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Diversity of serotypes and new *cps* loci variants among *Streptococcus suis* isolates from pigs in Poland and Belarus

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ABSTRACT

Streptococcus suis plays an important role in infections in pigs but information about the epidemiology of this pathogen in Poland and Belarus remains scarce. Ninety-six isolates from brain and lungs were studied by PCR-based serotyping, analysis of virulence-associated determinants and multilocus sequence typing (MLST). Selected six isolates were further analyzed by genomic sequencing and transmission electron microscopy (TEM). Serotype 2 was most prevalent, followed by serotypes 3, 4, 8 and 7. All isolates carried *fbpS*; 30, 74 and 79 isolates were positive for *epf, mrp* and *sao*, respectively. MLST revealed that while widely distributed clonal complexes, such as 1, 16, 25 and 28 circulate in both countries, a significant part of the population is composed of novel singletons. Six isolates, all positive for the capsule in TEM, harbored *cps* loci differing to a various degree from these previously described, including one with a novel *cps* locus (putative NCL21). In conclusion, our study provides first molecular data on *S. suis* from pigs in the Central/Eastern Europe and contributes to a better characterization of diversity of loci responsible for capsule production in this pathogen.

1. Introduction

Streptococcus suis is considered one of the major etiologic agents of infections in swine industry, responsible for considerable economic loses in pig production in many countries. Similarly to several other bacterial pathogens, *S. suis* produces a polysaccharide capsule, which protects the pathogen from host phagocytes (Segura et al., 2004). Serotype 2 (SS2) is the most prevalent in *S. suis* invasive infections of both humans and pigs, but also other serotypes, such as 9, 3, 1/2, 1, 7, 14, 8 and 4 are isolated from diseased pigs (Goyette-Desjardins et al., 2014). Capsular polysaccharide (CPS) of all known serotypes appears to be synthesized by the Wzx/Wzy-dependent pathway, determined by genes of the *cps* locus, typically located downstream *orfX* (Okura et al., 2013; Pan et al., 2015; Zheng et al., 2015; Qiu et al., 2016). Genetic methods, more and more widely used for serotyping typically target the *wzy* gene, considered to be specific for a given serotype (Okura et al., 2013). Isolates, non-typable with serological or genetic tests have been reported and recently 20 novel *cps* loci (NCLs) have been identified (Zheng et al., 2015; Qiu et al., 2016; Zheng et al., 2017) as well as a new serotype Chz (Pan et al., 2015).

Multi-locus sequence typing (MLST) provides an unambiguous identification of *S. suis* isolates and allows comparison of isolates from various countries (King et al., 2002). A given serotype of *S. suis* may be associated with several, even unrelated sequence types (STs) (Goyette-Desjardins et al., 2014). Apart from the capsule, *S. suis* produces a wide variety of factors, potentially associated with virulence, such as surface-located proteins Sao (surface antigen protein), OSF (serum opacity factor) and FbpS (fibronectin binding protein), as well as MRP (muramidase released protein), EF (extracellular protein factor), SLY (suilysin), pili and others (Gottschalk et al., 2010). The distribution of these factors among clones of *S. suis* is variable (Goyette-Desjardins et al., 2014) and divergent variants of these factors are observed (Feng et al., 2007; Fittipaldi et al., 2009).

Poland is an important producer of pork in Europe, moreover, human invasive infections, caused by *S. suis* have currently been observed in our country (Bojarska et al., 2016). The swine industry plays also important role in the neighboring country, Belarus. The aim of our study was to provide a detailed characterization of serotypes, virulence-

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associated factors, and clonal relationships of *S. suis* from diseased pigs in Poland and Belarus, with the focus on isolates that might represent new serotypes.

2. Material and methods

2.1. Isolate collection and DNA isolation

The study included 96 consecutive isolates of *S. suis* collected by the National Veterinary Research Institute (NVRI) from diseased pigs in 57 farms in Poland (61 isolates), located in 14 of 16 Polish provinces and in 24 farms in Belarus (35 isolates) over the period of 2003-2012. Forty-two and 54 isolates were obtained from brain and from lungs, respectively. Total DNA was purified using the Genomic DNA Prep Plus kit (A &A Biotechnology, Gdynia, Poland) with an additional initial step of mutanolysin (Sigma-Aldrich, Saint Louis, MO) digestion of bacteria. All isolates were tested by PCR for the presence of *recN*, specific for *S. suis* (Ishida et al., 2014).

2.2. Bacterial typing and sequence analysis

Serotypes were determined using the multiplex PCR scheme (Liu et al., 2013); isolates negative in this test were further studied by PCR for the presence of *wzy* characteristic for the Chz and the NCL1-NCL8 capsular loci (Pan et al., 2015; Zheng et al., 2015), whose sequences were available at the time of analysis. Serotypes 2 and 1/2, and serotypes 1 and 14 were distinguished by sequencing of the *cpsK* gene (Athey et al., 2016). MLST was performed as described (King et al., 2002); allele numbers and STs were obtained using the MLST database http://ssuis.mlst.net/ (26th September 2017, date last accessed). Sequence analysis was performed in the Lasergene package (DNASTAR, Madison, WI). MLST data were analysed using the eBURST software available at http://eburst.mlst.net/ (26th September 2017, date last accessed).

2.3. Virulence-associated phenotypes and detection of genes encoding potential virulence factors

The DNase test was performed with using DNase Test Agar with Methyl Green (Becton Dickinson, Franklin Lakes, NJ) with the *S. aureus* ATCC 29213 strain as a positive control. Haemolytic assay was performed on Columbia agar with 5 % horse blood (BioMerieux, Marcy l'Etoile, France). The *sly*, *mrp*, *epf*, *sao* and *fbpS* genes were detected and analysed as described (King et al., 2001; Feng et al., 2007; Zhu et al., 2008; Fittipaldi et al., 2009) with DNA from *S. suis* human isolates (Bojarska et al., 2016) as positive controls.

2.4. Genomic analyses

Genomic sequencing was performed with MiSeq (Illumina, San Diego, CA) as an external service (GENOMED, Warsaw, Poland) with the coverage of at least 50 × . Runs were assembled using the CLC software v9.0.1 (QIAGEN, Aarhus, Denmark). The 16S rRNA genes were compared to the 16S rRNA gene from the P1/7 strain (AM946016.1) by the EMBOSS Needle aligner (https://www.ebi.ac.uk/Tools/psa/emboss_needle; 25th October 2018, date last accessed). The ribosomal MLST (rMLST) database (https://pubmlst.org/rmlst/; 26th October 2018, date last accessed) was used for an additional species confirmation. Contigs encompassing the *cps* locus were identified using BLAST algorithm in CLC with the *cpsABCD* sequences from the P1/7 genome (AM946016) and sequences of the genes located downstream the *cps* locus (Okura et al., 2013) as queries. Gaps between contigs were bridged with PCR and Sanger sequencing. ORFs and their predicted functions were identified using Prokka (Seemann, 2014) and further analysed by comparison with known *cps* loci using BLAST-N at GenBank. The putative *wzy* gene was determined using TMHMM v2.0 (http://www.cbs.dtu.dk/services/TMHMM/). Wzy sequences of serotypes 1-34, Chz and NCL1-16 (Okura et al., 2013; Pan et al., 2015; Zheng et al., 2015; Qiu et al., 2016) were downloaded from GenBank and compared with sequences obtained in the study using MAFFT (Katoh et al., 2017) and Archaeopteryx.js for visualization of phylogenetic trees obtained by the NJ method (https://mafft.cbrc.jp/alignment/server/; 25th October 2018 date last accessed). The pairwise sequence comparisons of Wzy was performed with the EMBOSS Needle. The Artemis Comparison Tool (ACT) was used for comparisons of *cps* loci (Carver et al., 2005).

2.5. Capsule detection by transmission electron microscopy (TEM)

Isolates were grown overnight in BHI medium, embedded in agarose plugs and immediately fixed in 2 % paraformaldehyde and 2 % glutaraldehyde in 0.05 M sodium cacodylate buffer (pH 7.2; Merck, Darmstadt, Germany) (Karnovsky, 1965) for 2 h and then placed in 1 mg/ml ruthenium red for 3 h. After washing three times in cacodylate buffer, samples were post-fixed in 2 % osmium tetroxide in 0.05 M cacodylate buffer for 2 h at 4 °C and dehydrated in a graded series of acetone concentrations and in propylene oxide for 30 min, and embedded in epoxy resin (Epon 812, Merck). Ultrathin (70 − 80 nm) sections of the polymerized samples were taken on a UCT ultramicrotome (Leica Microsystems, Wetzlar, Germany) and mounted on formvar-coated slot copper grids. Sections were stained with 1.2 % uranyl acetate and 2.5 % lead citrate and examined with a 268D Morgagni transmission electron microscope (FEI, Hillsboro, OR) operating at 80 kV. The images were taken with a Morada digital camera (Olympus SIS, Münster, Germany) at 10 M pix resolution. The capsule thickness between the inner and the external edge of the capsular layer was measured by using the iTEM (SIS) computer program (version 5.0.1131, FEI). Each of the values was based on 20 measurements per experiment and each experiment was done three times independently. The NCTC 10234 strain of *S. suis* was used as a positive control of capsule presence.

2.6. Nucleotide sequence accession numbers

New sequences of *mrp* and *cps* loci were submitted to GenBank (MF171190 and KX785319, KX785320, MH763816, MH763818-MH763829, respectively).

3. Results

3.1. Serotyping, and other virulence-associated phenotypes and genotypes

Multiplex PCR and the *cpsK* gene sequencing allowed to detect 15 different serotypes and three known NCLs among 90 isolates (Table 1); two isolates were negative and four yielded ambiguous results. Serotype 2 was most prevalent (21 isolates). Other serotypes included 3, 4, 8 and 7 (16, 11, 7 and 6 isolates, respectively), followed by (in decreasing prevalence) 1/2, 31, 5, 9, 12, 13, 18, 1, 21 and 24. NCL3, NCL5 and NCL6 were specific for one, three and one isolate, respectively. Among 36 isolates positive in the DNase activity assay, 26 isolates demonstrated a complete clearing of the test medium and 10 isolates showed a weak reaction. Forty-six isolates showed various levels of hemolysis of horse blood; all these isolates carried the *sly* gene, present also in four non-hemolytic isolates. All isolates carried *fbpS* and 30 isolates were positive for *epf*. The *mrp* gene, present in 74 isolates revealed five *mrp* variants, with the most frequent mrp^{N} detected in 37 isolates, followed by *mrp**, mrp^{S}, and *mrp****, specific for 17, 14 and 4 isolates, respectively. Two isolates had a new *mrp* variant (*mrp*-Δ), which harbored a 90-bp internal deletion. Seventy-nine isolates contained the *sao* gene, including the *sao*-L, *sao*-M and *sao*-S variants, characteristic for 37, 28 and three isolates, respectively. Sequencing of the atypical length amplification products of the remaining seven

Table 1
STs, serotypes and virulence-associated factors among 96 *S. suis* pig isolates from Poland and Belarus.

CC (n;N)[a] ST (n)[a]	Country (n)[a]	Isolation site (n)[a]	Serotype (n)[a]	DNase (n)	HL (n)	*sly* (n)	*epf* (n)	*mrp* variant (n)[a]	*sao* variant (n)[a]
1 (14;14) 1 (13), 3	PL (13); BY	brain (12); lung (2)	1, 2 (13)	11	14	14	14	mrp^N (14)	*sao*-M (14)
16 (24;20) 15 (2), 16, 17 (10), 87 (4), 644, 645, 646, 648 (2), 700	PL (11); BY (13)	brain (6); lung (18)	3 (6), 4 (9), 5, 8 (6), 9, NCL5	10	17	24	12	*mrp** (8), *mrp**** (2), mrp^s (10), mrp^N (3)	*sao*-L (10), *sao*-M (7), *sao*-6, *sao*-8 (4), *sao*-10
25 (8;8) 29 (7), 642	PL (6); BY (2)	brain (3); lung (6)	5 (2), 7 (6)	3	0	0	4	*mrp**, *mrp**** (2), mrp^N (5)	*sao*-L (8)
28 (13;13) 27 (4), 28 (8), 640	PL (7); BY (6)	brain (2); lung (11)	1/2 (4), 2 (5), 3 (4)	11	0	0	6	*mrp** (4), mrp^N (9)	*sao*-L (9), *sao*-S, *sao*-6
94 (5;5) 94 (4), 108	PL (5)	brain (3); lung (2)	2, 3 (4)	0	5	5	0	mrp^N (5)	*sao*-L (3), *sao*-S (2)
30 singletons (32;30)									
54	PL	brain	4	0	0	1	0	*mrp**	*sao*-M
639	BY	lung	3	1	1	1	0	mrp^s	*sao*-L
641	BY	lung	3	1	1	1	0	mrp^s	*sao*-8
643 (2;2)	BY (2)	lung (2)	2 (2)	1	0	2	1	0	*sao*-L, *sao*-6
647	PL	brain	5-like	0	0	0	0	0	0
649	BY	lung	8	0	0	0	0	0	0
650	PL	brain	9	0	0	0	1	0	0
651	PL	brain	15-like	0	1	1	0	*mrp*_N	*sao*_L
652	PL	brain	12	0	0	0	1	*mrp*_S	*sao*_M
653	BY	lung	12	0	0	0	1	0	*sao*_M
654	PL	brain	18	0	0	0	1	0	*sao*_M
655	BY	lung	18	0	0	0	1	*mrp**	*sao*_L
656	PL	brain	31	0	0	0	1	*mrp*_N	0
657	PL	brain	31	0	0	0	0	0	0
688	PL	brain	31	0	0	0	0	0	0
689	PL	brain	NCL5	0	0	0	1	0	0
690	PL	brain	NCL5	0	0	0	0	0	0
691	PL	brain	31	0	0	0	0	0	0
692	PL	brain	17	0	0	0	1	0	0
693	PL	brain	NCL6	0	0	0	0	0	*sao*_M
694	PL	brain	30-like	0	0	0	1	0	*sao*_M
695	PL	brain	15-like	0	1	1	0	*mrp**	*sao*_M
696	BY	lung	9	0	0	0	0	*mrp*_S	*sao*-8
697	BY	lung	21	0	0	0	0	0	*sao*-6
698	BY	lung	12	0	0	0	1	0	*sao*_L
699	PL	lung	24	0	0	0	1	0	0
701	PL	lung	NCL21	1	0	0	0	0	0
702 (2;2)	PL, BY	lung (2)	13 (2)	1	0	0	2	0	0
703	BY	lung	NCL3	0	0	0	0	0	*sao*_L
704	BY	lung	NCL16-like	0	0	0	1	0	0

(n), number of isolates; (n) [a] number of isolates, if different from one given in brackets; N, number of farms (if different from one); PL, Poland; BY, Belarus; CC, clonal complex; S, singleton; *nd*, not determined; *mrp*-Δ, a *mrp* variant with a 90 nt deletion; *sao-10, sao-8* and *sao-6,* variants with 10, 8 and 6 repeats, respectively. HL, hemolysis; STs of isolates with the *cps* locus analyzed by genomic sequencing due to problematic results in PCR-based serotyping underlined; NCL21 proposed in this study.

isolates yielded variants of *sao* gene with ten, eight and six repeated regions (*sao-10, sao-8, sao-6*).

3.2. MLST analysis

MLST yielded 48 different STs (Table 1), with 36 new ones (639-657, 688-704). The eBURST analysis of 833 STs available in the MLST database (as of the 23rd April 2017) included 18 STs of 64 isolates from the current study into five clonal complexes (CCs) 1, 16, 28, 29 and 94 (Supplementary Fig. 1); representatives of these CCs typically showed a wide distribution in both Poland and Belarus, originating from several farms (Table 1). Among 30 remaining STs, characteristic for 32 isolates, the majority were singletons, two STs formed a single-locus variant (SLV) link and one ST represented an SLV of another ST from the MLST database (Supplementary Fig. 1); to simplify presentation, later in the text all these 30 STs are called “singletons”. Isolates representing CC1, derived chiefly from brain, almost exclusively had serotype 2, uniformly showed hemolysis and the presence of *sly, epf,* mrp^N and *sao*-M, and in majority produced DNase. Isolates belonging to CC16, the most numerous CC in the study, showed a remarkable diversity of STs, serotypes, *mrp*- and *sao*-types, and variable presence of DNase and hemolytic activities. CC16 isolates were obtained mostly from lungs, similarly to two related CCs, 25 and 28. Highly diverse group of singletons originated from both brain and lungs.

3.3. Genomic analyses of cps loci and capsule detection with TEM

The *cps* loci of six isolates from the singleton group (Table 1), problematic in PCR-based serotyping were analyzed in detail by genomic sequencing. The 16S rRNA genes of these isolates showed 99.2–99.9 % identity to the 16S rRNA gene from the P1/7 strain and rMLST identified all six isolates as *S. suis* (96 % identification for the ST704 isolate and 100 % identification for the remaining five isolates). All six isolates harbored complete *cps* loci located downstream *orfX*, with three different patterns of flanking regions, I-a, I-b and a new one, I-c (Fig. 1). The comparison of identified *cps* loci against nucleotide sequences at GenBank using BLAST-N revealed no complete matches for any of the studied isolates. The *cps* locus of the ST647 isolate (19.3 kb) was most similar to the *cps* characteristic for serotype 5 in the 11538 strain (BR001003.1; Okura et al., 2013), however it lacked distal *cps5RST* genes. The adjacent chromosomal genes downstream this locus represented the pattern I-b instead of I-a, suggesting a deletion event. This

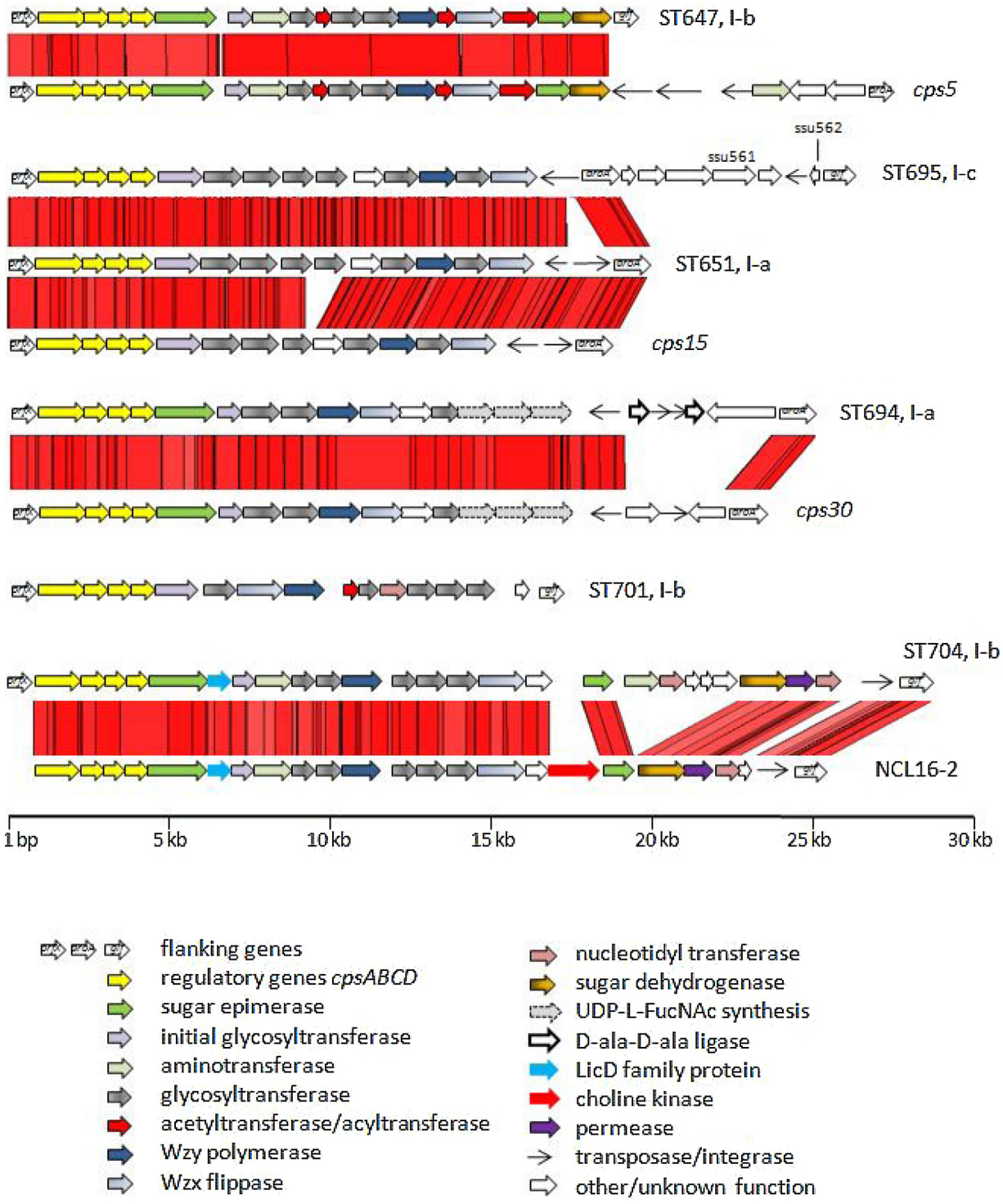

Fig. 1. Comparison of *cps* loci, analysed in this study, with their closest counterparts, including serotype 5, 15, 30 and NCL16-2 loci. STs of isolates and patterns of their genomic localization provided on the right; genes represented as arrows, with functions of gene products described in the legend; areas of homology in red. An approximate scale in 5-kb increments provided at the bottom.

locus demonstrated also an extensive similarity to the *cps* of the 1218846 strain (KX870061; Zheng et al., 2017) but the latter one harbored a different variant of the *cpsQ* gene (93 % identity) of a sugar dehydrogenase. The *cps* loci of ST651 and ST695 isolates (18.9 kb and 17.9 kb, respectively) shared the general structure but differed by the presence of ISs and the genetic neighborhood, with ST695 isolate representing the new pattern I-c, with two additional genes between the counterparts of SSU0561 and SSU0562. These two *cps* loci were most similar to the serotype 15 locus in the NCTC 10446 strain (AB737823.1; Okura et al., 2013), but they carried an additional glycosyltransferase (a putative alpha-1,2-fucosyltransferase) gene, without any hits in the nr/nt database of GenBank. In the ST694 isolate, the *cps* locus (24.2 kb) showed an extensive similarity to the *cps* of serotype 30 in the 92-1400 strain (AB737834.1; Okura et al., 2013) but lacked *cps30 P,* encoding a putative polyprenyl synthetase and *cps30Q* (a hypothetical protein gene). Instead, this locus harbored five additional genes, including two

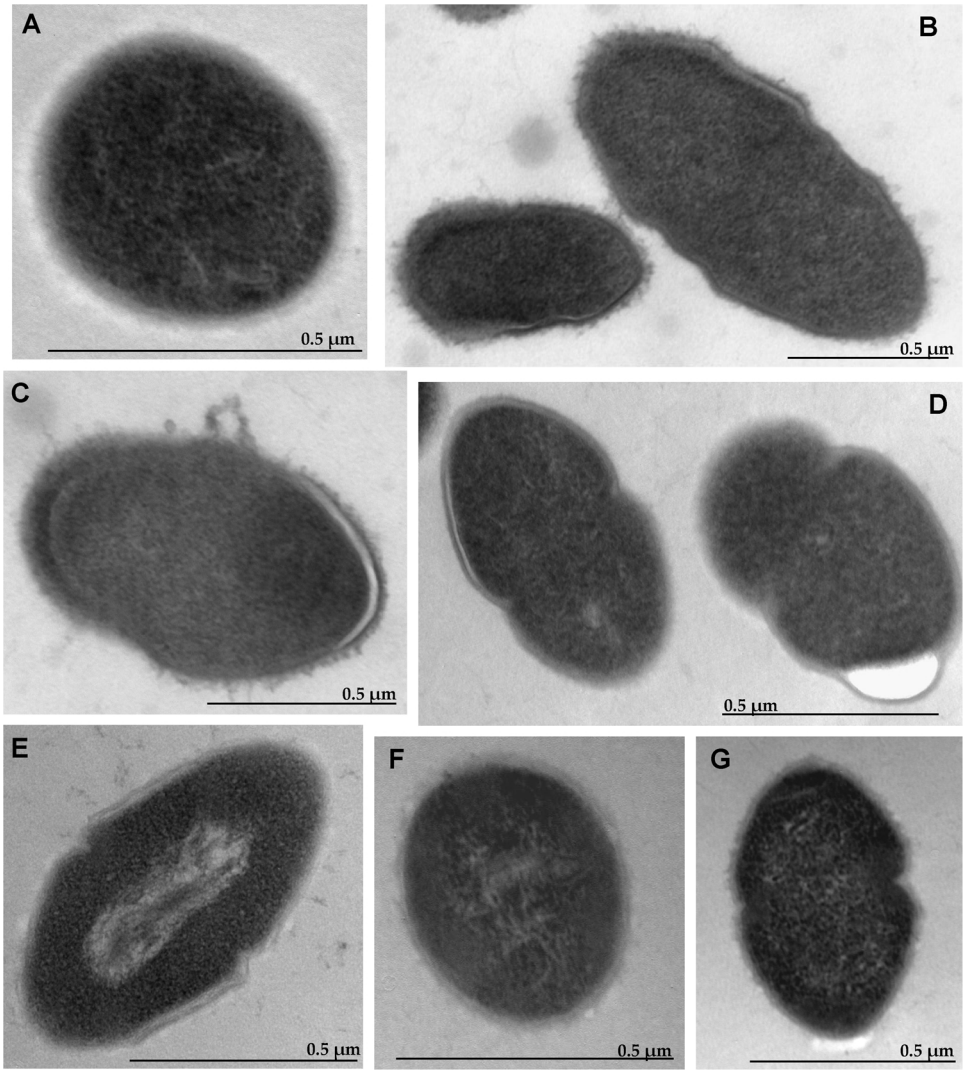

Fig. 2. Visualization of *the S. suis* capsule of representative strains TEM. (A) ST647; (B) ST695; (C) ST651; (D) ST694; (E) ST701; (F) ST704; (G) The NCTC 10234 positive control strain. Scale bar equal 0.5 μm.

D-alanine-D-alanine ligase genes that were nearly identical to 5' and 3' parts of the *cpsQ* gene from the 1336915 and 1336897 strains (KX870068.1 and KX870067.1, respectively) and most likely represented a single gene, interrupted by an insertion of two genes of putative mobile elements, with no homology to GenBank sequences. The fifth gene, located in an opposite orientation in the locus, encoded a putative DNA repair protein, found in *cps* loci of serotypes 1/2 and 2 (corresponding to SSU0556 in P1/7). Except for the first four regulatory genes, no close matches were observed for genes present in the *cps* locus of the ST701 isolate (17.0 kb). This *cps* locus (the localization pattern I-b and 32.4 % GC content) is proposed to constitute NCL21. The *cps* of ST704 isolate (20.1 kb) showed the highest similarity to the NCL16-2 of the YS511 strain (KU665280; Qiu et al., 2016), however the ST704 isolate lacked *cpsT* and *cpsY* genes, encoding choline kinase and a hypothetical protein, respectively, replaced by a ~5 kb region containing genes specifying an aminotransferase, with 98 % nucleotide identity to the aminotransferase gene *cps31 N* from the 43640 strain (KM576773.1), phosphocholine cytidiltransferase, in 96 % identical to the *cps31O* from the same strain, and three hypothetical proteins, with 97 % identity to *cpsWXY* in theYS46 strain, representing NCL7-2 (KM972271.1; Zheng et al., 2015). None of Wzy CPS polymerases encoded in six studied *cps* loci shared 100 % identity with the previously reported Wzy, but apart from Wzy characteristic for the NCL21, the other ones were clustered with Wzy from the related *cps* loci, differing by one to four amino acid substitutions (Supplementary Fig. 2). TEM demonstrated the presence of the capsule of different thickness for six investigated isolates (Fig. 2), with the thinnest capsule (15 – 20 nm) in ST704 isolate, thicker capsule (25 – 30 nm) characteristic for isolates of STs 647, 694 and 701, similarly to the control NCTC 10234 strain and the thickest capsule (30–45 nm) specific for isolates of ST695 and ST651.

4. Discussion

The aim of our study was to perform a molecular characterization of *S. suis* isolates from pigs in Poland and in the neighboring Belarus; for both countries the epidemiological and molecular data remain very limited. The prevalence of particular serotypes of *S. suis* shows geographical and temporal variability, and differs also depending from the isolation site (Wisselink et al., 2000; Gottschalk et al., 2013; Goyette-Desjardins et al., 2014). In the current study we observed the highest prevalence of serotype 2, especially among isolates from the brain. Serotype 2, the main serotype responsible for human infections, is commonly recognized as the most virulent and isolates of this serotype are found worldwide in diseased pigs, albeit with various frequency (Goyette-Desjardins et al., 2014). Serotype 3, the second frequent serotype in our collection was isolated mostly from lungs, in agreement with other observations (Wisselink et al., 2000; Wei et al., 2009). The

MLST analysis revealed a highly divergent structure, composed of a few main CCs, circulating in both countries, and several, mostly novel, singletons. A similar structure was reported in the study introducing the MLST scheme for *S. suis* (King et al., 2002) and for pig isolates from The Netherlands (Schultsz et al., 2012). The major observed CC in this study, CC1, is associated with human and pig disease in Europe, Asia and South America but is much less prevalent in North America (Goyette-Desjardins et al., 2014). Other CCs, such as CC16, initially described as CC87 (King et al., 2002), and related CCs 25 and 28 showed diverse serotypes and were mostly associated with lung infections, as found also by others (King et al., 2002; Schultsz et al., 2012).

Six isolates were selected for analysis of their *cps* loci, which differed from the known ones to various extend. Five isolates showed a partial homology to *cps* of serotypes 5, 15 (2 isolates), 30 and NCL16, with gene deletions (ST647 and ST704), gene acquisition (ST651/ST695), nucleotide substitutions and presumable recombination with other *cps* loci (ST694 and ST704). Such variability within *cps* loci was reported in other studies as well (Zheng et al., 2015; Qiu et al., 2016; Zheng et al., 2017). Importantly, some of such changes may have a significant impact on the serological properties of a strain, yielding it non-serotypeable with available sera (Zheng et al., 2017), presumably by affecting the composition of its CPS. In another species from the same genus, *Streptococcus pneumoniae,* even minor changes in *cps* locus are sufficient to generate a new serotype, e.g. a single-nucleotide polymorphism in *wciP* resulting in an amino acid substitution determines serotypes 6A and 6B (Mavroidi et al., 2004) and serotypes 15B and 15C differ by a number of TA repeats in *wciZ* (van Selm et al., 2003). How genetic differences influence the CPS structure and antigenic properties of the investigated isolates remains to be established.

The *cps* locus in the ST701 isolate is proposed to represent NCL21. This *cps* demonstrated the genomic localization typical for *S. suis* and the GC content lower than ~41 % characteristic for the *S. suis* genome, similarly to other *cps* loci (Okura et al., 2013). NCL21 shared with them an overall structure, harboring a conserved set of regulatory and processing genes *cpsABCD,* a putative initial glycosyltransferase gene, genes of glycosyltransferases, an acetyltransferase, a nucleotidyl transferase, a putative flippase Wzx and a unique polymerase Wzy. As hypothesized, other oral streptococci might represent source of such genes for *cps* loci in *S. suis* (Qiu et al., 2016). All the *cps* loci investigated in our study, including the NCL21 appear to be functional, as demonstrated by TEM.

5. Conclusion

In conclusion, our study provides the first insight to epidemiological situation of *S. suis* pig in Central/Eastern Europe, demonstrating a significant prevalence of CC1/serotype 2, responsible also for human invasive infections in Poland. Novel variants in the *cps* locus, that potentially may be associated with new serotypes, indicate that capsule diversity in *S. suis* remains only partly explored and our study contributes to a better understanding of the diversity and evolution of the major virulence factor of this pathogen.

Declaration of Competing Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.vetmic.2019.108534.

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