INTRODUCTION

Streptococcus suis is a Gram-positive bacterium with zoonotic potential, causing meningitis, septicaemia and arthritis (Wertheim, Nghia, Taylor, & Schultsz, 2009). Streptococcus suis is rarely found in healthy individuals, but is a commensal of pigs and is carried in the upper respiratory and the gastrointestinal tracts of up to 100% of pigs in the Netherlands. The S. suis serotype is determined by the antigenic properties of the polysaccharide capsule. Serotype 2 is responsible for infections in pigs and causes the majority of zoonotic infections (Goyette-Desjardins, Auger, Xu, Segura, & Gottschalk, 2014). In the Netherlands, zoonotic infections with S. suis are observed predominantly in persons who have been in close contact with pigs, such as hunters, butchers and farmers (van de Beek, Spanjaard, & Gans, 2008). Despite passive surveillance through the Netherlands Reference Laboratory of Bacterial Meningitis (NRLBM), underreporting of S. suis meningitis cases still occurs (van de Beek et al., 2008; Wertheim et al., 2009). Vaccines that protect against S. suis infection are not available for human use.

MATERIALS AND METHODS

The S. suis isolates were sequenced as described previously using paired-end MiSeq sequencing (Willemse et al., 2016). We performed MLST using (https://pubmlst.org/ssuis) and annotated the genomes using Prokka 1.9 (https://github.com/tseemann/prokka). Roary (Page et al., 2015) was used to calculate core and pan-genomes. For substitution rate calculation, SNPs were determined.
by mapping sequencing reads against the closely related reference genome of P1/7 using SMALT (https://sourceforge.net/projects/smalt), to include SNPs in intergenic regions, which are typically not included in a core genome, in the analysis. SNPs were extracted using Samtools (Li, 2011). Mappings were inspected using Artemis (https://www.sanger.ac.uk/science/tools/artemis). SNPs for phylogenetic analysis were extracted from the core genome alignment of Roary using SNP-sites (https://github.com/sanger-pathogens/snp-sites). Maximum likelihood trees were generated with RAxML 8.1.6 (https://github.com/stamatak/standard-RAxML) and run until convergence at the bootstopping criterion.

3 | RESULTS

Isolate 2071319 (ERS902349) was previously included in a genomic comparison of S. suis isolates from the Netherlands (Willemse et al., 2016) whilst isolate 2150651 (ERS1669548) was sequenced using identical methods. The 2071319 and 2150651 genomes were 2,048,581 and 2,036,490 nucleotides in length, respectively, and both belonged to ST1 and were serotype 2. GC contents were 41.17% and 41.23%, respectively. However, 2071319 contained 1988 coding sequences (CDS) whilst 2150651 contained 1970 CDS. The core genome of the two isolates comprised 1914 genes, leaving 31 genes in the accessory genome of which 24 genes belonged to 2071319 and seven genes to 2150651 (Table 1).

Part of the accessory genome of 2071319, spanning genes 937–948, encoded an integrase, replication initiator protein, transcriptional regulator, but mostly hypothetical proteins without predicted domains. The mean GC content of these contiguous genes was 30.6% and lower than the GC content of the whole genome. Among the remaining accessory genes is the sadP gene, encoding the Streptococcal Adhesion Protein (SadP), which had less than the 95% amino acid identity (84.8%) limit as set by Roary due to the presence of two fewer repeats. Mac family proteins also showed <95% identity due to different number of repeats, and the IS110 family transposases showed overlap, but had much lower protein identity. Other proteins did not show similarities.

Mapping of sequencing reads against the closely related reference genome of strain P1/7 yielded 74 SNPs and 24 indels in 2071319 and 100 SNPs and 15 indels in 2150651. There were 35 SNPs and seven indels shared between 2071319 and 2150651 against P1/7 resulting in 104 SNPs and 25 indels between 2071319 and 2150651. We did not identify regions of high SNP density, indicating these SNPs should be attributed to mutations instead of recombination. Using the SNPs, we estimated a required substitution rate of 6.36·10^{-6} substitutions per site per year (104 SNPs divided by the average genome size of 2071319 and 2150651, divided by 8 years), which is almost tenfold higher compared to the recently calculated substitution rate of 8.58 × 10^{-7} for related ST7 isolates in China (Du et al., 2017). It is also higher than the rates calculated for S. pneumoniae (1.57·10^{-5}) and S. aureus (3.3·10^{-6}) strains (Croucher et al., 2011).

Impacts

- We report the first genomic comparison between consecutive Streptococcus suis strains, isolated from a Dutch butcher with recurring meningitis.
- We conclude this reinfection was the result of similar yet unrelated strains based on genomic analyses.
- Professionals in continuous close contact with pigs should be vigilant of reinfection even after previous S. suis related illness as natural infection may not provide protection against future infections.

We compared isolates 2071319 and 2150651 with all complete genomes of serotype 2 isolates, belonging to MLST clonal complex 1 (CC1), and included available draft genomes from the Netherlands (Figure 1, Supporting Information Table S1). Single Locus Variants (SLV) of ST1 were included in this analysis because single SNPs in the MLST housekeeping genes may not be representative of variation across the genome. Four main clusters could be observed. Outliers in this tree are GZ1 as well as A7 and SC070731 (both ST7 isolates) with long diverging branches (Supporting Information Figure S1). The cluster consisting of 16 isolates including the isolates 2071319 and 2150651 resulted in a core genome of 1886 genes with an accessory genome of 129 genes, which was slightly smaller than the shared core genome between isolates 2071319 and 2150651. A maximum likelihood tree was again generated to create the highest resolution among the closest related isolates (Supporting Information Figure S2). Whilst isolates 2071319 and 2150651 clustered on the same branch, they cluster amongst other CC1 isolates from the Netherlands suggesting these two isolates are not more related to each other than to the other isolates. Using hierarchical clustering with the presence–absence matrix of the pangenome in R, we compared the accessory genome content of these 16 isolates (Supporting Information Figure S3). Isolates 2071319 and 2150651 clustered among other CC1 isolates from the Netherlands, but two main clusters of the accessory genome were separated in the dendrogram due to the presence or absence of the previously mentioned insertion in 2071319, indicative of co-evolution of two CC1 subclones circulating in the Netherlands.

4 | DISCUSSION

Our results suggest that it is unlikely that 2150651 was a descendant from 2071319 and indicate reinfection by two unrelated isolates belonging to different ST1 subclones. The isolates differed by an inserted region, with genes which were likely inserted as a whole, but the origin of this inserted sequence is not well understood. The isolates also had different SadP genes, previously characterized as an adhesin as well as a factor H binding protein which may contribute
to zoonotic potential (Ferrando et al., 2017) and is considered a putative virulence factor of S. suis (Kouki et al., 2011; Pian et al., 2012). Current evidence suggests that carriage of S. suis by humans in general is very rare (Nghia et al., 2011). Whilst potential carriage of S. suis due to continuous professional exposure to pigs (Bonifait, Veillette, Letourneau, Grenier, & Duchaine, 2014), combined with skin lesions related to his chronic dermatitis, cannot be ruled out in this patient, the genomic analysis does not suggest that infections occurred due to long-term carriage of a single strain as the estimated substitution rate would be too high.

Reinfection with encapsulated bacteria, such as Neisseria meningitidis and Streptococcus pneumoniae, has been associated with host complement and immunoglobulins deficiencies (Lewis & Ram, 2014). Studies in C3- and C5R-deficient mice indicated an increased susceptibility to S. suis resulting in severe infection in an intranasal mouse model (Seitz et al., 2014). A case of recurrent S. suis infections was reported in a patient after splenectomy (Francois, Gissot, Ploy, & Vignon, 1998). Whilst the patient did not have a medical history suggesting immunodeficiency, he did not consent to additional investigations to confirm or reject S. suis carriage or immunodeficiency, after his recovery.

In conclusion, we identified a patient with a S. suis reinfection on the basis of whole genome sequence analysis.

### Table 1

| Isolate   | Draft genome gene location | Predicted protein function | Protein length | Nearest BLASTP reference protein |
|-----------|---------------------------|---------------------------|----------------|----------------------------------|
| 2071319   | 220                       | Mac family protein        | 1,084          | WP_012775646.1                  |
|           | 380                       | Competence/damage-inducible protein A | 272   | WP_012774894.1                  |
|           | 753                       | IS110 family transposase  | 242            | CYX90486.1                      |
|           | 760                       | Minor spike protein H     | 202            | EQJ03522.1                      |
|           | 891                       | Serine protease           | 712            | WP_053866547.1                  |
|           | 937                       | Site-specific integrase   | 436            | WP_011922382.1                  |
|           | 938                       | Hypothetical protein      | 76             | WP_011922383.1                  |
|           | 939                       | Replication initiator protein | 412   | WP_014636592.1                  |
|           | 940                       | Hypothetical protein      | 174            | WP_011922385.1                  |
|           | 941                       | Hypothetical protein      | 101            | WP_012775097.1                  |
|           | 942                       | Hypothetical protein      | 433            | WP_012775098.1                  |
|           | 943                       | Transcriptional regulator | 68             | WP_012775099.1                  |
|           | 944                       | Membrane/hypothetical protein | 325   | WP_011922387.1                  |
|           | 945                       | Hypothetical protein      | 279            | WP_011922388.1                  |
|           | 946                       | Hypothetical protein      | 134            | WP_012028114.1                  |
|           | 947                       | Hypothetical protein      | 340            | WP_012775100.1                  |
|           | 948                       | Hypothetical protein      | 538            | WP_011922392.1                  |
|           | 1,018                     | Hypothetical protein      | 108            | WP_012775144.1                  |
|           | 1,052                     | Peptidase C26             | 67             | CYV04131.1                      |
|           | 1,083                     | Penicillinase repressor (89% ID) | 99    | WP_011921706.1                  |
|           | 1,213                     | Cell surface protein (SadP) | 902   | WP_074392131.1                  |
|           | 1,223                     | ABC transporter ATP-binding protein | 275   | WP_074411925.1                  |
|           | 1,362                     | N-acetylmuramoyl-l-alanine amidase | 373   | WP_074415670.1                  |
| 1578      | RNA helicase              |                           | 467            | WP_041179122.1                  |
| 2150651   | 220                       | IgM protease (Mac family protein) | 1,141 | WP_011922092.1                  |
|           | 754                       | Minor spike protein       | 328            | WP_000466547.1                  |
|           | 908                       | Hypothetical protein      | 100            | WP_012775088.1                  |
|           | 1,067                     | Transcriptional regulator | 156            | WP_011921706.1                  |
|           | 1,197                     | Cell surface protein (SadP) | 765   | WP_012775427.1                  |
|           | 1,240                     | Oxidoreductase            | 66             | WP_044764778.1                  |
|           | 1896                      | IS110 family transposase  | 276            | WP_061843547.1                  |
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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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