

### Introduction

Over the past decades, substantial diversity has been revealed among bacteria previously identified as *Streptococcus suis*. Distinct *Streptococcus* species have emerged both among the original 35 *S. suis* serotypes and among strains with newly identified capsular loci. The host specificity of some of these strains is not limited to pigs, as they are frequently isolated from other species, both healthy and diseased.

Here, we present an analysis of five bovine isolates collected between 2018 and 2020, originally identified as *S. suis*.

### Materials and Methods

The isolates were obtained from four cow mastitic milk samples and one lung sample from a ten-day-old calf suffering from bronchopneumonia. Each isolate originated from a different farm. All isolates were identified as *S. suis* using MALDI-TOF MS. Genomic DNA was extracted and subjected to Illumina whole genome sequencing. Genomes were annotated using Prokka. Subsequently, a phylogenetic tree based on core genome alignment was constructed (using neighbour joining method in Jalview software). The genome was searched for antimicrobial resistance genes (using the Resfinder database) and virulence factor genes (VFDB database). Average nucleotide identity (ANI) of the isolates were determined using fastANI 1.33 software.

### Results and discussion

Four of the isolates tested negative for the *recN* gene. One isolate repeatedly tested positive for *recN* by PCR, although the gene was not detected in the genome sequence data. Analysis of the *cps* locus revealed that each isolate harbored a unique composition of *cps* genes. Based on core genome alignment, all bovine isolates formed a distinct cluster closely related to *S. parasuis* serotypes 20, 22, and 26 (Figure 1). These isolates may therefore represent a novel species within the *S. suis* complex. Although the phylogenetic tree indicated a separate cluster of bovine isolates (Figure 1), the ANI results show a high similarity of bovine strains to reference *S. parasuis* strains (Table 1). Also when showing the differences in the presence of core and accessory genes in Figure 2, their great similarity is obvious, in comparison to other species. Only ant(6)-Ia and tet(M) resistance genes were detected in these bovine isolates (Table 2) and no virulence factor gene from VFDB database was identified. The identification and characterization of these isolates may open the way for improved discrimination of potential bovine pathogens.

Figure 1. Phylogenetic tree based on core genes alignment of all *S. suis* serotypes and close view to *S. parasuis* strains

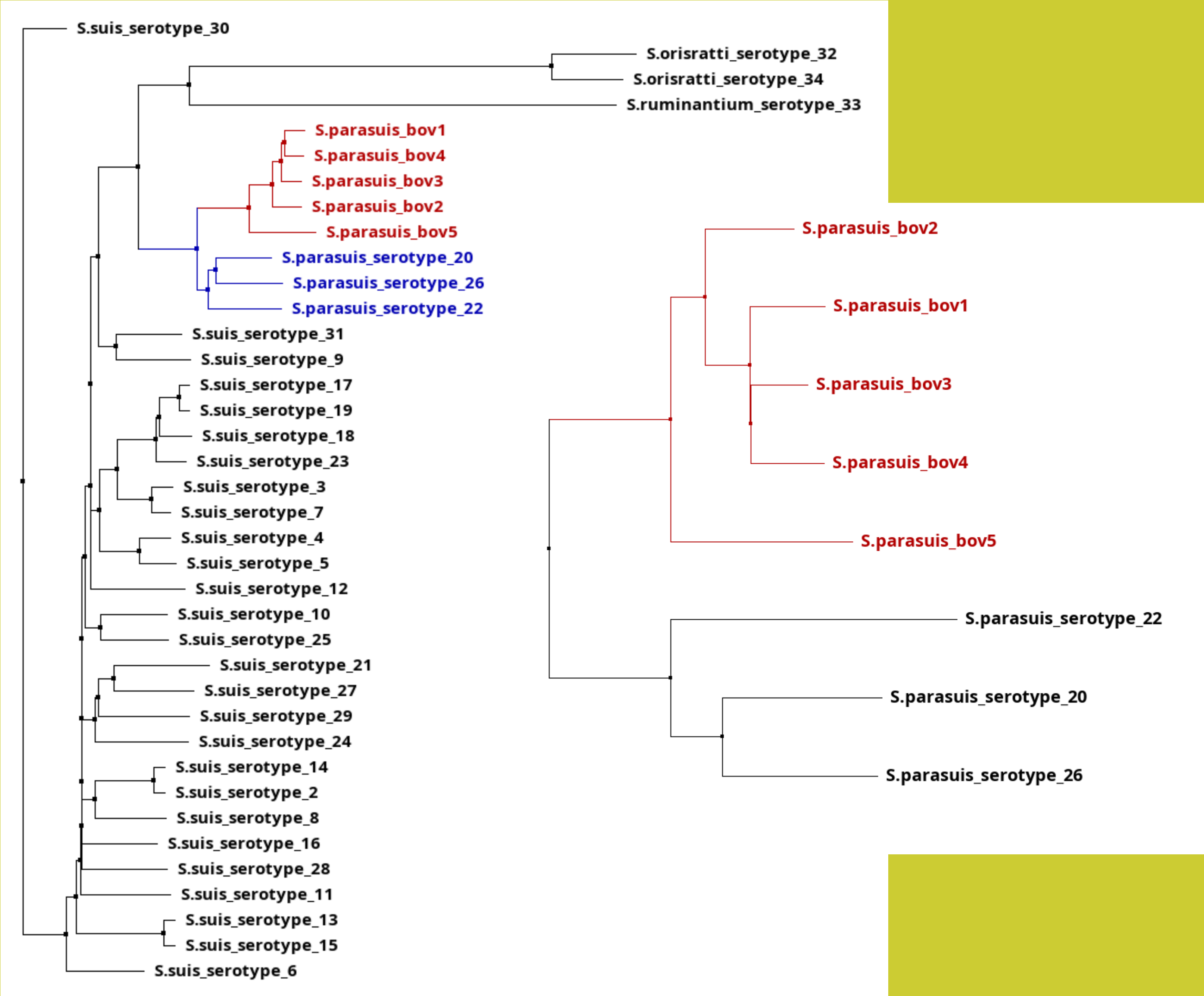


Figure 2. Schematic representation of genes presence between isolates

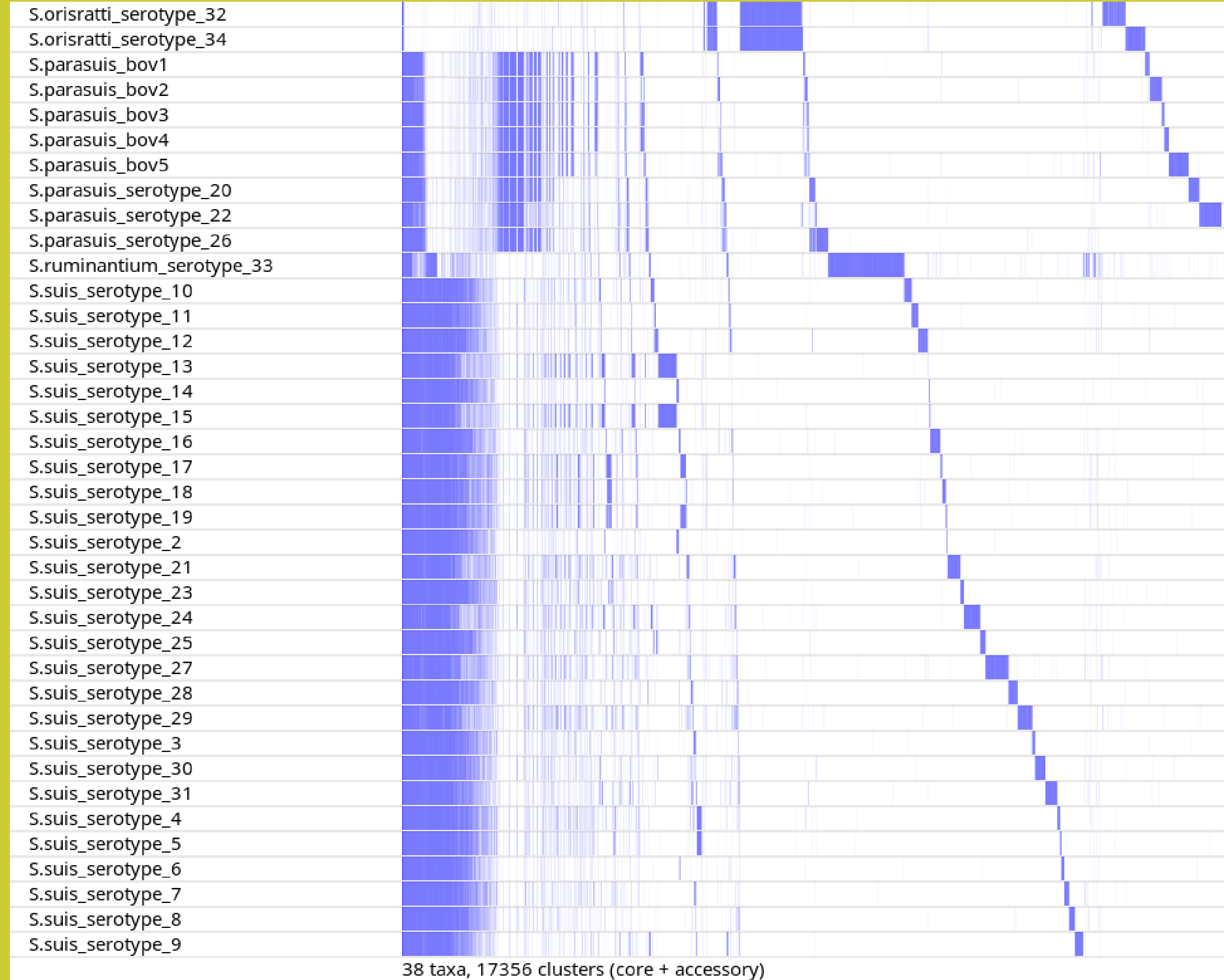


Table 1. Average nucleotide identity of the isolates (comparison of bovine *S. parasuis* strains with reference ones is highlighted in blue)

|                           | S. parasuis serotype 20 | S. parasuis serotype 22 | S. parasuis serotype 26 | S. parasuis bov1 | S. parasuis bov2 | S. parasuis bov3 | S. parasuis bov4 | S. parasuis bov5 | S. ruminantium serotype33 |
|---------------------------|-------------------------|-------------------------|-------------------------|------------------|------------------|------------------|------------------|------------------|---------------------------|
| S. parasuis serotype 20   | 100 %                   | 95.29%                  | 97.55%                  | 95.46%           | 95.26%           | 95.39%           | 95.35%           | 94.79%           | 79.81%                    |
| S. parasuis serotype 22   | 95.29%                  | 100 %                   | 95.46%                  | 94.44%           | 94.03%           | 94.42%           | 94.39%           | 94.04%           | 79.29%                    |
| S. parasuis serotype 26   | 97.60%                  | 95.41%                  | 100 %                   | 95.32%           | 95.19%           | 95.60%           | 95.46%           | 94.83%           | 79.52%                    |
| S. parasuis bov1          | 95.40%                  | 94.50%                  | 95.45%                  | 100 %            | 98.42%           | 99.14%           | 99.00%           | 97.48%           | 79.73%                    |
| S. parasuis bov2          | 95.39%                  | 94.16%                  | 95.24%                  | 98.30%           | 100 %            | 98.37%           | 98.35%           | 97.45%           | 79.62%                    |
| S. parasuis bov3          | 95.47%                  | 94.31%                  | 95.55%                  | 99.21%           | 98.36%           | 100 %            | 99.30%           | 97.54%           | 79.42%                    |
| S. parasuis bov4          | 95.40%                  | 94.25%                  | 95.60%                  | 99.05%           | 98.42%           | 99.23%           | 100 %            | 97.65%           | 79.54%                    |
| S. parasuis bov5          | 97.51%                  | 94.14%                  | 94.89%                  | 97.51%           | 97.31%           | 97.61%           | 97.56%           | 100 %            | 79.55%                    |
| S. ruminantium serotype33 | 79.48%                  | 79.51%                  | 79.76%                  | 79.61%           | 79.67%           | 79.63%           | 79.72%           | 79.66%           | 100 %                     |

Table 2. Antimicrobial resistance genes in bovine and reference *S. parasuis* strains

|                         | Gene        | Coverage | Identity | Product   | Resistance  |
|-------------------------|-------------|----------|----------|-----------|---|
| S. parasuis serotype 20 | ant(6)-Ia_3 | 100      | 100      | ant(6)-Ia | Streptomycin  |
|                         | erm(B)_18   | 100      | 99       | erm(B)    | Erythromycin, Lincomycin, Clindamycin, Quinupristin, Pristinamycin, Virginiamycin |
|                         | tet(O)_3    | 100      | 100      | tet(O)    | Doxycycline, Tetracycline, Minocycline  |
| S. parasuis serotype 22 | ant(6)-Ia_3 | 100      | 100      | ant(6)-Ia | Streptomycin  |
|                         | erm(B)_18   | 100      | 99       | erm(B)    | Erythromycin, Lincomycin, Clindamycin, Quinupristin, Pristinamycin, Virginiamycin |
|                         | tet(O)_3    | 100      | 100      | tet(O)    | Doxycycline, Tetracycline, Minocycline  |
| S. parasuis serotype 26 | ant(6)-Ia_3 | 100      | 100      | ant(6)-Ia | Streptomycin  |
|                         | erm(B)_18   | 100      | 99       | erm(B)    | Erythromycin, Lincomycin, Clindamycin, Quinupristin, Pristinamycin, Virginiamycin |
|                         | lnu(C)_1    | 100      | 99       | lnu(C)    | Lincomycin  |
| S. parasuis bov1        | ant(6)-Ia_2 | 100      | 100      | ant(6)-Ia | Streptomycin  |
| S. parasuis bov2        | ant(6)-Ia_2 | 100      | 100      | ant(6)-Ia | Streptomycin  |
| S. parasuis bov3        | -           |          |          |           |   |
| S. parasuis bov4        | -           |          |          |           |   |
| S. parasuis bov5        | tet(M)_12   | 100      | 100      | tet(M)    | Doxycycline, Tetracycline, Minocycline  |

**Acknowledgements:** The study was supported by the Ministry of Agriculture of the Czech Republic (Projects No. MZE-RO0523 and No. TN02000017).