

Molecular Epidemiology of Underreported Emerging Zoonotic Pathogen *Streptococcus suis* in Europe

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Learning Objectives

Upon completion of this activity, participants will be able to:

- Assess the worldwide epidemiology of *Streptococcus suis*.
- Distinguish the most common serotype of *S. suis* associated with zoonotic infection.
- Analyze clinical syndromes associated with infection with *S. suis*.
- Evaluate genetic characteristics of *S. suis* isolates in the current study.

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Streptococcus suis, a zoonotic bacterial pathogen circulated through swine, can cause severe infections in humans. Because human *S. suis* infections are not notifiable in most countries, incidence is underestimated. We aimed to increase insight into the molecular epidemiology of human *S. suis* infections in Europe. To procure data, we surveyed 7 reference laboratories and performed a systematic review of the scientific literature. We identified 236 cases of human *S. suis* infection from those sources and an additional 87 by scanning gray literature. We performed whole-genome sequencing to type 46 zoonotic *S. suis* isolates and combined them with 28 publicly available genomes in a core-genome phylogeny. Clonal complex (CC) 1 isolates accounted for 87% of typed human infections; CC20, CC25, CC87, and CC94 also caused infections. Emergence of diverse zoonotic clades and notable severity of illness in humans support classifying *S. suis* infection as a notifiable condition.

Streptococcus suis is an opportunistic bacterial porcine pathogen that can cause severe disease in humans, most commonly meningitis and sepsis (1). Human *S. suis* infections occur both through direct contact with infected pigs and consumption of undercooked contaminated pork (2). Human *S. suis* infections have become endemic in Thailand and Vietnam, driven by consumption of traditional raw pork dishes (1), and *S. suis* has caused multiple outbreaks in humans with high levels of illness and death in China and Thailand (3). In Europe, *S. suis* infections are considered an occupational hazard, mainly occurring among persons with skin lesions working closely with pigs or pork products (1). Human infections in Europe account for ≈10% of the global prevalence, but incidence in Europe is likely underestimated because *S. suis* infections are not a notifiable disease (4). Togo, Madagascar, Chile, and Indonesia have recently reported zoonotic *S. suis* infections, meaning all continents except Antarctica have now reported human infections (1,5–8).

S. suis is classified into 29 distinct serotypes based on its capsular polysaccharide, as well as 27 novel serotypes based on novel capsular polysaccharide loci. Serotypes 2, 4, 7, and 9 are the most common causes of porcine disease in Europe (3); serotype 2 isolates cause ≈95% of human infections and serotype 14 causes ≈4% (4). In addition, sporadic infections caused by serotypes 4, 5, 7, 9, 16, 21, 24, and 31 have been reported (3,9–11). *S. suis* genotypes are classified on the basis of sequence types (STs) determined through multilocus sequence typing (MLST), which are grouped into clonal complexes (CCs) (12). CC1 with a serotype 2 capsule is the main lineage causing human infections and has expanded worldwide (3). Emerging zoonotic

lineages, such as CC20, which emerged from CC16 in the Netherlands after acquiring a serotype 2 capsule, also been described (13).

We aimed to increase insight into the epidemiology of human *S. suis* infections in Europe and to assess the bacterial population structure and diversity of zoonotic *S. suis* clades (1). We assessed the frequency of human *S. suis* infections in Europe through a survey of reference laboratories in top pig-rearing countries in Europe, performed a systematic literature review and explored the gray literature (social media, news accounts, and government reports). In addition, we reconstructed a representative phylogeny of zoonotic *S. suis* isolates in Europe.

This study was not reviewed by an ethics review board, because it was based on anonymized surveillance data. In accordance with Dutch law, approval from a medical ethics committee was not deemed necessary because case-patients were not subject to any actions or rules of conduct. We did not obtain informed consent because our data collection processes were exempted under exceptions formulated in the Dutch Implementation of the European General Data Protection Regulation Act (2016/679).

Methods

Survey

We contacted national reference laboratories in 10 countries in Europe (Czech Republic, Denmark, France, Germany, Hungary, Italy, the Netherlands, Poland, Spain, and the United Kingdom) that included *S. suis* infections within their scope. We asked those laboratories to retrospectively collect data on cases of human *S. suis* infection during 1990–2018 because most human *S. suis* infections have been reported since 1990. We asked participating laboratories to complete a questionnaire collecting patient metadata and bacterial typing and metadata. Anonymized patient metadata were age, sex, clinical signs, and occupation. Bacterial typing encompassed serotype, sequence type (ST), and available whole-genome sequences. Bacterial metadata were date of isolation, source of isolation, and method of identification. In addition, we requested in the questionnaire that reference laboratories share their isolates for further genomic analysis (Appendix, <https://wwwnc.cdc.gov/EID/article/30/3/23-0348-App1.pdf>).

Systematic Review

We performed a systematic review according to PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines (14) to

identify cases of human *S. suis* infections in Europe in articles published from 1990 (survey start date) through 2022. We screened PubMed, Web of Science, and Scopus for key terms—*S. suis*, human, and ≥ 1 country in Europe (as defined by the World Health Organization)—in the titles or abstracts of articles published before April 1, 2022 (Appendix). We removed duplicate references by using Zotero version 6.0.8 (<https://www.zotero.org>) and manual checking. We included studies containing data on human *S. suis* isolates or case reports describing human *S. suis* infections in Europe; we extracted patient and bacterial metadata for further analysis. We excluded studies that did not include data on zoonotic *S. suis* isolates or human infections, reported isolates not collected in Europe, did not publish original data, were published before 1990, or lacked information on the origin of isolates (Figure 1). To avoid duplication, we excluded from the systematic review iso-

lates reported in both the survey and an article; in addition, if an isolate appeared in multiple articles, we included data only from the original article.

Gray Literature Search

Because *S. suis* is not a notifiable disease, there are no guidelines for reporting such infections. To identify additional cases, we performed a broad scan of gray literature to capture cases of human *S. suis* infections in Europe not identified in the scientific literature or the survey. However, we distinguished cases we identified in the survey, literature review, and official reports from unreported cases (all other cases). We searched X (previously Twitter) and the Google news section using the terms *S. suis*, infection, and human in Dutch, English, French, German, Italian, Portuguese, and Spanish. To complement those data, we scanned ministry of health websites from France, Germany, Italy, the Netherlands,

Identification of studies via databases and registers

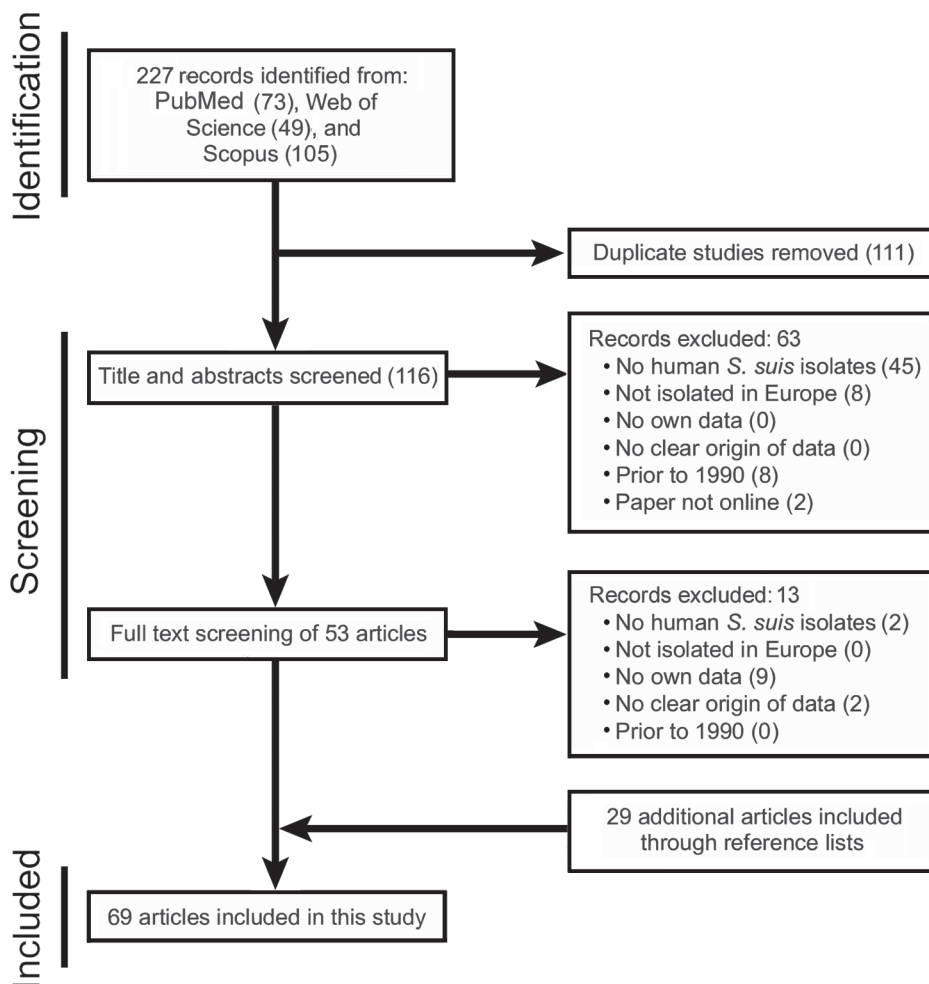


Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) search flowchart for systematic review of *Streptococcus suis* in Europe during 1990–2022.

Portugal, Spain, and the United Kingdom for reports on zoonotic bacterial infections related to human *S. suis* infections. To avoid duplication, we compared metadata, when available, with isolate data from the survey and systematic review.

In Silico Typing and Phylogenetic Analysis

We included 74 genomes for whole-genome sequencing (WGS) analysis, 67 from the survey (46 sequenced during this investigation and 21 previously sequenced) and 7 from the systematic review (Appendix Figure 1). We used MLST version 2.19.0 (<https://github.com/tseemann/mlst>) with the PubMLST database (<https://pubmlst.org>) to type the MLST profiles of the draft genomes. We submitted profiles for novel STs to PubMLST. We performed in silico serotyping by feeding processed Illumina reads into the *S. suis* serotyping pipeline (15). We reconstructed a core genome single-nucleotide polymorphism (SNP) phylogeny using Panaroo version 1.3.0 (16) to reconstruct the pangenome and align the core genome. We calculated the number of constant sites in the core genome alignment with SNP-sites version 2.5.1 (17) using the flag “-C.” We reconstructed the maximum-likelihood (ML) phylogeny by running IQ-TREE version 2.0.3 (18) with 1,000 bootstraps and used the general time-reversible plus gamma model with the flag “-fconst” to include the constant sites from SNP-sites. We investigated the presence of 46 accessory genes previously found to be overrepresented in zoonotic isolates (human-pig prevalence ratio >2) using ABRicate (<https://github.com/tseemann/abricate>) with a custom database and a minimum protein identity and coverage of 80%. We visualized the resulting gene presence/absence matrix in Phandango (19,20). Raw Illumina sequences can be found in the National Center for Biotechnology Information Short Read Archive (BioProject PRJNA853715). Genome assemblies have been deposited in GenBank and are available under the same BioProject number (Appendix Table 7).

Results

Geographic Distribution of Reported Human *S. suis* Infections across Europe, 1990–2022

Of 10 reference laboratories invited to participate in the survey, 7 laboratories (Spain, Germany, Netherlands, Denmark, Czech Republic, Poland, and United Kingdom) responded and reported 107 unique cases of human *S. suis* infections (Appendix Table 2). In the systematic review, of 119 screened titles and abstracts, we selected 53 articles mentioning human

S. suis infections in Europe for full-text reading. In addition, we included 29 studies identified by screening reference lists (Figure 1). In total, we extracted data from 129 cases of human *S. suis* infections reported in 69 research articles (Figure 1; Appendix Table 3). Combining both sources, we identified 236 unique cases of human *S. suis* infections across Europe during 1990–2022. Germany, Spain, and the Netherlands, the top pig-rearing countries in Europe (21), reported 114/236 (48%) of the cases (Figure 2). Furthermore, 203/236 (86%) of the reported cases originated from just 8 countries (Germany, Spain, the Netherlands, Denmark, Hungary, France, Poland, and the Czech Republic), 6 of which participated in the survey study; sporadic cases reported from 8 additional countries in Europe completed the dataset.

Epidemiology of Human *S. suis* Infections in Europe

Most patients were middle-aged men (Table 1). Of patients with a reported clinical syndrome, meningitis was the main clinical syndrome observed in both the survey (59/71 [83%]) and systematic review (59/86 [68%]), followed by sepsis, which affected 15/71 (21%) in the survey and 21/86 (24%) in the systematic review. Additional clinical signs and symptoms included hearing loss (n = 22), endocarditis (n = 6), and spondylodiscitis (n = 3); 11 patients died. Patient occupation was described as a potential risk factor in 19 cases in the survey and 72 cases in the systematic review (Table 1). Most infections, 78/92 (85%) in the survey and 43/48 (90%) in the systematic review, were caused by serotype 2 isolates, followed by serotype 14 isolates (Table 2). Most isolates (76/87 [78%] in the survey and 14/16 [88%] in the systematic review) belonged to zoonotic lineage CC1. In addition, 11/87 (13%) infections in the survey and 1 in the systematic review were caused by CC20 lineage isolates.

Year of isolation was collected for only 44/129 (34%) isolates from cases in the systematic review (Appendix Table 3). Nonetheless, average number of cases per year in the systematic review and survey increased after 1999, from 2.7 during 1990–1999 to 5.7 during 2000–2009 and 5.0 during 2010–2019 (Appendix Figure 2). Moreover, we calculated crude estimates of *S. suis* incidence in the at-risk population in 6 (Czech Republic, Germany, Hungary, the Netherlands, Poland, and Spain) countries with >5 cases reported in the survey or literature review during 2005–2013. We defined the population at risk as the proportion of the agricultural census involved in pig specialized holdings with a 10% upper margin to account for butchers, hunters, slaughterhouse workers, lorry drivers, and meat factory workers. Incidence

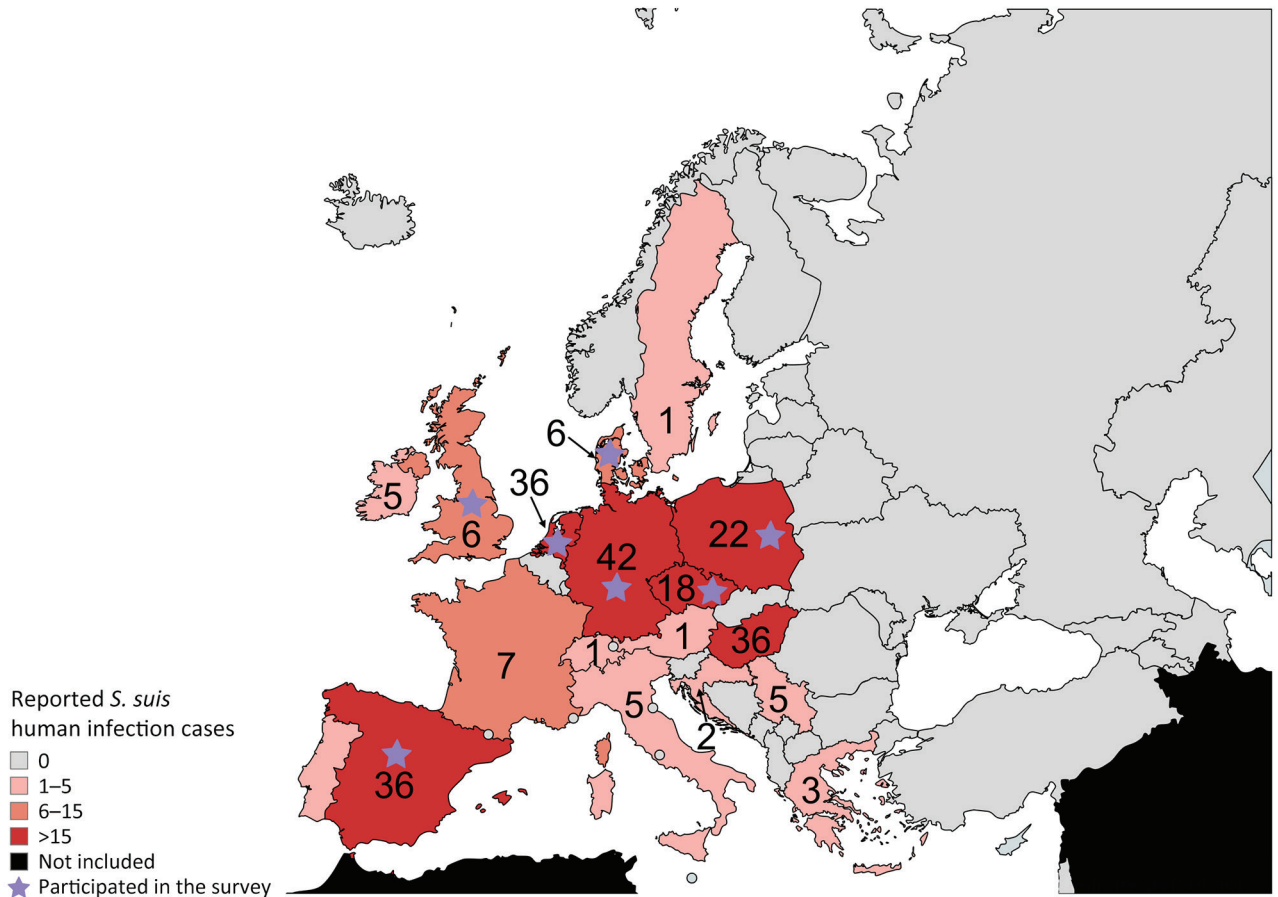


Figure 2. Reported cases of human *Streptococcus suis* infections across Europe during 1990–2022. We pooled reported cases collected in the survey study and systematic search study. The color of the countries represents the relative number of cases: the darker the tone, the higher the number of reported cases. Purple stars indicate reference laboratory participating in the survey study within that country. Scale bar indicated substitutions per site. Countries in black were not included in the study.

range in the at-risk population for those 6 countries during 2005–2013 averaged 0.161–4.945 cases/100,000 persons; Poland had the lowest incidence and the Netherlands the highest (Appendix Table 6).

Scan of Gray Literature

Because no centralized surveillance system exists for human *S. suis* infection and the disease is not notifiable in any country in Europe, the number of infections in Europe has likely been underestimated. We scanned gray literature in search of cases not identified through either the survey or systematic review. Public Health England (now the UK Health Security Agency) included human *S. suis* infections in their annual zoonosis official reports collected from the Veterinary Diagnostic Analysis database of the Animal and Plant Health Agency (22). During 1991–2017, those reports recorded 61 human *S. suis* infections in the United Kingdom, 10 times the number of cases identified from the survey and systematic review

combined (6 cases). However, those 61 cases might overlap with cases from the survey and systematic review because neither metadata nor identification method were provided (Appendix Table 4). The Netherlands Reference Laboratory for Bacterial Meningitis surveyed 57 medical microbiology laboratories in the Netherlands during 2013 with the aim of identifying cases not reported to the reference laboratory and collected an additional 25 unique cases isolated during 1990–2011 (Appendix Table 5) (23). We also found 1 case of *S. suis* meningitis in a butcher in Spain that was reported through X (24).

Population Structure of Zoonotic *S. suis* in Europe

To study the population structure of zoonotic *S. suis* isolates in Europe, we reconstructed a core-genome SNP phylogeny of 74 strains from 10 different countries (Figure 3). We identified 5 novel STs, 1660, 1602, 1663, 1707, and 1708. Most strains were part of the major zoonotic clade CC1, which has spread across

Table 1. Patient data collected in survey and systematic review for study of molecular epidemiology of underreported emerging zoonotic pathogen *Streptococcus suis*, Europe*

Patient data	Survey, n = 107	Systematic review, n = 129
Demographic information		
Sex		
M	73	93
F	16	24
NA	18	12
Age		
Median (range), y	52 (0–79)	48 (22–85)
NA	26	54
Clinical symptoms		
Meningitis	59	59
Sepsis	15	21
Hearing loss	0	22
Endocarditis	2	6
Spondylodiscitis	0	3
Death	0	11
NA	36	43
Occupational risk†		
Described	19	72
No risk	0	2
NA	88	55

*Values are no. patients except as indicated. NA, not available.

†Occupational risk: Any job involving close contact with pigs or pork products, including: farmer, butcher, abattoir worker, meat factory worker, hunter, livestock truck driver, or cook.

Europe; ≥1 strain from each country included in the phylogeny was CC1. Most of the CC1 strains had a serotype 2 capsule, and a small subset possessed the structurally similar serotype 14 capsule (25). We distinguished 2 subclades within CC1 in a genome-wide SNP phylogeny (Appendix Figure 3). The other zoonotic clades appeared to be more geographically restricted. For example, most of the CC20 strains were isolated in the Netherlands, where the lineage is thought to have emerged (13). Two additional CC20 strains were isolated in Germany, forming a serotype 5 outgroup to clonal CC20 serotype 2 strains from the Netherlands. All CC25 strains were recovered in the Czech Republic. The 3 ST25 serotype 2

Table 2. Bacterial isolate data collected in survey and systematic review for study of molecular epidemiology of underreported emerging zoonotic pathogen *Streptococcus suis*, Europe*

Isolate data	Survey, n = 107	Systematic review, n = 129
Serotype no.		
2	78	43
5	2	1
7	1	0
14	11	4
NA	15	81
Clonal complex no.		
1	68	14
20	11	1
25	4	0
87	3	1
94	1	0
NA	20	113

*NA, not available.

strains had only 73–116 SNPs across their core genomes, whereas the ST29 strain differed from the ST25 strains by 4,353–4,416 SNPs and had a serotype 7 capsule. Strains from the CC87 clade were identified in Germany and the Czech Republic and possessed a serotype 2 capsule. The 3 strains from Germany were ST19 and highly similar (81–118 SNPs), whereas the strain from the Czech Republic had novel ST1660 and differed from the ST19 clade by 9,411–9,434 SNPs.

CC1 and CC20 Isolates and Genes Associated with Zoonotic Potential

Overall, strains from clades CC1 and CC20 had a higher number of accessory genes overrepresented in zoonotic isolates than did strains from lineages CC25, CC87, and CC94 (Figure 4). Most genes associated with zoonoses were present in ≥1 of the lineages; only the 2-component signal transduction system *nisK/R* and the fimbria-like adhesin *sssP1* genes were absent from the dataset (Figure 4). Of note, despite its role in adhesion and virulence being extensively studied, muramidase-related protein (*mrrp*) was absent from the CC20 clade (26). Factor H binding protein (*fhb*), associated with binding factor H and increased translocation across the blood/brain barrier (27), was present only in CC1 strains. Differences could be observed within CC1 sublineages; 1 subclade had an additional factor H binding protein (*fhbp*). Last, suilysin (*sly*), a pore-forming hemolysin with a clear role in pathogenesis (20), was present in all clades except CC25, which instead carried the hyaluronate lysin A (*hyla*), associated with reduced virulence (Figure 4) (28).

Discussion

Despite having caused multiple outbreaks with high levels of illness and death in the past decade and reports of new zoonotic lineages arising on different continents, *S. suis* remains largely excluded from disease surveillance programs (29). Although neither carriage in healthy humans nor human-to-human transmission of *S. suis* have been reported to date, systematic surveillance is needed to follow the evolutionary trends of this pathogen in humans and pigs, the main reservoir from which zoonotic lineages emerge (2).

Our study included some potential sources of bias. Differences in number of cases between countries should not be attributed only to the size of pig populations. Other factors, such as government policy and disease monitoring and reporting, could contribute to observed differences in reported human *S. suis* cases between countries. For instance, although France has one of the largest pig populations in

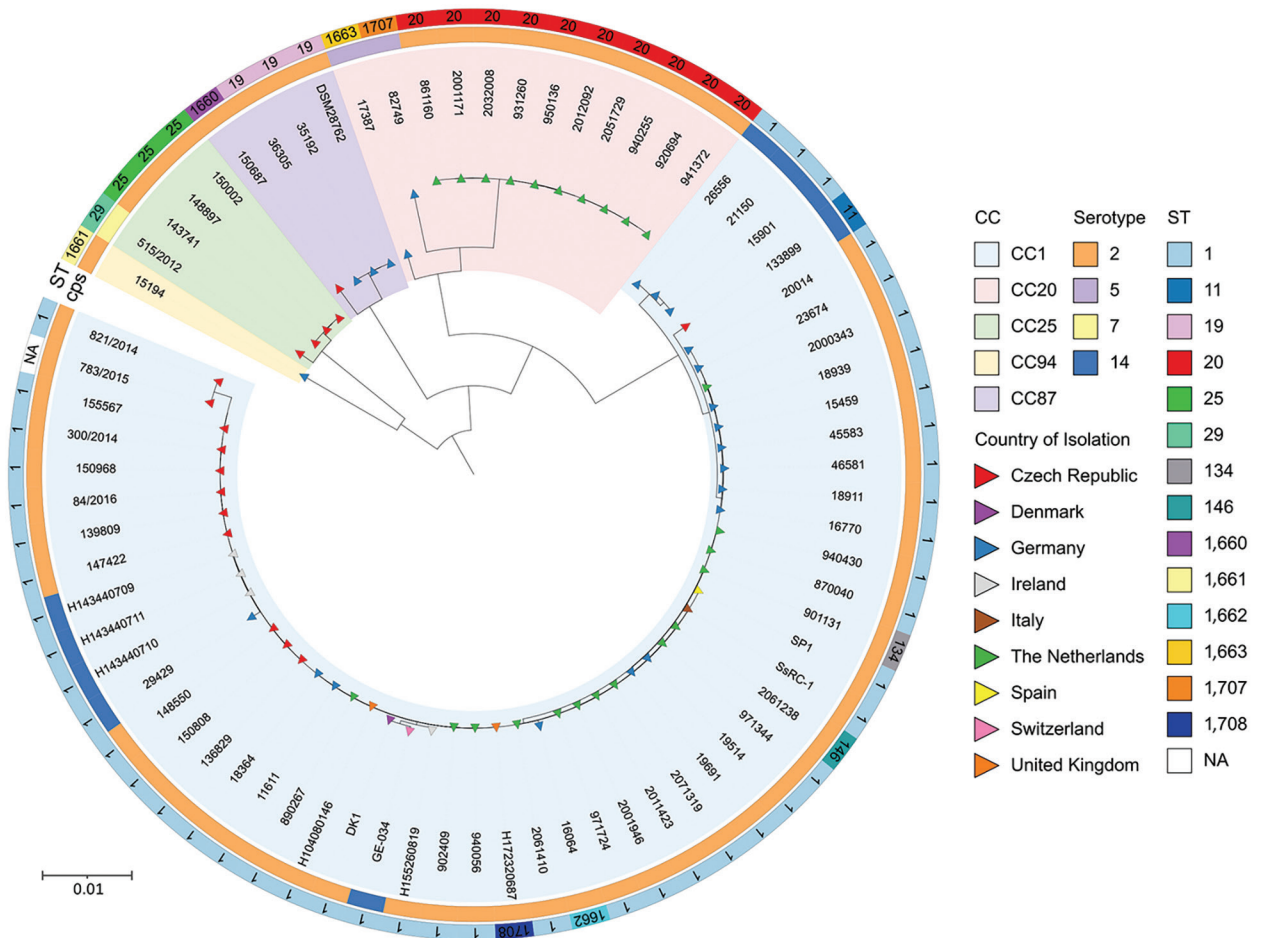


Figure 3. Genome population structure of zoonotic *Streptococcus suis* in Europe. The maximum-likelihood tree was reconstructed using IQ-TREE (18) with a core genome alignment produced with Panaroo (16). Color triangles at branch tips indicate country of collection; color rings indicate lineage (CC). The inner color ring indicates ST and is labeled accordingly. The outer color ring indicates the serotype as determined by the antigenic properties of the cps. We used iTOL (<https://itol.embl.de>) to visualize the tree. cps, capsular polysaccharide; NA, not available; ST, sequence type.

Europe, only 7 human cases have been reported. In contrast, although they have smaller pig populations, the Czech Republic reported 18 and Poland 22 cases (Figure 2) (30). The distribution of clinical symptoms aligns with previous regional and global estimates (1). However, clinical data gathered in the survey were potentially biased toward reporting meningitis because several of the surveyed laboratories are reference laboratories for bacterial meningitis (Table 1) (1). The systematic review yielded diverse article types (e.g., case reports, surveillance studies) and inconsistent quality of reported metadata. Often, year of isolation and bacterial typing was absent, making it difficult to establish meaningful time trends in the emergence of zoonotic *S. suis* in Europe. Finally, the time frames of the survey, 1990–2018, and systematic review, 1990–2022, were not identical.

The serotype 2 capsule is linked with zoonotic *S. suis* infections, and most worldwide *S. suis* cases are caused by serotype 2 (4). While investigating the emergence of the zoonotic clade CC20, 1 study (13) proposed that capsule-switching events leading to acquisition of a serotype 2 capsule may be necessary for pathogenic porcine strains to become zoonotic. We observed hints of capsule-switching events, with the CC20 strains from Germany carrying serotype 5 capsule instead of serotype 2, potentially representing an intermediate step in the emergence of zoonotic CC20 from CC16 (13). Furthermore, zoonotic strains from CC87 and CC94 lineages were serotype 2, whereas most porcine CC87 strains described in the literature carried a serotype 8 capsule; porcine CC94 strains displayed a wide range of capsules, serotypes 3, 7, and 23 being the most common (31). However,

the low number of samples collected for CC25, CC87, and CC94 in our study and the fact that they were collected more than a decade ago make it difficult to conclude whether or not these CCs are emerging as zoonotic lineages or are geographically restricted (Appendix Tables 2, 3).

The presence of genes associated with zoonotic potential varied across lineages. Differences in the accessory genome of the zoonotic *S. suis* population, with some well-studied virulence factors such as *sly*, *mrp*, and *fhb* missing from certain pathogenic clades, suggest that, although individual genes might contribute to virulence and zoonotic potential, those genes are not individually essential for *S. suis* to infect humans (20,26,27) (Figure 4). Moreover, simply because a gene is overrepresented in zoonotic isolates does not mean it plays an active role in zoonotic potential, and its role in zoonosis should be explored experimentally. For example, some genes, such as *zmp* and *sp1*, more common in human than porcine *S. suis* isolates, have been shown not to be critical for virulence (32,33), and others, such as *igdE* and *ideS*, only play a role in evading porcine, not human, immune response (34,35).

Estimated cumulative prevalence of human *S. suis* infection is substantially higher in southeastern Asia than Europe and the epidemiology of human *S. suis*

infections differs significantly between continents (1). In Europe, skin injuries and abrasions are thought to be the main point of entry for *S. suis* (3), whereas in countries in southeastern Asia with a tradition of raw pork product consumption, the intestinal tract is a notable entry point for infection (2,36). Differences in exposure routes have led to differences in epidemiology; multiple foodborne human *S. suis* outbreaks with high levels of illness and death have occurred in southeastern Asia in the past 2 decades (36,37). In Thailand, educational campaigns targeted toward at-risk populations have been shown to reduce incidence of human infections (36). Educational campaigns in Europe should be tailored to the different at-risk populations there. Our crude estimates of incidence of *S. suis* human infections in the population at risk for the Czech Republic, Germany, Hungary, the Netherlands, Poland, and Spain are comparable to the incidence of other pathogens causing similar infections in the general population (Appendix). Our estimated incidence in the population at risk for *S. suis*, range 0.161–4.945 cases/100,000 persons across the different countries, was generally higher (except in Poland) than population-wide incidence for *Neisseria meningitidis* (0.42–1.09) and lower than that of *S. pneumoniae* (1.52–14.86) reported by the European Centre for Disease Prevention and Control (38) (Appendix Table 6).



Figure 4. Presence/absence matrix of 46 genes putatively associated with zoonotic potential in study of zoonotic *Streptococcus suis* in Europe. The same phylogenetic tree presented in Figure 3 was used. Blue squares indicate presence of the gene while red squares indicate absence. The colored branches indicate CCs and follow the same pattern as in Figure 3 (blue, CC1; red, CC20; purple, CC87; yellow, CC94; green, CC25). We defined gene presence with 80% protein identity and coverage. We used Phandango (19) to visualize the tree. Bios, biosynthesis; CC, clonal complex; CS, complement system evasion.

Furthermore, we found evidence of underreporting in the Netherlands; 25 cases were not reported to the Netherlands Reference Laboratory for Bacterial Meningitis or described in published articles (23). The United Kingdom was the only country where human *S. suis* infections were included in official government reports. Those UK reports contained 10 times as many cases within the same timeframe than UK cases from the survey and systematic review combined (22) because the survey and systematic review did not capture many unpublished cases. This finding suggests that the number of cases collected in other countries through the survey might also be underestimated. We observed an increase in reported cases after 1999 (Appendix Figure 2); however, this increase could have been caused by heightened awareness after a severe outbreak in China in 2005 and by more precise bacterial identification techniques (37). Moreover, in Thailand, a country where *S. suis* is a notifiable disease, reported infections have increased in the past few years (10).

In conclusion, despite not being a notifiable disease in Europe, novel zoonotic *S. suis* lineages, including multidrug-resistant lineages, have been detected recently both in Europe and worldwide (13,29). Moreover, our likely underestimated incidence estimates suggest that risk for *S. suis* infection for the at-risk population is greater than that of *N. meningitidis* and comparable to that of *S. pneumoniae* in the general population. Given the severity of the disease it causes, we propose making *S. suis* infections notifiable in Europe to improve surveillance of emerging zoonotic lineages and evolutionary trends and better detect potential human-to-human transmission.

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About the Author

Mr. Brizuela is a PhD student at the Amsterdam UMC, within the context of the Netherlands Centre for One Health project CANVAS, which aims to identify vaccine candidates to prevent *Streptococcus suis* infections in pigs and humans.

References

- Huong VTL, Ha N, Huy NT, Horby P, Nghia HDT, Thiem VD, et al. Epidemiology, clinical manifestations, and outcomes of *Streptococcus suis* infection in humans. *Emerg Infect Dis*. 2014;20:1105–14. <https://doi.org/10.3201/eid2007.131594>
- Ho DTN, Le TPT, Wolbers M, Cao QT, Nguyen VMH, Tran VTN, et al. Risk factors of *Streptococcus suis* infection in Vietnam. A case-control study. *PLoS One*. 2011;6:e17604.
- Segura M, Aragon V, Brockmeier SL, Gebhart C, Greeff A, Kerdsin A, et al. Update on *Streptococcus suis* research and prevention in the era of antimicrobial restriction: 4th International Workshop on *S. suis*. *Pathogens*. 2020;9:374. <https://doi.org/10.3390/pathogens9050374>
- Goyette-Desjardins G, Auger JP, Xu J, Segura M, Gottschalk M. *Streptococcus suis*, an important pig pathogen and emerging zoonotic agent—an update on the worldwide distribution based on serotyping and sequence typing. *Emerg Microbes Infect*. 2014;3:e45. <https://doi.org/10.1038/emi.2014.45>
- Tall H, Njanpop-Lafourcade BM, Mounkoro D, Tidjani L, Agbenoko K, Alassani I, et al. Identification of *Streptococcus suis* meningitis through population-based surveillance, Togo, 2010–2014. *Emerg Infect Dis*. 2016;22:1262–4. <https://doi.org/10.3201/eid2207.151511>
- Raberahona M, Rasoanandrasana S, Rahajamanana VL, Ranaivo-Rabetokotany F, Andriananja V, Rakotomalala FA, et al. Novel *Streptococcus suis* sequence type 834 among humans, Madagascar. *Emerg Infect Dis*. 2018;24:391–2. <https://doi.org/10.3201/eid2402.171138>
- Alarcón L P, Araya R P, Aguayo C, Fernández J, Illesca V, Zaror A, et al. Laboratory confirmation of *Streptococcus suis* in Chile [in Spanish]. *Rev Chilena Infectol*. 2013;30:539–40. <https://doi.org/10.4067/S0716-10182013000500011>
- Susilawathi NM, Tarini NMA, Fatmawati NND, Mayura PIB, Suryapraha AAA, Subrata M, et al. *Streptococcus suis*-associated meningitis, Bali, Indonesia, 2014–2017. *Emerg Infect Dis*. 2019;25:2235–42. <https://doi.org/10.3201/eid2512.181709>
- Kerdsin A, Hatrongjit R, Wongsurawat T, Jenjaroenpun P, Chopjitt P, Boueroy P, et al. Genomic characterization of *Streptococcus suis* serotype 24 clonal complex 221/234 from human patients. *Front Microbiol*. 2021;12:812436. <https://doi.org/10.3389/fmicb.2021.812436>
- Kerdsin A. Human *Streptococcus suis* infections in Thailand: epidemiology, clinical features, genotypes, and susceptibility. *Trop Med Infect Dis*. 2022;7:359. <https://doi.org/10.3390/tropicalmed7110359>
- Liang P, Wang M, Gottschalk M, Vela AI, Estrada AA, Wang J, et al. Genomic and pathogenic investigations of *Streptococcus suis* serotype 7 population derived from a human patient and pigs. *Emerg Microbes Infect*. 2021;10:1960–74. <https://doi.org/10.1080/22221751.2021.1988725>
- King SJ, Leigh JA, Heath PJ, Luque I, Tarradas C, Dowson CG, 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. <https://doi.org/10.1128/JCM.40.10.3671-3680.2002>
- Willemsse N, Howell KJ, Weinert LA, Heuvelink A, Pannekoek Y, Wagenaar JA, et al. An emerging zoonotic clone in the Netherlands provides clues to virulence and zoonotic potential of *Streptococcus suis*. *Sci Rep*. 2016;6:28984. <https://doi.org/10.1038/srep28984>
- Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020

- statement: an updated guideline for reporting systematic reviews. *BMJ*. 2021;372:n71. <https://doi.org/10.1136/bmj.n71>
15. Athey TBT, Teatero S, Lacouture S, Takamatsu D, Gottschalk M, Fittipaldi N. Determining *Streptococcus suis* serotype from short-read whole-genome sequencing data. *BMC Microbiol*. 2016;16:162. <https://doi.org/10.1186/s12866-016-0782-8>
 16. Tonkin-Hill G, MacAlasdair N, Ruis C, Weimann A, Horesh G, Lees JA, et al. Producing polished prokaryotic pangenomes with the Panaroo pipeline. *Genome Biol*. 2020;21:180. <https://doi.org/10.1186/s13059-020-02090-4>
 17. Page AJ, Taylor B, Delaney AJ, Soares J, Seemann T, Keane JA, et al. SNP-sites: rapid efficient extraction of SNPs from multi-FASTA alignments. *Microb Genom*. 2016;2:e000056. <https://doi.org/10.1099/mgen.0.000056>
 18. Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol Biol Evol*. 2015;32:268–74. <https://doi.org/10.1093/molbev/msu300>
 19. Hadfield J, Croucher NJ, Goater RJ, Abudahab K, Aanensen DM, Harris SR. Phandango: an interactive viewer for bacterial population genomics. *Bioinformatics*. 2018;34:292–3. <https://doi.org/10.1093/bioinformatics/btx610>
 20. Roodsant TJ, Van Der Putten BCL, Tamminga SM, Schultsz C, Van Der Ark KCH. Identification of *Streptococcus suis* putative zoonotic virulence factors: a systematic review and genomic meta-analysis. *Virulence*. 2021;12:2787–97. <https://doi.org/10.1080/21505594.2021.1985760>
 21. Augère-Granier M-L. European Parliamentary Research Service. The EU pig meat sector [cited 2022 Nov 8]. [https://www.europarl.europa.eu/RegData/etudes/BRIE/2020/652044/EPRS_BRI\(2020\)652044_EN.pdf](https://www.europarl.europa.eu/RegData/etudes/BRIE/2020/652044/EPRS_BRI(2020)652044_EN.pdf)
 22. Public Health England, Department for Environment, Food and Rural Affairs. Zoonoses: UK annual reports: 2017 [cited 2022 Nov 8]. <https://www.gov.uk/government/publications/zoonoses-uk-annual-reports>
 23. National Institute for Public Health and the Environment. Zoonotic infections with *Streptococcus suis* in the Netherlands/IB 11–2013 [in Dutch] [cited 2022 Nov 8]. <https://www.rivm.nl/weblog/zoonotische-infecties-met-streptococcus-suis-in-nederland-ib-11-2013>
 24. @mrvr79. Meningitis due to *Streptococcus suis* in a butcher [in Spanish]. 2020 Dec 19 [cited 2022 Apr 1]. <https://twitter.com/mrvr79/status/1340282705439801345>
 25. Goyette-Desjardins G, Auger JP, Dolbec D, Vinogradov E, Okura M, Takamatsu D, et al. Comparative study of immunogenic properties of purified capsular polysaccharides from *Streptococcus suis* serotypes 3, 7, 8, and 9: the serotype 3 polysaccharide induces an opsonizing IgG response. *Infect Immun*. 2020;88:e00377–20. <https://doi.org/10.1128/IAI.00377-20>
 26. Wang J, Kong D, Zhang S, Jiang H, Zheng Y, Zang Y, et al. Interaction of fibrinogen and muramidase-released protein promotes the development of *Streptococcus suis* meningitis. *Front Microbiol*. 2015;6:1001. <https://doi.org/10.3389/fmicb.2015.01001>
 27. Kong D, Chen Z, Wang J, Lv Q, Jiang H, Zheng Y, et al. Interaction of factor H-binding protein of *Streptococcus suis* with globotriaosylceramide promotes the development of meningitis. *Virulence*. 2017;8:1290–302. <https://doi.org/10.1080/21505594.2017.1317426>
 28. Haas B, Vaillancourt K, Bonifait L, Gottschalk M, Grenier D. Hyaluronate lyase activity of *Streptococcus suis* serotype 2 and modulatory effects of hyaluronic acid on the bacterium's virulence properties. *BMC Res Notes*. 2015;8:722. <https://doi.org/10.1186/s13104-015-1692-9>
 29. Brizuela J, Kajeekul R, Roodsant TJ, Riwoad A, Boueroy P, Pattanapongpaibool A, et al. *Streptococcus suis* outbreak caused by an emerging zoonotic strain with acquired multi-drug resistance in Thailand. *Microb Genom*. 2023;9:mgen000952. <https://doi.org/10.1099/mgen.0.000952>
 30. Bojarska A, Molska E, Janas K, Skoczyńska A, Stefaniuk E, Hryniewicz W, et al. *Streptococcus suis* in invasive human infections in Poland: clonality and determinants of virulence and antimicrobial resistance. *Eur J Clin Microbiol Infect Dis*. 2016;35:917–25. <https://doi.org/10.1007/s10096-016-2616-x>
 31. Estrada AA, Gottschalk M, Rossow S, Rendahl A, Gebhart C, Marthaler DG. Serotype and genotype (multilocus sequence type) of *Streptococcus suis* isolates from the United States serve as predictors of pathotype. *J Clin Microbiol*. 2019;57:e00377–19.
 32. Dumesnil A, Auger JP, Roy D, Vötsch D, Willenborg M, Valentin-Weigand P, et al. Characterization of the zinc metalloprotease of *Streptococcus suis* serotype 2. *Vet Res*. 2018;49:109. <https://doi.org/10.1186/s13567-018-0606-y>
 33. Roy D, Fittipaldi N, Dumesnil A, Lacouture S, Gottschalk M. The protective protein sao (surface antigen one) is not a critical virulence factor for *Streptococcus suis* serotype 2. *Microb Pathog*. 2014;67-68:31–5. <https://doi.org/10.1016/j.micpath.2014.02.002>
 34. Sperry C, Seele J, Valentin-Weigand P, Baums CG, von Pawel-Rammingen U. Identification and characterization of IgdE, a novel IgG-degrading protease of *Streptococcus suis* with unique specificity for porcine IgG. *J Biol Chem*. 2016;291:7915–25. <https://doi.org/10.1074/jbc.M115.711440>
 35. Seele J, Beineke A, Hillermann LM, Jaschok-Kentner B, von Pawel-Rammingen U, Valentin-Weigand P, et al. The immunoglobulin M-degrading enzyme of *Streptococcus suis*, IdeS_{suis}, is involved in complement evasion. *Vet Res*. 2015;46:45. <https://doi.org/10.1186/s13567-015-0171-6>
 36. Takeuchi D, Kerdsin A, Akeda Y, Chiranairadul P, Loetthong P, Tanburawong N, et al. Impact of a food safety campaign on *Streptococcus suis* infection in humans in Thailand. *Am J Trop Med Hyg*. 2017;96:1370–7. <https://doi.org/10.4269/ajtmh.16-0456>
 37. Yu H, Jing H, Chen Z, Zheng H, Zhu X, Wang H, et al.; *Streptococcus suis* study groups. Human *Streptococcus suis* outbreak, Sichuan, China. *Emerg Infect Dis*. 2006;12:914–20. <https://doi.org/10.3201/eid1206.051194>
 38. European Centre for Disease Prevention and Control. Surveillance atlas of infectious diseases [cited 2023 Jul 27]. <https://www.ecdc.europa.eu/en/surveillance-atlas-infectious-diseases>

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Molecular Epidemiology of Underreported Emerging Zoonotic Pathogen *Streptococcus suis*, Europe

Appendix

I. Questionnaire requesting patient and isolate data of *S. suis* infections.

Below are listed the questionnaire and supplied response options. Variables included region of coverage of the laboratory and population demographics (general information) as well as information on isolate and patient metadata. If predetermined response was not provided, responder was able to fill out the adequate response. If willing, laboratories were requested to send their isolates for future research.

1. General information
 - a. What is the region covered by your reference laboratory?
 - b. What is the population size for the above stated region?
 - c. If known, what is the size of the population at risk* for the above stated region?
 - d. If known, what is the size of the pig population for the above stated region?
- *The population at risk in Europe is defined as the population that includes all pig farmers, butchers, slaughterhouse workers, and hunters of wild boar.
2. Information on *Streptococcus suis*
 - a. Is *S. suis* infection a notifiable disease in your country? (Y/N)
 - b. What is the total number of *S. suis* cases notified to your laboratory from 1990 to date?
 - c. If applicable, when was the first notified case of *S. suis* in your country? (dd/mm/yyyy)
3. Patient information

Isolate information							
Isolate ID	Date of isolation	Isolated from	Isolate available	Identification method	Serotype	MLST sequence type	Whole genome sequenced
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							

Variables including definitions and provided response options:

- Isolate ID
Definition: ID or number assigned to an incoming patient sample, i.e. liquor, blood or other.
Response input: variable according to reference laboratory-specific format.
- Date of isolation
Definition: Date the sample arrived at the ref lab
Response format: dd-mm-yyyy
- Isolated from
Definition: source of harvest of isolate or sample from patient.
Response options: CSF/ Blood / Other, namely
- Isolate available
Definition: is the isolate stored in the ref lab and available to eventually send for follow-up.
Response options: Yes/ No
- Identification method
Definition: method via which bacterial typing was done.
Response options: API Strep/ MALDI-TOF/ PCR, please specify target/ VITEK/ WGS/ If other, specify.
- Serotype
Definition: known S. suis serotypes to date, i.e. 1, 1/2, 2, ..., 34.
Response options: Please enter numerical value; options: 1, ½, 2, ..., 34/ Undetermined.
- Sequence type
Definition: S. suis sequence type determined by MLST
Response format: If determined, fill out value.
- Whole genome sequenced
Definition: if whole genome sequencing has been performed
Response options: Yes/ N

5. Follow-up

Would you be willing to ship *S. suis* isolates to the Dutch National Reference Laboratory for further analysis through whole genome sequencing? (Y/N)

II. Searches

Search Queries used in the Systematic review

PubMed Query

("Streptococcus suis" [MESH] OR "Streptococcus suis" [TIAB]) AND (human [MESH] OR human [TIAB]) AND ((Albania [MESH] OR Albania [TIAB]) OR (Andorra [MESH] OR Andorra [TIAB]) OR (Armenia [MESH] OR Armenia [TIAB]) OR (Austria [MESH] OR Austria [TIAB]) OR (Azerbaijan [MESH] OR Azerbaijan [TIAB]) OR (Belarus [MESH] OR Belarus [TIAB]) OR (Belgium [MESH] OR Belgium [TIAB]) OR ("Bosnia and Herzegovina" [MESH] OR "Bosnia and Herzegovina" [TIAB]) OR (Bulgaria [MESH] OR Bulgaria [TIAB]) OR (Croatia [MESH] OR Croatia [TIAB]) OR (Cyprus [MESH] OR Cyprus [TIAB]) OR (Czechia [MESH] OR Czechia [TIAB]) OR ("Czech Republic" [MESH] OR "Czech Republic" [TIAB]) OR (Denmark [MESH] OR Denmark [TIAB]) OR (Estonia [MESH] OR Estonia [TIAB]) OR (Finland [MESH] OR Finland [TIAB]) OR (France [MESH] OR France [TIAB]) OR (Germany [MESH] OR Germany [TIAB]) OR (Georgia [MESH] OR Georgia [TIAB]) OR (Greece [MESH] OR Greece [TIAB]) OR (Hungary [MESH] OR Hungary [TIAB]) OR (Iceland [MESH] OR Iceland [TIAB]) OR (Ireland [MESH] OR Ireland [TIAB]) OR (Israel [MESH] OR Israel [TIAB]) OR (Italy [MESH] OR Italy [TIAB]) OR (Kazakhstan [MESH] OR Kazakhstan [TIAB]) OR (Kosovo [MESH] OR Kosovo [TIAB]) OR (Latvia [MESH] OR Latvia [TIAB]) OR (Liechtenstein [MESH] OR Liechtenstein [TIAB]) OR (Lithuania [MESH] OR Lithuania

[TIAB]) OR (Luxembourg [MESH] OR Luxembourg [TIAB]) OR (Macedonia [MESH] OR Macedonia [TIAB]) OR (Malta [MESH] OR Malta [TIAB]) OR (Moldova [MESH] OR Moldova [TIAB]) OR (Monaco [MESH] OR Monaco [TIAB]) OR (Montenegro [MESH] OR Montenegro [TIAB]) OR (Netherlands [MESH] OR Netherlands [TIAB]) OR (Norway [MESH] OR Norway [TIAB]) OR (Poland [MESH] OR Poland [TIAB]) OR (Portugal [MESH] OR Portugal [TIAB]) OR (Romania [MESH] OR Romania [TIAB]) OR (Russia [MESH] OR Russia [TIAB]) OR ("Russian Federation" [MESH] OR "Russian Federation" [TIAB]) OR ("San Marino" [MESH] OR "San Marino" [TIAB]) OR (Serbia [MESH] OR Serbia [TIAB]) OR (Slovakia [MESH] OR Slovakia [TIAB]) OR (Slovenia [MESH] OR Slovenia [TIAB]) OR (Spain [MESH] OR Spain [TIAB]) OR (Sweden [MESH] OR Sweden [TIAB]) OR (Switzerland [MESH] OR Switzerland [TIAB])) OR (Tajikistan [MESH] OR Tajikistan [TIAB]) OR (Turkey [MESH] OR Turkey [TIAB]) OR (Turkmenistan [MESH] OR Turkmenistan [TIAB]) OR (Ukraine [MESH] OR Ukraine [TIAB]) OR ("United Kingdom" [MESH] OR "United Kingdom" [TIAB]) OR (England [MESH] OR England [TIAB]) OR (Wales [MESH] OR Wales [TIAB]) OR (Scotland [MESH] OR Scotland [TIAB]) OR ("Northern Ireland" [MESH] OR "Northern Ireland" [TIAB]) OR (Faroes [MESH] OR Faroes [TIAB]) OR ("Vatican City" [MESH] OR "Vatican City" [TIAB])

Web of Science Query

("Streptococcus suis" and human) AND (Albania OR Andorra OR Armenia OR Austria OR Azerbaijan OR Belarus OR Belgium OR "Bosnia and Herzegovina" OR Bulgaria OR Croatia OR Cyprus OR Czechia OR "Czech Republic" OR Denmark OR Estonia OR Finland OR France

OR Germany OR Georgia OR Greece OR Hungary OR Iceland OR Ireland OR Israel OR Italy
OR Kazakhstan OR Kosovo OR Latvia OR Liechtenstein OR Lithuania OR Luxembourg OR
Macedonia OR Malta OR Moldova OR Monaco OR Montenegro OR Netherlands OR Norway
OR Poland OR Portugal OR Romania OR Russia OR "Russian Federation" OR "San Marino"
OR Serbia OR Slovakia OR Slovenia OR Spain OR Sweden OR Switzerland OR Tajikistan OR
Turkey OR Turkmenistan OR Ukraine OR "United Kingdom" OR England OR Wales OR
Scotland OR "Northern Ireland" OR Faroes OR "Vatican City")

Scopus Query

TITLE-ABS-KEY ("Streptococcus suis") AND TITLE-ABS-KEY (human) AND (TITLE-
ABS-KEY (Albania OR Andorra OR Armenia OR Austria OR Azerbaijan OR Belarus
OR Belgium OR "Bosnia and Herzegovina" OR Bulgaria OR Croatia OR Cyprus OR
Czechia OR "Czech Republic" OR Denmark OR Estonia OR Finland OR France OR
Germany OR Georgia OR Greece OR Hungary OR Iceland OR Ireland OR Israel OR
Italy OR Kazakhstan OR Kosovo OR Latvia OR Liechtenstein OR Lithuania OR
Luxembourg OR Macedonia OR Malta OR Moldova OR Monaco OR Montenegro OR
Netherlands OR Norway OR Poland OR Portugal OR Romania OR Russia OR "Russian
Federation" OR "San Marino" OR Serbia OR Slovakia OR Slovenia OR Spain OR
Sweden OR Switzerland OR Tajikistan OR Turkey OR Turkmenistan OR Ukraine OR
"United Kingdom" OR England OR Wales OR Scotland OR "Northern Ireland" OR
Faroes OR "Vatican City")

III. Whole Genome Sequencing and Genome Assembly

Whole-genome sequencing

Out of 110 *S. suis* isolates identified in the reference laboratory survey, 24 had been previously sequenced, 40 isolates were not available for sequencing and 46 isolates were available and shared for sequencing (Appendix Figure 1). Isolates were initially grown overnight on sheep blood agar plates to test for contaminations and cultured overnight in Todd Hewitt Broth with 0.5% Yeast extract (THY), both at 37 °C. Genomic DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA) and DNA concentration was measured with Qubit using the dsDNA HS Assay Kit as well as the BR Assay Kit (Thermo Fisher Scientific Corporation, Eugene, OR, USA). Library preparation was performed using the KAPA HTP Library preparation kit (Roche, Basel, Switzerland). Pooled libraries were sequenced with the Reagent Kit v3 using paired-end Illumina MiSeq.

Genome Assembly

Unless otherwise specified, bioinformatic tools were used with the default settings. Raw sequencing reads were preprocessed using fastp v0.20.0 with the “--disable_length_filtering” flag (1) and Kraken2 with the MiniKraken2 database was used to confirm the taxonomical identification of the *S. suis* isolates (2). Draft genomes were assembled from the filtered Illumina reads using Shovill (v1.0.9 <https://github.com/tseemann/shovill>) with SPAdes v3.14.1 (3) and the quality of the draft genomes was evaluated with QUAST v5.0.2 (4). Genome annotation was carried out using Prokka v1.14.6 (5). *In silico* serotyping was performed feeding the processed Illumina reads into the *S. suis* serotyping pipeline (6). For isolate H143440710, the pipeline

could not distinguish between serotypes 1 and 14, therefore, the *cpsK* gene was Sanger sequenced using the BigDye™ Terminator v1.1 Cycle Sequencing Kit (Thermo Fisher Scientific Corporation, Eugene, OR, USA) to resolve its serotype. Primers used to distinguish between serotypes 1 and 14 are in Appendix Table 1.

IV. Incidence estimates of human *S. suis* infections in European countries with at least 5 cases between 2005 and 2013.

The incidence of human *S. suis* infections was estimated for all European countries with at least 5 *S. suis* cases reported in the survey or literature review cases between 2005 and 2013. The size of the population at risk was estimated based on the Eurostat Agricultural census (81). The agricultural census contained data regarding the number of agricultural holdings, pig specialised holdings and the size of the agricultural labour force for 2005, 2007, 2010 and 2013. The estimated population at risk for each year was calculated by multiplying the proportion of pig specialized agricultural holds by the total agricultural labour force (formula below). A 10% upper margin of error was then added to the average population at risk between 2005 and 2013 to account for butchers, hunters, slaughterhouse and meat factory workers not included in the agricultural census. The average size of the general population for each country between 2005 and 2013 was calculated by averaging the national census figures for 2005, 2007, 2010 and 2013 (82–91). When population size estimates were not available on national databases, the estimates from the OECD were used (92). The estimated incidence was calculated for both the total population and the population at risk (Appendix 4 Table). The data on *S. pneumoniae* and *N.*

meningitidis incidence was reported by the European CDC (93). The average incidence between 2010 and 2013 (*S. pneumoniae*) and 2005 and 2013 (*N. meningitidis*) is displayed on (Appendix Table 6).

Formulas

Estimates on population at risk =

$$\left(\frac{\text{number of pig holdings in a given country}}{\text{number of agricultural holdings in a given country}} \right) \times \text{no. of persons in agricultural labour force}$$

Estimated incidence of S. suis in the population

$$\left(\frac{\text{number of confirmed cases of human S. suis during specified period}}{\text{size of population in specified period}} \right) \times 100,000 \text{ persons}$$

Appendix Table 1. Primers used in this study to distinguish between serotype 1 and 14.

Primer	DNA sequence
cps14K_forward	GCTATCAGATGCCCGTAATTATGGC
cps14K_reverse	GCCTAAAACAAAACAGCAAAGGC

Appendix Table 2. Collected *S. suis* cases and metadata during the Survey study

Isolate Metadata									Patient Metadata				Ref
Isolate	Country	Year isolated	Year reported	Source	Test method	Serotype	ST	WGS	Sex	Age range, y	Clinical symptoms	Occupation	
133899	Czech Republic	NA	NA	NA	API Strep, PCR	2	11	This study	NA	NA	NA	NA	NA
136829	Czech Republic	NA	NA	NA	API Strep, PCR	2	1	This study	NA	NA	NA	NA	NA
139809	Czech Republic	NA	NA	NA	API Strep, PCR	2	1	This study	M	NA	NA	Works with pigs	NA
143741	Czech Republic	2001	NA	CSF	API Strep, PCR	2	25	This study	M	66–70	Meningitis	Works with pigs	NA
147422	Czech Republic	2003	NA	CSF	API Strep, PCR	2	1	This study	M	56–60	Meningitis	Works with pigs	NA
148550	Czech Republic	2003	NA	CSF	API Strep, PCR	2	1	This study	M	61–65	Meningitis	Works with pigs	NA
148897	Czech Republic	2003	NA	CSF	API Strep, PCR	2	25	This study	F	61–65	Meningitis	NA	NA
150002	Czech Republic	2004	NA	Blood	API Strep, PCR	2	25	This study	M	31–35	NA	NA	NA
150808	Czech Republic	2005	NA	CSF	API Strep, PCR	2	1	This study	F	36–40	Meningitis	Works with pigs	NA
150968	Czech Republic	2011	NA	Blood	API Strep, PCR	2	1	This study	F	51–55	Meningitis	Work with pigs	NA
155567	Czech Republic	2012	NA	CSF	API Strep, PCR	2	1	This study	M	56–60	Meningitis/septicemia	NA	NA
515/2012	Czech Republic	2011	NA	Blood	API Strep, PCR	7	29	This study	M	51–55	Septicemia	NA	NA
300/2014	Czech Republic	2012	NA	CSF	API Strep, PCR	2	1	This study	F	41–45	Septicemia	NA	NA
821/2014	Czech Republic	2014	NA	CSF	API Strep, PCR	2	1	This study	M	0–5	Meningitis/septicemia	NA	NA
783/2015	Czech Republic	2015	NA	Blood	API Strep, PCR	2	NA*	This study	M	61–65	NA	NA	NA
84/2016	Czech Republic	2016	NA	NA	API Strep, PCR	2	1	This study	F	61–65	Septicemia	NA	NA
150687	Czech Republic	2005	NA	CSF	API Strep, PCR	2	1660	This study	F	51–55	Meningitis	NA	NA
16770	Germany	1998	NA	CSF	NA	2	1	This study	NA	NA	NA	NA	NA
18911	Germany	2001	NA	Blood	NA	2	1	This study	M	31–35	NA	NA	NA
19691	Germany	2003	NA	Blood	NA	2	1	This study	M	46–50	NA	NA	NA
20014	Germany	2004	NA	CSF	NA	2	1	This study	F	36–40	NA	NA	NA

Isolate Metadata									Patient Metadata				Ref
Isolate	Country	Year isolated	Year reported	Source	Test method	Serotype	ST	WGS	Sex	Age range, y	Clinical symptoms	Occupation	
23674	Germany	2005	NA	Blood	NA	2	1	This study	M	26–30	NA	NA	NA
26556	Germany	2005	NA	CSF	NA	14	1	This study	M	56–60	NA	NA	NA
29429	Germany	2004	NA	NA	NA	14	1	This study	NA	NA	NA	Pig farmer	NA
35192	Germany	2008	NA	CSF	NA	2	19	This study	M	66–70	NA	NA	NA
36305	Germany	2008	NA	Blood	NA	2	19	This study	M	61–65	NA	NA	NA
45583	Germany	2010	NA	Blood	NA	2	1	This study	F	71–75	NA	NA	NA
46581	Germany	2010	NA	Blood	NA	2	1	This study	F	46–50	NA	NA	NA
15194	Germany	1990	NA	CSF	NA	2	1661	This study	NA	NA	NA	NA	NA
15459	Germany	NA	NA	NA	NA	2	1	This study	NA	NA	NA	NA	NA
15901	Germany	1994	NA	NA	NA	14	1	This study	NA	NA	NA	NA	NA
16064	Germany	1994	NA	NA	NA	2	1662	This study	NA	NA	NA	NA	NA
17387	Germany	1999	NA	CSF	NA	5	1663	This study	NA	NA	NA	NA	NA
18364	Germany	2000	NA	CSF	NA	2	1	This study	M	36–40	NA	NA	NA
18939	Germany	2001	NA	Blood	NA	2	1	This study	M	NA	NA	NA	NA
19514	Germany	2002	NA	NA	NA	2	1	This study	NA	NA	NA	NA	NA
21150	Germany	2004	NA	CSF	NA	14	1	This study	M	26–30	NA	NA	NA
82749	Germany	2018	NA	Blood	NA	5	1707	This study	M	41–45	NA	Pig farmer	NA
16318	Germany	1996	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
19395	Germany	2002	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
19396	Germany	2002	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
48919	Germany	2011	NA	Blood	NA	NA	NA	NA	F	46–50	NA	NA	NA
4249	Denmark	NA	NA	Blood	Phenotypic methods	14	NA	NA	NA	NA	Sepsis	NA	NA
H48896	Denmark	2002	NA	Blood	Phenotypic methods	14	NA	NA	M	56–60	Sepsis	NA	NA
None	Denmark	NA	NA	CSF	Phenotypic methods	14	NA	NA	M	NA	Meningitis/ sepsis	NA	NA

Isolate Metadata									Patient Metadata				Ref
Isolate	Country	Year isolated	Year reported	Source	Test method	Serotype	ST	WGS	Sex	Age range, y	Clinical symptoms	Occupation	
H55346	Denmark	2002	NA	Blood	Phenotypic methods	14	NA	NA	M	51–55	Sepsis	NA	NA
DK1	Denmark	2016	NA	Blood	NA	2	1	This study	F	51–55	NA	NA	NA
901131	Netherlands	1990	2012	CSF/ Blood	NA	2	134	7	M	51–55	Meningitis	NA	7
902409	Netherlands	1990	2012	NA	API Strep	2	1	7	M	36–40	Meningitis	NA	7
920694	Netherlands	1992	2012	CSF	API Strep	2	20	7	M	56–60	Meningitis	NA	7
931260	Netherlands	1993	2012	CSF	API Strep	2	20	7	M	NA	Meningitis	NA	7
940056	Netherlands	1994	2012	CSF/ Blood	API Strep	2	1	7	M	56–60	Meningitis	NA	7
940255	Netherlands	1994	2012	Blood	API Strep	2	20	7	M	51–55	Meningitis	Pig breeder	7
940430	Netherlands	1994	2012	CSF/ Blood	API Strep	2	1	7	M	51–55	Meningitis	NA	7
941372	Netherlands	1994	2012	CSF	API Strep	2	20	7	M	61–65	Meningitis	NA	7
950136	Netherlands	1995	2012	CSF	API Strep	2	20	7	M	51–55	Meningitis	NA	7
971344	Netherlands	1997	2012	CSF/ Blood	API Strep	2	1	7	M	41–45	Meningitis	Pig keeper	7
971724	Netherlands	1997	2012	Blood	API Strep	2	1	7	M	51–55	Meningitis	Butcher	7
2000343	Netherlands	2000	2008	CSF	API Strep	2	1	7	M	61–65	Meningitis	Pig farmer	8
2001171	Netherlands	2000	2012	Blood	API Strep	2	20	7	M	61–65	Meningitis	Pig keeper	7
2001946	Netherlands	2000	2008	CSF/ Blood	MALDI-TOF	2	1	7	M	56–60	Meningitis	Pig farmer	8
2011423	Netherlands	2001	2008	CSF	MALDI-TOF	2	1	7	M	46–50	Meningitis	Pig farmer	8
2012092	Netherlands	2001	2012	CSF	API Strep	2	20	7	M	31–35	Meningitis	Meat processing	7
2032008	Netherlands	2003	2012	CSF/ Blood	API Strep	2	20	7	M	56–60	Meningitis	Pig keeper	7
2051729	Netherlands	2005	2012	CSF/ Blood	API Strep	2	20	7	M	66–70	Meningitis	NA	7
2061238	Netherlands	2006	2012	CSF	API Strep	2	146	7	M	26–30	Meningitis	NA	7
2061410	Netherlands	2006	2012	CSF/ Blood	API Strep	2	1	7	M	36–40	Meningitis	NA	7
2071319	Netherlands	2007	2012	CSF	API Strep	2	1	7	M	46–50	Meningitis	Butcher	7
902035	Netherlands	1990	2012	CSF	API Strep	NA	NA	NA	F	0–5	Meningitis/ sepsis	NA	7
20120669	Netherlands	2007	2012	Blood	API Strep	NA	NA	NA	M	51–55	Meningitis	NA	7
2121473	Netherlands	2012	2012	CSF/ Blood	API Strep	NA	NA	NA	M	41–45	Meningitis/ sepsis	NA	7
2150651	Netherlands	2015	2012	Blood	API Strep	NA	NA	NA	M	56–60	Meningitis	NA	7
H17232068 7	United Kingdom	2017	NA	CSF	API RAPID ID32/ 16S rDNA seq	2	1708	This study	M	46–50	Meningitis	NA	NA

Isolate Metadata									Patient Metadata				Ref
Isolate	Country	Year isolated	Year reported	Source	Test method	Serotype	ST	WGS	Sex	Age range, y	Clinical symptoms	Occupation	
H143440709	Ireland	2014	NA	CSF	API RAPID ID32/ 16S rDNA seq	14	1	This study	M	NA	Meningitis	NA	NA
H155260819	Ireland	2015	NA	Blood	API RAPID ID32/ 16S rDNA seq	2	1	This study	M	41-45	Septicemia	NA	NA
H143440711	Ireland	2014	NA	Blood	API RAPID ID32/ 16S rDNA seq	14	1	This study	M	NA	Meningitis	NA	NA
H104080146	United Kingdom	2010	NA	Blood	API RAPID ID32/ 16S rDNA seq	2	1	This study	M	76-80	Septicemia	NA	NA
H143440710	Ireland	2014	NA	Blood	API RAPID ID32/ 16S rDNA seq	14	1	This study	M	NA	Meningitis	NA	NA
SP1	Spain	2014	NA	NA	NA	2	1	This study	M	46-50	NA	Abattoir worker	NA
Spain2	Spain	NA	NA	NA	NA	NA	NA	NA	NA	NA	UTI	NA	NA
Spain3	Spain	NA	NA	Blood	NA	NA	NA	NA	NA	NA	Bacteremia	NA	NA
Spain4	Spain	NA	NA	Blood	NA	NA	NA	NA	NA	NA	Bacteremia	NA	NA
Spain5	Spain	2014	NA	NA	NA	NA	NA	NA	M	46-50	NA	NA	NA
Spain6	Spain	2018	NA	NA	NA	NA	NA	NA	M	61-65	NA	NA	NA
Spain7	Spain	NA	NA	Semen	NA	NA	NA	NA	M	NA	NA	NA	NA
Spain8	Spain	NA	NA	CSF	NA	NA	NA	NA	NA	NA	NA	NA	NA
2309	Poland	2000	2016	CSF	Vitek MS	2	1	NA	M	46-50	Meningitis	NA	9
3177	Poland	2003	2016	CSF	Vitek MS	2	1	NA	M	46-50	Meningitis	NA	9
3006	Poland	2004	2016	CSF/ Blood	Vitek MS	2	1	NA	M	46-50	Meningitis	Butcher	9
3899	Poland	2004	2016	CSF	Vitek MS	2	1	NA	M	46-50	Meningitis	Pig farmer	9
2610	Poland	2005	2016	CSF/ Blood	Vitek MS	2	1	NA	F	51-55	Meningitis	NA	9
3828	Poland	2005	2016	CSF	Vitek MS	2	1	NA	F	36-40	Meningitis	NA	9
626	Poland	2006	2016	Blood	Vitek MS	2	1	NA	M	36-40	Endocarditis	NA	9
584	Poland	2007	2016	CSF/ Blood	Vitek MS	2	1	NA	M	26-30	Meningitis	NA	9
3586	Poland	2008	2016	CSF/ Blood	Vitek MS	2	1	NA	M	66-70	Meningitis	NA	9
6571	Poland	2009	2016	CSF	Vitek MS	2	1	NA	M	36-40	Meningitis	NA	9
41	Poland	2010	2016	Blood	Vitek MS	2	1	NA	M	56-60	Meningitis	NA	9
1738	Poland	2010	2016	CSF	Vitek MS	2	1	NA	M	51-55	Meningitis	NA	9
4524	Poland	2010	2016	CSF	Vitek MS	2	1	NA	M	56-60	Meningitis	NA	9
6021	Poland	2010	2016	CSF	Vitek MS	2	1	NA	F	51-55	Meningitis	NA	9
5228	Poland	2011	2016	CSF/ Blood	Vitek MS	2	1	NA	M	31-35	Meningitis	NA	9
8719	Poland	2011	2016	CSF	Vitek MS	2	1	NA	M	56-60	Meningitis	NA	9

Isolate Metadata									Patient Metadata				Ref
Isolate	Country	Year isolated	Year reported	Source	Test method	Serotype	ST	WGS	Sex	Age range, y	Clinical symptoms	Occupation	
174	Poland	2012	2016	CSF	Vitek MS	2	1	NA	M	61–65	Meningitis	NA	9
6708	Poland	2012	2016	CSF	Vitek MS	2	1	NA	M	46–50	Meningitis	NA	9
4011	Poland	2013	2016	CSF	Vitek MS	2	1	NA	M	56–60	Meningitis	NA	9
4364	Poland	2013	2016	CSF	Vitek MS	2	1	NA	F	51–55	Meningitis	NA	9
5604	Poland	2013	2016	CSF	Vitek MS	2	1	NA	M	61–65	Meningitis	NA	9

*To avoid case identification, the age of each patient is shown as a 5 year range. One of the 7 core genes used for MLST typing did not assemble; the other 6 genes had allele 1 suggesting this isolate belongs to CC1. CC, clonal complex; MLST, multilocus sequence typing; NA, not available; ST, sequence type; WGS, whole genome sequencing.

Appendix Table 3. Collected *S. suis* cases and metadata during the Systematic search study. To avoid case identification, the age of each patient is shown as a 5 year range*

Isolate Metadata								Patient Metadata				
Isolate	Country	Year isolated	Year reported	Source	Serotype	ST	WGS	Sex	Age range, y	Clinical symptoms	Occupation	Ref
AUS01	Austria	NA	1999	CSF	NA	NA	NA	F	61–65	Meningitis; hearing loss	Pig farmer	10
CRO01	Croatia	2000	2002	Blood	NA	NA	NA	M	NA	Septic shock; Died	NA	11
CRO02	Croatia	2000	2002	CSF	NA	NA	NA	M	NA	Meningitis	NA	11
CZ01	Czech Republic	NA	2013	Blood	NA	NA	NA	M	61–65	Sepsis; spondylodiscitis	Pig farmer	12
MAC 724	Germany	NA	2002	Blood	2	1	NA	M	NA	Died	Butcher	13
BK52339	Germany	2012	2015	Blood	2	1	NA	M	NA	Septicemia; died	Hunter	14
TiHo441	Germany	2011	2015	Blood	2	1	NA	NA	NA	Septicemia	NA	14
TiHo224	Germany	NA	2005	Blood/heart	2	NA	NA	M	41–45	Endocarditis	NA	15
199	Germany	NA	2003	CSF/blood	NA	NA	NA	M	51–55	Sepsis; meningitis; hearing loss	Hunter	16
DE06	Germany	NA	2002	Blood	2	NA	NA	M	36–40	Septic shock	Truck driver (pork)	17
DE07	Germany	NA	2002	Pharyngeal swab	2	NA	NA	NA	NA	NA	Abattoir	17
DE08	Germany	NA	2002	Pharyngeal swab	2	NA	NA	NA	NA	NA	Abattoir	17
DE09	Germany	NA	2002	Pharyngeal swab	2	NA	NA	NA	NA	NA	Abattoir	17
DE10	Germany	NA	2002	Pharyngeal swab	2	NA	NA	NA	NA	NA	Meat processing factory	17
DE11	Germany	NA	2002	Pharyngeal swab	2	NA	NA	NA	NA	NA	Meat processing factory	17
DE12	Germany	NA	2002	Pharyngeal swab	2	NA	NA	NA	NA	NA	Meat processing factory	17

Isolate Metadata								Patient Metadata				
Isolate	Country	Year isolated	Year reported	Source	Serotype	ST	WGS	Sex	Age range, y	Clinical symptoms	Occupation	Ref
DE13	Germany	NA	2002	Pharyngeal swab	2	NA	NA	NA	NA	NA	Meat processing factory	17
DE14	Germany	NA	2007	CSF/ blood	2	NA	NA	M	41–45	Meningitis; hearing loss	Butcher	18
DE15	Germany	NA	1997	CSF/ blood	NA	NA	NA	M	51–55	Meningitis, hearing loss	Hunter	19
DSM28762	Germany	2002	2021	Blood	2	19	Yes	NA	NA	NA	NA	20
11611	Germany	NA	2013	NA	2	1	Yes	NA	NA	NA	NA	21
DK01	Denmark	2006	2009	CSF/ blood	14	NA	NA	M	51–55	Meningitis; spondylodiscitis, bacteremia	Pig farmer	22
FR01	France	NA	1996	CSF/ blood	NA	NA	NA	M	26–30	Meningitis	Butcher	23
FR02	France	1991	1992	CSF	NA	NA	NA	M	56–60	Meningitis	Meat processing factory	24
FR03	France	NA	2001	CSF	2	NA	NA	M	56–60	Meningitis, hearing loss	Pig breeder and hunter	25
FR04	France	1994	1996	Blood	2	NA	NA	M	36–40	Endocarditis	Butcher	26
FR05	France	1996	1998	CSF/ blood	2	1	NA	M	46–50	Meningitis; septic shock;	Butcher	27
FR06	France	NA	2008	CSF	NA	NA	NA	M	31–35	Meningitis; visual impairment	Lorry driver	28
FR07	France	NA	2003	Blood	NA	NA	NA	NA	NA	Bacteremia; septic shock; died	Hunter	29
GRE01	Greece	NA	2015	CSF/ blood	NA	NA	NA	M	31–35	Fever; partial hearing loss	Pig farmer	30
GRE02	Greece	NA	2005	CSF/ blood	2	NA	NA	F	61–65	Meningitis; sepsis; hearing loss	NA	31
GRE03	Greece	NA	2006	Blood	NA	NA	NA	M	56–60	Endocarditis	Butcher and farmer	32
HUN01	Hungary	NA	2020	Blood	2	NA	NA	M	31–35	Died; sepsis	Butcher	33
HUN02	Hungary	NA	2020	Blood	NA	NA	NA	M	NA	NA	NA	34
HUN03	Hungary	NA	2020	Blood	NA	NA	NA	M	NA	NA	NA	34
HUN04	Hungary	NA	2020	Blood	NA	NA	NA	M	NA	NA	NA	34
HUN05	Hungary	NA	2020	Blood	NA	NA	NA	M	NA	NA	NA	34
HUN06	Hungary	NA	2020	Blood	NA	NA	NA	M	NA	NA	NA	34
HUN07	Hungary	NA	2020	Blood	NA	NA	NA	M	NA	NA	NA	34
HUN08	Hungary	NA	2020	Blood	NA	NA	NA	M	NA	NA	NA	34
HUN09	Hungary	NA	2020	Blood	NA	NA	NA	M	NA	NA	NA	34
HUN10	Hungary	NA	2020	Blood	NA	NA	NA	M	NA	NA	NA	34
HUN11	Hungary	NA	2020	Blood	NA	NA	NA	M	NA	NA	NA	34
HUN12	Hungary	NA	2020	Blood	NA	NA	NA	M	NA	NA	NA	34
HUN13	Hungary	NA	2020	Blood	NA	NA	NA	M	NA	NA	NA	34
HUN14	Hungary	NA	2020	Blood	NA	NA	NA	M	NA	NA	NA	34

Isolate Metadata								Patient Metadata				
Isolate	Country	Year isolated	Year reported	Source	Serotype	ST	WGS	Sex	Age range, y	Clinical symptoms	Occupation	Ref
HUN15	Hungary	NA	2020	Blood	NA	NA	NA	M	NA	NA	NA	34
HUN16	Hungary	NA	2020	Blood	NA	NA	NA	M	NA	NA	NA	34
HUN17	Hungary	NA	2020	Blood	NA	NA	NA	M	NA	NA	NA	34
HUN18	Hungary	NA	2020	Blood	NA	NA	NA	M	NA	NA	NA	34
HUN19	Hungary	NA	2020	Blood	NA	NA	NA	M	NA	NA	NA	34
HUN20	Hungary	NA	2020	Blood	NA	NA	NA	M	NA	NA	NA	34
HUN21	Hungary	NA	2020	Blood	NA	NA	NA	M	NA	NA	NA	34
HUN22	Hungary	NA	2020	CSF/ blood	NA	NA	NA	F	NA	NA	NA	34
HUN23	Hungary	NA	2020	CSF/ blood	NA	NA	NA	F	NA	NA	NA	34
HUN24	Hungary	NA	2020	CSF/ blood	NA	NA	NA	F	NA	NA	NA	34
HUN25	Hungary	NA	2020	CSF/ blood	2	NA	NA	F	NA	NA	NA	34
HUN26	Hungary	NA	2020	CSF/ blood	2	NA	NA	F	NA	NA	NA	34
HUN27	Hungary	NA	2020	CSF/ blood	2	NA	NA	F	NA	NA	NA	34
HUN28	Hungary	NA	2020	CSF/ blood	2	NA	NA	F	NA	NA	NA	34
HUN29	Hungary	NA	2020	CSF/ blood	2	NA	NA	F	NA	NA	NA	34
HUN30	Hungary	NA	2020	CSF/ blood	2	NA	NA	F	NA	NA	NA	34
HUN31	Hungary	NA	2020	CSF/ blood	2	NA	NA	F	NA	NA	NA	34
HUN32	Hungary	NA	2020	CSF/ blood	2	NA	NA	F	NA	NA	NA	34
HUN33	Hungary	NA	2020	CSF/ blood	2	NA	NA	F	NA	NA	NA	34
HUN34	Hungary	NA	2020	CSF/ blood	2	NA	NA	F	NA	NA	NA	34
HUN35	Hungary	NA	2020	CSF/ blood	14	NA	NA	F	NA	NA	NA	34
HUN36	Hungary	NA	2019	CSF/ blood	NA	NA	NA	M	46–50	Meningitis	Hunter	35
IRE01	Ireland	NA	1995	NA	NA	NA	NA	M	21–25	Sepsis, died	Pig farmer	36
ITA01	Italy	NA	2007	CSF	NA	1	NA	M	26–30	Meningitis	Butcher	37
SsRC-1	Italy	2016	2016	Blood	2	1	Yes	M	51–55	Septic shock, died	Pig farmer	38
ITA03	Italy	2007	2008	CSF	2	1	NA	M	66–70	Meningitis	NA	39
ITA04	Italy	NA	1995	CSF	NA	NA	NA	NA	51–55	Meningitis	Farmer	40
ITA05	Italy	NA	2010	CSF	NA	NA	NA	F	46–50	Meningitis	Caregiver	41
861160	Netherlands	1986	2012	CSF	2	20	Yes	M	36–40	Meningitis	NA	42
870040	Netherlands	1987	2012	CSF	2	1	Yes	M	26–30	Meningitis	NA	42
890267	Netherlands	1989	2012	CSF	2	1	Yes	M	46–50	Meningitis	NA	42
NL01	Netherlands	2006	2012	CSF/ blood	NA	NA	NA	M	56–60	Meningitis, hearing loss	Meat processing factory	43
NL26	Netherlands	2001	2008	CSF	NA	NA	NA	F	31–35	Meningitis	Butcher	44
NL27	Netherlands	2007	2015	CSF/ blood	NA	NA	NA	M	61–65	Meningitis, hearing loss	Pig farmer	45
NL28	Netherlands	2008	2015	CSF/ blood	NA	NA	NA	M	36–40	Meningitis, hearing loss	Pig farmer	45
NL29	Netherlands	2011	2015	CSF/ blood	NA	NA	NA	M	56–60	Meningitis, hearing loss	Abattoir	45
NL30	Netherlands	2012	2015	CSF/ blood	NA	NA	NA	M	76–80	Meningitis, hearing loss	Pig farmer	45
NL31	Netherlands	2015	2015	Blood	2	NA	NA	M	56–60	Meningitis	Butcher	45

Isolate Metadata								Patient Metadata				
Isolate	Country	Year isolated	Year reported	Source	Serotype	ST	WGS	Sex	Age range, y	Clinical symptoms	Occupation	Ref
NL32	Netherlands	1989	1990	Blood	NA	NA	NA	M	26–30	Sepsis	Pig weigher	46
POL22	Poland	NA	2013	CSF/ blood	NA	NA	NA	M	31–35	Meningitis	Butcher	47
POR01	Portugal	2011	2014	CSF/ blood	NA	NA	NA	M	41–45	Meningitis	Pork griller	48
POR02	Portugal	NA	2008	CSF	NA	NA	NA	M	31–35	Meningitis	NA	49
POR03	Portugal	NA	2005	NA	NA	NA	NA	M	46–50	Meningitis	Butcher	50
POR04	Portugal	NA	2017	CSF/ blood	NA	NA	NA	M	46–50	Meningitis, hearing loss	Butcher	51
SER01	Serbia	2003	2005	CSF/ blood	NA	NA	NA	M	NA	Meningitis, hearing loss	Pig farmer	52
SER02	Serbia	2003	2005	CSF	NA	NA	NA	M	NA	Meningitis, hearing loss	Pig farmer	52
SER03	Serbia	2003	2005	CSF	NA	NA	NA	M	NA	Meningitis, hearing loss	Pig farmer	52
SER04	Serbia	2003	2005	CSF	NA	NA	NA	M	NA	Meningitis, hearing loss	NA	52
SER05	Serbia	2003	2005	CSF	NA	NA	NA	M	NA	Meningitis	NA	52
SP01	Spain	NA	2006	Blood	NA	NA	NA	M	56–60	Endocarditis, spondylodiscitis	Restaurant owner	53
SP02	Spain	NA	2009	Blood	2	NA	NA	M	66–70	Endocarditis	Helped at pig farm	54
SP03	Spain	NA	2012	Blood	NA	NA	NA	M	61–65	Meningitis	NA	55
SP04	Spain	1999	2001	CSF/ blood	NA	NA	NA	M	26–30	Meningitis	Butcher	56
SP05	Spain	1999	2001	CSF	NA	NA	NA	M	21–25	Meningitis, hearing loss	Butcher	56
SP06	Spain	2012	2014	CSF/ blood	2	3	NA	M	56–60	Meningitis, septic shock	NA	57
SP07	Spain	NA	2007	CSF/ blood	NA	NA	NA	M	31–35	Meningitis, hearing loss	Chicken farmer	58
SP08	Spain	NA	2008	CSF/ blood	NA	NA	NA	F	26–30	Meningitis	Butcher	59
SP09	Spain	NA	2016	Blood	2	NA	NA	M	51–55	Septic shock	Pig farmer	60
SP10	Spain	2004	2006	CSF/ blood	NA	NA	NA	M	36–40	Meningitis	Pig farmer	61
SP11	Spain	NA	2012	Blood	NA	NA	NA	M	31–35	Meningitis, hearing loss	Pig farmer	62
SP12	Spain	NA	2009	CSF	NA	NA	NA	F	81–85	Meningitis, spondylodiscitis	NA	63
SP13	Spain	NA	1994	NA	NA	NA	NA	F	56–60	Sacroilitis	NA	64
SP14	Spain	NA	2011	CSF/ blood	2	NA	NA	F	31–35	Meningitis, hearing loss	Pig farmer	65
SP15	Spain	NA	2005	CSF/ blood	2	NA	NA	M	26–30	Meningitis	Meat processing factory	66
SP16	Spain	NA	2012	Blood	2	3	NA	M	36–40	Meningitis, visual impairment	Pig farmer	67
SP17	Spain	1998	1998	CSF/ blood	NA	NA	NA	M	46–50	Meningitis, hearing loss	Pig farmer	68

Isolate Metadata								Patient Metadata				
Isolate	Country	Year isolated	Year reported	Source	Serotype	ST	WGS	Sex	Age range, y	Clinical symptoms	Occupation	Ref
SP18	Spain	1998	1998	CSF	NA	NA	NA	M	46–50	Meningitis	Butcher	68
SP19	Spain	1997	1997	CSF/ blood	NA	NA	NA	M	51–55	Meningitis	Pig farmer	69
SP20	Spain	2014	2018	NA	2	1	NA	M	46–50	Meningitis, arthritis	Hunter	70
SP21	Spain	2013	2021	CSF/ blood	NA	NA	NA	M	46–50	Meningitis	Butcher	71
SP22	Spain	2014	2021	CSF	NA	NA	NA	M	46–50	Meningitis	Pig farmer	71
SP23	Spain	2018	2021	Blood	NA	NA	NA	M	61–65	Endocarditis	Pig farmer	71
SP24	Spain	2018	2021	Semen	NA	NA	NA	M	46–50	Orchitis	NA	71
SP25	Spain	2019	2021	CSF	NA	NA	NA	M	41–45	Meningitis	Pig farmer	71
SP26	Spain	2019	2021	Blood/ synovial fluid	NA	NA	NA	M	61–65	Sepsis/ arthritis	Pig farmer	71
SP27	Spain	2019	2021	Synovial fluid	NA	NA	NA	M	36–40	Prosthetic hip infection	Pig farmer	71
SP28	Spain	NA	2014	CSF	NA	NA	NA	M	61–65	Meningitis	NA	72
SWE01	Sweden	NA	2014	Blood/ synovial fluid	5	NA	NA	M	61–65	Arthritis	Pig farmer	73
GE-034	Switzerland	NA	2020	Blood	14	1	Yes	F	41–45	Septic shock	NA	74
UK01	United Kingdom	NA	1991	CSF/ blood	NA	NA	NA	M	56–60	Meningitis, died	Meat porter	75
UK02	United Kingdom	NA	1992	CSF/ blood	NA	NA	NA	M	46–50	Meningitis, visual impairment	Pig farmer	76
UK03	United Kingdom	1999	2001	Blood	14	NA	NA	M	26–30	Septicemia, died	Pig farmer	77
UK04	United Kingdom	NA	1990	Blood	NA	NA	NA	F	41–45	Sepsis	Pig farmer	78

*NA, not available; ST, sequence type; WGS, whole genome sequencing.

Appendix Table 4. Number of reported human *S. suis* infections in the Public Health England zoonosis reports (79)

Years	No. of isolates
1991–2000	20
2001	1
2002	1
2003	1
2004	0
2005	2
2006	2
2007	2
2008	7
2009	2
2010	4
2011	1
2012	3
2013	3
2014	3
2015	4
2016	1
2017	4
Total	61

Appendix Table 5. Number of reported human *S. suis* infections in the National Institute for Public Health and Environment of the Netherlands. To avoid case identification, the age of each patient is shown as a 5 year range NA stands for missing information

Isolate	Isolate Metadata		Patient Metadata			Ref
	Year isolated	Source	Sex	Age range, y	Clinical symptoms	
NL33	1990	CSF	M	41–45	NA	80
NL34	1990	Blood	M	66–70	NA	80
NL35	1992	Blood	M	71–75	NA	80
NL36	1993	Blood	NA	51–55	NA	80
NL37	1996	Throat swab	M	36–40	NA	80
NL38	1996	NA	M	71–75	NA	80
NL39	1996	Blood	M	51–55	Meningitis	80
NL40	1999	Blood	M	66–70	NA	80
NL41	2004	Blood	M	0–5	NA	80
NL42	2006	CSF/ blood	M	61–65	NA	80
NL43	2006	Blood	M	56–60	NA	80
NL44	2007	Urine	F	0–5	Urinary tract infection	80
NL45	2008	Dialysate	F	56–60	Peritonitis	80
NL46	2008	Urine	F	41–45	NA	80
NL47	2008	Bilious abdominal fluid	M	86–90	NA	80
NL48	2009	Wound culture	F	51–55	NA	80
NL49	2009	Wound culture	F	36–40	NA	80
NL50	2009	Wound culture	M	36–40	NA	80
NL51	2010	Urine	F	21–25	Urinary tract infection	80
NL52	2010	Abscess fluid	M	26–30	NA	80
NL53	2011	Urine	F	66–70	NA	80
NL54	2011	Pus	M	71–75	NA	80
NL55	2011	CSF/ blood	M	46–50	NA	80
NL56	2011	Urethra swab	M	66–70	NA	80
NL57	NA	Wound culture	F	NA	Sepsis	80

Appendix Table 6. Incidence estimates of human *S. suis* infections in European countries with at least 5 cases during 2005–2013*

Country	Population, thousands†	Estimated at-risk population, thousands	No. of <i>S. suis</i> infections	Incidence in total population /100,000 persons‡	Incidence in at-risk population, /100,000 persons‡	<i>S. pneumoniae</i> incidence in total population /100,000 persons	<i>N. meningitidis</i> incidence in total population /100,000 persons
Czech Republic	10,393.68	46.05	7	0.0075	1.689	3.19	0.69
Germany	81,793.72	189.53	12	0.0016	0.703	NA	0.55
Hungary	10,021.71	509.01	36	0.0399	0.786	1.52	0.42
Netherlands	16,507.50	24.72	11	0.0074	4.945	14.86	0.93
Poland*	38,324.00	1,242.46	18	0.0052	0.161	1.10	0.67
Spain	45,323.88	189.92	12	0.0029	0.702	5.84	1.09

*NA, not available.

†Used OECD estimates for total population

‡Rough estimates of incidence and demographics of human *Streptococcus suis* infection reported to national reference laboratories during 2005–2013.

Appendix Table 7. Sequences used in this study and their accession numbers.

Strain	Bioproject	Genbank	Biosample	SRA Illumina	Reference
This study					
133899	PRJNA917482	JARATX000000000	SAMN32539671	SRR26047323	This study
136829	PRJNA917482	JAVMBI000000000	SAMN32539672	SRR26047322	This study
139809	PRJNA917482	JARATW000000000	SAMN32539673	SRR26047311	This study
143741	PRJNA917482	JARATV000000000	SAMN32539674	SRR26047300	This study
147422	PRJNA917482	JARATU000000000	SAMN32539675	SRR26047289	This study
148550	PRJNA917482	JARATT000000000	SAMN32539676	SRR26047282	This study
148897	PRJNA917482	JARATS000000000	SAMN32539677	SRR26047281	This study
150002	PRJNA917482	JARATR000000000	SAMN32539678	SRR26047280	This study
150808	PRJNA917482	JARATQ000000000	SAMN32539679	SRR26047279	This study
150968	PRJNA917482	JARATP000000000	SAMN32539680	SRR26047278	This study
155567	PRJNA917482	JARATO000000000	SAMN32539681	SRR26047321	This study
515/2012	PRJNA917482	JARATN000000000	SAMN32539682	SRR26047320	This study
300/2014	PRJNA917482	JARATM000000000	SAMN32539683	SRR26047319	This study
821/2014	PRJNA917482	JARATL000000000	SAMN32539684	SRR26047318	This study
783/2015	PRJNA917482	JAVMBJ000000000	SAMN32539685	SRR26047317	This study
84/2016	PRJNA917482	JARATK000000000	SAMN32539686	SRR26047316	This study
150687	PRJNA917482	JAVMBK000000000	SAMN32539687	SRR26047315	This study
16770	PRJNA917482	JARATJ000000000	SAMN32539688	SRR26047314	This study
18911	PRJNA917482	JARATI000000000	SAMN32539689	SRR26047313	This study
19691	PRJNA917482	JAVMBL000000000	SAMN32539690	SRR26047312	This study
20014	PRJNA917482	JARATH000000000	SAMN32539691	SRR26047310	This study
23674	PRJNA917482	JARATG000000000	SAMN32539692	SRR26047309	This study
26556	PRJNA917482	JARATF000000000	SAMN32539693	SRR26047308	This study
29429	PRJNA917482	JAVMBM000000000	SAMN32539694	SRR26047307	This study
35192	PRJNA917482	JARATE000000000	SAMN32539695	SRR26047306	This study
36305	PRJNA917482	JARATD000000000	SAMN32539696	SRR26047305	This study
45583	PRJNA917482	JARATC000000000	SAMN32539697	SRR26047304	This study
46581	PRJNA917482	JARATB000000000	SAMN32539698	SRR26047303	This study
15194	PRJNA917482	JARATA000000000	SAMN32539699	SRR26047302	This study
15459	PRJNA917482	JARASZ000000000	SAMN32539700	SRR26047301	This study
15901	PRJNA917482	JARASY000000000	SAMN32539701	SRR26047299	This study
16064	PRJNA917482	JARASX000000000	SAMN32539702	SRR26047298	This study
17387	PRJNA917482	JARASW000000000	SAMN32539703	SRR26047297	This study
18364	PRJNA917482	JAVMBN000000000	SAMN32539704	SRR26047296	This study
18939	PRJNA917482	JARASV000000000	SAMN32539705	SRR26047295	This study
19514	PRJNA917482	JARASU000000000	SAMN32539706	SRR26047294	This study
21150	PRJNA917482	JARAST000000000	SAMN32539707	SRR26047293	This study
82749	PRJNA917482	JARASS000000000	SAMN32539708	SRR26047292	This study
DK1	PRJNA917482	JARASR000000000	SAMN32539709	SRR26047291	This study
H172320687	PRJNA917482	JARASQ000000000	SAMN32539710	SRR26047290	This study
H143440709	PRJNA917482	JARASP000000000	SAMN32539711	SRR26047288	This study
H155260819	PRJNA917482	JARASO00000000	SAMN32539712	SRR26047287	This study
H143440711	PRJNA917482	JARASN000000000	SAMN32539713	SRR26047286	This study
H104080146	PRJNA917482	JARASM000000000	SAMN32539714	SRR26047285	This study
H143440710	PRJNA917482	JARASL000000000	SAMN32539715	SRR26047284	This study

Strain	Bioproject	Genbank	Biosample	SRA Illumina	Reference
SP1	PRJNA917482	JARASK000000000	SAMN32539716	SRR26047283	This study
Public Genomes					
861160	PRJEB11219	CZEH000000000	SAMEA3595177	ERR1055554	94
870040	PRJEB11219	CZDK000000000	SAMEA3595178	ERR1055555	94
890267	PRJEB11219	CZDM000000000	SAMEA3595179	ERR1055556	94
901131	PRJEB11219	CZEW000000000	SAMEA3595180	ERR1055557	94
902409	PRJEB11219	CZDT000000000	SAMEA3595181	ERR1055558	94
920694	PRJEB11219	CZES000000000	SAMEA3595182	ERR1055559	94
931260	PRJEB11219	CZDW000000000	SAMEA3595183	ERR1055560	94
940056	PRJEB11219	CZEU000000000	SAMEA3595184	ERR1055561	94
940255	PRJEB11219	CZDN000000000	SAMEA3595185	ERR1055562	94
940430	PRJEB11219	CZDX000000000	SAMEA3595186	ERR1055563	94
941372	PRJEB11219	CZEJ000000000	SAMEA3595187	ERR1055564	94
950136	PRJEB11219	CZDZ000000000	SAMEA3595188	ERR1055565	94
971344	PRJEB11219	CZDL000000000	SAMEA3595189	ERR1055566	94
971724	PRJEB11219	CZEG000000000	SAMEA3595190	ERR1055567	94
2000343	PRJEB11219	CZET000000000	SAMEA3595191	ERR1055568	94
2001171	PRJEB11219	CZDY000000000	SAMEA3595192	ERR1055569	94
2001946	PRJEB11219	CZDO000000000	SAMEA3595193	ERR1055570	94
2011423	PRJEB11219	CZEO000000000	SAMEA3595194	ERR1055571	94
2012092	PRJEB11219	CZEK000000000	SAMEA3595195	ERR1055572	94
2032008	PRJEB11219	CZEV000000000	SAMEA3595196	ERR1055573	94
2051729	PRJEB11219	CZEE000000000	SAMEA3595197	ERR1055574	94
2061238	PRJEB11219	CZDQ000000000	SAMEA3595198	ERR1055575	94
2061410	PRJEB11219	CZDV000000000	SAMEA3595199	ERR1055576	94
2071319	PRJEB11219	CZEQ000000000	SAMEA3595200	ERR1055577	94
SsRC-1	PRJNA445803	PYUF000000000	SAMN08799542	NA	95
GE-034	pubMLST	pubMLST	pubMLST	pubMLST	96
DSM28762	PRJEB45445	NA	SAMEA8942198	ERR6131134	97
11611	PRJNA171412	ALKR000000000	SAMN02470636	NA	98

References

- Chen S, Zhou Y, Chen Y, Gu J. fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics*. 2018;34:i884–90. [PubMed https://doi.org/10.1093/bioinformatics/bty560](https://doi.org/10.1093/bioinformatics/bty560)
- Wood DE, Lu J, Langmead B. Improved metagenomic analysis with Kraken 2. *Genome Biol*. 2019;20:257. [PubMed https://doi.org/10.1186/s13059-019-1891-0](https://doi.org/10.1186/s13059-019-1891-0)
- Prjibelski A, Antipov D, Meleshko D, Lapidus A, Korobeynikov A. Using SPAdes de novo assembler. *Curr Protoc Bioinformatics*. 2020;70:e102. [PubMed https://doi.org/10.1002/cpbi.102](https://doi.org/10.1002/cpbi.102)
- Gurevich A, Saveliev V, Vyahhi N, Tesler G. QUAST: quality assessment tool for genome assemblies. *Bioinformatics*. 2013;29:1072–5. [PubMed https://doi.org/10.1093/bioinformatics/btt086](https://doi.org/10.1093/bioinformatics/btt086)

5. Seemann T. Prokka: rapid prokaryotic genome annotation. *Bioinformatics*. 2014;30:2068–9. [PubMed](#)
<https://doi.org/10.1093/bioinformatics/btu153>
6. Athey TBT, Teatero S, Lacouture S, Takamatsu D, Gottschalk M, Fittipaldi N. Determining *Streptococcus suis* serotype from short-read whole-genome sequencing data. *BMC Microbiol*. 2016;16:162. [PubMed](#) <https://doi.org/10.1186/s12866-016-0782-8>
7. Willemse N, Howell KJ, Weinert LA, Heuvelink A, Pannekoek Y, Wagenaar JA, et al. An emerging zoonotic clone in the Netherlands provides clues to virulence and zoonotic potential of *Streptococcus suis*. *Sci Rep*. 2016;6:28984. [PubMed](#) <https://doi.org/10.1038/srep28984>
8. van de Beek D, Spanjaard L, de Gans J. *Streptococcus suis* meningitis in the Netherlands. *J Infect*. 2008;57:158–61. [PubMed](#) <https://doi.org/10.1016/j.jinf.2008.04.009>
9. Bojarska A, Molska E, Janas K, Skoczyńska A, Stefaniuk E, Hryniewicz W, et al. *Streptococcus suis* in invasive human infections in Poland: clonality and determinants of virulence and antimicrobial resistance. *Eur J Clin Microbiol Infect Dis*. 2016;35:917–25. [PubMed](#)
<https://doi.org/10.1007/s10096-016-2616-x>
10. Spiss HK, Kofler M, Hausdorfer H, Pfausler B, Schmutzhard E. *Streptococcus suis* meningitis and neurophysiology of the acoustic system. First case report from Austria [in German]. *Nervenarzt*. 1999;70:738–41. [PubMed](#) <https://doi.org/10.1007/s001150050503>
11. Kopic J, Paradžik MT, Pandak N. *Streptococcus suis* infection as a cause of severe illness: 2 cases from Croatia. *Scand J Infect Dis*. 2002;34:683–4. [PubMed](#)
<https://doi.org/10.1080/00365540210147769>

12. Smetana J, Machac J, Prasil P, Bostikova V, Plisek S, Kosina P, et al. Spondylodiscitis with Abscess Formation in Paravertebral Muscles due to *Streptococcus suis*—a Case Report. *Ces Slov Neurol Neurochir.* 2013;76:769–72.
13. Baums CG, Verkühlen GJ, Rehm T, Silva LMG, Beyerbach M, Pohlmeier K, et al. Prevalence of *Streptococcus suis* genotypes in wild boars of Northwestern Germany. *Appl Environ Microbiol.* 2007;73:711–7. [PubMed https://doi.org/10.1128/AEM.01800-06](https://doi.org/10.1128/AEM.01800-06)
14. Eisenberg T, Hudemann C, Hossain HM, Hewer A, Tello K, Bandorski D, et al. Characterization of Five Zoonotic *Streptococcus suis* Strains from Germany, Including One Isolate from a Recent Fatal Case of Streptococcal Toxic Shock-Like Syndrome in a Hunter. *J Clin Microbiol.* 2015;53:3912–5. [PubMed https://doi.org/10.1128/JCM.02578-15](https://doi.org/10.1128/JCM.02578-15)
15. Heidt MC, Mohamed W, Hain T, Vogt PR, Chakraborty T, Domann E. Human infective endocarditis caused by *Streptococcus suis* serotype 2. *J Clin Microbiol.* 2005;43:4898–901. [PubMed https://doi.org/10.1128/JCM.43.9.4898-4901.2005](https://doi.org/10.1128/JCM.43.9.4898-4901.2005)
16. Rosenkranz M, Elsner HA, Stürenburg HJ, Weiller C, Röther J, Sobottka I. *Streptococcus suis* meningitis and septicemia contracted from a wild boar in Germany. *J Neurol.* 2003;250:869–70. [PubMed https://doi.org/10.1007/s00415-003-1103-3](https://doi.org/10.1007/s00415-003-1103-3)
17. Strangmann E, Fröleke 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. [PubMed https://doi.org/10.1078/1438-4639-00165](https://doi.org/10.1078/1438-4639-00165)

18. Braun S, Jechart G, Emmerling U, Ehret W. Bakterielle Meningitis durch *Streptococcus suis*. Dtsch Med Wochenschr. 2007;132:1098–100. [PubMed https://doi.org/10.1055/s-2007-979385](https://pubmed.ncbi.nlm.nih.gov/10.1055/s-2007-979385/)
19. Grebe T, Bergenthal D, Fahr AM, Scheja HW. Erwachsenen-Meningitis durch *Streptococcus suis* Typ 2. Dtsch Med Wochenschr. 1997;122:1244–7. [PubMed https://doi.org/10.1055/s-2008-1047754](https://pubmed.ncbi.nlm.nih.gov/10.1055/s-2008-1047754/)
20. Werinder A, Aspán A, Söderlund R, Backhans A, Sjölund M, Guss B, et al. Whole-Genome Sequencing Evaluation of MALDI-TOF MS as a Species Identification Tool for *Streptococcus suis*. J Clin Microbiol. 2021;59:e0129721. [PubMed https://doi.org/10.1128/JCM.01297-21](https://pubmed.ncbi.nlm.nih.gov/10.1128/JCM.01297-21/)
21. Chen C, Zhang W, Zheng H, Lan R, Wang H, Du P, et al. Minimum core genome sequence typing of bacterial pathogens: a unified approach for clinical and public health microbiology. J Clin Microbiol. 2013;51:2582–91. [PubMed https://doi.org/10.1128/JCM.00535-13](https://pubmed.ncbi.nlm.nih.gov/10.1128/JCM.00535-13/)
22. Poggenborg R, Gaïni S, Kjaeldgaard P, Christensen JJ. *Streptococcus suis*: meningitis, spondylodiscitis and bacteraemia with a serotype 14 strain. Scand J Infect Dis. 2008;40:346–9. [PubMed https://doi.org/10.1080/00365540701716825](https://pubmed.ncbi.nlm.nih.gov/10.1080/00365540701716825/)
23. Beziau MC, Diallo BS, Chachia A, Gabinski C, Beylot J. Fulminant purpura during *Streptococcus suis* meningitis and septicaemia. Med Mal Infect. 1996;26:349–51. [https://doi.org/10.1016/S0399-077X\(96\)80212-5](https://doi.org/10.1016/S0399-077X(96)80212-5)
24. Dupas D, Vignon M, Géraut C. *Streptococcus suis* meningitis. A severe noncompensated occupational disease. J Occup Med. 1992;34:1102–5. [PubMed https://doi.org/10.1097/00043764-199211000-00013](https://pubmed.ncbi.nlm.nih.gov/10.1097/00043764-199211000-00013/)

25. Durand F, Périno CL, Recule C, Brion JP, Kobish M, Guerber F, et al. Bacteriological diagnosis of *Streptococcus suis* meningitis. Eur J Clin Microbiol Infect Dis. 2001;20:519–21. [PubMed](#)
<https://doi.org/10.1007/PL00011299>
26. Tayoro J, Besnier JM, Laudat P, Cattier B, Choutet P. Infective endocarditis due to *Streptococcus suis* serotype 2. Eur J Clin Microbiol Infect Dis. 1996;15:765–6. [PubMed](#)
<https://doi.org/10.1007/BF01691970>
27. François B, Gissot V, Ploy MC, Vignon P. Recurrent septic shock due to *Streptococcus suis*. J Clin Microbiol. 1998;36:2395–2395. [PubMed](#) <https://doi.org/10.1128/JCM.36.8.2395-2395.1998>
28. Bahloul H, Mofredj A, Mrabet A, Gineyt G, Rousselier P. Méningite à *Streptococcus suis* secondaire à une contamination orale? Med Mal Infect. 2008;38:281–2. [PubMed](#)
<https://doi.org/10.1016/j.medmal.2008.01.002>
29. Pedroli S, Kobisch M, Beauchet O, Chaussinand JP, Lucht F. *Streptococcus suis* bacteremia [in French]. Presse Med. 2003;32:599–601. [PubMed](#)
30. Chatzopoulou M, Voulgaridou I, Papalas D, Vasiliou P, Tsiakalou M. Third Case of *Streptococcus suis* Infection in Greece. Case Rep Infect Dis. 2015;2015:505834. [PubMed](#)
<https://doi.org/10.1155/2015/505834>
31. 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. [PubMed](#) <https://doi.org/10.1111/j.1468-1331.2005.00998.x>

32. Papatsiros V, Vourvidis D, Tzitzis A, Meichanetsidis P, Stougiou D, Papaioannou D. *Streptococcus suis*: an important zoonotic pathogen for human prevention aspects. *Vet World*. 2011;216. <https://doi.org/10.5455/vetworld.2011.216-221>
33. Ágoston Z, Terhes G, Hannauer P, Gajdács M, Urbán E. Fatal case of bacteremia caused by *Streptococcus suis* in a splenectomized man and a review of the European literature. *Acta Microbiol Immunol Hung*. 2020;67:148–55. [PubMed https://doi.org/10.1556/030.2020.01123](https://doi.org/10.1556/030.2020.01123)
34. Gajdács M, Németh A, Knausz M, Barrak I, Stájer A, Mestyán G, et al. *Streptococcus suis*: An Underestimated Emerging Pathogen in Hungary? *Microorganisms*. 2020;8:1292. [PubMed https://doi.org/10.3390/microorganisms8091292](https://doi.org/10.3390/microorganisms8091292)
35. Németh A, Knausz M, Schmidt P. *Streptococcus suis* okozta purulens meningitis különleges esete. *Orv Hetil*. 2019;160:30–4. [PubMed https://doi.org/10.1556/650.2019.31243](https://doi.org/10.1556/650.2019.31243)
36. Baddeley PG. *Streptococcus suis* infection. *Occup Med (Lond)*. 1995;45:222. [PubMed https://doi.org/10.1093/occmed/45.4.222-a](https://doi.org/10.1093/occmed/45.4.222-a)
37. Camporese A, Tizianel G, Bruschetta G, Cruciatti B, Pomes A. Human meningitis caused by *Streptococcus suis*: the first case report from north-eastern Italy. *Infez Med*. 2007;15:111–4.
38. Mancini F, Adamo F, Creti R, Monaco M, Alfarone G, Pantosti A, et al. A fatal case of streptococcal toxic shock syndrome caused by *Streptococcus suis* carrying tet (40) and tet (O/W/32/O), Italy. *J Infect Chemother*. 2016;22:774–6. [PubMed https://doi.org/10.1016/j.jiac.2016.05.011](https://doi.org/10.1016/j.jiac.2016.05.011)

39. Manzin A, Palmieri C, Serra C, Saggi B, Princivalli MS, Loi G, et al. *Streptococcus suis* meningitis without history of animal contact, Italy. *Emerg Infect Dis*. 2008;14:1946–8. [PubMed](#)
<https://doi.org/10.3201/eid1412.080679>
40. Perseghin P, Bezzi G, Troupioti P, Gallina M. *Streptococcus suis* meningitis in an Italian blood donor. *Lancet*. 1995;346:1305–6. [PubMed](#) [https://doi.org/10.1016/S0140-6736\(95\)91912-0](https://doi.org/10.1016/S0140-6736(95)91912-0)
41. Parisi G, Spanu T, Mariani B, Rianda A, Tronci M. Meningitis caused by *Streptococcus suis*: a case report. *Microbiol Med*. 2010;25. 10.4081/mm.2010.2414 <https://doi.org/10.4081/mm.2010.2414>
42. Schultsz C, Jansen E, Keijzers W, Rothkamp A, Duim B, Wagenaar JA, et al. Differences in the population structure of invasive *Streptococcus suis* strains isolated from pigs and from humans in The Netherlands. *PLoS One*. 2012;7:e33854. [PubMed](#)
<https://doi.org/10.1371/journal.pone.0033854>
43. de Ceuster LME, van Dillen JJ, Wever PC, Rozemeijer W, Louwse ES. [*Streptococcus suis* meningitis in a meat factory employee]. *Ned Tijdschr Geneesk*. 2012;156:A5080. [PubMed](#)
44. van de Beek D, Spanjaard L, de Gans J. *Streptococcus suis* meningitis in the Netherlands. *J Infect*. 2008;57:158–61. [PubMed](#) <https://doi.org/10.1016/j.jinf.2008.04.009>
45. van Samkar A, Brouwer MC, Schultsz C, van der Ende A, van de Beek D. *Streptococcus suis* meningitis in the Netherlands. *J Infect*. 2015;71:602–4. [PubMed](#)
<https://doi.org/10.1016/j.jinf.2015.07.001>

46. van Jaarsveld BC, van Kregten E, van Kesteren RG, Rozenberg-Arska M, Bartelink AK. Fulminant sepsis caused by *Streptococcus suis* [in Dutch]. Ned Tijdschr Geneesk. 1990;134:1462–4. [PubMed](#)
47. Zalas-Więcek P, Michalska A, Grąbczewska E, Olczak A, Pawłowska M, Gospodarek E. Human meningitis caused by *Streptococcus suis*. J Med Microbiol. 2013;62:483–5. [PubMed](#)
<https://doi.org/10.1099/jmm.0.046599-0>
48. Seixas D, Lebre A, Crespo P, Ferreira E, Serra JE, Saraiva da Cunha JG. Meningite bacteriana aguda como doença ocupacional. Acta Med Port. 2014;27:519–21. [PubMed](#)
<https://doi.org/10.20344/amp.4735>
49. Taipa R, Lopes V, Magalhães M. *Streptococcus suis* meningitis: first case report from Portugal. J Infect. 2008;56:482–3. [PubMed](#) <https://doi.org/10.1016/j.jinf.2008.03.002>
50. Pinto IV, Caixinha JP, Castro V, Marques A, Fonseca A, Vera J. Infecção por *Streptococcus suis*: uma causa rara de meningite no homem. Rev Port Doenc Infec. 2005;2:34–5.
51. Sena Esteves S, Carvalho de Almeida J, Abrunhosa J, Almeida E Sousa C, Arshad Q. *Pig's ear*: *Streptococcus suis* Meningitis and its associated inner ear implications. IDCases. 2017;10:55–7. [PubMed](#) <https://doi.org/10.1016/j.idcr.2017.08.015>
52. Dragojlović J, Milosević B, Sasić N, Pelemis M, Sasić M. *Streptococcus suis* infection—clinical manifestations. Med Pregl. 2005;58:236–9. [PubMed](#) <https://doi.org/10.2298/MPNS0506236D>

53. Alonso-Socas MM, Alemán-Valls R, Roldán-Delgado H, Gómez-Sirvent JL. Endocarditis y espondilodiscitis por *Streptococcus suis*. Enferm Infecc Microbiol Clin. 2006;24:354–5. [PubMed](#)
<https://doi.org/10.1157/13089675>
54. Aspiroz C, Vela AI, Pascual MS, Aldea MJ. Endocarditis aguda por *Streptococcus suis* serogrupo 2 en España. Enferm Infecc Microbiol Clin. 2009;27:370–1. [PubMed](#)
<https://doi.org/10.1016/j.eimc.2008.11.010>
55. Baena IM, Fernández C, Sánchez J, Calvente M, Aguadero V. *Streptococcus suis* type 2: emerging pathogen producer of meningitis [in Spanish]. Rev Esp Quimioter. 2012;25:293–4. [PubMed](#)
56. Geffner Sclarsky DE, Moreno Muñoz R, Campillo Alpera MS, Pardo Serrano FJ, Gómez Gómez A, Martínez-Lozano MD. Meningitis por *Streptococcus suis*. An Med Interna (Madrid). 2001 Jun;18(6) [cited 2022 Aug 24]. http://scielo.isciii.es/scielo.php?script=sci_arttext&pid=S0212-71992001000600007&lng=en&nrm=iso&tlng=en
57. Gómez-Zorrilla S, Ardanuy C, Lora-Tamayo J, Cámara J, García-Somoza D, Peña C, et al. *Streptococcus suis* infection and malignancy in man, Spain. Emerg Infect Dis. 2014;20:1067–8. [PubMed](#) <https://doi.org/10.3201/eid2006.131167>
58. Hidalgo A, Ropero F, Palacios R, García V, Santos J. Meningitis due to *Streptococcus suis* with no contact with pigs or porcine products. J Infect. 2007;55:478. [PubMed](#)
<https://doi.org/10.1016/j.jinf.2007.02.013>

59. Riquelme E, Escribano E, Blanch JJ, Crespo MD. Meningitis aguda por *Streptococcus suis* en una carnicera. *Enferm Infecc Microbiol Clin*. 2008;26:256–7. [PubMed https://doi.org/10.1016/S0213-005X\(08\)72700-9](https://doi.org/10.1016/S0213-005X(08)72700-9)
60. López-Mestanza C, Bolaño-Navarro A, Sánchez-Sánchez A, Aldea-Mansilla C. Primer caso de shock séptico por *Streptococcus suis* de posible etiología alimentaria en España. *Med Intensiva*. 2016;40:516–8. [PubMed https://doi.org/10.1016/j.medin.2016.01.006](https://doi.org/10.1016/j.medin.2016.01.006)
61. Luengo-Alvarez J, Martín-Ruiz C, Sánchez Muñoz-Torrero JF, Iñiguez-Ovando R. Meningitis due to *Streptococcus suis*: a case report [in Spanish]. *Enferm Infecc Microbiol Clin*. 2006;24:352–4. [PubMed https://doi.org/10.1157/13089674](https://doi.org/10.1157/13089674)
62. Corrales-Arroyo MJ, Real-Francia MAD, Hernandez-Gonzalez A, Lopez-Gallardo G, Puebla JMM, Caston-Osorio JJ. Acute Meningitis by *Streptococcus suis*. *Journal of Microbiology and Infectious Diseases*. 2015;2:160–2. <https://doi.org/10.5799/jmid.123130>
63. Galbarro J, Franco-Álvarez de Luna F, Cano R, Angel Castaño M. Meningitis aguda y espondilodiscitis por *Streptococcus suis* en paciente sin contacto previo con cerdos o productos porcinos derivados. *Enferm Infecc Microbiol Clin*. 2009;27:425–7. [PubMed https://doi.org/10.1016/j.eimc.2008.07.012](https://doi.org/10.1016/j.eimc.2008.07.012)
64. Martínez Avilés P, Jurdado Ruiz-Capillas JJ, Gómez Rodrigo J, Solís Villa J. [Sacroiliitis caused by *Streptococcus suis* type II]. *An Med Interna*. 1994;11:309. [PubMed](https://pubmed.ncbi.nlm.nih.gov/)

65. Fernández-Ferro J, López-González FJ, Pardo F, Pías-Peleteiro JM. Meningitis aguda por *Streptococcus suis* en una criadora de cerdos. *Enferm Infecc Microbiol Clin*. 2011;29:396–7. [PubMed https://doi.org/10.1016/j.eimc.2010.12.013](https://doi.org/10.1016/j.eimc.2010.12.013)
66. de la Hoz Adame ME, de la Rubia Martín F, Domínguez Fuentes B, García Gil D. Meningitis aguda por *Streptococcus suis*: a propósito de un caso en un paciente esplenectomizado. *An Med Interna (Madrid)*. 2005 Oct;22(10) [cited 2022 Aug 24]. http://scielo.isciii.es/scielo.php?script=sci_arttext&pid=S0212-71992005001000020&lng=en&nrm=iso&tlng=en
67. Vela AI, Aspiroz C, Fortuño B, Tirado G, Sierra J, Martínez R, et al. Meningitis caused by an unusual genotype (ST3) of *Streptococcus suis*. *Infection*. 2013;41:701–3. [PubMed https://doi.org/10.1007/s15010-012-0382-y](https://doi.org/10.1007/s15010-012-0382-y)
68. María Asensi J, Asensi V, Arias M, Moreno A, Pérez F, Navarro R. Meningitis por *Streptococcus suis*. A propósito de dos casos y revisión de la literatura. *Enferm Infecc Microbiol Clin*. 2001;19:186–8. [https://doi.org/10.1016/S0213-005X\(01\)72606-7](https://doi.org/10.1016/S0213-005X(01)72606-7)
69. Juncal AR, Pardo F, Rodríguez I, Pérez del Molino ML. [Meningitis by *Streptococcus suis*]. *Enferm Infecc Microbiol Clin*. 1997;15:120–1. [PubMed https://doi.org/10.1016/S0213-005X\(97\)70120-1](https://doi.org/10.1016/S0213-005X(97)70120-1)
70. Fernández-Aguilar X, Gottschalk M, Aragon V, Càmara J, Ardanuy C, Velarde R, et al. Urban Wild Boars and Risk for Zoonotic *Streptococcus suis*, Spain. *Emerg Infect Dis*. 2018;24:1083–6. [PubMed https://doi.org/10.3201/eid2406.171271](https://doi.org/10.3201/eid2406.171271)

71. Díez de Los Ríos J, Reynaga E, García-González M, Càmara J, Ardanuy C, Cuquet J, et al. Clinical and Epidemiological Characteristics of *Streptococcus suis* Infections in Catalonia, Spain. *Front Med (Lausanne)*. 2021;8:792233. [PubMed https://doi.org/10.3389/fmed.2021.792233](https://doi.org/10.3389/fmed.2021.792233)
72. Broullón-Dobarro A, Rey-Martínez M, Cabadas-Avió R. Meningitis aguda por *Streptococcus suis*. *Rev Esp Anestesiol Reanim*. 2014;61:223–4. [PubMed https://doi.org/10.1016/j.redar.2013.06.004](https://doi.org/10.1016/j.redar.2013.06.004)
73. Gustavsson C, Rasmussen M. Septic arthritis caused by *Streptococcus suis* serotype 5 in pig farmer. *Emerg Infect Dis*. 2014;20:489–90. [PubMed https://doi.org/10.3201/eid2003.130535](https://doi.org/10.3201/eid2003.130535)
74. Olearo F, Marinosci A, Stephan R, Cherkaoui A, Renzi G, Gaia N, et al. First case of *Streptococcus suis* infection in Switzerland: An emerging public health problem? *Travel Med Infect Dis*. 2020;36:101590. [PubMed https://doi.org/10.1016/j.tmaid.2020.101590](https://doi.org/10.1016/j.tmaid.2020.101590)
75. Clarke D, Almeyda J, Ramsay I, Drabu YJ. Primary prevention of *Streptococcus suis* meningitis. *Lancet*. 1991;338:1147–8. [PubMed https://doi.org/10.1016/0140-6736\(91\)92005-M](https://doi.org/10.1016/0140-6736(91)92005-M)
76. Meecham JS, Worth RC. Persistent diplopia following *streptococcus suis* type 2 meningitis. *J R Soc Med*. 1992;85:579–80. [PubMed https://doi.org/10.1177/014107689208500927](https://doi.org/10.1177/014107689208500927)
77. Watkins EJ, Brooksby P, Schweiger MS, Enright SM. Septicaemia in a pig-farm worker. *Lancet*. 2001;357:38. [PubMed https://doi.org/10.1016/S0140-6736\(00\)03570-4](https://doi.org/10.1016/S0140-6736(00)03570-4)
78. Maher D. *Streptococcus suis* septicaemia presenting as severe acute gastro-enteritis. *J Infect*. 1991;22:303–4. [PubMed https://doi.org/10.1016/S0163-4453\(05\)80022-2](https://doi.org/10.1016/S0163-4453(05)80022-2)
79. Public Health England. Zoonoses Report UK 2017 [cited 2022 Aug 24]]. <https://www.gov.uk/government/publications/zoonoses-uk-annual-reports>

80. Schultsz C, van Dijk D, Wagenaar JA, van der Ende A. Zoonotic infections with *Streptococcus suis* in the Netherlands / IB 11-2013 | RIVM [cited 2022 Nov 8].
<https://www.rivm.nl/weblog/zoonotische-infecties-met-streptococcus-suis-in-nederland-ib-11-2013>
81. Main tables—Agriculture—Eurostat [cited 2023 Jan 26].
<https://ec.europa.eu/eurostat/web/agriculture/data/main-tables>
82. Population Censuses. Population Censuses. [cited 2023 Jan 23].
<https://www.czso.cz/csu/czso/population-censuses>
83. Current population. Federal Statistical Office. [cited 2023 Jan 23].
https://www.destatis.de/EN/Themes/Society-Environment/Population/Current-Population/_node.html
84. Demography—Population at the beginning of the month—France (including Mayotte since 2014) | Insee. [cited 2023 Jan 25]. <https://www.insee.fr/en/statistiques/serie/001641607>
85. Population. [cited 2023 Jan 23]. <https://www.dst.dk/en/Statistik/emner/borgere/befolkning>
86. Greece Population 1950–2023. [cited 2023 Jan 25].
<https://www.macrotrends.net/countries/GRC/greece/population>
87. Hungarian Central Statistical Office. [cited 2023 Jan 25]. <https://www.ksh.hu/?lang=en>
88. Estimated resident population—Years 2002–2019. [cited 2023 Jan 25].
<http://dati.istat.it/Index.aspx?QueryId=12386&lang=en>

89. INEbase. CONSULINE. [cited 2023 Jan 26].

<https://www.ine.es/consul/serie.do?d=true&s=CP335&c=2&>

90. StatLine—Population; key figures. [cited 2023 Jan 23].

<https://opendata.cbs.nl/statline/#/CBS/en/dataset/37296eng/table?ts=1530690320120>.

91. Population estimates—Office for National Statistics. [cited 2023 Jan 26].

<https://www.ons.gov.uk/peoplepopulationandcommunity/populationandmigration/populationestimates#timeseries>

92. Demography—Population—OECD Data. theOECD. [cited 2023 Jan 25].

<http://data.oecd.org/pop/population.htm>

93. Surveillance Atlas of Infectious Diseases [cited 2023 Jan 27].

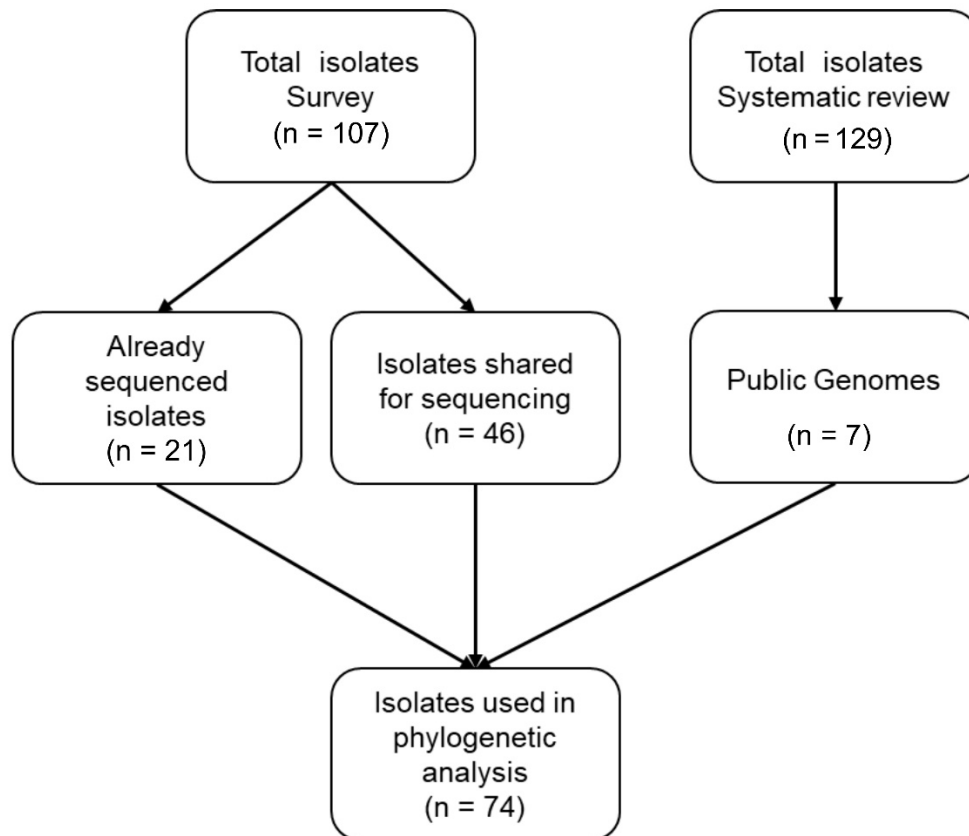
<https://www.ecdc.europa.eu/en/surveillance-atlas-infectious-diseases>

94. Willemse N, Howell KJ, Weinert LA, Heuvelink A, Pannekoek Y, Wagenaar JA, et al. An emerging zoonotic clone in the Netherlands provides clues to virulence and zoonotic potential of *Streptococcus suis*. *Sci Rep*. 2016;6:28984. [PubMed <https://doi.org/10.1038/srep28984>](https://doi.org/10.1038/srep28984)

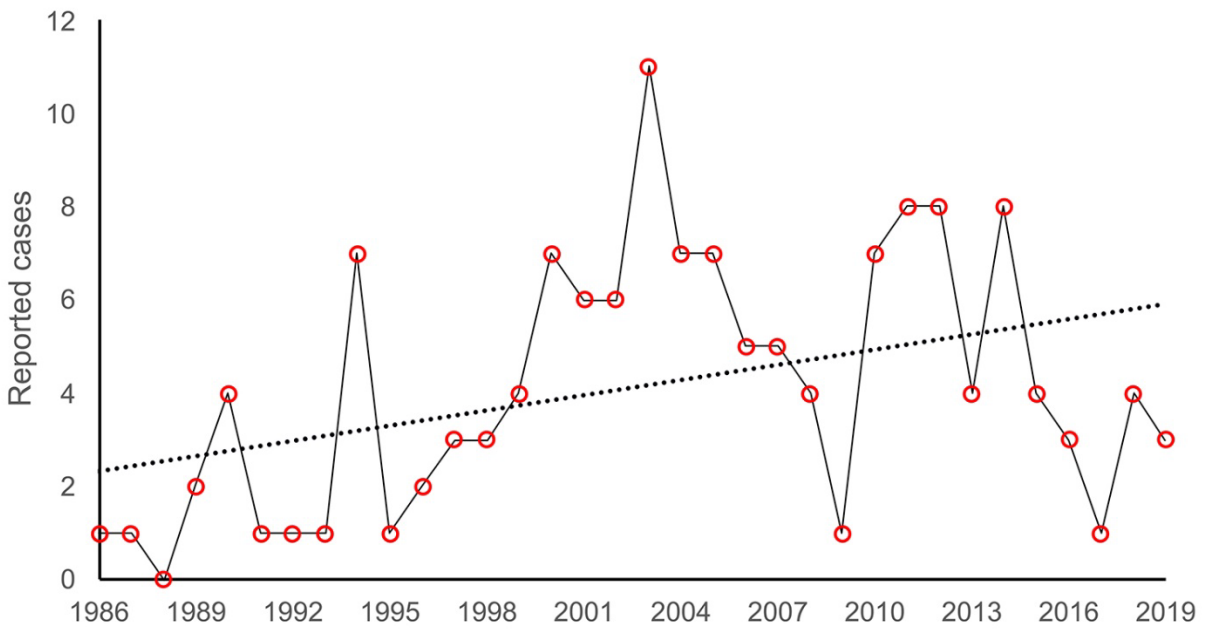
95. Fillo S, Mancini F, Anselmo A, Fortunato A, Rezza G, Lista F, et al. Draft Genome Sequence of *Streptococcus suis* Strain SsRC-1, a Human Isolate from a Fatal Case of Toxic Shock Syndrome. *Genome Announc*. 2018;6:6. [PubMed <https://doi.org/10.1128/genomeA.00447-18>](https://doi.org/10.1128/genomeA.00447-18)

96. Olearo F, Marinosci A, Stephan R, Cherkaoui A, Renzi G, Gaia N, et al. First case of *Streptococcus suis* infection in Switzerland: An emerging public health problem? *Travel Med Infect Dis*. 2020;36:101590. [PubMed <https://doi.org/10.1016/j.tmaid.2020.101590>](https://doi.org/10.1016/j.tmaid.2020.101590)

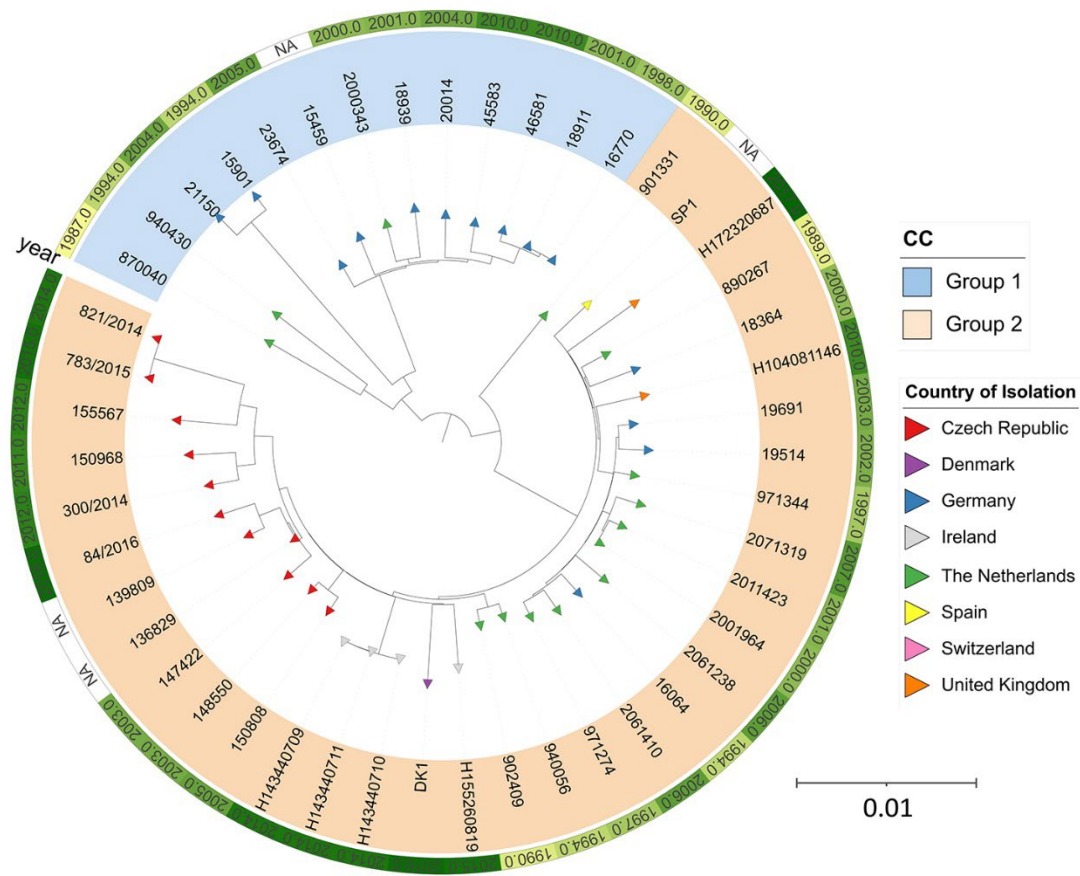
97. Werinder A, Aspán A, Söderlund R, Backhans A, Sjölund M, Guss B, et al. Whole-genome sequencing evaluation of MALDI-TOF MS as a species identification tool for *Streptococcus suis*. J Clin Microbiol. 2021;59:e0129721. PubMed <https://doi.org/10.1128/JCM.01297-21>
98. Chen C, Zhang W, Zheng H, Lan R, Wang H, Du P, et al. Minimum core genome sequence typing of bacterial pathogens: a unified approach for clinical and public health microbiology. J Clin Microbiol. 2013;51:2582–91. PubMed <https://doi.org/10.1128/JCM.00535-13>



Appendix Figure 1. Human *Streptococcus suis* isolates included in phylogenetic analysis.



Appendix Figure 2. Reported human *Streptococcus suis* infections by year in Europe, combining survey and systematic review data.



Appendix Figure 3. The 2 sublineages identified within zoonotic CC1 clade in Europe. Colored triangles indicate country of isolation. Colored ranges mark the 2 different groups: group 1 in blue and group 2 in orange. Outer ring indicates year of isolation; darker tones are associated with more recent and paler tones with more distant dates.