ฟีโนไทป์หลากหลายของเชื้อสเตรปโตคอคคัส ซูอิส ซีโรไทป์ 2 ที่แยกได้จากผู้ป่วยในภาคเหนือ ประเทศไทย โดยศึกษาจากการอยู่รอดในเลือด และซีรัม

Phenotypic variation of *Streptococcus Suis* Serotype 2 isolated from Northern Thai patients characterized by survival in human blood and serum

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บทคัดย่อ

บทนำ: สเตรปโตคอคคัส ซูอิสเป็นหนึ่งในเชื้อก่อโรคจากสัตว์สู่คนที่สำคัญและพบทั่วโลก แม้มีการศึกษามากมายเกี่ยวข้องกับการก่อโรคของเชื้อแต่ก็ยังไม่สามารถระบุปัจจัยก่อโรคที่จำเพาะได้ อีกทั้งความสัมพันธ์ระหว่างจีโนไทป์กับความสามารถในการก่อโรคยังไม่มีความชัดเจน และมีความแตกต่างกันในแต่ละพื้นที่ทางภูมิศาสตร์

วัตถุประสงค์: เพื่อศึกษาการอยู่รอดในเลือดครบส่วนและในซีรัมมนุษย์ของเชื้อสเตรปโตคอคคัส ซูอิส ซีโรไทป์ 2 สายพันธุ์ต่างๆ

วัสดุและวิธีการ: เชื้อสเตรปโตคอคคัส ซูอิส ซีโรไทป์ 2 จำนวนทั้งสิ้น 10 สายพันธุ์ แบ่งเป็นสายพันธุ์ที่แยกได้จากผู้ป่วยในภาคเหนือ ประเทศไทย จำนวน 9 สายพันธุ์ และจากสุกรปกติ 1 สายพันธุ์ นำมาศึกษาการอยู่รอดของเชื้อเมื่ออยู่ในเลือดครบส่วนและในซีรัมมนุษย์

ผลการศึกษา: พบว่าเชื้อที่แยกได้จากผู้ป่วย 3 สายพันธุ์ และจากสุกรปกติสามารถทนอยู่ในเลือดครบส่วนหลังจากบ่มเป็นเวลา 4 ชั่วโมง โดยเชื้อมีอัตราการรอดชีวิตร้อยละ 93-208 ส่วนอีก 6 สายพันธุ์พบการเจริญลดลงโดยมีอัตราการรอดชีวิตร้อยละ 59 จนถึงตรวจไม่พบการเจริญ ที่น่าสนใจคือมีเชื้อ 5 สายพันธุ์ที่ถูกทำลายเมื่อบ่มในซีรัมเป็นเวลา 1 ชั่วโมง โดยมีอัตราการรอดชีวิตร้อยละ 37.12-64.31 และเชื้อมีอัตราการรอดชีวิตเพิ่มขึ้นเมื่อบ่มร่วมกับซีรัมที่ผ่านความร้อน และพบว่าอัตราการอยู่รอดของเชื้อในเลือดครบส่วนหรือในซีรัมไม่มีความสัมพันธ์กับจีโนไทป์ หรืออาการแสดงของผู้ป่วย

สรุปผลการศึกษา: การศึกษาแสดงให้เห็นว่าการอยู่รอดในเลือดครบส่วนและซีรัมของเชื้อขึ้นกับสายพันธุ์ของเชื้อ ส่วนจีโนไทป์ของยีนที่เกี่ยวข้องกับการก่อโรคเพียง 3 ยีน (*sly, epf, และ mrp*) ไม่สามารถบอกได้ถึงฟีโนไทป์ของเชื้อโดยเฉพาะการอยู่รอดในเลือดครบส่วนและซีรัมเนื่องจากเชื้อบางสายพันธุ์ถูกทำลายเมื่ออยู่ในซีรัมจึงควรมีการศึกษาเพิ่มเติมถึงองค์ประกอบในซีรัมที่สามารถฆ่าเชื้อสเตรปโตคอคคัส ซูอิสได้

วารสารเทคนิคการแพทย์เชียงใหม่ 2559; 49(3): 377-388. Doi: 10.14456/jams.2016.31

คำหัส: สเตรปโตคอคคัส ซูอิส เลือดครบส่วน ซีรัม การทดสอบการฆ่าเชื้อแบคทีเรีย

Abstract

Introduction: *Streptococcus suis* is one of the most important zoonotic pathogens worldwide. It can cause a wide range of diseases both in pigs and human including meningitis, endocarditis, arthritis and pneumonia. Although several virulence-associated factors have been characterized, specific virulence factors were not clearly defined. Moreover, the genotype of virulence-associated genes involved in virulence of *S. suis* was uncertain and varied between geographic areas.

Objective: To study phenotype of *S. suis* serotype 2 strains by human whole blood and serum bactericidal assays.

Materials and materials: Nine clinical *S. suis* serotype 2 strains isolated from Northern Thai infected individuals and one isolated from a healthy pig were used in this study. The viability of *S. suis* strains was determined after incubation in human whole blood or fresh serum.

Results: We found that three clinical strains and a pig strain resisted human blood after a four-hour incubation with survival rate ranging from 93-208%. In contrast, growth of the other six clinical strains was inhibited with a survival rate ranging from undetectable to 59%. Surprisingly, we found that five *S. suis* strains were killed in fresh serum after one hour of exposure with survival rates ranging from 37.12-64.31% and the survival of those strains were restored when incubated with heat-inactivated serum. Additionally, the survival of *S. suis* in human blood or serum was not associated with their virulence-associated genotypes or symptoms of the patients.

Conclusion: Our results suggested that survival of *S. suis* in human blood and serum was strain dependent. The only three virulence-associated genes (*sly, epf,* and *mrp*) could not determine the phenotypic character of *S. suis* particularly survival in human blood or serum. Since some *S. suis* strains loss their viability in human serum, further investigation of substances in serum involved in *S. suis* killing is warranted.

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Keywords: *Streptococcus suis*, human blood, serum, bactericidal assay

Introduction

Streptococcus (S.) suis is one of the most important zoonotic pathogens worldwide. *S. suis* can cause serious infections in humans, such as septicemia, endocarditis, and meningitis, especially for those who have occupationally closed contact to pigs or swine products.[1] In a recent global review, the greatest numbers of reported cases were found in Asia (90%). Thailand had the second highest number of worldwide reported cases (34%).[2] Human cases of *S. suis* infection in Thailand were first reported in 1987[3] and streptococcal toxic shock-like syndrome (STSLS) was first identified in 1997.[4] During 2013-2015, more than 700 cases have been reported and most of the cases were found in Northern Thailand.[5] Among 33 serotypes described, *S. suis* serotype 2 is the most frequently associated with human disease.[6] Most of the serotype 2 human cases in Thailand were identified ST1 as found in Vietnam but different from the outbreak strains in Mainland China which were identified as ST7.[2]

S. suis transmits to humans via closed or direct contact with diseased pigs, consumption of pork-derived products or undercooked meat, and through abrasions of skin. However, human to human transmission has not yet been reported.[7,8] Although many molecules including cell-associated proteins or extracellular proteins has been proposed as virulence factors, the association with pathogenesis remains poorly understood.[9] Polysaccharide capsule (CPS) is one of the known virulence factors described. It protects *S. suis* against killing by macrophages and neutrophils *in vitro*.[10,11] *S. suis* can produce a haemolysin

named suilysin (Sly) that can damage epithelial cells, endothelial cells, and macrophages. However, isogenic suilysin-negative mutants were still virulent in pig model of infection.[12] Moreover, *S. suis* can also produce extracellular protein factor (EF) and muraminidase-released protein (MRP) which have been proposed as additional virulence markers.[9] Recently, MRP was found to improve anti-phagocytic ability of virulent strains from certain countries.[13] However, the absence of this protein does not necessarily result in lack of virulence.[13,14]

Many studies were attempted to demonstrate the correlation between virulence factors and pathogenesis. The European virulent strain, 31533 (*sly+, mrp+, epf+*) had a highly induced proinflammatory cytokine release during septic shock phase and was associated with meningitis development after septic shock phase in mouse model.[15] Another European virulent strain, P1/7 (*sly+, mrp+, epf+*) was associated with porcine meningitis.[16] The Chinese outbreak strains (*sly+, mrp+, epf+*), also primarily caused STSLS and meningitis in human.[17] In addition, fifty percent of meningitis cases caused by *S. suis* in Vietnam were also infected with *sly+, mrp+, epf+* genotypes.[18] It was likely that this genotype was correlated with STSLS and meningitis. However, piglets infected with the Canadian virulent strain, 89-1591 (*sly-, mrp-, epf-*) showed severe nervous signs correlated with CNS infection.[14] In Thailand, most of meningitis cases were correlated with European and Chinese outbreak genotypes (*sly+, mrp+, epf+*) but non-meningitis cases were most related with *sly+, mrp-, epf-* genotypes.[19] Interestingly, two STSLS cases from Chiang Mai and Lamphun hospitals in Thailand were infected with *sly-, mrp-, epf+* genotype which were different from Chinese outbreak strains.[20] These suggested that the relationship between virulence factors and pathogenesis remains unclear.

To cause severe diseases, *S. suis* must avoid the host immune system to survive in blood and disseminate to other tissues. The aim of this study was to phenotypically characterize the *S. suis* serotype 2 isolated from hospitalized patients in Northern Thailand by measuring the ability to survive in human blood and serum samples *in vitro*. We found that *S. suis* strains showed heterogeneous growing patterns in human blood despite the presence of a similar virulence associated genetic background. Interestingly, growth of some clinical strains was inhibited by fresh human serum. These results indicated that the ability of Thai clinical *S. suis* serotype 2 strains to survive in human blood and resistant to human serum were different in a strain dependent manner.

Materials and methods

1. Bacterial strains and growth condition

A total of 10 *S. suis* serotype 2 strains including 9 human strains isolated from human patients hospitalized in Maharaj Nakorn Chiang Mai Hospital and Lamphun Hospital, Northern region of Thailand, and one isolated from a healthy pig, were used in this study (Table 1). Bacteria, maintained as stock culture in 20% glycerol–Todd–Hewitt broth at -70 °C, was grown overnight on Brain-Hearth Infusion (BHI) agar (Becton, Dickinson and Company, USA) at 37 °C with 5% CO_2. Isolated colonies were inoculated in BHI broth and incubated for 16 hours at 37 °C, 5% CO_2. The overnight cultures were subsequently grown in fresh BHI to mid log phase (OD_{600} of 0.4-0.5). The cultures were then adjusted to OD_{600} of 0.4 (approximately 10^8 CFU/mL) with fresh BHI broth, washed twice and suspended in cold-phosphate buffer saline (PBS) pH 7.4.

Table 1 Clinical background, sequence type and profile of virulence-associated genes of *S. suis* strains Investigated in this Study.[20]

Strain1	Origin	Site of isolation	Virulence-associated genes2	ST3 (ST complex)
MNCM 06	Meningitis	Blood, CSF	*cps2J+/sly+/epf+/mrp+*	1 (1)
MNCM 16	Meningitis	CSF	*cps2J+/sly+/epf+/mrp+*	1 (1)
MNCM 21	Meningitis	CSF	*cps2J+/sly+/epf-/mrp-*	101 (27)#
MNCM 50	Pulmonary Edema	Blood	*cps2J+/sly+/epf-/mrp-*	104 (27)#
MNCM 55	Septic Shock	Blood	*cps2J+/sly-/epf-/mrp+*	25 (27)#
LPH 03	Meningitis	Blood	*cps2J+/sly-/epf-/mrp+*	103 (27)#
LPH 12	Septic Shock	Blood	*cps2J+/sly-/epf-/mrp+*	25 (27)#
LPH 210/53	Septicemia	Blood	*cps2J+/sly+/epf+/mrp+*	ND
H 131/53	Septicemia	Blood	*cps2J+/sly+/epf-/mrp-*	ND
TSK 10.4	Healthy Pig	Tonsil	*cps2J+/sly-/epf-/mrp+*	ND

[1] MNCM were isolated from patients at Maharaj Nakorn Chiang Mai Hospital, Thailand. LPH and H were isolated from patients at Lamphun Hospital, Thailand.

[2] sly, suilysin gene; epf, extracellular protein factor gene; mrp, muraminidase-released protein gene; +, positive; –, negative.

[3] ST, sequence type #ST25, ST101, ST103, and ST104 belong to the ST27 complex, only with a less-stringent approach that defines an ST complex by sharing of alleles at >5 of the 7 loci. ND; Not determined; CSF, cerebrospinal fluid

2. Bactericidal assay by human blood

Viability of *S. suis* in human blood was performed as previously described.[13] Briefly, working cultures of *S. suis* (100 µL, 10^4 CFU/mL) were combined with fresh heparinized human blood (900 µL) and incubated in shaking incubator at 37 °C. At which time (1, 2 and 4 hours), aliquots (20 µL) were incubated on ice for 20 minutes in hypotonic solution to lyse eukaryotic cells. Viable cell count was determined by plating diluted samples onto BHI agar. Percentage of live bacteria was subsequently calculated as [CFU on plate (hr_x)/CFU in original inoculum (hr_0)] × 100%.

3. Serum bactericidal activity

Serum bactericidal activity of normal human serum against *S. suis* serotype 2 was performed in a microtiter system.[15] Briefly, working cultures of *S. suis* were diluted to approximately 10^6 CFU/mL in BHI broth. Bacterial suspension (12.5 µL) was added to microtiter plate wells. Fresh or heat-inactivated (56 °C, 30 min) serum (25.0 µL) was added. Finally, 12.5 µL of 0.85% NaCl was added to a final concentration of 50% (v/v). Microtiter plate was rotated to complete mixing and incubated at 37 °C, 5% CO_2 for one hour. Twenty µL of samples was diluted, spread onto BHI agar, incubated overnight at 37 °C, 5% CO_2 and bacterial colonies were counted. Percent of live bacteria was calculated as described above.

4. Statistical analysis

Data and statistical analysis were performed by Graphpad Prism software, and statistical significance of the survival in human serum was determined by unpaired two-tailed Student's t-test. Differences were considered statistically significant if the p value was <0.05.

Results

1. Survival of S. suis in whole blood

Since survival in bloodstream is an initial step to disseminate and cause systemic diseases during infection, we first investigated the growth of different *S. suis* strains in whole blood *in vitro*. At 1 hour post incubation, survival rates of *S. suis* could be divided into two groups as shown in Figure 1 and Table 2. The first group showed a survival rate higher than 80 percent (83-97%). These included MNCM06 (97.58%), TSK10.4 (87.11%) and MNCM16 (83.83%). The survival rate of MNCM06 and TSK10.4 were increased at two hours of incubation, and at four hours the survival rates were continuously increased to more than 150 percent. In contrast, the growth of MNCM16 was steady throughout four hours of incubation.

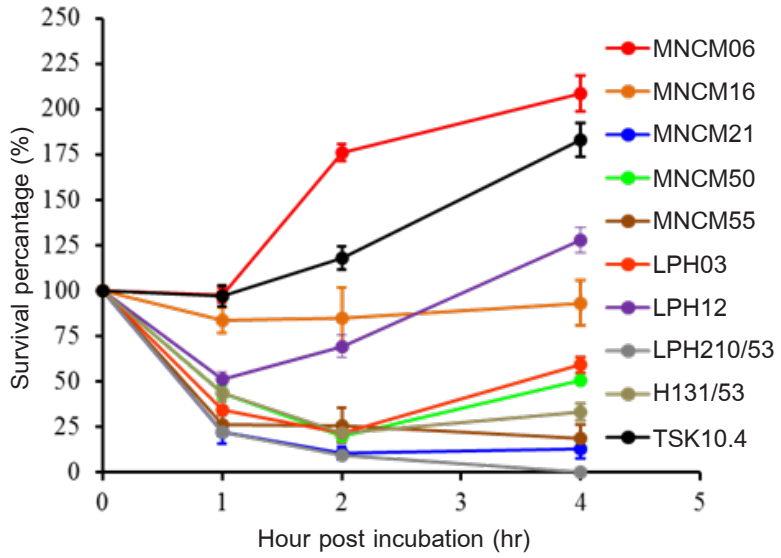


Figure 1 *Survival rates of S. suis strains in human blood. Survival rate was evaluated by bactericidal assay. Data are expressed as percentage of survival (±standard error of mean; SEM) in blood from 5 donors compared to total inoculum (n=5).*

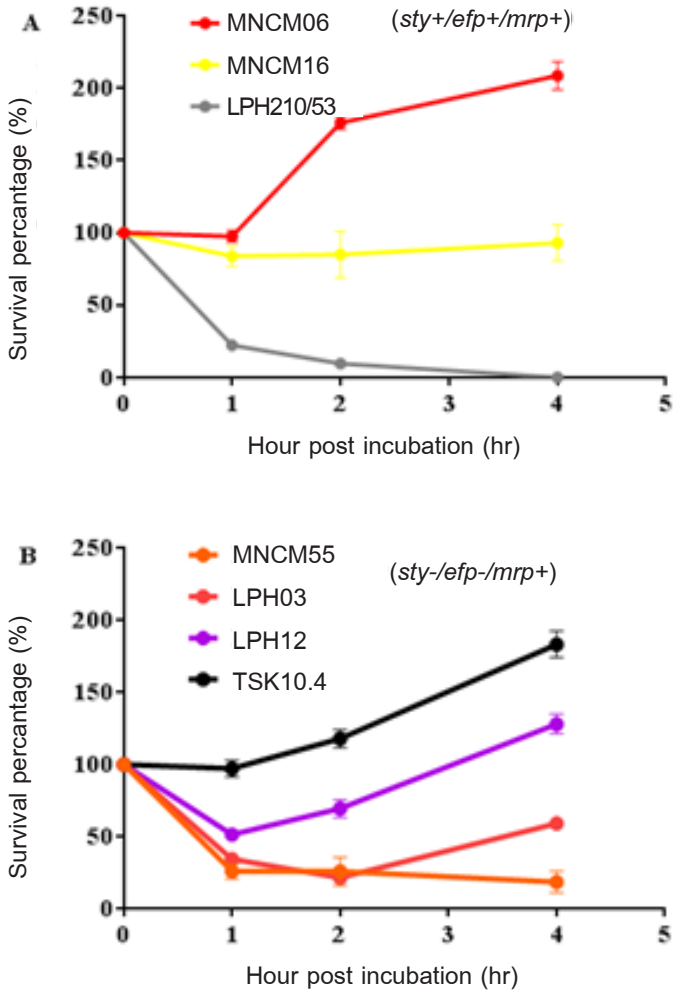
Table 2 Survival of *S. suis* strains in human blood and serum.

Strains	Survival percentage in human blood at time point (hr.) (%, mean±SEM) (n=5)			Survival percentage in human serum (%, mean±SEM) (n=6)	
	1	2	4	Fresh Serum	Heat-Inactivated Serum
MNCM06	97.58±4.05	175.96±4.49	208.55±9.75	99.31±2.81	143.92±9.67
MNCM16	83.83±7.03	85.04±16.94	93.21±12.47	83.45±4.97	163.95±3.56
MNCM21	21.93±6.29	10.59±3.59	12.63±5.24	66.02±3.60	158.99±8.06
MNCM50	43.57±3.05	19.64±1.85	50.58±1.64	96.63±3.72	154.76±7.63
MNCM55	26.11±5.13	25.93±9.76	18.64±7.46	46.89±6.67	139.38±4.92
LPH03	34.54±1.58	21.63±1.96	59.17±4.42	61.48±3.74	106.45±3.59
LPH12	51.47±3.85	69.47±6.07	127.98±6.84	48.08±4.23	130.09±2.29
LPH210/53	22.29±2.56	9.58±1.80	0.00±0.00	39.51±3.09	69.84±8.30
H131/53	43.53±4.67	21.34±3.00	33.02±4.57	88.85±3.87	124.68±2.89
TSK10.4	97.11±5.85	117.85±6.34	183.07±9.22	85.93±3.50	189.08±7.05

The second group with survival rate of approximately 51 percent or lower (21-51%) after one hour post incubation were LPH12 (51.47%), MNCM50 (43.57%), H131.53 (43.53%), LPH03 (34.54%), MNCM55 (26.11%), LPH210/53 (22.29%) and MNCM21 (21.93%). At 2 hours post incubation, survival rate of almost all strains, except LPH12, was slightly decreased to less than 25 percent (9-25%). After 4 hours of human blood incubation, survival rate of H131/53, MNCM50 and LPH03 was recovered to 33.02, 50.58 and 59.17%, respectively. In contrast, survival rates of other strains including MNCM55 (18.64%), MNCM21 (12.63%), and LPH210/53 (0.00%) was still decreased to lower than 20%. Interestingly, survival rate of LPH12 was increased to 69.47% at the second hour and rose to 127.98% at the fourth hour. However, survival in human blood of all strains was not different when concentration of *S. suis* was increased to 10^5 and 10^6 CFU/mL (data not shown).

2. Survival of S. suis in whole blood is genotype independent

A total of 10 *S. suis* strains can be divided into 3 groups based on presence or absence of virulence associated genes including *sly, epf* and *mrp* as shown in Figure 2. Three strains, MNCM06, MNCM16 and LPH210/53, were positive for all virulence associated genes (*sly+/epf+/mrp+*). The strains with *sly-/epf-/mrp+* genotype were MNCM55, LPH03, LPH12 and TSK10.4. *S. suis* strains in these two groups showed various survival patterns with a wide range from 0 to 208% during the one to four hours post incubation as shown in Figures 2A and B. Interestingly, the last group including MNCM21, MNCM50, and H131/53 which were only sly positive (*sly+/epf-/mrp-*) exhibited a similar growth pattern during the experiment. Their survival rates decreased to lower than 50% at the first hour post incubation and stayed low until the fourth hour post incubation as shown in Figure 2C.



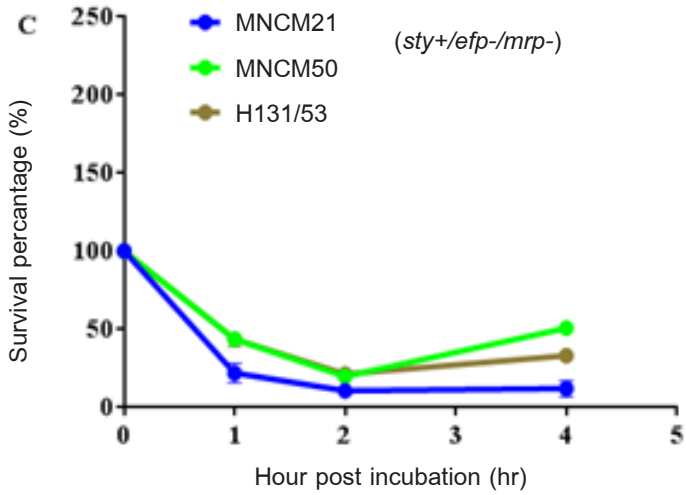


Figure 2 *Survival of S. suis in whole blood is genotype independent. S. suis was divided into 3 groups based on virulence-associated gene including (sly+/epf+/mrp+, panel A), (sly-/epf-/mrp+, panel B) and (sly+/epf-/mrp-, panel C) (n=5). Data are expressed as mean±SEM of survival rate in blood samples compared to total inoculum.*

3. S. suis was resistant to human serum

Complement or other soluble substances in serum are important innate immune components to protect bacterial infection. Resistance of *S. suis* to human serum was then examined by performing a serum bactericidal activity test. Results showed that *S. suis* strains exhibited different growth in fresh human serum as shown in Figure 3. By means of the percentage of survival, *S. suis* strains can be divided into 2 groups, with more than 80% and less than 80% survival. *S. suis* strains with a survival rate more than 80% were MNCM06 (96.47%), MNCM50 (94.87%), H131/53 (86.81%), TSK10.4 (86.76%), and MNCM16 (80.86%). The other five strains in which survival rate was less than 80% were MNCM21 (64.32%), LPH03 (59.27%), LPH12 (44.56%), MNCM55 (42.41%), and LPH210/53 (37.12%). Survival of all strains was restored when combined with heat-inactivated serum as shown in Figure 3.

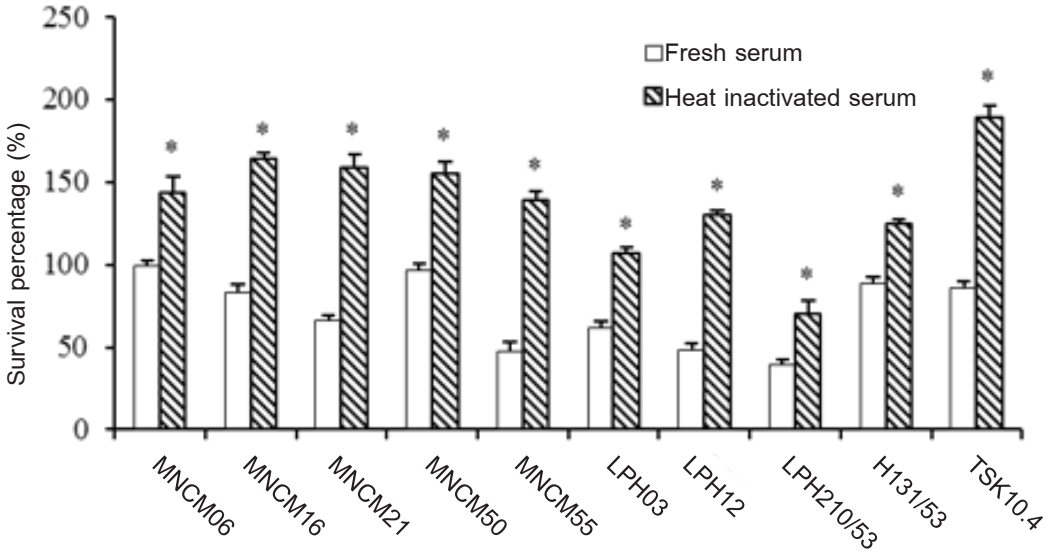


Figure 3 *Survival rate of S. suis strains in human serum. Data are expressed as mean percentage of survival (±SEM) in serum from 6 donors compared to total inoculum (n=6). Asterisk indicates that survival value of S. suis in heat inactivated serum was significantly higher than that of S. suis in fresh serum, at a p<0.05.*

Discussion

S. suis is a zoonotic pathogen responsible for a various kinds of infections including septicemia, endocarditis, meningitis and septic shock. The incidence of human cases in Thailand has been increasing especially in the Northern region.[5] Investigations of *S. suis* showed that not all serotype 2 strains were virulent, and degree of virulence varies among strains.[22] Several *S. suis* putative virulence factors including CPS, MRP, EF and suilysin have been previously reported.[9-14] However, many virulent strains lacked these factors.[23] Consistent with previous reports, *Kerdsin et al*. showed that clinical *S. suis* strains from Thailand were associated with various genotypes of virulence-associated genes.[19]

To cause systemic disease, *S. suis* must enter and survive in the bloodstream. Many virulence factors may support the ability to escape a variety of immune response such as phagocytosis and complement responses.[9] In this study, we evaluated the ability of *S. suis* serotype 2 in surviving in human blood. Results showed that thee human isolates and one pig isolate were resistant in human blood after a 4-hour incubation with high growth rates. In contrast, growth of other human isolates was inhibited by human blood which ranged from undetectable to 59% despite sharing of some virulence-associated genes. These results suggested that ability to survive in human blood may be dependent on virulence factors other than those 3 identified virulence-associated factors.[11]

S. suis CPS plays an important role in protection against the immune system. It has poor immunogenicity and prevents *S. suis* from phagocytosis.[24,25] CPS was also involved in the intracellular survival inside dendritic cells.[26] In addition, *S. suis* non-encapsulated mutant strains increased susceptibility to being killed by neutrophils, the most abundant type of granulocytes in blood circulation.[10,11] In this study, all *S. suis* strains used were positive for *cps2J* gene but there were only four strains of *S. suis* that could survive more than 80% in human blood after 4 hours of incubation. A previous study showed that lower survival in human blood of Chinese STSLS isolate (05ZYH33) than that of German STSLS isolate (BK52339) was not due to reduction of capsule expression.[27] Screening for capsule formation by microscopic capsule staining method,[28] indicated that all strains were encapsulated (data not shown). Thus, it seems that capsule was not involved in growth of *S. suis* in human blood in our experimental setting.

Suilysin, a cholesterol-binding cytolysin, is involved in modulation of the interaction with various host cells.[24,29] It is not only cytotoxic but reduces C3 deposition on bacteria.[10,30] The latter is involved in complement-dependent killing by phagocytes. In this study, growth of *S. suis* in human blood varied among the *sly*+ strains. We found that 2 *sly*+ strains, MNCM06 and MNCM16, exhibited a higher proliferation rate in human blood than other *sly*+ strains. A previous study demonstrated that increased SLY production in *S. suis* strain 05ZYH33 involved in higher resistance in bloodstream than non-epidemic strain 1940, a lower producing SLY strain, in murine model.[31] Additionally, SLY producing strains were more resistant to neutrophils and macrophages than their isogenic mutant strain, and as a result, had a higher survival rate in bloodstream.[10] However, there were some *sly*- strains that could also proliferate in human blood. Thus, the differential expression of SLY could only explain the distinct survival in human blood of *sly*+ strains.

MRP is a fibrinogen binding protein with an LPxTG motif. It is not only found on cell wall surface but is detected in culture supernatant.[32] MRP is involved in the survival in human blood of most Chinese and European clinical isolates. This process is independent of the inhibition of complement deposition on bacterial surface.[13] Interesting, we found that the three *mrp*-, *sly*+ strains similarly showed a low survival in human blood. These results were agreed with findings of *Pian et al*. in which MRP improved the anti-phagocytic ability of *S. suis* and this ability was decreased in a MRP mutant strain.[13] However, the three *mrp*+ strains also exhibited low growth in human blood. Thus, expression of MRP should be further verified.

Other factors supporting growth of *S. suis* in blood have been reported. The intracellular survival of *S. suis* was associated with arginine-ornithine antiporter (ArcD), a part of arginine deiminase system. This protein has supported resistance in a low pH environment and survival inside host cell. In contrast, ArcD mutant strain has a reduced ability to neutralize acidified environment

and correlated with a lower intracellular survival rate.[33] The acid resistance mechanism may support growth of *S. suis* inside phagolysosome and may lead to various proliferation in whole blood. Moreover, *S. suis* also produces other proteins which have been proposed as virulence factor/markers.[9] For instance, DNase contributes to degradation of neutrophil extracellular trap and evasion of antimicrobial activity of the traps.[34] *S. suis* can digest host antimicrobial peptide by protease[35] and superoxide dismutase support the resistance to toxic substances from host cells.[36] Expression of these putative virulence markers could affect survival of *S. suis* resulting in a distinct growth of *S. suis* strains that share the same *sly, epf*, and *mrp* genes.

Serum bactericidal activity is one of the most important host defense mechanisms for preventing systemic invasion of serum sensitive bacteria. It can be distinguished into 2 groups, complement dependent and complement independent mechanism.[37,38] In complement dependent mechanism, there are 3 different pathways to activate a complement cascade including classical (immune complex), alternative (pathogen surface, LPS) and lectin (mannose, ficolin) pathways.[39] Complement independent mechanism is associated with a group of anti-microbial peptides such as cathelicidin, alpha-defensin or protegrins produced by granulocytes, lysozyme, iron-binding protein, and beta-lysin.[40-42] Antimicrobial peptides inhibit or kill organism by direct and indirect mechanisms.[43] It is widely accepted that gram-negative bacteria in general are killed by complement mechanism.[38,39] In contrast, gram-positive bacteria have often resisted direct killing by a complement because gram-positive cell walls are thick and the peptidoglycan layer constitutes almost 95% of the cell wall.[38,39,44] Complement independent mechanism plays a key role in protection of gram positive bacteria.[38,39] Peptidoglycan of gram-positive bacteria can be degraded by lysozyme and growth of bacteria is blocked by chelating iron transferrin.[41,42]

We have evaluated the susceptibility of *S. suis* to human serum and found that 5 strains were serum resistant. Interestingly, other 5 strains were killed by fresh human serum and their viability was restored when combined with heat-inactivated serum. *Evelien et al*. have demonstrated unidentified heat-labile components in serum other than complement that were responsible for killing serum sensitive bacteria at a high serum concentration.[45] In this study, *S. suis* was incubated with 50% human serum. Thus, it is possible that other known factors in serum such as lysozyme or undefined heat-labile factors may be involved in direct *S. suis* killing.

Conclusion

Our study indicated that ability of *S. suis* to survive in human blood and serum was strain dependent. The only 3 virulence-associated genes (*sly, epf,* and *mrp*) could not determine phenotypic character of *S. suis* particularly the survival in human blood or serum. Since some *S. suis* strains loss their viability in human serum, further investigation of substances in serum involving in *S. suis* killing is warranted.

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