Clonal dissemination of human isolates of *Streptococcus suis* serotype 14 in Thailand

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Most cases of *Streptococcus suis* infection in humans are caused by serotype 2 strains, and only a few cases caused by other serotypes have been reported. Among 177 human isolates of *S. suis* in Thailand, 12 (6.8 %) were identified as being of serotype 14, and an occurrence of sporadic *S. suis* serotype 14 infection was noted during 2006–2008, particularly in northern Thailand. Clinical presentations of the 12 patients (median age 62.9 years) included meningitis (58.3 %), septic arthritis (25 %) and sepsis (16.7 %). These clinical features were similar to those previously reported for *S. suis* infections, except that there were no fatal cases. All of the 12 serotype 14 strains belonged to the multilocus sequence types (ST) 105 (n=11) and the novel ST127 (n=1). Molecular typing by PFGE revealed four different pulsotypes, including an identical pattern for nine ST105 strains and three closely related patterns for two ST105 strains and one ST127 strain. Our PFGE data suggested clonal dissemination of ST105 strains in Thailand. Because serotype 14 is becoming a more common cause of *S. suis* infections in humans, diagnostic tests for serotype 14 should be performed in South-East Asian countries.

**INTRODUCTION**

*Streptococcus suis*, an important zoonotic pathogen, causes meningitis and sepsis including streptococcal toxic shock syndrome in humans who are in close contact with infected pigs or contaminated pork-derived products (Lun et al., 2007). Based on capsular polysaccharides, 33 serotypes of *S. suis* have been identified. Serotype 2 is the most common cause of human disease (Lun et al., 2007; Wertheim et al., 2009). Currently, only seven human cases worldwide have been attributed to serotype 14 (Gottschalk et al., 1989; Haleis et al., 2009; Mai et al., 2008; Poggenborg et al., 2008; Takamatsu et al., 2008; Watkins et al., 2001; Ye et al., 2008).

In 1987, two human cases of *S. suis* infection were first reported in Thailand (Phuapradit et al., 1987). Since the occurrence of a large outbreak of *S. suis* serotype 2 infection in Sichuan Province, China, in 2005 (Ye et al., 2006), this disease has been increasingly recognized worldwide. During the past decade, the number of reported human cases has increased, particularly in South-East Asia, with more than 700 *S. suis* infections reported worldwide (Wertheim et al., 2009). To date, the number of *S. suis* infections in humans reported in previous studies from Thailand (Wertheim et al., 2009; Wangkaw et al., 2006; Rusmechean & Sribusara, 2008; Wongsomboonsiri et al., 2008; Khadthasrima et al., 2009) and on the website of the Bureau of Epidemiology, Ministry of Public Health, Thailand, exceeds 300 cases. Therefore, *S. suis* is an emerging human pathogen in Thailand.

Because of the variable biochemical characteristics (Lun et al., 2007; Ma et al., 2008), *S. suis* infection is often either undiagnosed or misdiagnosed by the local hospital laboratories in South-East Asian countries, including...
Thailand. In 2006, therefore, the Miscellaneous Bacteriology Laboratory, National Institute of Health (NIH), initiated a microbiological service for the identification of S. suis in clinical isolates from laboratories of local hospitals in Thailand. Through this microbiological service, 177 clinical isolates were confirmed as S. suis and 12 were determined to be serotype 14.

Herein, we report on both the clinical features of 12 human cases of S. suis serotype 14 infection that occurred in 2006–2008 in Thailand and the clonal dissemination of the serotype 14 isolates.

**METHODS**

**Bacterial isolates and identification.** A total of 1154 unidentified streptococcal isolates from blood or cerebrospinal fluid were collected from hospitals in all 76 provinces of Thailand by the Miscellaneous Bacteriology Laboratory, NIH, between January 2006 and September 2008 for species identification. Biochemical tests including API Strep (bioMérieux), and specific PCR amplification of the S. suis 16S rRNA gene generating a 294 bp PCR product, confirmed 177 isolates of S. suis from 34 hospitals in 25 provinces of Thailand (Marmois et al., 2004). A previous study reported that four genes of S. suis serotype 1 specifically hybridized with serotype 1 and 14 strains only, while five genes of S. suis serotype 2 specifically hybridized with serotype 2 and 1/2 strains only (Smith et al., 1999). Based on these findings, the 177 isolates of S. suis were designed to be serotyped for 1 or 14 and 2 or 1/2 using duplex PCR. The following primers were used for duplex PCR: SS-cps1 J-F, 5’-gatatagatgttagttattgaagctg-3’; SS-cps2 J-F, 5’-gtttagcttataacccqtp-3’; and SS-cps-J-R, 5’-acattaGtacgctaataaa-3’. The PCR products detected for reference strains of serotype 1 (NIAH 10227) or 14 (NIAH 13730) were 217 bp in size, while those for serotype 2 (CCUG 7984, provided by the US Centers for Disease Control) or 1/2 (NIAH 11318) were 515 bp (Fig. 1). NIAH 10227, 13730 and 11318 were kindly provided by the National Institute of Animal Health (NIAH), Japan. No PCR products were detected for the reference strains of other serotypes such as 3, 4, 5, 6, 7, 8, 9 or 16. Of the 177 strains, 165 (93.2 %) had PCR products of 515 bp, while 12 strains (6.8 %) had 217 bp PCR products. Coagglutination tests using rabbit antisera (Statens Serum Institute, Copenhagen, Denmark) showed that the strains that gave 515 bp PCR products were of serotype 2 and the strains that gave 217 bp PCR products belonged to serotype 14. These results were confirmed by Dr M. Gottschalk at the International Reference Laboratory, Université de Montréal, Canada.

**Molecular characterization of isolates.** The 12 serotype 14 strains were examined using multilocus sequence typing (MLST) as previously described with some modifications (King et al., 2002). eBURST was used to identify clonal complexes in the MLST database, which can be accessed at http://ssuis.mlst.net (Feil et al., 2004). The 12 serotype 14 strains were subjected to PFGE using the restriction enzyme Smal (Luey et al., 2007). PCR was used to test the serotype 14 strains for the virulence-associated gene profile, including the extracellular factor gene (epf), the suilysin gene (sly) and the muramidase-released protein gene (mrp) (Silva et al., 2006), as well as for an ~89 kb candidate pathogenicity island (89K PAI), which were identified in serotype 2 isolates obtained from a previous outbreak of infections in Sichuan, China (Chen et al., 2007).

**Patients.** The medical records of the 12 patients whose cultures were positive for S. suis serotype 14 were retrospectively reviewed by attending physicians at local hospitals in Thailand. Meningitis was defined as a presentation of nuchal rigidity of acute onset and a positive culture from either cerebrospinal fluid or blood. Sepsis was defined as a presentation of systemic inflammatory response syndrome without localized infection and a positive blood culture (Muckart & Bhagwanjee, 1997). Septic arthritis was defined as a presentation of acute arthritis and a positive blood culture.

**RESULTS AND DISCUSSION**

**Patients**

Of the 12 serotype 14 strains, 11 were isolated on different occasions in northern Thailand (Table 1). Another strain was isolated in central Thailand. S. suis serotype 14 infections that occurred sporadically were noted, especially in northern Thailand. The median age (range) of the 12 patients was 62.9 (40–79) years and 58.3 % were male. Two patients (16.7 %) had a history of eating raw pork or blood. No patients had occupational contact with raw pork. Clinical presentations included meningitis (58.3 %), septic arthritis (25.0 %) and sepsis (16.7 %). Hearing loss was a complication in five cases (41.7 %) and four of these cases were associated with meningitis. Acute respiratory distress syndrome and extradural and subdural abscesses were also found as a complication for each patient with meningitis.
Table 1. Clinical and microbiological features of 12 cases of *S. suis* serotype 14 infection in Thailand

M, Male; F, female; CSF, cerebrospinal fluid; ST, sequence type; VAG, virulence-associated gene; 89K PAI, a candidate pathogenicity island ~89 kb in length.

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Age</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Complication</th>
<th>Outcome</th>
<th>Duration of admission (days)</th>
<th>Strain no.*</th>
<th>Isolation site</th>
<th>Province/region†</th>
<th>ST</th>
<th>VAG profile</th>
<th>89K PAI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>48</td>
<td>F</td>
<td>Meningitis</td>
<td>Hearing loss</td>
<td>Survived</td>
<td>18</td>
<td>21928</td>
<td>CSF</td>
<td>Lampang/North</td>
<td>105</td>
<td>epf⁺/sly⁺/mrp⁺</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>79</td>
<td>M</td>
<td>Septic arthritis</td>
<td>None</td>
<td>Survived</td>
<td>8</td>
<td>27964</td>
<td>Blood</td>
<td>Sukhothai/North</td>
<td>105</td>
<td>epf⁺/sly⁺/mrp⁺</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>63</td>
<td>F</td>
<td>Meningitis</td>
<td>Hearing loss</td>
<td>Survived</td>
<td>31</td>
<td>27578</td>
<td>CSF</td>
<td>Phechabul/North</td>
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<td>epf⁺/sly⁺/mrp⁺</td>
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<tr>
<td>4</td>
<td>68</td>
<td>M</td>
<td>Meningitis</td>
<td>Acute respiratory</td>
<td>Survived</td>
<td>5</td>
<td>27071</td>
<td>Blood</td>
<td>Lampang/North</td>
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<td>epf⁺/sly⁺/mrp⁺</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>78</td>
<td>F</td>
<td>Septic arthritis</td>
<td>None</td>
<td>Survived</td>
<td>12</td>
<td>26012</td>
<td>Blood</td>
<td>Phechabul/North</td>
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<td>epf⁺/sly⁺/mrp⁺</td>
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<tr>
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<td>Sepsis</td>
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<td>Survived</td>
<td>16</td>
<td>25780</td>
<td>Blood</td>
<td>Tak/North</td>
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<td>epf⁺/sly⁺/mrp⁺</td>
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<tr>
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<td>None</td>
<td>Survived</td>
<td>14</td>
<td>24524</td>
<td>Blood</td>
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<td>epf⁺/sly⁺/mrp⁺</td>
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<tr>
<td>8</td>
<td>70</td>
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<td>Meningitis</td>
<td>Hearing loss</td>
<td>Survived</td>
<td>28</td>
<td>24451</td>
<td>CSF</td>
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<td>epf⁺/sly⁺/mrp⁺</td>
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<tr>
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<td>62</td>
<td>F</td>
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<td>Hearing loss</td>
<td>Survived</td>
<td>37</td>
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<td>Blood</td>
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<td>–</td>
</tr>
<tr>
<td>11</td>
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<td>Meningitis</td>
<td>Extradural and subdural abscess</td>
<td>Survived</td>
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<td>26390</td>
<td>Blood</td>
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<td>–</td>
</tr>
<tr>
<td>12</td>
<td>40</td>
<td>M</td>
<td>Meningitis</td>
<td>Hearing loss</td>
<td>Survived</td>
<td>15</td>
<td>27024</td>
<td>CSF</td>
<td>Lampang/North</td>
<td>127</td>
<td>epf⁺/sly⁺/mrp⁺</td>
<td>+</td>
</tr>
</tbody>
</table>

*DMST (Department of Medical Science, Thailand) number.
†Province and region of Thailand where the serotype 14 strain was isolated.
The median duration (range) of admission was 15.5 (1–45) days and no fatalities were reported.

Patients with serotype 14 infection in the present study were approximately 10 years older than those with *S. suis* infections in previous reports (Wertheim *et al.*, 2009; Mai *et al.*, 2008; Wangkaew *et al.*, 2006; Wongsomboonsiri *et al.*, 2008). However, the clinical manifestations of patients with *S. suis* serotype 14 infection, including hearing loss, were similar to previously reported findings of *S. suis* infections that were caused mostly by serotype 2, with the noted exception of a lack of fatalities (Wertheim *et al.*, 2009; Mai *et al.*, 2008; Wangkaew *et al.*, 2006; Wongsomboonsiri *et al.*, 2008). Complications of subdural abscess and acute respiratory distress syndrome found in this study were also reported in previous studies in Thailand (Wangsomboonsiri *et al.*, 2008) and China (Tang *et al.*, 2006), respectively.

A recent outbreak of *S. suis* infection, including 29 laboratory confirmed cases, occurred in Phayao Province during May 2007 (Khadthasrima *et al.*, 2009). A major route of transmission during this outbreak was consumption of raw blood from infected pigs. A retrospective study of 66 cases of *S. suis* infection in humans living in northern Thailand also found that the majority (59%) had a history of eating undercooked pork (Wangsomboonsiri *et al.*, 2008). The general public should be made aware of the risks associated with the traditional Thai custom of consuming uncooked pork products. In the present study, only two (16.7%) of the 12 patients with *S. suis* serotype 14 infection reported a history of eating raw pork or blood and no occupational exposure to raw pork was found. However, these results might be due to inadequate evaluation of traditional dietary practice at the time of admission. Although previous studies reported low proportions of female patients with *S. suis* infections (13.3–28.1%) (Wangkaew *et al.*, 2006; Wongsomboonsiri *et al.*, 2008; Ma *et al.*, 2008), a relatively high proportion of female patients (41.7%) was found in this study. Collectively, these findings indicate that local residents, including housewives, may have unintentional exposure during cooking to contaminated pork products that are sold at markets in northern Thailand, because a recent study from Hong Kong demonstrated a dense contamination of *S. suis* in raw pork meats available in local supermarkets or at wet markets (Cheung *et al.*, 2008).

### Molecular characterization of isolates

MLST analysis using seven selected housekeeping genes (*aroA*, *cpn60*, *dpr*, *gki*, *mutS*, *recA* and *thrA*) confirmed that 11 of the 12 strains had identical sequence type (ST) profiles (1,1,1,52,1,1,1,1) (King *et al.*, 2002). The ST profile of the strain that differed from the others was 1,1,1,1,1,1,1,18. These strains were assigned to ST105 and the novel ST127 (Table 1). Snapshots of all isolates of *S. suis* generated by eBURST, including those of our 12 serotype 14 isolates and all 408 isolates available in the MLST dataset (Feil *et al.*, 2004), suggested six major clonal complexes: ST1, ST17, ST27, ST29, ST87 and ST94 (Fig. 2). The ST105 and ST127 strains in this study were derived from ST1, which is the primary strain of the ST1 complex.

All 12 serotype 14 strains were positive for three virulence-associated genes (Table 1). However, the 11 ST105 serotype 14 strains were negative for the 89K PAI, while the ST127 strain was positive for the 89K PAI since three sets of PCR for the 89K PAI were positive (Chen *et al.*, 2007). Because all of the ST105 serotype 14 strains were positive for the three virulence-associated genes, but negative for the 89K PAI, the ST105 human isolates may have another PAI. However, the role of the 89K PAI remains to be determined. PFGE typing revealed four different DNA profiles among the 12 serotype 14 isolates (Fig. 3). An identical pattern, type A, was found for nine of the ST105 strains. Closely related patterns – A1 and A2 – were also noted for each of the ST105 serotype 14 isolates. The pulsotypes of ST105 strains were assigned to clusters of isolates with >70% similarity in the dendrogram. Other closely related patterns – A3 and A4 – were found for the ST127 strain of serotype 14 as well as a serotype 14 reference strain (NIAH 13730).

Of the 177 *S. suis* isolates identified in the present study, 12 strains (6.8%) were serotype 14 with STs 105 and 127. By contrast, in southern Vietnam, only one (1.1%) of 92 human isolates of *S. suis* was of serotype 14; all other strains belonged to serotype 2 (Mai *et al.*, 2008). Our data on PFGE suggest that clonal dissemination of serotype 14 with ST105 occurred in Thailand, and an involvement of three pulsotypes in 11 ST105 strains indicates that PFGE is more discriminatory than MLST, which corresponds to a previous report (Ma *et al.*, 2008). Of the seven serotype 14 isolates previously reported, the STs of five strains were determined as follows: ST1 from China (Ye *et al.*, 2008) and England (http://ssuis.mlst.net), ST6 from the Netherlands (Ye *et al.*, 2008; King *et al.*, 2002), ST11 from Thailand (Takamatsu *et al.*, 2008) and ST105 from Vietnam (Mai *et al.*, 2008). Collectively, all of the serotype 14 strains reported in our and previous studies belonged to the ST1 complex (Fig. 2). Interestingly, the pulsotype of the serotype 14 strain with ST105 isolated from Vietnam in 2004 appears to be identical or closely related to the type A of serotype 14 strains with ST105 in the present study (Mai *et al.*, 2008).

The results of the present study and earlier reports (Lun *et al.*, 2007; Ma *et al.*, 2008) indicate that this pathogen is often either undiagnosed or misdiagnosed by the laboratories of local hospitals in South-East Asian countries, including Thailand. Given the increased awareness of this disease on the part of clinicians and the availability of diagnostic tests for this pathogen at local hospitals, the true prevalence of this disease in this region will be determined.

In conclusion, over the past 3 years, 12 cases of *S. suis* serotype 14 infection have occurred sporadically in Thailand. The clinical features of these patients were...
Fig. 2. Entire S. suis MLST database displayed as a single eBURST diagram. The primary founders of ST1 are located in the centre of the cluster, and subgroup founders are shown in closed circles, except for STs 1, 11, 25, 28, 101, 103, 104, 105 and 127. ST105 for 11 serotype 14 strains in this study and one strain of serotype 14 from Vietnam (Mai et al., 2008) and ST127 for one strain of serotype 14 in this study are shown in grey circles. STs 1, 11, 25, 28, 101, 103 and 104 for human isolates of S. suis serotypes 2 and 14 in Thailand previously reported by Takamatsu et al. (2008) are shown in open circles. The size of each circle in the diagram corresponds to the abundance of the isolates of the ST in the input data.

Fig. 3. Dendrogram generated from PFGE profiles after Smal digestion of 12 human isolates of S. suis serotype 14 obtained from humans living in Thailand and reference strains of serotype 14 (NIAH 13730) and serotype 1 (NIAH 10227). The numbers in the dendrogram indicate the percentage similarity. Arrows indicate molecular size; * indicates DMST (Department of Medical Science, Thailand) strain number; † indicates NIAH (National Institute of Animal Health) strain number.
similar to those previously reported for \( S. \text{suis} \) infections, except that there were no fatal cases. Clonal dissemination of serotype 14 with ST105 was demonstrated in 11 of the 12 isolates. To our knowledge, this is the first report of clonal dissemination of serotype 14 in humans. Because this serotype is becoming more common in human infections, continuous surveillance of this disease using the diagnostic tests, which include serotyping PCR and a coagglutination test, for both serotypes 2 and 14 should be required at hospital laboratories in South-East Asian countries.

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