Streptococcus suis Meningitis in Adults in Vietnam

Nguyen Thi Hoang Mai,[1,a] Ngo Thi Hoa,[2,a] Tran Vu Thieu Nga,[2] Le Dieu Linh,[2] Tran Thi Hong Chau,[1] Dinh Xuan Sinh,[1] Nguyen Hoan Phu,[1] Ly Van Chuong,[1] To Song Diep,[1] James Campbell,[2,3] Ho Dang Trung Nghia,[1] Tran Ngoc Minh,[2] Nguyen Van Vinh Chau,[1] Menno D. de Jong,[2,3,4] Nguyen Tran Chinh,[1] Tran Tinh Hien,[1] Jeremy Farrar,[2,3] and Constance Schultsz[2,3]

[1]Hospital for Tropical Diseases, and [2]Oxford University Clinical Research Unit, Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam; [3]Nuffield Department of Clinical Medicine, John Radcliffe Hospital, Oxford, United Kingdom; and [4]Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

Background. *Streptococcus suis* infection is an emerging zoonosis in Asia. We determined the detailed epidemiological, clinical, and microbiological characteristics of *S. suis* meningitis in adults.

Methods. We prospectively studied 450 patients with suspected bacterial meningitis. Four hundred thirty-five (96.7%) of the patients participated in a trial to determine the effect of adjunctive dexamethasone treatment. For patients with *S. suis* infection, bacterial DNA load at hospital admission and during treatment was analyzed in cerebrospinal fluid specimens using quantitative real-time polymerase chain reaction. *S. suis* strains were characterized using pulsed-field gel electrophoresis and multilocus sequence typing. Putative virulence factors, including extracellular protein factor, suilysin, and muramidase released protein, were detected using polymerase chain reaction and Western blot assay. Predictors of outcome were identified using logistic regression analysis.

Results. *S. suis* was the most common pathogen and was detected in 151 (33.6%) of the patients. Fifty (33.1%) of these 151 patients reported exposure to pigs or pork. Mortality was low (2.6%; 4 of 151 patients died), but mild to severe hearing loss occurred in 93 (66.4%) of 140 patients. Severe deafness at hospital discharge was associated with age >50 years (odds ratio, 3.65; 95% confidence interval, 1.15–11.6), a strain carrying the *epf* gene (odds ratio, 3.42; 95% confidence interval, 1.02–11.4), and dexamethasone therapy (odds ratio, 0.23; 95% confidence interval, 0.06–0.78) but was not associated with cerebrospinal fluid bacterial DNA load. Bacterial DNA was still detectable in 58 (63%) of 92 cerebrospinal fluid samples after 6–10 days of antimicrobial treatment. Ninety-one of 92 *S. suis* strains had serotype 2. Thirty-three (36%) of these epidemiologically unrelated strains belonged to 1 pulsed-field gel electrophoresis cluster of multilocus sequence type 1, indicating clonal spread.

Conclusion. *S. suis* serotype 2 is the most frequent cause of bacterial meningitis in adults in southern Vietnam and is associated with substantial morbidity attributable to hearing loss.

Streptococcus suis is a gram-positive facultatively anaerobic coccus of which 34 serotypes have been described. The pig is considered to be the natural reservoir of *S. suis* and the main source of human infection. A recent increase in the number of reports of *S. suis* infection from China, Thailand, Hong Kong, Taiwan, and Singapore indicates that *S. suis* is an important cause of adult meningitis, endocarditis, septicemia, and arthritis in Asia [1–4]. The emergence of *S. suis* as a human pathogen is particularly illustrated by the major outbreak of severe illness that caused high morbidity and mortality attributable to infection with *S. suis* serotype 2 in Sichuan province, China, in 2005 [5, 6]. Data on human *S. suis* infections are restricted to case series, retrospective studies, or outbreak reports [1, 6–10], and prospective studies on the epidemiology, clinical presentation, and outcome of *S. suis* infection in humans are lacking. In addition, we are uninformed about the molecular epidemiology and the distribution of putative virulence factors, such as extracellular protein factor (EF and EF*) [11] and muramidase released protein (MRP) [12] in human *S. suis* strains. Here, we present, to our knowledge, the first large prospective study of *S. suis* infection in humans.

Received 29 August 2007; accepted 15 October 2007; electronically published 29 January 2008.

Reprints or correspondence: Dr. Constance Schultsz, Oxford University Clinical Research Unit, Hospital for Tropical Diseases, 190 Ben Ham Tu, District 5, Ho Chi Minh City, Vietnam (schultsz@gmail.com).

Clinical Infectious Diseases 2008; 46:659–67

1058-4838/2008/4605-0003$15.00
DOI: 10.1086/527385

[a] N.T.H.M. and N.T.H. contributed equally to this article.

METHODS

Patients and clinical investigations. The Hospital for Tropical Diseases in Ho Chi Minh City serves the local community and acts as a tertiary referral hospital for infectious diseases in southern Vietnam. We conducted a randomized, double-blind, placebo-controlled trial of adjuvant treatment with dexamethasone in patients with bacterial meningitis who were admitted to the hospital from November 1996 through June 2005 [13]. Demographic data, including occupation, history, and exposure to pigs, were recorded for all patients aged >14 years who had suspected bacterial meningitis. All patients underwent detailed clinical and laboratory assessment at hospital admission. CSF samples were obtained at hospital admission, 48 h after hospital admission, and after 6–10 days of therapy (or when indicated), in accordance with standard Vietnamese clinical practice. For patients participating in the trial, audiogram testing and neurological examination were performed at hospital discharge and 6 months after hospital admission. Severe hearing loss was defined as hearing loss >80 dB in at least 1 ear. Any hearing loss greater than the normal threshold (30 dB) but <80 dB in at least 1 ear was defined as nonsevere hearing loss. Disability was assessed by the modified Rankin score as described elsewhere [13]. Outcome was defined as (1) fully recovered, (2) mild sequelae, or (3) severe disability. All patients were initially treated with ceftriaxone (2 g every 12 h), and those included in the randomized trial received either intravenous dexamethasone sodium phosphate (0.4 mg/kg every 12 h) or placebo during the first 4 days of hospitalization. The Ethical and Scientific Committees of the Hospital for Tropical Diseases and the Health Services of Ho Chi Minh City approved the study protocol.

Laboratory investigations. Aliquots of CSF samples were sent for biochemical and microbiological investigations, and an aliquot was stored at −70°C in a dedicated freezer. CSF cell count and protein, lactate, and glucose concentrations were determined using standard methods. CSF samples were cultured on blood and chocolate agar plates and were inoculated in brain heart infusion broth for enrichment. Plates were incubated at 37°C in 5% CO_2. The broth was incubated aerobically and subcultured if growth was present. Bacteria were identified using standard identification methods. *S. suis* was identified on the basis of colony morphology, negative katalase reaction, optochin resistance, and APIStrep (bioMérieux). Serotyping was performed by slide agglutination with use of specific antisera (Statens Serum Institute). Antimicrobial susceptibility to penicillin, ceftriaxone, chloramphenicol, erythromycin, tetracycline, and vancomycin was tested using Etest (AB-Biodisk) and Clinical Laboratory Standards Institute breakpoints. Blood culture was performed at hospital admission using the BACTEC 9050 system, and positive culture results were identified as described above. Isolates were stored at −20°C.

DNA was extracted from stored CSF samples [14]. DNA was submitted to monoplex real-time PCR for detection of *Streptococcus pneumoniae, Haemophilus influenzae, Neisseria meningitidis,* and *S. suis.* Primers and probes for detection of *S. pneumoniae, H. influenzae,* and *N. meningitidis* were described by Corless et al. [15]. An *S. suis* PCR was designed and was targeted at the *cps2J* gene with primers cps2JF (GGTTACTTGCTACTTTTGATGGAAATT) and cps2JR (CGCACCTCTTTTATCTCTTCCAA) and probe (FAM-TCAAGAATCTGAGCTGCAAAAGTGTCAAATTGA-TAMRA). The detection limit was 5 copies per PCR. Efficiency of extraction and amplification was monitored in all reactions by inclusion of an internal control that consisted of a serial dilution of phocid herpes virus (provided by B. Niesters) and by detection by specific primers and probes, as described elsewhere [16]. The *S. suis* PCR fragment was cloned in plasmid pGEMT (Promega), and a serial dilution of the plasmid was used as an external standard for quantitative real-time PCR. Extensive precautions to avoid specimen contamination were taken, including the use of physically separated laboratories for preparation, extraction, and amplification.

Nonduplicate *S. suis* isolates from culture-positive specimens were analyzed by PFGE with *Sma*I [17, 18]. Gels were analyzed using Bionumerics software (Applied Maths). Multilocus sequence typing (MLST) was performed on 48 randomly chosen isolates [19]. The sequence type assignment was based on the sequence of the alleles at each locus of 7 housekeeping genes included in the MLST scheme, using the MLST database [20]. MLST results were analyzed using eBURST [21]. The presence of the genes encoding EF or the high molecular weight variant EF* *(epf* or *epf*)* and suilysin *(sly)* [22] was determined by PCR. MRP and EF expression was determined by Western blot. *S. suis* serotype 2 strains 31533 and 89–1591 (provided by M. Gottschalk) were used as positive and negative controls, respectively. Polyclonal antibodies against EF and MRP were provided by H. Smith.

Data analysis. Data were entered into an electronic database when follow-up was completed. An epidemic curve was created for each province to assess potential clustering of cases. χ^2 test or Fisher's exact test was used to compare categorical outcomes. Kruskal-Wallis test was used for comparison of bacterial DNA loads between groups. Multivariate analysis was used to identify baseline variables that were independently associated with severe hearing loss at hospital discharge (outcome) and included patients with observed outcome only. Of the variables age, sex, duration of symptoms, pretreatment with antimicrobial agents, Glasgow Coma Scale, dexamethasone use, bacterial DNA load at hospital admission, PFGE cluster, and *epf* genotype, those with $P \leq .1$ in univariate analysis were included in multivariate analysis. The distribution of these variables was compared between patients with and without out-

Table 1. Results of microbiological investigations of all patients admitted to the hospital during the study period.

Microorganism	No. (%) of positive specimens			
	Blood culture (n = 450)	CSF culture (n = 450)	Real-time PCR (n = 445)	Total (n = 450)
Streptococcus suis	73 (16.2)	117 (26)	149 (33.5)	151 (33.6)[a]
Streptococcus pneumoniae	25 (5.6)	50 (11.1)	79 (17.8)	81 (18)[b]
Neisseria meningitidis	5 (1.1)	11 (2.4)	29 (6.5)	29 (6.5)
Klebsiella pneumoniae	5 (1.1)	12 (2.7)	Not available	12 (2.7)
Escherichia coli	3 (0.7)	8 (1.8)	Not available	9 (2)
Staphylococcus aureus	8 (1.8)	3 (0.7)	Not available	9 (2)
Haemophilus influenzae	0 (0)	4 (0.9)	5 (1.1)	7 (1.6)[c]
Other[d]	8 (1.8)	18 (4)	Not available	22 (4.9)

[a] One culture-positive CSF sample was not available, and 1 sample contained *S. suis* serotype 14, which is not detected by the real-time PCR used.
[b] Two CSF samples had negative PCR results but positive culture results.
[c] Three CSF samples had negative PCR results but positive latex antigen test results.
[d] Includes *Pseudomonas aeruginosa* (n = 1), *Proteus mirabilis* (n = 1), *Bacteroides* species (n = 2), *Streptococcus* species (n = 15), *Neisseria* species (n = 1), *Haemophilus* species (n = 1), and *Campylobacter* species (n = 1).

come missing. In addition, the distribution of the outcome was compared between patients with missing data and patients without missing data for each variable.

All analyses were performed using SPSS (Microsoft) and Stata (StataCorp). All reported P values were 2-sided, and $P < .05$ was considered to be statistically significant.

RESULTS

We included 450 patients with presumed bacterial meningitis in the study. *S. suis* was detected in specimens from 151 of these patients by culture, PCR, or both, and *S. pneumoniae* and *N. meningitidis* were detected less frequently (table 1). The annual number of cases of *S. suis* infection increased gradually during the first few years of the study, although the total number of patients included in the study remained stable each year (figure 1). One hundred three patients (68.2%) originated from provinces in southern Vietnam, and 48 patients (31.8%) originated from Ho Chi Minh City. Clustering of cases in time and place, suggestive of outbreaks, was not observed. The proportion of male patients with *S. suis* infection (116 [76.8%] of 151 patients) was similar to the proportion of patients with other causes of bacterial meningitis (208 [69.6%] of 299 patients). Fifty patients (33.1%) with *S. suis* infection recalled exposure to pigs or pork within 1 week before the start of their illness. The occupations of 13 (25%) of these patients included butcher, abattoir worker, and seller of raw pork; 38 patients (75%) kept pigs at home. For 101 patients (66.9%), exposure to pigs or pork was not evident. Three patients had a history of splenectomy.

Patients presented with a median duration of illness of 4 days (range, 1–21 days; interquartile range, 3–5 days). The majority of patients presented with symptoms and signs of bacterial meningitis, including fever, headache, neck stiffness, and CSF leukocytosis. Clinical characteristics and details of neurological and CSF examinations at hospital admission are presented in table 2. Nine patients had widespread skin abnormalities, ranging from petechia or purpura to large hemorrhages with central necrosis (with bullae in 1 patient) and/or conjunctival hemorrhages. Four of these patients had necrotic fingers; amputation was required for 1 patient. Two patients presented with septic shock with jaundice, renal failure,

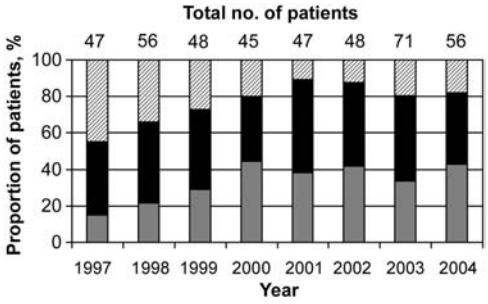

Figure 1. Annual proportion of patients admitted to the Hospital for Tropical Diseases (Ho Chi Minh City, Vietnam) with suspected bacterial meningitis who received a diagnosis of *Streptococcus suis* infection *(gray),* other bacterial causes *(black),* or unconfirmed bacterial meningitis *(dashed).* Diagnosis was based on positive results of culture and/or PCR of CSF and/or positive blood culture results. The study started in November 1996 and ended in May 2005. In November and December 1996, 8 patients were included, 1 of whom had *S. suis* infection and 4 of whom had unconfirmed bacterial meningitis. From January through May 2005, 24 patients were included, 11 of whom had *S. suis* infection and 6 of whom had unconfirmed bacterial meningitis.

Table 2. Clinical and laboratory characteristics at hospital admission for patients with *Streptococcus suis* meningitis.

Characteristic	Patients with *S. suis* meningitis (n = 151)
Age, median years (range)	46.5 (19–84)
Male sex	117 (77.5)
Duration of symptoms, median years (range)	4 (1–21)
No. of immunocompromised patients[a]	3
Symptoms	
Headache	142 (94.0)
Neck stiffness	142 (94.0)
Vomiting	100 (66.2)
Body temperature, ⩾38°C	148 (98.0)
Skin hemorrhages/rash	9 (6.0)
Pneumonia	6 (4.0)
Diarrhea	9 (6.0)
Glasgow Coma Score	
Mean (range)	12 (5–15)
<15	104 (68.9)
<11	47 (31.1)
Focal neurological signs	
Monoplegia, hemiplegia	15 (9.9)
Cranial nerve palsy	
3rd nerve	3 (2.0)
6th nerve	3 (2.0)
7th nerve	7 (4.6)
Indices of CSF inflammation	
WBC count	
Median $\times 10^9$ cells/L (range)	2.1 (0.001–64)
$<0.1 \times 10^9$ cells/L	4 (2.6)
$0.1–0.999 \times 10^9$ cells/L	40 (26)
$>0.999 \times 10^9$ cells/L	104 (70.9)
Median percentage neutrophils (range)	84 (1–99)
Protein level, median g/L (range)	2.06 (0.2–10.19)
Lactate level, median mmol/L (range)	11.2 (2–27)
CSF:plasma glucose level, median % (range)	13.76 (0.07–71)
Blood test result	
WBC count, median $\times 10^9$ cells/L (range)	16.8 (3.75–57.0)
Hematocrit, % (range)	39.7 (21–55)
Platelet count, $\times 10^{12}$ median platelets/L (range)	159 (18–933)

NOTE. Data are no (%) of patients, unless otherwise indicated.

[a] Defined as the use of immunosuppressive drugs, history of splenectomy, and presence of diabetes mellitus, alcoholism, and HIV infection. All 3 patients had a history of splenectomy.

and pneumonia; 1 of these 2 patients required mechanical ventilation and hemofiltration. Seventy-six patients received dexamethasone, 72 received placebo, and 3 received neither. The median duration of hospital stay was 14 days (range, 1–43 days; interquartile range, 12–16 days). Four patients (2.6%) died, all of whom had no history of splenectomy. Ninety-three (66.4%) of 140 patients with *S. suis* meningitis developed hearing loss, ranging from tinnitus to complete deafness (table 3), compared with 11 (23.9%) of 46 of the patients with *S. pneumoniae* meningitis who developed hearing loss. Fifteen patients complained of dizziness. The median bacterial DNA load in CSF at hospital admission was 1.48×10^6 copies/mL (range, 1.0×10^3–1.1×10^8 copies/mL). The median DNA load at hospital admission was not significantly higher in older patients, patients who did not receive pretreatment, patients with a low Glasgow Coma Score, or patients with severe hearing loss (table 4). Clearance of bacterial DNA was gradual, with 58 (63%) of 92 of the CSF samples having positive PCR results but negative culture results after 6–10 days of treatment. There was no difference in clearance of bacterial DNA between patients treated with dexamethasone and patients treated with placebo (figure 2).

S. suis could be isolated in samples from 115 patients. All isolates were *S. suis* serotype 2, except 1 isolate, which was serotype 14. All isolates tested were susceptible to penicillin, ceftriaxone, and vancomycin, but 79 (83.2%) of 95 isolates were resistant to tetracycline (MIC_{50}, 16 mg/L; MIC_{90}, 32 mg/L), 19 (20.2%) of 94 were resistant to erythromycin (MIC_{50}, 0.064 mg/L; MIC_{90}, >256 mg/L), and 3 (3.3%) of 92 were resistant to chloramphenicol. Ninety-two strains were available for molecular typing. PFGE identified 30 band patterns and 6 clusters (figure 3). The strains within these clusters were epidemiologically unrelated, because they were isolated in different years and from patients living in different provinces in southern Vietnam (figure 3). Thirty-three strains (35.9%) belonged to a single cluster (cluster D). MLST was performed for 47 serotype 2 strains representative of all clusters identified by PFGE and showed that 46 strains had sequence type 1. One strain had a single mutation in the *dpr* gene and was assigned sequence type 107. The strain with serotype 14 had a new *gki* allele and was assigned sequence type 105. All strains belonged to clonal complex 1. All serotype 2 strains and the serotype 14 strain were

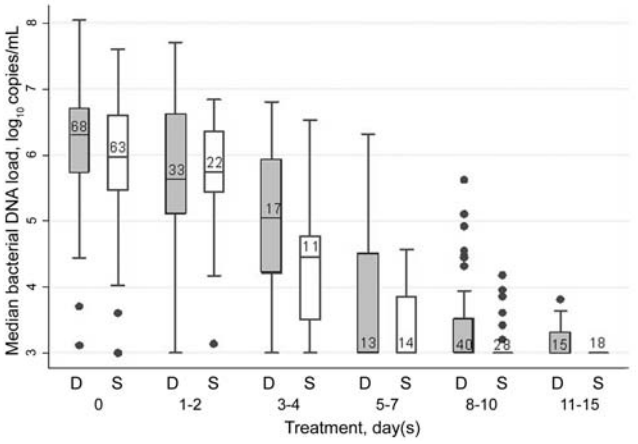

Figure 2. Median (interquartile range) and range of *Streptococcus suis* DNA load during treatment with dexamethasone adjuvant treatment (D) or placebo (S). The limit of detection was 10^3 DNA copies/mL. Numbers in the bars indicate the total number of samples tested at the respective time. No statistically significant differences between dexamethasone- and placebo-treated patients were observed at any of the times (Kruskall-Wallis test).

Table 3. Outcome and neurological findings in patients with *Streptococcus suis* meningitis at hospital discharge and at a 6-month follow-up visit, according to adjuvant treatment with either dexamethasone sodium phosphate (0.4 mg/kg every 12 h) or placebo/no adjuvant therapy.

Characteristic	Proportion (%) of patients		
	All (n = 151)	Dexamethasone group (n = 76)	Placebo/no adjuvant therapy group (n = 75)
At hospital discharge			
Outcome[a]			
Death	4/151 (2.6)	1/76 (1.3)	3/75 (4.0)
Fully recovered	57/151 (37.7)	28/76 (36.8)	29/75 (38.7)
Mild sequelae	65/151 (43.0)	35/76 (46.1)	30/75 (40.0)
Severe disability	25/151 (16.6)	12/76 (15.8)	13/75 (17.3)
Neurological findings			
Cerebellar syndrome	1/151 (0.07)	1	…
Third nerve palsy	1/151 (0.07)	…	1
Hemiplegia	1/151 (0.07)	1	…
Paraparesis	1/151 (0.07)	…	1
Hearing loss			
>80 dB[b]	34/140 (24.3)	11/71 (15.5)	23/69 (33.3)
<80 dB and/or tinnitus	59/140 (42.1)	34/71 (47.9)	25/69 (36.2)
At the 6-month follow-up visit			
Outcome[a]			
Death after hospital discharge	0/91	…	…
Fully recovered	51/91 (56.0)	27/45 (60.0)	24/46 (52.2)
Mild sequelae	28/91 (30.8)	12/45 (26.7)	16/46 (34.8)
Severe disability	12/91 (13.2)	6/45 (13.3)	6/46 (13.0)
Neurological findings			
Cerebellar syndrome	1/91	1/45	…
Hemiplegia	1/91	1/45	…
Hearing loss			
>80 dB	14/86 (16.3)	4/41 (9.8)	10/46 (21.7)
<80 dB and/or tinnitus	27/86 (31.4)	11/41 (26.8)	16/46 (34.8)

[a] Disability was assessed by the modified Rankin score, as follows: 0, no symptoms; 1, minor symptoms not interfering with lifestyle; 2, symptoms that may restrict lifestyle, but the patients can look after themselves; 3, symptoms restrict lifestyle and prevent independent living; 4, symptoms clearly prevent independent living, although constant care and attention is not required; 5, totally dependent on others, requiring constant help day and night. Outcome was defined as fully recovered (score, 0), mild sequelae (score, 1 or 2), or severe disability (score, 3, 4, or 5).

[b] Severe hearing loss at hospital discharge was significantly associated with the use of dexamethasone, age >50 years, and infection with an *epf*-positive strain (see text).

sly positive. Forty-six strains (50%) were *epf* positive, 46 strains were *epf** positive, and protein expression was detected in all strains. MRP production was detected in 64 strains (69.6%). Strains in cluster A produced predominantly EF* and MRP, and strains in cluster D produced predominantly EF with or without MRP (figure 3).

Deafness at hospital discharge was assessed in 140 (92.7%) of 151 patients, and for 87 (62%) of these patients, characteristics of infecting strains were available. For all 9 variables tested in univariate analysis, the distribution of severe deafness at hospital discharge among patients with missing data was similar to that among patients without missing data. The variables sex, age >50 years, dexamethasone therapy, and infection with a strain carrying the *epf* gene were entered in multivariate analysis. Severe deafness at hospital discharge was independently associated with age >50 years (OR, 3.66; 95% CI, 1.15–11.6; $P = .028$), infection with a strain carrying the *epf* gene (OR, 3.43; 95% CI, 1.03–11.4; $P = .045$), and dexamethasone therapy (OR, 0.23; 95% CI, 0.06–0.78; $P = .019$).

DISCUSSION

Our study identified *S. suis* serotype 2 as the most important cause of acute bacterial meningitis in adults in southern Vietnam. *S. suis* was detected at higher rates than *S. pneumoniae* or *N. meningitidis*, the 2 major causes of bacterial meningitis

Table 4. Baseline, clinical, and microbiological characteristics and bacterial DNA load in patients at hospital admission.

Characteristic	Bacterial DNA load, median $\log_{10}$ copies/mL (range)	No. of patients[a]	P[b]
Age			
⩾50 years	6.17 (3.0–8.04)	77	.89
>50 years	6.12 (3.13–7.6)	53	
Glasgow Coma Score			
15	6.04 (3.13–7.66)	41	.22
<15	6.21 (3.0–8.04)	90	
Previous antibiotic therapy			
Yes	6.1 (3.13–7.66)	81	.15
No	6.38 (3.0–8.04)	46	
Severe deafness			
Dexamethasone group	6.25 (3.13–7.1)	11	.83[d]
Placebo group	5.84 (3.6–7.02)	20	.48[d]
No mild hearing loss			
Dexamethasone group	6.25 (3.11–8.04)	49	
Placebo group	5.97 (3.0–7.6)	41	
Extracellular protein factor gene			
epf Positive	6.39 (3.11–7.42)	39	.067
*epf** Positive	5.87 (3.0–7.45)	34	

[a] Admission samples from 131 patients were available for quantitative real-time PCR.
[b] By Kruskall-Wallis test.
[d] Severe deafness versus no hearing loss or mild hearing loss.

in adults in most countries [23]. The data indicate that *S. suis* serotype 2 infection is endemic in southern Vietnam. Our study was limited to the adult population. To our knowledge, only 1 case of *S. suis* infection in children has been reported [24]. In addition, we did not detect *S. suis* in 145 children with clinical signs of meningitis in Ho Chi Minh City in 2006 using real-time PCR (C.S., unpublished observation), suggesting that *S. suis* infection is rare in children.

The clinical characteristics of *S. suis* meningitis were similar to those observed in patients with bacterial meningitis caused by other encapsulated microorganisms, such as *N. meningitidis* and *S. pneumoniae,* but the mortality associated with *S. suis* meningitis was lower than the mortality associated with bacterial meningitis caused by these other pathogens [23]. The proportion of patients with *S. suis* meningitis (49.3%) who had positive blood culture results was higher than the proportion of patients with *S. pneumoniae* meningitis (30.8%) who had positive blood culture results. Skin rash, distal necrosis, jaundice, and renal failure were observed in a number of patients. These symptoms and signs were also observed during the outbreak of *S. suis* infection in China in 2005 and were suggested to form part of a streptococcal toxic shock syndrome [6]. The most striking feature of *S. suis* meningitis is the progressive hearing loss, resulting in mild-to-severe deafness in two-thirds of patients. The pathogenesis of the hearing loss in *S. suis* meningitis is unknown. Studies in guinea pigs have shown direct invasion of the cochlea by *S. suis* [25]. Detailed imaging studies (using MRI) in humans with bacterial meningitis also suggested involvement of the cochlea; the severity of this involvement was related to the degree of hearing loss [26]. Adjuvant therapy with dexamethasone sodium phosphate has been shown to reduce the risk of severe hearing loss and neurological sequelae in adults with bacterial meningitis [13, 27]. Dexamethasone therapy was associated with protection against severe hearing loss in our study and, therefore, is strongly indicated in the treatment of *S. suis* meningitis.

The EF protein, the function of which is unknown, is associated with virulence of *S. suis* serotype 2 in pigs in certain geographic areas [11, 28, 29]. The *epf* genotype was associated with severe deafness and a higher bacterial DNA load at hospital admission, compared with the *epf** genotype, although the difference in bacterial DNA load did not reach statistical significance. These data suggest that, despite the fact that strains with *epf* and *epf** genotypes are both able to invade and pass the blood-brain barrier, serotype 2 strains carrying *epf* may be the more virulent strain in humans, similar to the situation in pigs.

Infected pigs are considered to be the main source of *S. suis* infection in humans [7]. The exact route of transmission from pigs to humans is not known. Cases have been linked to accidental inoculation through skin injuries, inhalation of aerosols, and ingestion of contaminated food [6, 7, 30]. Although a proportion of our patients had evidence of exposure to pigs or pork, such exposure was absent in two-third of the patients. However, the possibility cannot be excluded that these patients were unaware of exposure (e.g., through consumption of undercooked pork, including pig intestine, pig tonsil, and raw pig blood, as is common in Vietnam). Intestinal translocation of EF-positive *S. suis* serotype 2 in piglets has been demonstrated under experimental conditions [31], and ingestion of *S. suis* may be an important route of entry in humans. Asymptomatic pharyngeal carriage of *S. suis* has been observed in slaughterhouse workers in Germany [32], and asymptomatic nasopharyngeal or intestinal carriage of *S. suis* may contribute to transmission of *S. suis* among humans. However, it is unknown how common asymptomatic carriage of *S. suis* is in humans in endemic areas and whether it increases the probability of infection.

S. suis serotypes 1, 1/2, 2, 9, 7, and 14 are the most important serotypes responsible for disease in pigs. Although serotype 2 is often considered to be the most virulent serotype, the frequency of isolation of certain serotypes in pigs may vary according to geographic region and over time [33]. All reported human *S. suis* infections were caused by serotype 2 strains, except 1 infection caused by serotype 1, 1 caused by serotype 4, 2 caused by serotype 14, and 1 caused by serotype 16 [7, 9, 24, 34, 35]. In accordance with this, serotype 2 is also the most important serotype in patients with meningitis in Vietnam,

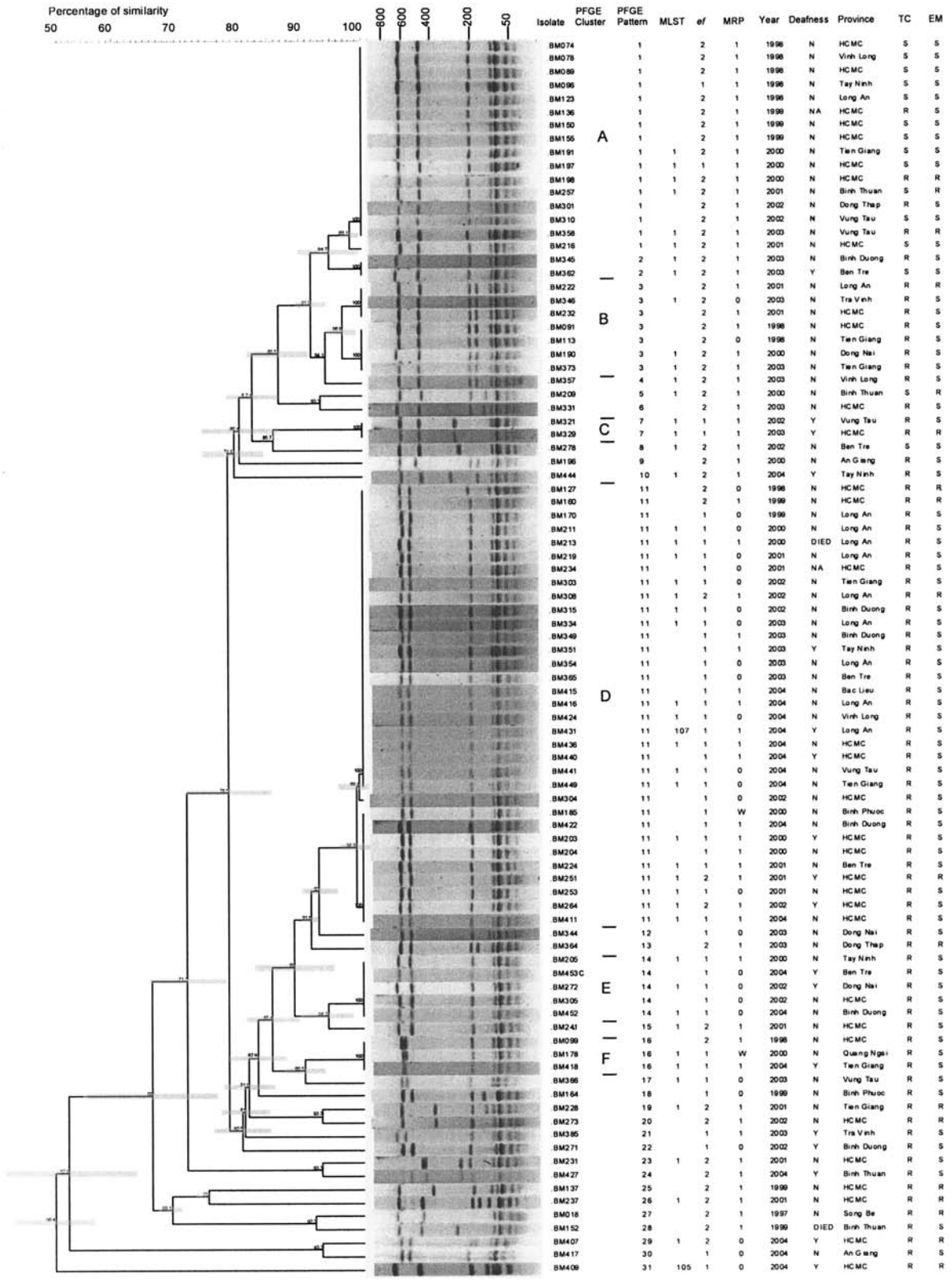

Figure 3. PFGE after *Sma*I digestion of *Streptococcus suis* serotype 2 strains isolated from patients with meningitis in southern Vietnam. A dendrogram was generated by Dice analysis (optimization, 0.5%; band tolerance, 1.5%) and cluster analysis with unweighted pair group method with arithmetic mean, using Bionumerics software (Applied Maths). Bars indicate 95% CIs. Numbers above gel picture indicate molecular size (kb). Deafness was defined as severe hearing loss at hospital discharge. *ef, epf* gene (1) or *epf** gene (2) positive; EM, erythromycin; MLST, sequence type (obtained after multilocus sequence typing); MRP, muramidase released protein (with 1 indicating positivity and 0 indicating negativity); N, absent; NA, not available; R, resistant; S, susceptible; TC, tetracycline; Y, present.

because 91 of 92 isolates were serotype 2. We cannot rule out infection with *S. suis* serotype 1/2 for samples that had negative culture results but positive PCR results, because PCR does not distinguish between serotypes 2 and 1/2. However, such infections have never been reported in humans.

Previous studies on the genetic diversity of *S. suis* revealed that strains of serotype 2 were genetically diverse but also suggested clustering of strains with a certain virulence-associated phenotype or clinical syndrome. Human isolates clustered in patterns similar to those in pig isolates, although PFGE showed some specific clustering of human isolates [18]. However, the number of human strains studied to date, outside outbreak situations, has been small (range, 1–27 strains), with limited epidemiological data available [18, 36–38]. PFGE typing of our strains identified 30 band patterns, but 36% of strains belonged to a single cluster (cluster D) (figure 2). Ninety-eight percent of our strains characterized by MLST had sequence type 1, and all strains belonged to clonal complex 1. The strain that caused an outbreak of infection in pigs and humans in China in 2005 had sequence type 7 and also belonged to clonal complex 1 [5]. Our results indicate that, despite their epidemiological unrelatedness, the population of *S. suis* infecting humans in southern Vietnam derives from a single lineage and is highly homogenous. Although divergence has occurred—as reflected in the presence of 30 band patterns on PFGE and the variability in the presence of EF/EF* and MRP—a predominance of 2 clusters (clusters A and D) exists, suggesting the prevalence of stable clones. Although it is tempting to speculate that these clones may have an increased propensity to infect humans, molecular typing of strains from the appropriate pig population is needed to conclude this.

In addition to recent and current health threats caused by severe acute respiratory syndrome and avian influenza H5N1, *S. suis* infection represents yet another zoonotic disease emerging in Asia. The threat of human *S. suis* infections is likely to increase because of the expansion of pig farming in Asia.

Acknowledgments

We thank Nguyen Minh Hoang and Tran Thi Thu Nga, for laboratory assistance; Paul Savelkoul and Narisara Chantratita, for their help with PFGE; and Tuan Phung Quoc, for his advice about statistics. This publication made use of the Multi Locus Sequence Typing Web site (http://www.mlst.net) at Imperial College London that was developed by David Aanensen and funded by the Wellcome Trust.

Financial support. Wellcome Trust UK and Wellcome Trust International Traveling Fellowship (to N.T.H.).

Potential conflicts of interest. All authors: no conflicts.

References

1. Huang YT, Teng LJ, Ho SW, Hsueh PR. *Streptococcus suis* infection. J Microbiol Immunol Infect **2005**; 38:306–13.
2. Kay R, Cheng AF, Tse CY. *Streptococcus suis* infection in Hong Kong. QJM **1995**; 88:39–47.
3. Mazokopakis EE, Kofteridis DP, Papadakis JA, Gikas AH, Samonis GJ. First case report of *Streptococcus suis* septicaemia and meningitis from Greece. Eur J Neurol **2005**; 12:487–9.
4. Wangkaew S, Chaiwarith R, Tharavichitkul P, Supparatpinyo K. *Streptococcus suis* infection: a series of 41 cases from Chiang Mai University Hospital. J Infect **2006**; 52:455–60.
5. Ye C, Zhu X, Jing H, et al. *Streptococcus suis* sequence type 7 outbreak, Sichuan, China. Emerg Infect Dis **2006**; 12:1203–8.
6. Yu H, Jing H, Chen Z, et al. Human *Streptococcus suis* outbreak, Sichuan, China. Emerg Infect Dis **2006**; 12:914–20.
7. Arends JP, Zanen HC. Meningitis caused by *Streptococcus suis* in humans. Rev Infect Dis **1988**; 10:131–7.
8. Lun ZR, Wang QP, Chen XG, Li AX, Zhu XQ. *Streptococcus suis:* an emerging zoonotic pathogen. Lancet Infect Dis **2007**; 7:201–9.
9. Watkins EJ, Brooksby P, Schweiger MS, Enright SM. Septicaemia in a pig-farm worker. Lancet **2001**; 357:38.
10. Willenburg KS, Sentochnik DE, Zadoks RN. Human *Streptococcus suis* meningitis in the United States. N Engl J Med **2006**; 354:1325.
11. Smith HE, Reek FH, Vecht U, Gielkens AL, Smits MA. Repeats in an extracellular protein of weakly pathogenic strains of *Streptococcus suis* type 2 are absent in pathogenic strains. Infect Immun **1993**; 61:3318–26.
12. Smith HE, Vecht U, Gielkens AL, Smits MA. Cloning and nucleotide sequence of the gene encoding the 136-kilodalton surface protein (muramidase-released protein) of *Streptococcus suis* type 2. Infect Immun **1992**; 60:2361–7.
13. Nguyen TH, Tran TH, Thwaites G, et al. Dexamethasone in Vietnamese adolescents and adults with bacterial meningitis. N Engl J Med **2007**; 357:2431–40.
14. Boom R, Sol C, Beld M, Weel J, Goudsmit J, Wertheim-van Dillen P. Improved silica-guanidiniumthiocyanate DNA isolation procedure based on selective binding of bovine alpha-casein to silica particles. J Clin Microbiol **1999**; 37:615–9.
15. Corless C, Guiver M, Borrow R, Edwards-Jones V, Fox A, Kaczmarski E. Simultaneous detection of *Neisseria meningitidis, Haemophilus influenzae,* and *Streptococcus pneumoniae* in suspected cases of meningitis and septicemia using real-time PCR. J Clin Microbiol **2001**; 39:1553–8.
16. van Doornum GJ, Guldemeester J, Osterhaus AD, Niesters HG. Diagnosing herpesvirus infections by real-time amplification and rapid culture. J Clin Microbiol **2003**; 41:576–80.
17. Allgaier A, Goethe R, Wisselink HJ, Smith HE, Valentin-Weigand P. Relatedness of *Streptococcus suis* isolates of various serotypes and clinical backgrounds as evaluated by macrorestriction analysis and expression of potential virulence traits. J Clin Microbiol **2001**; 39:445–53.
18. Berthelot-Herault F, Marois C, Gottschalk M, Kobisch M. Genetic diversity of *Streptococcus suis* strains isolated from pigs and humans as revealed by pulsed-field gel electrophoresis. J Clin Microbiol **2002**; 40:615–9.
19. King SJ, Leigh JA, Heath PJ, et al. Development of a multilocus sequence typing scheme for the pig pathogen *Streptococcus suis:* identification of virulent clones and potential capsular serotype exchange. J Clin Microbiol **2002**; 40:3671–80.
20. Multilocus sequence typing database. *Streptococcus suis*—database. Available at: http://www.ssuis.mlst.net. Accessed 28 August 2007.
21. Multilocus sequence typing database. Unique profile treedraw. **2006**. Available at: http://ssuis.mlst.net/eburst/. Accessed 28 August 2007.
22. King SJ, Heath PJ, Luque I, Tarradas C, Dowson CG, Whatmore AM. Distribution and genetic diversity of suilysin in *Streptococcus suis* isolated from different diseases of pigs and characterization of the genetic basis of suilysin absence. Infect Immun **2001**; 69:7572–82.
23. van de Beek D, de Gans J, Tunkel AR, Wijdicks EF. Community-acquired bacterial meningitis in adults. N Engl J Med **2006**; 354:44–53.
24. Vilaichone RK, Vilaichone W, Nunthapisud P, Wilde H. *Streptococcus suis* infection in Thailand. J Med Assoc Thai **2002**; 85(Suppl 1):109–17.
25. Kay R. The site of the lesion causing hearing loss in bacterial meningitis: a study of experimental streptococcal meningitis in guinea-pigs. Neuropathol Appl Neurobiol **1991**; 17:485–93.
26. Dichgans M, Jager L, Mayer T, Schorn K, Pfister HW. Bacterial men-

ingitis in adults: demonstration of inner ear involvement using high-resolution MRI. Neurology **1999**; 52:1003–9.
27. van de Beek D, de Gans J, McIntyre P, Prasad K. Corticosteroids for acute bacterial meningitis. Cochrane Database Syst Rev **2007**: CD004405.
28. Vecht U, Wisselink HJ, van Dijk JE, Smith HE. Virulence of *Streptococcus suis* type 2 strains in newborn germfree pigs depends on phenotype. Infect Immun **1992**; 60:550–6.
29. Wisselink HJ, Smith HE, Stockhofe-Zurwieden N, Peperkamp K, Vecht U. Distribution of capsular types and production of muramidase-released protein (MRP) and extracellular factor (EF) of *Streptococcus suis* strains isolated from diseased pigs in seven European countries. Vet Microbiol **2000**; 74:237–48.
30. Sriskandan S, Slater JD. Invasive disease and toxic shock due to zoonotic *Streptococcus suis:* an emerging infection in the East? PLoS Med **2006**; 3:187.
31. Swildens B, Stockhofe-Zurwieden N, van der Meulen J, Wisselink HJ, Nielen M, Niewold TA. Intestinal translocation of *Streptococcus suis* type 2 EF^+ in pigs. Vet Microbiol **2004**; 103:29–33.
32. Strangmann E, Froleke H, Kohse KP. Septic shock caused by *Streptococcus suis:* case report and investigation of a risk group. Int J Hyg Environ Health **2002**; 205:385–92.
33. Gottschalk M, Segura M. The pathogenesis of the meningitis caused by *Streptococcus suis:* the unresolved questions. Vet Microbiol **2000**; 76:259–72.
34. Gottschalk M, Higgins R, Jacques M, Mittal KR, Henrichsen J. Description of 14 new capsular types of *Streptococcus suis.* J Clin Microbiol **1989**; 27:2633–6.
35. Nghia HDT, Hoa NT, Linh LD, et al. Human case of *Streptococcus suis* serotype 16 infection. Emerg Infect Dis **2008**. Available at: http://www.cdc.gov/EID/content/14/1/155.htm. Accessed 21 January 2008.
36. Chatellier S, Gottschalk M, Higgins R, Brousseau R, Harel J. Relatedness of *Streptococcus suis* serotype 2 isolates from different geographic origins as evaluated by molecular fingerprinting and phenotyping. J Clin Microbiol **1999**; 37:362–6.
37. Hampson DJ, Trott DJ, Clarke IL, Mwaniki CG, Robertson ID. Population structure of Australian isolates of *Streptococcus suis.* J Clin Microbiol **1993**; 31:2895–900.
38. Luey CK, Chu YW, Cheung TK, et al. Rapid pulsed-field gel electrophoresis protocol for subtyping of *Streptococcus suis* serotype 2. J Microbiol Methods **2007**; 68:648–50.