Fatal case of bacteremia caused by *Streptococcus suis* in a splenectomized man and a review of the European literature

ZSUZSANNA ÁGOSTON¹, GABRIELLA TERHES², PÉTER HANNAUER¹, MÁRIÓ GAJDÁCS³ and EDIT URBÁN¹⁴,⁵

¹ Department of Anaesthesiology and Intensive Therapy, University of Szeged, Faculty of Medicine, Szeged, Hungary
² Department of Clinical Microbiology, University of Szeged, Faculty of Medicine, Szeged, Hungary
³ Department of Pharmacodynamics and Biopharmacy, Faculty of Pharmacy, University of Szeged, 6720, Eötvös utca 6, Szeged, Hungary
⁴ Department of Public Health, Faculty of Medicine, University of Szeged, 6720, Dóm tér 10, Szeged, Hungary
⁵ Institute of Translational Medicine, University of Pécs, Medical School, 7624, Szigeti utca 12, Pécs, Hungary

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ABSTRACT

*Streptococcus suis* is an emerging zoonotic human pathogen, which is a causative agent of invasive infections in people who are in close contact with infected pigs or contaminated pork products. It is associated with severe systemic infections, most commonly meningitis and sepsis, which may lead to high rates of morbidity and mortality. Serotype 2 is the most prevalent type in *S. suis* infections in humans. We have reported a case of a very rapidly proceeding fatal human *S. suis* infection in a splenectomized, but otherwise immunocompetent patient in Hungary. We would like to highlight the attention for this pathogen for the risk group patients, not only pig breeders, veterinarians, abattoir workers, meat processing and transport workers, butchers and cooks, that those persons who are immunocompromised including those with spleen removed, persons with diabetes mellitus, cancer and alcoholism, are also at greater risk of infection.

KEYWORDS

zoonosis, *Streptococcus suis*, sepsis, DIC, fatal infection

INTRODUCTION

*Streptococcus suis* is a group of heterogeneous Gram-positive bacteria that were earlier classified into the Lancefield groups R, S, and T. These bacteria are facultative anaerobes with a spherical/ovoid shape which exist in short chains and/or pairs or as single bacteria. The microorganisms show narrow zones of α-hemolysis when growing on selective plates of on sheep or bovine blood agar plates. *S. suis* is an important zoonotic pathogen in mostly swines and wild boars, but occurs in a wide range of animals and humans [1]. Clinical presentations associated with this species in human infections are most frequently manifest as acute purulent bacterial meningitis, but include sepsis, streptococcal toxic shock-like syndrome (STTLS) with multiple organ failure, endocarditis, and uveitis-endophthalmitis [2–4]. Generalized septicemia caused by *S. suis* may transform into arthritis, which affects different types of joints such as hips, elbows, wrists, sacroiliac, spine, and thumbs. Permanent hearing
loss and vestibular dysfunction are commonly noted sequelae of these infections [5]. Such infections are typically sporadic, and in the majority of cases, occur in particular occupational groups, such as pig breeders, veterinarians, hunters, meat processing and transport workers, butchers and cooks. According to literature data, skin injury in the presence of pig/pork contact was described in 20–25% of the published cases of S. suis infections [6]. S. suis may directly pass into the bloodstream after exposure to pigs or pork/bones in the presence of skin injuries, even without visible wound infections. Infections may also be acquired by contact with raw or undercooked meat products, traditionally consumed in the Far East of Asia and, thus, S. suis should be considered there as a food-borne pathogen. S. suis as a pathogenic bacteria was initially reported by veterinarians in 1954, after outbreaks of meningitis, septicemia, and purulent arthritis occurred among piglets [7]. The first published case of S. suis meningitis in a human patient demonstrated as an etiological agent for this kind of bacterial infection was in Denmark in 1988 [8] and soon, some other cases were reported in different Northern European countries and Hong Kong. The interest in human infections caused by S. suis has grown exponentially after the high morbidity-mortality rates observed in Eastern-Asia countries, and human infections have been now described worldwide [9–16]. Epidemiology of infections differ among European–American countries and different regions of Asia and the role of high-risk eating habits (i.e., ingesting raw or undercooked pig parts, including pig blood, organs) in some Asian communities has been recently recognized [9–17]. According to Goyette-Desjardins et al. [17], 1,642 cases of S. suis infections were identified between 2002 and 2013 worldwide in humans, in a total of 34 countries. Rates of S. suis infection are low in the general population of Europe and North America, and cases are concentrated among the exposed groups with other risk factors. Hungary is a country in Central Europe, where the breeding of pigs has long-lasting traditions, due to the proclivity and preference of the general population to consume pig meat. Therefore, there are a lot of people working with these animals (farmers breeding or involved in the transportation of pigs, dealing with the processing of pork meat for consumption, e.g., butchers or workers in meat plants), however, until this time only one case of human S. suis infection has been previously described in Hungary, but only in the Hungarian-language literature in Hungary [18].

S. suis a complex population consisting of heterogeneous strains, which can be classified into 35 serotypes (1–34, 1/2) based on the differentiation of capsule polysaccharide (CPS), antigens, but most clinical isolates from reported human cases were serotype 2 strains [19–21]. However, sporadic human cases of other serotypes such as 1, 4, 5, 9, 14, 16, and 24 have also been reported, mostly in Southeast-Asian countries. Still now, little is known factually about the pathogenesis of S. suis infections and how the bacteria crosses the blood-brain barrier. Inflammation is likely to contribute to the manifestations of disease in pigs and probably also in humans, as has been summarized in the literature previously [22–24]. Bacteria may be transmitted to patients through contamination, minor cuts or abrasions are enough for the pathogen to be transmitted through the skin of hands and/or arms causing a breach of skin integrity. Some authors believe that the presence of multiple scars or cuts on the hands of the patients facilitates the developing the septic shock syndrome caused by S. suis after handling animals [22–24]. Until now, more than 20 bacterial virulence-associated factors have been identified, including capsular polysaccharides (CPS), DNase (SsnA), extracellular protein factor (EPF), suilysin (Sly), serum opacity factor (OFS), some various adhesins: include fibronectin-binding protein (FBP), muramidase-released protein (MRP), hyaluronate lyase, surface antigen enolase (Eno) and surface antigen one (Sao) [22–31]. Streptococcal toxic shock syndrome (STSS) and high mortality of patients, observed in both big Chinese outbreaks, was attributed to the presence of the 89K putative pathogenicity island (89K PAI) found in strains which were responsible for these outbreaks [29]. Further studies suggested that 89K PAI with a transposon-like essence can undergo GI-type T4SS-mediated horizontal transfer in epidemic SS2 species, and to identify genes encoding a two-component signal transduction system SalK/SalR, a type IV-like secretion system and a novel hemolysis-related gene hly3, located within this element and presumably involved in the STSS development [22–31]. Capsular polysaccharide (CPS) a determinant of serotypes, but it is also very important virulence factor and has high anti-phagocytic effects against monocytes, neutrophils and dendritic cells [19–21].

CASE DESCRIPTION

A 34-year-old obese (BMI > 30) male patient was admitted to the Emergency Unit of the University Hospital in Szeged, due to very high fever (body temperature of 39.9 °C) and unconsciousness. In his past medical history, splenectomy was recorded 10 years ago due to a motorcycle accident. The patient worked as a butcher in a local meat processing plant, where he was injured on his hand by a pig bone one day before hospital admission. On arrival to the emergency room, he exhibited no neck stiffness or meningism, nor peripheral stigmata of infective endocarditis; precordial examination revealed no audible cardiac murmur. At home, the patient had high fever, chills, abdominal pain and watery diarrhea. Relatives found the unconscious patient on the floor of the sanitary room. XBLS (Extended Basic Life Support) and later ALS (Advanced Life Support) were started because of circulatory and respiratory failure. After intubation and administration of epinephrine, spontaneous circulation returned. Because of the possibility of subarachnoid bleeding or pulmonary embolism, urgent cranial and abdominal CT scans and pulmonary angiography were carried out, and the patient was transferred to the ICU. CT scans could not reveal any circulatory disorders, while cranial CT showed right-sided, multiple, acute ischemic lesions.
In his past medical history, beside the splenic rupture, hypotension was also noted. On admission to the ICU, severe hypotension, sinus tachycardia and high fever were observed in spite of intravenous dopamine. Using invasive cardiovascular monitoring, hydration and noradrenaline administration were applied. In spite of the PEEP (positive end-expiratory pressure), adequate oxygenisation could not be achieved, values for hypercapnic ventilator drives were measured using blood gas analysis. The development of respiratory and metabolic acidosis, severe hypoglycaemia and hypokalemia were noted, thus supportive therapy (ventilation, glucose, and potassium substitutions) were introduced. High-dose vasopressor therapy was supplemented with epinephrine, but only transient increase in blood pressure has been achieved. Renal function deteriorated and bleeding from each puncture site was noticed. Simultaneously, petechiae in skin and mucous membranes throughout the body could be observed. Laboratory investigations revealed severe coagulopathy, thrombocytopenia and fibrinolysis, thus alterations in hemostasis, for which therapy has been initiated. Leukocyte cell count and C-reactive protein were 17.0 x 10^9 L^-1 and 0.65 mg dL^-1, respectively. Samples for microbiological investigation, including duplicate blood culture pairs, urine samples, faeces, sera for serology were immediately sent to the microbiological laboratory. Empiric antibiotic therapy was introduced (ceftriaxone and vancomycin), but in spite of this, the patient’s condition did not improve; his blood pressure was not stable, the oxygenisation did not improve and the acidosis was persistent. Laboratory results showed disseminated intravascular coagulation (DIC), acute renal, and hepatic failures, while the result of a total microbiological investigation of blood culture was given after the patient died. S. suis was cultured from one pair of blood culture bottles after 1 h of incubation and from the other bottles after 4 h. Viral serology only revealed a past EBV infection. The patient died within 12 h after hospital admission from complications, such as DIC, acute renal failure (ARF), and acute respiratory distress syndrome (ARDS). Other toxic causes of metabolic catastrophe were not verified. Postmortem examination of patients showed features of DIC. Evidence of multiple organ damage was observed, primarily involving lungs, liver, kidneys, histologic findings included microthrombosis in organ capillaries; necrosis of parenchymal cells; and congestion, exudate, and hemorrhage of interstitial vessels of kidneys, lungs, and other organs. The patient’s blood culture proved to be positive for S. suis which was identified using matrix-assisted laser desorption/ionization time of flight method (MALDI-TOF) with a high log-score (2.324; score >2.3 indicates “highly probable species identification”). The cultures were identified as S. suis serotype 2 by polymerase chain reaction (PCR) and specific sera [16]. The strain isolated from our patient was resistant to clindamycin, erythromycin, clari-thromycin and tetracycline, but susceptible to all tested β-lactam antibiotics and vancomycin (note: susceptible/ resistant classifications employed for the breakpoints were for viridans group streptococci, as neither the Clinical and Laboratory Standards Institute guidelines nor EUCAST provides breakpoints for S. suis) and susceptible to the other antimicrobial agents tested. Species identification was further confirmed by sequence analysis of the 16S rRNA gene. Polymerase chain reaction amplification of the nearly complete 16S rRNA gene (1 475 bp) with two primers (8FPL and 1492) was performed, as described previously [16, 32]. The amplification products were purified and sequenced. The BLAST (Basic Local Alignment Search Tool) program was used to compare the sequence of our isolates with those in the GenBank and Ribosomal Database Project databases. Sequencing of the 16S rRNA gene confirmed that the strain belonged to the species S. suis with 99% nucleotide identity compared with GenBank accession no. NR036918, AF009487 and AM946016. The PCR-based results indicated that our isolate possessed both tet(Q) the TC-resistance determinant, and erm(B), the ML-resistance determinant genes together.

**DISCUSSION AND REVIEW OF EUROPEAN LITERATURE**

*S. suis* is a Gram-positive, facultative anaerobic coccus, that has been implicated as the cause of a wide range of clinical diseases in swine and can be found in wide range of animal species, including wild boars, horses, dogs, raccoon dogs, cats, and birds [1–6]. In pigs, the natural habitat of *S. suis* is the upper respiratory tract, particularly tonsils (tonsil carriage rate in piglets aged 4–6 months was 32–50%), nasal cavities, genital and alimentary tracts [33, 34], they could be healthy carriers or afflicted. In contrast to the serotypes infecting pigs, *S. suis* serotype 2 is the most common cause of this disease in humans [33, 34]. *S. suis* infection normally occurs among certain risk population particularly veterinarians, hunters, farmers, and abattoir works involving meat processing [1–6]. There is a wide clinical variation in the presentation of *S. suis* infections in humans, but meningitis is the most common clinical manifestation; *S. suis* can cause a very rare but serious infection: there was a description of a large-scale STSS outbreak among humans in China in 2005 caused by a single clonal *S. suis* type 2 strain [35]. While pig-related occupation is a main risk factor for human *S. suis* infection, exposure to pigs or pig meat is not present in all of the cases of *S. suis* infection, in Western countries. Although substantial new data on the incidence, clinical and microbiological characteristics, and risk factors for *S. suis* infection has been accumulated during recent years, the prevalence of this infection has not yet been described in Europe. In most European countries, the infection rates among the exposed groups are poorly known because *S. suis* infection is not a notifiable disease, while only the United Kingdom and France consider *S. suis* infections in humans as an industrial-risk disease. During the last decade, the number of reported human cases due to *S. suis* has dramatically increased, and most sporadic human cases of infection appear to be due to close contact with pigs/pork products, particularly in Western countries.
parameters of STSS, the case was characterized by multi-organ splenectomized, and in accordance with the clinical protection of community-acquired bacterial meningitis in Hong Kong [35–37]. In Western countries, infections in humans mostly occur sporadically.

We searched the PubMed/MEDLINE database using the MeSH terms “Streptococcus suis” OR “Streptococcus suis AND infection” with searching for human studies only, without any time or language restrictions: we have found 1709 and 736 articles, respectively. If we narrowed our search “Streptococcus suis infection Europe” we found only 58 articles. In addition to its first documentation from Denmark, cases have been reported in The Netherlands [8, 38, 39], Italy [40–42], Spain [43–45], the United Kingdom [46], Belgium [47], Croatia [48], Serbia [49], Austria [50], Sweden [51], Germany [52–55], Poland [56], Hungary [18], France [57], Greece [58–60], and Portugal [61], but some of them are only available in their national languages. Unfortunately, no cases have yet been published in Russia, despite being a country with significant animal husbandry and considerable pig production. Mancini et al. reported the first human fatal case of STSS in Italy caused by S. suis serotype 2. The isolated strain was carrying a rare variation: tetracycline efflux tet gene and the tetracycline ribosomal protection tet (O/W/32/O) gene together [62]. Their patient was splenectomized, and in accordance with the clinical parameters of STSS, the case was characterized by multi-organ dysfunction (MOD) and DIC [62].

Strangmann et al. published a case of septic shock from Germany caused by S. suis type 2 in a 36-year-old man, who was working as a truck driver transporting pigs preceding the incident [63]. In the same study, 132 workers were involved in pig slaughtering, pork disecting and processing industries, to assess the potential risk for an infection with S. suis [63]. In their cross-sectional study, the otherwise healthy workers were examined for the occurrence of S. suis in their pharynx, and compared with an age and sex matched control group. The colonization rate of case group with S. suis amounted to 5.3% [63]. In a study by Gustavsson et al., two patients infected with the rare S. suis serotype 5 strains have been documented: one of them was a 65-year-old pig farmer who had cut his hand at work (while no cases were noted of severe illness among his pigs, he had a history of benign hyperplasia of the prostate gland he later developed septic arthritis in Sweden [64]. Another interesting case was a pig farmer without wounds or injuries in the US, who developed an arthroplasty infection and STSLS [64]. According to Schultzz et al., the S. suis serotype 2 is the main cause of zoonotic S. suis infection in Netherlands despite the fact that other serotypes are frequently isolated in their country from pig-diseases [65]. Unfortunately, studies comparing concurrent pathogenic invasive human and different pig-isolates (healthy colonizers or infected) from a single geographical location are lacking. In the abovementioned Dutch study, the population structures of invasive human S. suis strains isolated between 1986 and 2008 (n = 24) and from pigs with invasive disease (n = 124) were characterized by serotyping and multilocus sequence typing (MLST) [65]. Most of the pig isolates (56%) were of serotype 9, belonging to 15 clonal complexes (CCs) or singleton sequence types (ST), but all of the human isolates were of serotype 2 and they only belonged into two non-overlapping clonal complexes CC1 (58%) and CC20 (42%) [65]. Their results concluded that the rate of serotype 2 isolates among S. suis strains from human patients were significantly higher than among strains isolated from pigs (24/24 vs. 29/124; P < 0.0001). They also established, that the S. suis strain population isolated from infected pigs was more diverse than the one isolated from human patients. The S. suis serotype 2 strains of CC20 (which consisted 42% of all human strains) were all negative for PCR detection of genes encoding extracellular protein factor (EF) variants [65]. These data indicate that the polysaccharide capsule was an important factor regarding human S. suis infection, irrespective of other genotypes and virulence factors of S. suis strains. Princivali et al. published a report in 2009, where two strains from human meningitis in Sardinia and Northeastern Italy was characterized for their genetics and antimicrobial susceptibility [66]. The abovementioned S. suis strains (the n = 2 recent human strains and n = 57 swine clinical isolates were collected between 2003 and 2007 from different Italian herds and regions), were tested for their susceptibility patterns, for virulence factors and antibiotic resistance genes using PCR and they were also subjected to molecular typing. Their study demonstrated an overall high genetic diversity among isolates, the majority of which were resistant to macrolides (78%) and tetracyclines (90%). The erm(B), tet(O), mosaic tet(O/W/32/O), tet(W), and tet(M) genes were detected [66]. The tet(O/ W/32/O) gene was first described in the genus Streptococcus in this publication which was the most frequent tet gene after tet(O) [66]. A virulent clone belonging to sequence type 1 (ST1) of the ST1 complex was found to be prevalent and persistent in Italian swine herds, but the two human isolates (both ST1) carrying cps2, erm(B) and tet(W) were seen to be closely related to each other. The very interesting finding that invasive and non-invasive isolates share identical virulence profiles seems to support the conclusions of earlier publications that some other, yet unknown virulence factors are responsible for the pathogenic potential of S. suis [66]. In one of the few available retrospective report from Eastern-European countries, Bojarska et al. performed a retrospective analysis of S. suis human invasive isolates, collected by the National Reference Centre for Bacterial Meningitis in Poland [67]. In this report, isolates obtained from 21 patients during 2000–2013 were investigated by phenotypic biochemical tests and multilocus sequence typing (MLST): 48% of cases were misdiagnosed/misidentified by a microbiological laboratory who submitted the strains. All investigated isolates were members of the ST1 and were suggested to be serotype 2, most of the isolates were α-hemolytic, DNase-positive and little less, than half of them could form biofilm. Genes encoding sluylsin, EPF, FBP, MRP, surface antigen one, enolase, OFS, and pili were ubiquitous in the studied group of
isolates, while none of the isolates carried sequences characteristic for the 89R pathogenicity island [67]. In this report, fatal hyperacute infections with shock have also been described and a high mortality rate, especially in the case of the STSLS, closely associated with the 2005 Chinese epidemic, but also observed independently. The authors expressed the opinion that the real morbidity due to S. suis in Poland, a country with a well-developed swine industry (over 10 million units), is very likely underestimated [67]. Manzin et al. described a severe case in a 68-year-old retired welder of S. suis menigitis from Sardinia, Italy, who interestingly had no reported contact with swine, other animals or any animal products [68]. However, as described in the case, the patient had cancer, which was discovered incidentally during the workup: a computer tomography scan, bronchoscopy and histopathologic findings led to diagnosis of the mass as an advanced-stage squamous cell carcinoma [68]. As reported already for Group B Streptococcus, splenectomy, alcoholism, diabetes mellitus and malignancy have been suggested as important predisposing factors for the development of serious, often rapid, fatal S. suis diseases [45, 68, 69–72]. Indeed, many S. suis cases have been described in individuals who had suffered splenectomy, furthermore, the mortality rate of S. suis infection after splenectomy is nearly 80% [1–6, 45, 69–72].

Although the colonization rate of S. suis in swine and wild boars is well-known and characterized (85–100%), the reports on the epidemiology of invasive S. suis infections in these animals in Europe (especially Hungary) are scarce [73]. Interestingly, while there is significant geographical variation in the prevalence of human S. suis cases (cases from Europe only account for about 8–9% of cases), the epidemiological variations in pig infections are far less pronounced [74]. Transmission of this pathogen is usually through the respiratory system in the form of direct nose-to-nose contact (horizontal transmission), however, vertical transmission through the genital tract during farrowing has also been described [75, 76]. Susceptible pigs (especially piglets during their weaning, may suffer from meningitis, septicaemia, pneumonia, endocarditis, or polyarthritis; the molecular pathogenesis of these diseases in pigs is similar to their human counterparts [73, 77]. A study reporting on the significant factors of piglet mortality in Europe (including Hungary) has already highlighted the role of S. suis infections as an economic burden for the industry [78], while later a report noted the relevance of this bacterium as an occupational hazard [79]. In a Hungarian large breeding unit of >3,000 sows, an intervention study was performed regarding S. suis Serotype 2 vaccination because of severe animal losses due to S. suis disease; as a result, pre-weaning mortality was significantly reduced the vaccinated groups of pigs (both in groups vaccinated 3 or 6 weeks after parturition) [80]. Similar antibiotics may be used for the therapy of S. suis disease in pigs like in humans, additionally, a 3rd generation cephalosporin (cefotiofur) for veterinary use only is licenced for this exact indication [73].

In conclusion, we have described the first fatal human case of S. suis infection in Hungary (with a rapidly-progrediating disease course), where the pathogen was extensively characterized both microbiologically and genetically. To our knowledge, this is the second Hungarian published case, however due to Hungary’s pronounced traditional agriculture, which primarily includes pork, prevalent pig-meat consumption customs and disadvantageous epidemiological characteristics (high levels of alcohol consumption, diabetes mellitus, obesity and one of the highest incidence of malignancies worldwide), which have all been described as important predisposing factors for the development of serious, often fatal outcome in this infection. So far, some reports of S. suis infections have only been presented at national congresses. In the database of the National Bacteriological Surveillance (NBS) in Hungary, there have been 17 reports of these bacteria in invasive samples (i.e., blood cultures, cerebrospinal fluids) over a 15-year period [81, 82]. Therefore, one invasive case should be expected annually, based on national surveillance data.

It seems that our patient acquired the infection through his skin wounds caused by a pig bone. As the patient had splenectomy several years before this injury, he was more prone to rare infections. His clinical symptoms presented as an invasive deep-tissue infection with STSS, characterized by acute high fever, vascular collapse, hypotension, DIC, shock, multiple organ failure, and despite the fact that he was a young, otherwise healthy strong man, finally resulted in a very rapid deterioration and at last death. Although the isolation rate for these bacteria is still very low in European countries, clinicians should be aware of the emergence of S. suis infections especially in immunocompromised patients. Since first described in Denmark in 1968, over 1,600 human cases of S. suis infection have been reported however on the basis of European data about the prevalence of S. suis, we assume that this infection is underdiagnosed in most European countries. Misidentification of S. suis is very common: this bacterium is frequently misidentified as Streptococcus viridans [14] and has also been misidentified as Streptococcus bovis, Streptococcus pneumoniae, Streptococcus faecalis, and Streptococcus acidominimus [2, 6, 21, 83]. Misidentification of S. suis also has been reported in Canada, which raises suspicion that human S. suis infections might be underdiagnosed in North America [84, 85]. In the report by Tarini et al., a previously healthy 50-year-old man was admitted to one of the district hospitals in Bali Province, Indonesia, due to meningitis. The patient had a history of consuming homemade raw pork product two days before the onset of illness. Streptococcus mitis was identified from the cerebrospinal fluid culture by using VITEK 2 COMPACT (Biomerieux) with a 99% probability score. This patient had clinical symptoms and risk factor identical to S. suis infection, so they performed confirmation tests for the cerebrospinal fluid by PCR (using primer specific for gdh and recN) and sequencing of those PCR products, both of the confirmation tests showed a positive result for S. suis [86]. Finally, the use of modern diagnostic methods, such as MALDI-TOF, targeted directly from clinical isolates could identify correctly the strain, as well as PCR can
provide better possibilities to identify unusual or biochemically inactive species [87].

Our study highlights that in non-endemic areas, infection with this pathogen, although not frequently reported, should be considered when diagnosis is made for patients who work in pigeries. In conclusion, clinicians should be aware of this microorganism when examining and treating patients with fever, who are handling raw pork or having close contact with infected pigs even if they are immunocompetent. People in risk groups should be educated and made aware of this infection. Patients with an increased risk of infection, e.g., because of splenectomy or use of immunosuppressive medication, should avoid direct pig or pork contact when skin lesions-particularly on the hands—are present. Skin protection has been suggested to reduce the incidence of S. suis infection. On the basis of this case, the necessity of vaccination should have arisen in the case of patients with splenectomy.

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