Mean		*p*-value
	TSS (*n*=20)	Non-TSS (*n*=33)	
Age (years)	44.10 ± 1.79	54.00 ± 2.33	0.004
Incubation period (days)	2.00 ± 0.94	5.86 ± 4.72	0.001
Treatment duration in hospital (days)	2.85 ± 0.84	15.30 ± 2.14	<0.001
Creatinine	2.89 ± 1.17	1.63 ± 1.81	0.010
Serum TCO_2	11.59 ± 0.84	21.67 ± 0.95	<0.001
Total protein	5.16 ± 1.27	6.53 ± 1.41	0.013
SGOT	440.36 ± 314.24	63.18 ± 34.01	<0.001
SGPT	201.73 ± 238.43	60.65 ± 34.96	0.023
Total birilubin	4.11 ± 0.89	1.33 ± 2.29	<0.001
Blood sugar	59.84 ± 25.86	131.46 ± 51.28	<0.001

Table 4
IgG and IgM antibody levels in patients with *S. suis* infection with different clinical problems.

TSS (*n*=5)		Meningitis (*n*=9)		Septicemia (*n*=4)		SBE (*n* =1)	
IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM
1,600	800	>12,800	1,600	>12,800	1,600	>12,800	>12,800
6,400	1,600	>12,800	3,200	>12,800	>12,800		
6,400	800	>12,800	>12,800	>12,800	1,600		
6,400	1,600	>12,800	>12,800	3,200	1,600		
1,600	600	1,600	6,400				
		>12,800	3,200				
		>12,800	>12,800				
		>12,800	800				
		>12,800	6,400				

Normal human serum titer for IgG = 3,200; IgM = 1,600

and IgM levels in the TSS group were significantly lower than levels in the non-TSS group, (statistics significant at $p = 0.003$ for IgG and $p = 0.008$ for IgM using Mann – Whitney *U* test at 95% confidence interval), as shown in Table 4.

Fifteen cases (93.8%) with meningitis had both positive CSF and blood cultures. The mean CSF protein level was 451.6 mg/dl, the mean CSF sugar was 14.3 mg/dl (extremely low), and the mean CSF WBC count was 632.8 cells/mm^3.

DISCUSSION

At least 20 cases of *S.suis* infection occurred each year at the hospital in Lamphun, the smallest northern province of Thailand.

There are 17 provinces in northern Thailand where the local residents tradition of raw pork and blood consumption. In this study, most of cases contracted the infection orally, unlike other studies which found a mainly cutaneous route of infection (Tang *et al*, 2006; Lun *et al*, 2007; Ma *et al*, 2008). In order to prevent this disease, behavior modification is essential though may be difficult to achieve.

S. suis is usually reported as viridans streptococci or occasionally as *S. pneumoniae* (Donsakul *et al*, 2003). In our study about 70% of viridians streptococci cases were determined to be *S. suis* after confirmation testing. Some cases of *S. suis* infection may be misdiagnosed, leading to inadequate treatment. Some patients had clinical symptoms suggestive of *S .suis* infection and had a risk factor of raw pork consumption, but their blood or CSF cultures were negative due to previous antibiotic treatment. The setting up of a screening protocol and diagnostic criteria would be useful in the diagnosis of probable cases of *S. suis* while awaiting culture results.

There were two possible features of *S. suis* infection in this study: an outbreak, as was seen in the year 2000 and sporadic infections. The rate of infection peaked during the hot, rainy months similar to a study from Hong Kong (Kay *et al*, 1995; Ma *et al*, 2008).

Outbreaks of *S. suis* infection can occur in the same manner as other food-borne infections. It can be contracted by consuming infected pork and infection is more common in hot and humid climates. Epidemiologic investigation is important for the detection and prevention of outbreaks of *S. suis* infection in humans and pigs. Since the outbreak in Lamphun Province 2000, we have used the Avian Flu surveillance rapid response team(SRRT) to aid in *S. suis* surveillance.

Our study differed from others *S. suis* infection in that it was a prospective study. Variations in the clinical presentation of the disease were noted. Several people ate the contaminated pork but only one or two became sick with varying symptoms. In this study, 83.0% of patients had an underlying disease perhaps making them more susceptible to *S. suis* infection than people without underlying disease. This was not seen in other studies (Clarke *et al*, 1991; Kay *et al*, 1995; Vilaichone *et al*, 2002; Rusmeechan *et al*, 2008). Further study is warranted to evaluate risk factors among cases and contacts who do not fall ill.

Five clinical syndromes of *S. suis* infection were identified in this study. TSS had the highest mortality but meningitis was the most common. This is similar to an outbreak in China (Tang *et al*, 2006). A report from Chiang Mai University Hospital found that SBE was the most common presentation (Wangkaew *et al*, 2006). In the TSS patients, the common symptoms were diarrhea, hypothermia and leukopenia, which differed from the Chinese outbreak (Tang *et al*, 2006). Our study found no significant differences in sex, occupation, underlining disease, timing of illness, alcohol and smoking history or route of infection between the TSS and non-TSS groups. However, in the TSS group the mean age was lower and the incubation period was shorter than in the non-TSS group. Rising levels of serum creatinine, SGOT, SGPT, serum bilirubin and low serum TCO_2 may be explained by the shock. A low blood sugar level was a common characteristic in patients with TSS and resembled the low CSF sugar levels found in those with meningitis. Many studies have tried to explain the severity of *S. suis* infection through various virulence factors, including virulence factor genes (gdh, mrp, epf, suilysin, and cps-2J), superantigens, and specific strains of the organism, but the cause re-

mains unclear(Sriskandan and Slater, 2006; Tang *et al*, 2006; Poggenborg *et al*, 2008). In our study we found lower antibody levels of both IgG and IgM in the TSS group than in the non-TSS group, which supports host factors as a cause of clinical symptoms, severity and the outcomes of the disease.

A limitation of this study was selection bias since this study only included positive blood or CSF culture cases. Future studies should include all positive clinical specimens, including pus, ascitic fluid, joint fluid and other body fluids which could be infected with *S. suis*.

Subsequent to this study, the Lamphun Public Health Organization and the Lamphun Provincial Hospital have established a *S. suis* prevention program. This includes the training of health care personnel, the provision of screening guidelines, diagnostic criteria, epidemiological and laboratory investigations, a reporting system and treatment recommendations. We also give public health education for the general population and in coordination with the regional veterinary department, assist in investigation and control of the disease in pigs.

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