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Prevalence of *Streptococcus suis* Genotypes in Wild Boars of Northwestern Germany▽

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Invasive serotype 2 (*cps2*$^{+}$) strains of *Streptococcus suis* cause meningitis in pigs and humans. Four case reports of *S. suis* meningitis in hunters suggest transmission of *S. suis* through the butchering of wild boars. Therefore, the objective of this study was to investigate the prevalence of potentially human-pathogenic *S. suis* strains in wild boars. *S. suis* was isolated from 92% of all tested tonsils ($n = 200$) from wild boars. A total of 244 *S. suis* isolates were genotyped using PCR assays for the detection of serotype-specific genes, the hemolysin gene *sly*, and the virulence-associated genes *mrp* and *epf*. The prevalence of the *cps2*$^{+}$ genotype among strains from wild boars was comparable to that of control strains from domestic pig carriers. Ninety-five percent of the *cps2*$^{+}$ wild boar strains were positive for *mrp*, *sly*, and *epf, the large variant of *epf*. Interestingly, *epf** was significantly more frequently detected in *cps2*$^{+}$ strains from wild boars than in those from domestic pigs; *epf** is also typically found in European *S. suis* isolates from humans, including a meningitis isolate from a German hunter. These results suggest that at least 10% of wild boars in Northwestern Germany carry *S. suis* strains that are potentially virulent in humans. Additional amplified fragment length polymorphism analysis supported this hypothesis, since homogeneous clustering of the *epf** *mrp*$^{+}$ *sly*$^{+}$ *cps2*$^{+}$ strains from wild boars with invasive human and porcine strains was observed.**

Streptococcus suis is one of the major pathogens in the modern swine industry (13), causing mainly meningitis, septicemia, polyarthritis, endocarditis, and pneumonia. A number of biotic and abiotic factors are thought to play an important role in the epidemiologies of these diseases. Other pathogens of the porcine respiratory tract, such as the porcine reproductive and respiratory virus, may increase the host's susceptibility (9). Abiotic factors that may predispose piglets to *S. suis* infection are, for example, corrosive gases and weaning, transport, and crowding of piglets.

S. suis has also been identified as a causative agent of meningitis, septicemia, arthritis, endocarditis, hearing loss, and ocular diseases in humans (2, 16, 18, 19, 21, 27, 33, 35). This zoonosis appears to be rather common in Hong Kong and Thailand (8, 21, 35) but less common in Central Europe (2) and almost unknown in North America (11). Recently, in an unusual outbreak of 215 cases of *S. suis* diseases in humans in Sichuan, People's Republic of China, 38 deaths occurred (43). The high lethality was related to a toxic shock-like syndrome, which was yet unknown for *S. suis* infections, except in one case (34). Based on the histories of numerous human *S. suis* cases including the outbreak in Sichuan, the processing of pork is considered to be a major risk factor for this zoonosis (2, 22, 43). The histories of previously described *S. suis* diseases suggest that injured human skin is a major entry site for the pathogen (2, 43).

S. suis is a heterogeneous species that can be divided into at least 33 serotypes (13, 17). Worldwide, capsular serotype 2 is the most prevalent one among invasive porcine and human isolates (23, 29, 42). Smith et al. previously demonstrated protection against phagocytosis through the expression of the capsule in serotype 2 strains (30). However, it is well known that strains within serotype 2 may differ substantially in virulence (31, 38, 42). The muramidase-released protein (MRP) (*mrp* gene) and the extracellular factor (EF) (*epf* gene) have been proven to be suitable markers for virulence of serotype 2 strains (38, 42). However, their function is still unknown, and mutational inactivation of both genes did not result in an attenuation of virulence (32). Different size variants of MRP and EF can be distinguished. In serotype 2 strains, MRP and EF, with sizes of 136 and 110 kDa, respectively, are expressed by highly virulent strains (MRP^{+} and EF^{+}) that have been shown to induce meningitis and septicemia in experimentally infected piglets (32). Serotype 2 strains that express a large variant of EF (EF*) are considered to be less virulent in piglets (31). However, European human *S. suis* isolates frequently belong to this genotype (*mrp*$^{+}$ *epf** *cps2* strains) (31). Similar to EF, MRP is also highly variable. In addition to serotype 2 strains, serotype 9 strains, which carry a large version of the *mrp* gene (*mrp**), are epidemiologically important for invasive porcine, but not human, *S. suis* infections in Europe (29, 42).

A number of other putative virulence factors in *S. suis* have been described. These include suilysin, a hemolysin that is expressed by most European and Asian *S. suis* isolates but is very rarely expressed by those from North America (12, 20, 28). Suilysin has cytolytic functions, may be involved in the invasion of eucaryotic cells, and can affect complement-mediated opsonization (6). A putative *S. suis* survival factor recently

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TABLE 1. Prevalence of different *S. suis* genotypes among wild boars and domestic pigs in Northwestern Germany

Genotype	*S. suis* isolates from: Wild boars — Prevalence (no. of animals with genotype/total no. of animals)[a]	Wild boars — No. of regions with positive animal/total no. of regions investigated[b]	Wild boars — AFLP cluster A (no. of strains with cluster A/total no. of strains investigated)[c]	Domestic pigs—prevalence (no. of animals with genotype/total no. of animals)[a]
cps1	0/200	0/12	0/0	1/92
sly$^+$ *mrp*$^+$*epf** *cps1*	0/200	0/12	0/0	1/92
cps2	22/200	4/12	19/20	13/92
sly$^+$ *mrp*$^+$ *epf*$^+$ *cps2*	0/200	0/12	0/0	5/92
sly negative *mrp*$^+$ *epf* negative *cps2*	1/200	1/12	0/1	5/92
sly$^+$ *mrp*$^+$ *epf* negative *cps2*	0/200	0/12	0/0	1/92
mrp$^+$ *epf** *sly*$^+$ *cps2*[d]	21/200[e]	4/12	19/19	2/92[e]
cps7	4/200	3/12	0/4	3/92
sly$^+$ *mrp*** *cps7*	1/200	1/12	0/1	0/92
sly$^+$ *mrp* negative *cps7*	3/200	2/12	0/3	3/92
cps9	44/200[e]	10/12	1/22	2/92[e]
sly$^+$ *mrp*$^+$ *cps9*	1/200	1/12	0/1	0/92
sly$^+$ *mrp** *cps9*	1/200	1/12	0/1	0/92
sly$^+$ *mrp*** *cps9*	1/200	1/12	0/1	0/92
sly$^+$ *mrp**** *cps9*	2/200	2/12	1/2	1/92
sly$^+$ *mrp* negative *cps9*	8/200	6/12	0/4	0/92
sly negative *mrp* negative *cps9*	31/200	10/12	0/13	1/92
Nontypeable[f]	174/200	12/12	3/24	73/92

[a] Number of investigated animals from which this genotype was isolated.
[b] Number of investigated regions with at least one wild boar from which this genotype was isolated.
[c] Number of strains investigated by AFLP with this genotype that were assigned to cluster A. Note that cluster A is defined by a 68% similarity cutoff and contains all five investigated human European isolates.
[d] All five investigated human European isolates showed this genotype.
[e] Significant difference between wild boars and domestic pigs.
[f] Isolates were not *cps1*, *cps2*, *cps7*, or *cps9*.

identified by our group is arginine deiminase (AdiS), encoded by the gene *arcA* (3, 40). Almost all *S. suis* strains isolated from pigs carry this gene (23).

In addition to domesticated pigs, wild boars (both *Sus scrofa*) have been proposed to be a reservoir for virulent *S. suis* strains. This hypothesis is based on cases of *S. suis* diseases in hunters after butchering wild boars (5, 14, 15, 27). In Germany, wild boars have become very abundant, and during the last few years, more than 400,000 animals were shot annually (www.jagd-online.de). The butchering of these animals is very often performed under limited light conditions and without gloves, thus increasing the risk of infection through skin wounds. However, knowledge of the epidemiological distribution of *S. suis* in wild boars is very limited.

In this study, we addressed the hypothesis that wild boars are carriers of putative human-pathogenic *S. suis* strains. We assumed that *S. suis* strains with the same, or similar, virulence-associated gene profiles and DNA fingerprints as those isolated from humans should be detectable in wild boars. Therefore, isolates from wild boars in Northwestern Germany were characterized by virulence-associated gene and amplified fragment length polymorphism (AFLP) typing. As domestic pigs are known to be a reservoir of virulent *S. suis* strains, we included not only isolates from humans but also isolates from domestic pigs for comparison. Our results suggest that wild boars in Northwestern Germany frequently carry putative zoonotic *S. suis* strains.

MATERIALS AND METHODS

Samples and bacterial strains. Tonsils from 200 wild boars shot in Northwestern Germany were collected and processed for bacterial culture within 24 h after the death of the animal. Samples were collected in 12 different regions. No region contained more than 14% of the samples used. As two highways intersect the area of sample collection, it is very likely that at least four populations of wild boars with little exchange are represented. Also included in this study were 20 wild boars shot in the Saupark Springe game park, which has been surrounded by a wall since 1839. This park has a size of 16 km^2 and harbors a large population of wild boars (www.saupark-springe.de).

More than one isolate per animal was included only if it showed a distinct multiplex PCR (MP-PCR) genotype with regard to the first isolate(s). For comparison, *S. suis* isolates from tonsils of pigs (n = 92) that were either healthy or dissected for reasons other than *S. suis* infection in Northwestern Germany were investigated as well (domestic carrier group). In both groups, 70 to 80% of the animals were between 4 weeks and 8 months of age. Both sexes were represented. Samples were streaked onto Columbia and *Streptococcus*/*Staphylococcus* selective agar plates (Oxoid, Wesel, Germany) with 6% sheep blood. From each sample, up to four subcultures (depending on the different colony morphologies) of alpha-hemolytic streptococci were used for the preparation of chromosomal DNA. Isolates were further cultivated on Columbia agar with sheep blood and in Todd-Hewitt broth (Oxoid).

Preparation of chromosomal DNA. Chromosomal DNA was prepared according to standard procedures (39).

PCR. A previously described MP-PCR was used to identify and differentiate *S. suis* isolates in a single-step procedure (29). The identification of *S. suis* was based on the detection of a specific *gdh* amplification product. The MP-PCR allowed the identification of four capsule types (types 1, 2, 7, and 9) through oligonucleotide primers targeting the *cps* locus and the detection of four virulence-associated genes (*mrp*, *epf*, *sly*, and *arcA*).

Differentiation of *mrp* and *epf* variants was done by monoplex PCR assays as described previously (29).

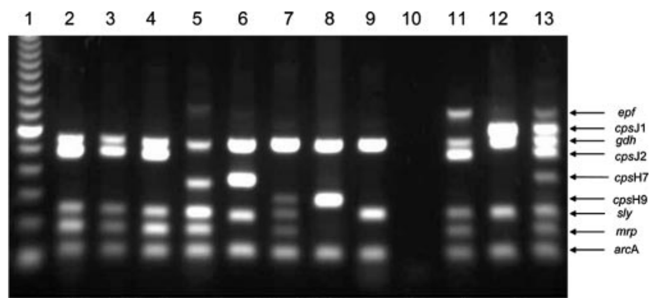


FIG. 1. Representative MP-PCR of *S. suis* strains. Lanes: 1, 100-bp ladder; 2, meningitis isolate from a German hunter (strain 199) (27); 3 to 9, isolates from tonsils of wild boars (W183.1, W168.1, W102.2, W31.3, W162.1, W151.2, and W184.1); 10, water; 11, reference strain P1/7; 12, serotype 1 reference strain DSM 9683; 13, mixture of different reference strains (29).

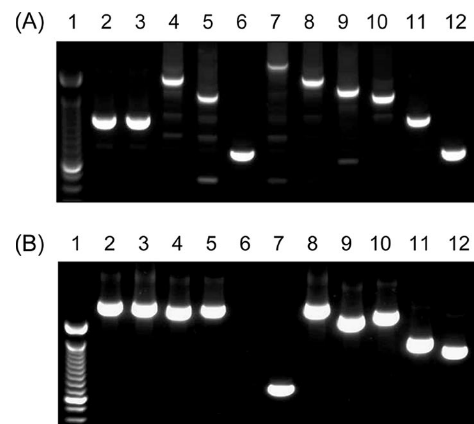


FIG. 2. Representative *mrp* (A) and *epf* (B) PCR of *S. suis* strains for differentiation of size variants. (A) Lane 1, 100-bp ladder; lane 2, meningitis isolate from a German hunter (strain 199) (27); lanes 3 to 6, isolates from tonsils of wild boars (W183.1, W123.3, W131.1, and W108.2); lanes 7 to 12, *mrp* reference strains (V7353/1, 90-2741-7, A5373/4, A3286/94, D282, and A5140/3/96) (29). (B) Lane 1, 100-bp ladder; lane 2, strain 199 (see A); lane 3, isolate from a butcher (MAC 724); lanes 4 and 5, *cps2*$^+$ isolates from tonsils of wild boars (W50.2 and W50.3); lane 6, negative control (A5683/93); lane 7, *epf*$^+$ control strain (P1/7); lanes 8 to 12, *epf** reference strains (lane 8, 1890; lane 9, 2840; lane 10, 3921; lane 11, 3988; lane 12, 3995) (29, 31).

For comparison, 39 selected tonsils from wild boars in 10 of the 12 different regions were also investigated using a multiplex PCR recently described by Marois et al. (24). Template DNA for this MP-PCR (100 ng per reaction) was prepared from a 10-ml Todd-Hewitt broth culture with Streptococci Selective Supplement (Oxoid) inoculated with the tonsil specimen.

AFLP. Single-enzyme (HindIII) AFLP typing was done with 70 selected strains from wild boars, 8 porcine reference strains, and 5 human strains. Selection was based on the results of virulence-associated gene profiling. All except two *cps2*$^+$ isolates were included (the two *cps2*$^+$ strains not investigated had exactly the same virulence-associated gene profile and were from the same region as at least three other strains that were included). Each of the 22 different genotypes (regarding *cps1/2/7/9*, *sly*, *mrp*, *epf*, and *arcA*) identified among *S. suis* isolates from wild boars in this study was represented. Additional strains were selected randomly. AFLP was performed as described previously for *Helicobacter pylori* (10), with the following modifications. Briefly, 1 μg of digested DNA was used in the ligation reaction mixture containing 30 μM of annealed adapter ADH1/ADH2, 1$\times$ T4 DNA ligase buffer, and 1 U T4 DNA ligase (both from Promega, Mannheim, Germany) in a final volume of 20 μl. Subsequently, PCR amplification products were generated with 1 μM primer HI-G (10) and separated by 2.5% (wt/vol) agarose gel electrophoresis. AFLP patterns were analyzed using BioNumerics software 4.0 (AppliedMath, Sint-Martens Latem, Belgium). The pairwise comparison of band patterns was performed using the Pearson product-moment correlation coefficient, and the dendrogram was calculated by the unweighted-pair group method analysis using average linkages.

Sequencing. The *epf*^{3004} amplification product of strain W50.2 generated with the *epf*-specific primer pair was cloned and sequenced as described previously (29). The obtained *epf*^{3004} sequence and the previously published sequence of *epf*^{1890} (GenBank accession no. A24024) were used for additional primer design to generate overlapping PCR amplification products of *epf*^{3004} of strain W50.2. These amplification products were cloned and sequenced by primer walking.

Statistical evaluation. Fisher's two-sided exact test was used to compare the prevalences of strains with a specific gene (e.g., *sly*$^+$) or genotype (e.g., *sly*$^+$ *mrp*$^+$ *epf** *cps2*) in the two different groups. Differences were estimated as significant when probabilities (P) were lower than 0.05.

Nucleotide sequence accession number. The full-length sequence of *epf*^{3004} has been deposited in the GenBank database under accession no. DQ372915.

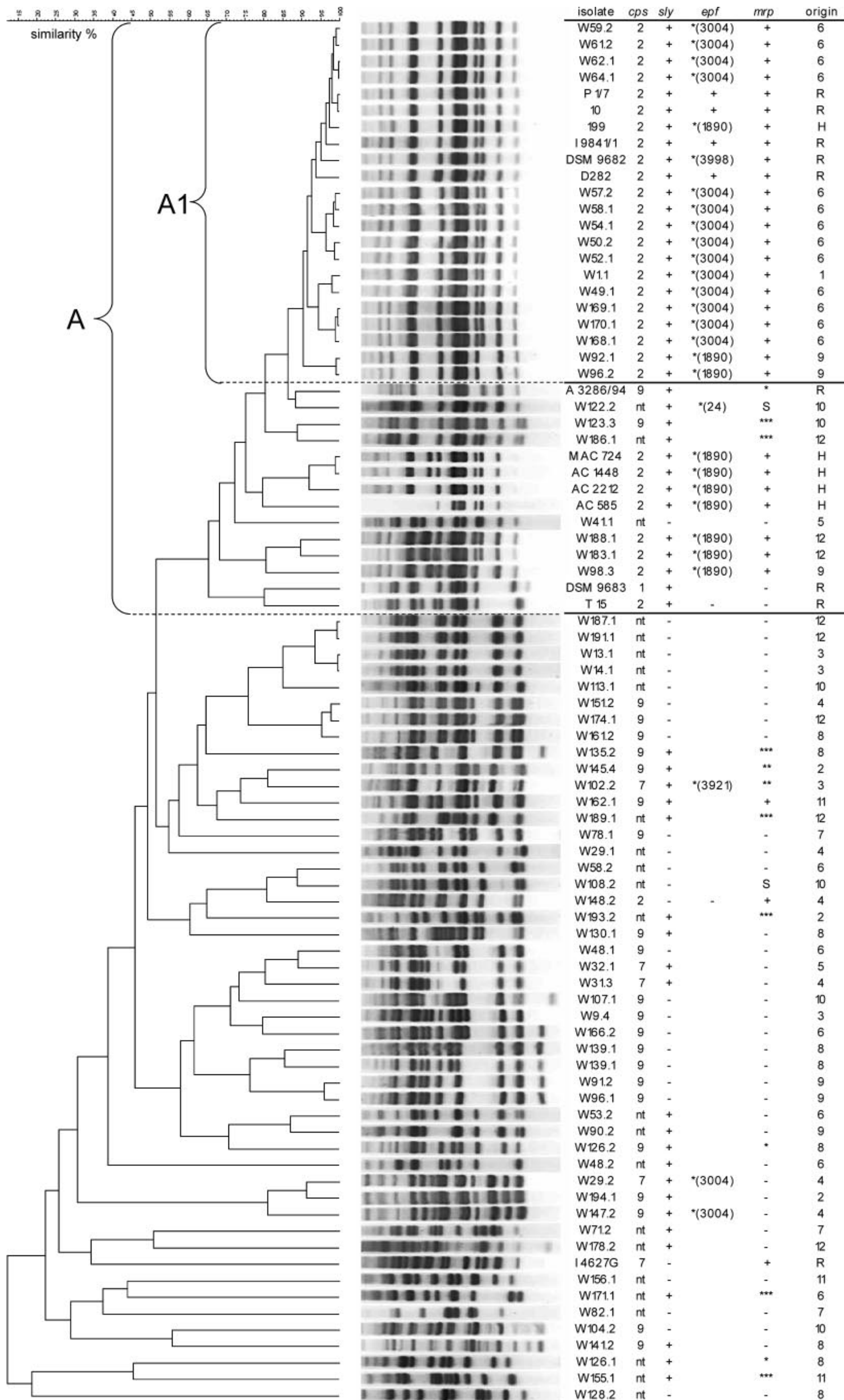
RESULTS

Prevalence of *S. suis* and detection of serotype-specific genes. *S. suis* was isolated from 92% of all 200 tested tonsils from wild boars based on the generation of the *gdh*-specific PCR amplification product from alpha-hemolytic streptococci (26). More than one genotype of *S. suis* was isolated from 61 (30.5%) wild boars. Among the total 244 *S. suis* isolates, 22 different genotypes could be differentiated using previously described (29) MP-PCR and *mrp* and *epf* variant PCR, respectively. Eighteen different genotypes were detected in the control group of 92 *S. suis* isolates from domestic pigs. Three of the four investigated capsular genotypes were detected in isolates from wild boars, namely, *cps2*, *cps7*, and *cps9* but not *cps1* (Table 1). The prevalence of *cps2* strains was similar in wild boars (11%) and domestic pigs (14%). In contrast, *cps9* strains were significantly (P < 0.0001) more often detected in wild boars (22%) than in domestic pigs (2%). More than 70% of the genotyped *S. suis* isolates from both groups (wild boars and domestic pigs) did not belong to the genotypes *cps1*, *cps2*, *cps7*, or *cps9*.

Thirty-nine tonsils from wild boars from 10 regions were also investigated for *cps2* strains by an approach based on the MP-PCR described previously by Marois et al. (24). This approach avoids the isolation of strains. In 10 of the 39 samples, a *cps2*-specific fragment was generated. However, correlation with the results of genotyping as described above was rather low. In six samples, *cps2* was detected by both methods, four samples were positive only by the MP-PCR described previously by Marois et al. (24), and four samples did not show a *cps2* amplification product in the latter, although a *cps2*$^+$ strain was isolated and detected with the MP-PCR described above by Silva et al. (29).

The prevalences of *cps2*$^+$ wild boars differed among the 13 regions investigated. One region had a very high carrier rate of *cps2*$^+$ strains (58%). No *cps2* strain was detectable with either of the two approaches in wild boars from 6 of the 13 regions.

Prevalence of *arcA* and *sly*. Each of the four additional virulence-associated genes investigated (*arcA*, *sly*, *mrp*, and *epf*) was present in isolates from wild boars (Fig. 1 and Table 1). All isolates from wild and domestic pigs were positive for *arcA*. In general, the gene encoding suilysin, *sly*, was significantly less frequently detected in isolates from wild boars (39% positive) than in isolates from domestic pigs (66% positive; $P < 0.0001$). However, all except 1 of the 22 *cps2* strains from wild boars were positive for *sly* (Table 1).


similarity %
A
A1
isolate cps sly epf mrp origin
W59.2 2 + *(3004) + 6
W61.2 2 + *(3004) + 6
W62.1 2 + *(3004) + 6
W64.1 2 + *(3004) + 6
P 1/7 2 + + + R
10 2 + + + R
199 2 + *(1890) + H
I 9841/1 2 + + + R
DSM 9682 2 + *(3998) + R
D282 2 + + + R
W57.2 2 + *(3004) + 6
W58.1 2 + *(3004) + 6
W54.1 2 + *(3004) + 6
W50.2 2 + *(3004) + 6
W52.1 2 + *(3004) + 6
W1.1 2 + *(3004) + 1
W49.1 2 + *(3004) + 6
W169.1 2 + *(3004) + 6
W170.1 2 + *(3004) + 6
W168.1 2 + *(3004) + 6
W92.1 2 + *(1890) + 9
W96.2 2 + *(1890) + 9
A 3286/94 9 + * R
W122.2 nt + *(24) S 10
W123.3 9 + *** 10
W186.1 nt + *** 12
MAC 724 2 + *(1890) + H
AC 1448 2 + *(1890) + H
AC 2212 2 + *(1890) + H
AC 585 2 + *(1890) + H
W41.1 nt - - 5
W188.1 2 + *(1890) + 12
W183.1 2 + *(1890) + 12
W98.3 2 + *(1890) + 9
DSM 9683 1 + - R
T 15 2 + - - R
W187.1 nt - - 12
W191.1 nt - - 12
W13.1 nt - - 3
W14.1 nt - - 3
W113.1 nt - - 10
W151.2 9 - - 4
W174.1 9 - - 12
W161.2 9 - - 8
W135.2 9 + *** 8
W145.4 9 + ** 2
W102.2 7 + *(3921) ** 3
W162.1 9 + + 11
W189.1 nt + *** 12
W78.1 9 - - 7
W29.1 nt - - 4
W58.2 nt - - 6
W108.2 nt - S 10
W148.2 2 - - + 4
W193.2 nt + *** 2
W130.1 9 + - 8
W48.1 9 - - 6
W32.1 7 + - 5
W31.3 7 + - 4
W107.1 9 - - 10
W9.4 9 - - 3
W166.2 9 - - 6
W139.1 9 - - 8
W139.1 9 - - 8
W91.2 9 - - 9
W96.1 9 - - 9
W53.2 nt + - 6
W90.2 nt + - 9
W126.2 9 + * 8
W48.2 nt + - 6
W29.2 7 + *(3004) - 4
W194.1 9 + - 2
W147.2 9 + *(3004) - 4
W71.2 nt + - 7
W178.2 nt + - 12
I 4627G 7 - + R
W156.1 nt - - 11
W171.1 nt + *** 6
W82.1 nt - - 7
W104.2 9 - - 10
W141.2 9 + - 8
W126.1 nt + * 8
W155.1 nt + *** 11
W128.2 nt - - 8

Prevalence and variability of *mrp*. The gene encoding the muramidase-released protein, *mrp* (including all variants), was significantly less frequently detected in isolates from wild boars (18%) than in isolates from domestic pigs (43%; $P < 0.0001$). Different size variants of MRP have been described previously (31, 42). PCR assays with primers targeting sequences flanking the variable region of the *mrp* gene were used to distinguish these size variants. Thus, MP-PCR was used in combination with the specific monoplex PCR for discrimination of *mrp* variants (27). All *cps2*$^+$ isolates from wild boars and domestic pigs investigated were positive for the 136-kDa-protein-encoding *mrp* gene (*mrp*$^+$) (Table 1). *S. suis* isolates from wild boars other than *cps2* isolates showed size variations of *mrp* similar to those of isolates from domestic pigs (Fig. 2A). In *cps9*$^+$ strains from wild boars, four different *mrp* variants were distinguishable. However, only one wild boar *cps9* isolate was positive for the *mrp** variant, which is frequently detected in invasive serotype 9 isolates from domestic pigs, as represented by strain A3286/94 (1, 29, 42).

Analysis of the *epf* genotype in *cps2* strains isolated from wild boars. None of the *cps2* isolates from wild boars generated the *epf* amplification product specific for the gene (*epf*$^+$) coding for the 110-kDa EF protein. This important virulence marker was also rarely found in isolates from domestic pigs (Table 1). The *cps2*$^+$ strains were screened with *epf* variant PCR (27) to detect large variants of *epf*, generally named *epf** (29, 31). Sixteen strains from wild boars generated an amplification product that was different in size from those of the products of all known *epf* variants (Fig. 2B, lane 4). The particular gene, called *epf*^{3004}, of strain W50.2 was sequenced completely and was found to be very similar to *epf*^{1890} (96% identity). The only differences were one single nucleotide exchange and the deletion of repeat 6 in *epf*^{1890} (nucleotides 4021 to 4248). Ninety-five percent of serotype 2 wild boar strains, which were shot in four different regions, were positive for one of these two very similar size variants of *epf** (*epf*^{1890} and *epf*^{3004}) (Fig. 2). Importantly, all five European human isolates investigated, including the meningitis isolate from a German hunter (Fig. 2B, lane 2), also showed the genotype *sly*$^+$ *mrp*$^+$ *epf** (and in particular *epf*^{1890}) *cps2*$^+$. The prevalence of *sly*$^+$ *mrp*$^+$ *epf** *cps2*$^+$ strains was significantly higher in wild boars than in domestic pigs (Table 1).

AFLP typing. In a preliminary study, we developed an AFLP typing procedure for *S. suis* and identified a homogeneous cluster (A1) associated with *sly*$^+$ *mrp*$^+$ *epf*$^+$ (or *epf**) *cps2*$^+$ strains of porcine and human origin with an invasive clinical background (26a). In the present study, 70 wild boar isolates, 5 European human isolates, and 9 porcine reference strains were typed by this AFLP approach. Of the wild boar strains, 20 *cps2*$^+$, all 4 *cps7*$^+$, 22 *cps9*$^+$, and 24 *cps*-nontypeable strains representing all regions were included. As shown in Fig. 3, 16 (80%) of the AFLP-typed *cps2*$^+$ wild boar isolates clustered at a linkage level of 90% within cluster A1, which additionally included only the hunter isolate, strain 199 (27), and the 5 highly virulent *sly*$^+$ *mrp*$^+$ *epf*$^+$ serotype 2 reference strains, 10 (30, 32), P1/7 (20), I9841/1 (1, 40), DSM 9682, and D282 (38). Closely associated with this cluster (≥68% similarity, cluster A) were seven additional wild boar strains (three *cps2*$^+$, one *cps*9$^+$, and three *cps* nontypeable), four European human strains (strains 122, 126, 124, and 127), and three porcine reference strains (*mrp** serotype 9 reference strain A3286/94 [1, 29], serotype 1 strain DSM 9683, and serotype 2 strain T15). All other wild boar strains were very heterogeneous (Fig. 3). Some *S. suis* strains, such as strains w48.2 and w128.2, showed a similarity to any other strain of less than 50%. Neither of the investigated *cps9*$^+$ and *cps7*$^+$ wild boar strains formed an AFLP cluster comparable in similarity and number of strains to the described cluster A1 (A). Interestingly, there was a very high diversity among *cps9* strains. Only one of the 44 *cps9* strains from wild boars (w123.3) generated a pattern with a similarity level of more than 60% compared to the pattern of invasive *cps9* reference strain A3286/94 (1).

DISCUSSION

S. suis is a zoonotic pathogen that has received only limited attention with respect to epidemiology in humans, except for the recent outbreak in Sichuan, People's Republic of China, in 2005 (11, 34, 43). The current knowledge about the zoonotic aspect of *S. suis* is based on a few larger case series (2, 21, 33, 43), numerous single case reports, and few epidemiological studies of pigs that included human isolates (4, 7, 23, 31). Altogether, more than 100 human *S. suis* isolates have been serotyped (2, 4, 7, 23, 27, 31, 33). The results strongly suggest that serotype 2 (*cps2*) and probably also serotype 1 (*cps1*) strains have a higher zoonotic potential than other *S. suis* serotypes. This is in accordance with the high prevalence of serotype 2 strains among invasive porcine isolates (42). However, the zoonotic potential of other (non-serotype 2) invasive porcine *S. suis* pathotypes is unknown. In particular, serotype 9 strains have a high prevalence among *S. suis* isolates from diseased pigs with meningitis and other invasive diseases in Europe (42). However, to our knowledge, no human isolate belonging to serotype 9 has been described so far.

In this study, we demonstrated by two different approaches that tonsils of wild boars in Northwestern Germany are frequently colonized with *cps2*$^+$ strains (more than 10%). We propose that the majority, if not all, of the *cps2*$^+$ strains from wild boars detected in this study are virulent and putative zoonotic agents. Firstly, all except one of these strains were also positive for the virulence-associated factors *sly* and *mrp*. In addition, 96% of these strains carried a large variant of the

FIG. 3. AFLP dendrogram of 70 *S. suis* isolates from wild boars (indicated by "W" under isolate), 5 human strains (indicated by "H" under origin), and 9 porcine reference strains (indicated by "R" under origin). The pairwise comparison of band patterns was performed using the Pearson product-moment correlation coefficient. The numbers in the parentheses specifying the *epf** variant are equivalent to the names of the reference strains with the same-sized *epf** (29, 31), except for the variant found only in wild boars (*epf*^{3004}). The "+" under *epf* and *mrp* refers to the variants that encode the 110-kDa EF and 136-kDa MRP, respectively. The numbers under origin refer to the 12 regions of sample collection in Northwestern Germany.

virulence-associated factor *epf* (*epf**). These large variants are found in serotype 2 strains of moderate virulence in piglets but are also typically detected in human European isolates (25, 29, 31). In agreement with this, the invasive human European isolates genotyped in this study were all positive for the particular large variant epf^{1890}. The same variant and a closely related variant, epf^{3004}, were detected among the wild boar isolates investigated in this study. The putative zoonotic potential of these $cps2^+$ wild boar strains (with the exception of strain w148.2) is supported by our finding that 6 of 19 strains were completely, and the remaining strains were nearly, indistinguishable from a meningitis isolate from a hunter who was infected with *S. suis* after butchering a wild boar in Northern Germany (27). Furthermore, the 19 $cps2^+$ wild boar isolates belonged to AFLP cluster A, which showed a high overall similarity level (>60%) and, in addition, harbored only highly virulent porcine and human *S. suis* strains. Homogeneous clustering of invasive *cps2* (and *cps1*) *S. suis* strains has previously been observed using the same typing method (26a) and other typing methods (23).

AFLP allowed the differentiation of two populations of *cps2* strains that showed a similarity of less than 45% to each other. The cluster of invasive *cps2* strains is represented by virulent mrp^+ epf^+ sly^+ $cps2^+$ reference strains, such as strains P1/7, 10, and D282 (26a). These strains clustered with a very high similarity together with the $cps2^+$ wild boar isolates, except for strain W148.2. Thus, these $cps2^+$ wild boar isolates can be considered to belong to cluster A and not to cluster C as defined previously by Rehm et al. (26a). Accordingly, the *cps2* strains of cluster C were all negative for *sly* and *epf*. Strains of AFLP cluster A most probably belong to the multilocus sequence type 1 complex described previously by King et al. (23), since strains investigated with both typing methods, especially invasive *cps2* strains, formed similar clusters with both methods (26a). Interestingly, the vast majority (87%) of the human isolates investigated with multilocus sequence typing also belonged to the sequence type 1 complex (23).

Despite the large number (n = 244) of genotyped *S. suis* isolates from wild boars, sly^+ mrp^+ epf^+ (coding for the 110-kDa EF protein) $cps2^+$ genotypes were not detected in this group, in contrast to the smaller control group of isolates from domestic pigs (Table 1) and the high prevalence of this genotype among invasive porcine isolates (29, 42). In addition, only one wild boar isolate belonged to the genotype *mrp** sly^+ *cps9*, which is also very common among clinical isolates from pigs in Europe (29, 42). In domestic pigs, these two important invasive *S. suis* genotypes are detectable not only in specimens from diseased pigs but also in tonsillar specimens from healthy carriers, which are very frequent in swine herds with *S. suis* problems (41). Based on our results, it may be speculated that factors associated with modern swine production, such as early weaning, high concentrations of animals, and corrosive gases, led to the selection of highly virulent *S. suis* strains that are less frequent or even absent in wild boars. The high virulence of these strains might, however, be specific for domestic pigs and less adapted to the human host. The aspect of host-specific virulence of *S. suis* strains has been demonstrated in experimental infections of mice and pigs (36, 37). In contrast to sly^+ mrp^+ epf^+ *cps2* strains, sly^+ mrp^+ *epf** *cps2* strains might express virulence factors that are less host specific. Reduced host specificity of virulence factors might be disadvantageous for survival in modern swine production but advantageous for the zoonotic potential of these strains. Though *epf* is not an essential virulence factor for *S. suis* serotype 2 strains (32), it is probably associated with other factors that play a more crucial role in determining virulence and host specificity in *S. suis* strains. Their identification and functional characterization are an important goal of future studies of the pathogenesis and epidemiology of *S. suis* in pigs and humans.

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