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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

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A *Streptococcus suis* isolate from a German hunter with streptococcal toxic shock-like syndrome (STSLS) and four additional zoonotic isolates were genotyped as *mrp*$^+$ *epf*$^+$ (variant 1890) *sly*$^+$ *cps2*$^+$. All five zoonotic German strains were characterized by high multiplication in human blood samples *ex vivo*, but induction of only low levels of proinflammatory cytokines compared to a Chinese STSLS strain.

Streptococcus suis is an important porcine and human pathogen causing septicemia, meningitis, and other pathologies (1, 2). More than 90% of the strains isolated from humans belong to serotype 2 (3–5), although other serotypes, such as 9 and 7, are epidemiologically also very important in pigs (6). In addition to domesticated pigs, wild boars are an important reservoir for *S. suis* serotype 2 strains in Germany (7). Accordingly, zoonotic cases have been described in hunters (8–12).

In August 2005, a zoonotic outbreak of *S. suis* diseases occurred in China, including at least 37 cases of streptococcal toxic shock-like syndrome (STSLS) (13). Multilocus sequence typing (MLST) revealed that the Chinese STSLS isolates belong to sequence type (ST) 7 within clonal complex (CC) 1 (14). An 89-kb region designated a pathogenicity island was described as a hallmark of the genome of the Chinese STSLS isolates (15) but was later identified as an integrative conjugative element present also in a similar form in *S. suis* isolates in Vietnam not associated with STSLS (16).

This study was initiated after a fatal case of STSLS in a German hunter. The hunter showed rapid clinical deterioration marked by hypotension (70/50 mm Hg) and multiorgan failure, including severe hepatic and renal impairment despite hospitalization in an intensive care unit. Furthermore, cardiomyopathy and coagulopathy with severe thrombocytopenia (platelets of 14/nl) as well as disseminated intravascular coagulation associated with petechiae were diagnosed (Quick value of 5% and partial thromboplastin time of >200 s). *S. suis* (strain BK52339) was isolated in pure culture from this patient's blood sample. The diagnosis of STSLS is in accordance with the clinical criteria defined by others (17).

The MLST analysis (18) revealed that the German STSLS isolate BK52339, four additional German zoonotic strains, and 3 of 4 *cps2*$^+$ *S. suis* strains from wild boars belonged to ST1 (Table 1). Differentiation of virulence-associated genes using a multiplex (MP)-PCR (19) and a PCR for detection of the 89-kb region demonstrated that BK52339 is an *mrp*$^+$ *sly*$^+$ *cps2*$^+$ strain lacking the 89-kb region present in the genome of Chinese STSLS strains like O5ZYH33 (results not shown). The European serotype 2 strains causing problems in the pig industry belong mainly to ST1 and carry, in addition to *mrp* and *sly*, an *epf* gene encoding a 110-kDa extracellular factor (EF) (20). The genotypic analysis of the zoonotic German strains also included an *epf* PCR (19) for determination of specific variants of this highly variable gene (Fig. 1). Interestingly, the STSLS strain BK52339 as well as the other 4 German zoonotic strains generated *epf* amplification products of the same size as reference strain 1890 (Fig. 1) (21). This variant was also found in 2 wild boar isolates (W183.1 and W188.1) (Fig. 1) (7). In conclusion, the case of STSLS described in a hunter and the other four German zoonotic *S. suis* cases investigated were caused by a specific *mrp*$^+$ *sly*$^+$ *epf*$^+$ (1890) *cps2*$^+$ ST1 strain. At least one of the *cps2*$^+$ strains (W183.1) previously isolated from wild boars in Germany also showed this genotype.

To phenotypically characterize the German zoonotic isolates, we determined their survival in human blood samples *ex vivo* in comparison to that of various other strains by inoculating 1 ml of freshly drawn heparinized blood with approximately 1.5×10^5 CFU. Importantly, all 5 human zoonotic strains from Germany that we investigated, including the STSLS strain from the hunter, at least tripled the mean specific bacterial load during 2 h of incubation at 37°C in human blood samples (Fig. 2A). In contrast, the Chinese STSLS strain O5ZYH33 and the CC27 strain B2441/96 were efficiently killed (mean survival factor [SF], 0.05 [SD, 1.18] and mean SF, 0.14 [SD 0.21], respectively). The survival factors of the four *cps2*$^+$ strains isolated from wild boars were significantly lower than those of the German zoonotic strains (analysis of vari-

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TABLE 1 Clinical background, sequence type, and profile of virulence-associated genes of *S. suis* strains investigated in this study

S. suis strain	Source		*cps*	MLST		Profile of virulence-associated genes
	Clinical background	Reference or study		Sequence type	Clonal complex	
BK52339	Human (hunter): STSLS	This study	*cps2*	1	1	*mrp*$^+$ *epf** (1890) *sly*$^+$
MAC 724	Human: sudden death	7	*cps2*	1	1	*mrp*$^+$ *epf** (1890) *sly*$^{+a}$
199	Human (hunter): meningitis	7;11	*cps2*	1	1	*mrp*$^+$ *epf** (1890) *sly*$^{+a}$
441	Human: septicemia		*cps2*	1	1	*mrp*$^+$ *epf** (1890) *sly*$^+$
224	Human: endocarditis	24	*cps2*	1	1	*mrp*$^+$ *epf** (1890) *sly*$^+$
O5ZYH33	Human: STSLS	15;25	*cps2*	7	1	*mrp*$^+$ *epf*$^+$ *sly*$^+$
W57.2	Wild boar carrier	7	*cps2*	1	1	*mrp*$^+$ *epf** (3004) *sly*$^{+a}$
W59.2	Wild boar carrier	7	*cps2*	1	1	*mrp*$^+$ *epf** (3004) *sly*$^{+a}$
W183.1	Wild boar carrier	7	*cps2*	1	1	*mrp*$^+$ *epf** (1890) *sly*$^{+a}$
W188.1	Wild boar carrier	7	*cps2*	156	1	*mrp*$^+$ *epf** (1890) *sly*$^{+a}$
P1/7	Pig: meningitis	26	*cps2*	1[b]	1	*mrp*$^+$ *epf*$^+$ *sly*$^+$
T15	Pig: pneumonia	27	*cps2*			
B2441/96	Pig: pneumonia		*cps2*	28[c]	27[c]	
A3286/94	Pig: meningitis		*cps9*	99[c]	16 (87)[c]	*mrp** *sly*$^+$
B2663/96	Pig		*cps9*			*mrp** *sly*$^+$
B2795/96	Pig: pneumonia		*cps7*	29	27[d]	
451	Pig: meningitis		*cps7*			

[a] Determined by Baums et al. (7).
[b] Determined by King et al. (18).
[c] Determined by Rehm et al. (28). Clonal complex 16 was previously called CC87.
[d] ST29 belongs to CC27, defining a CC by sharing alleles at ≥5 of the 7 loci.

ance [ANOVA], $P < 0.05$), although the wild boar strain W57.2 doubled its number during 2 h of incubation in human blood samples (mean SF, 2.1 [SD, 1.18]). In conclusion, the STSLS isolate BK52339 and the other 4 German zoonotic strains investigated share a specific profile of virulence-associated factors [*mrp*$^+$ *sly*$^+$ *epf** (1890) *cps2*$^+$] and the ability to proliferate efficiently in human blood samples *ex vivo* in contrast to the Chinese STSLS strain O5ZYH33, the CC27 strain B2441/96, and the two *cps7* and two *cps9* strains. However, the zoonotic strains of other clonal complexes are negative for *epf* but also belong to serotype 2, which indicates that the capsule is a major zoonotic determinant (5). Of note, transmission electron microscopy using lysine-ruthenium red (LRR) staining as described previously (22) confirmed that the two STSLS isolates, O5ZYH33 and BK52339, are both encapsulated, which makes it unlikely that reduced encapsulation is the explanation for the lower survival in human blood samples of the former (Fig. 2).

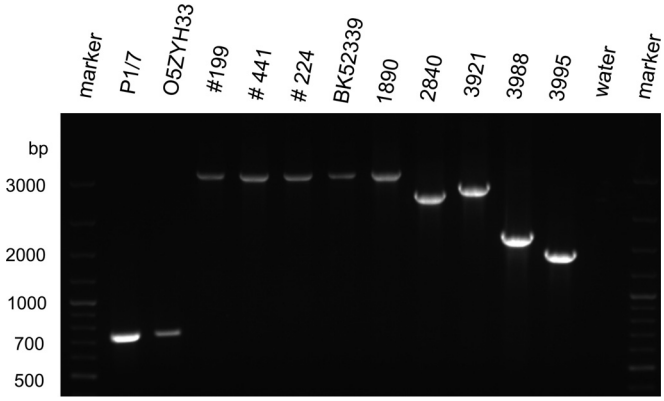

FIG 1 Differentiation of *epf** variants by PCR (19) revealed that the German STSLS strain BK52339 carries the 1890 *epf** variant, which was also found in other German zoonotic isolates (199, 441, and 224). Strains 1890, 2840, 3921,3988, and 3995 were included for determination of *epf* variants as in previous studies (7, 21).

A cytokine storm has been discussed as a distinct feature of the pathogenesis of STSLS caused by *S. suis* as patients with *S. suis*-associated STSLS showed high serum concentrations of interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and IL-1β (17). Furthermore, infections of mice demonstrated that a Chinese ST7 STSLS strain induced higher systemic levels of these proinflammatory cytokines than the virulent European *mrp*$^+$ *epf*$^+$ *sly*$^+$ *cps2*$^+$ strain P1/7 (23). Accordingly, we hypothesized that induction of cytokines in human blood samples *ex vivo* might show associations with the different clinical backgrounds of our strains under investigation. The cytokine concentrations were determined using human cytometric bead array flex sets (BD Biosciences, San Jose, CA, USA) with an Accuri C6 flow cytometer (BD Biosciences). As shown in Fig. 3 infection of human blood samples with the Chinese strain O5ZYH33 for 2 h led to levels of IL-1β (mean 41 pg/dl; SD, 30 pg/dl), IL-6 (mean, 227 pg/dl; SD, 300 pg/dl), TNF-α (mean, 515 pg/dl; SD, 356 pg/dl), and macrophage inflammatory protein 1 alpha (MIP-1α) (mean, 557 pg/dl; SD, 444 pg/dl) that were significantly higher than the concentrations recorded after incubation with the STSLS isolate BK52399 for IL-1β (mean, 6 pg/dl; SD, 8 pg/dl), IL-6 (mean, 63 pg/dl; SD, 95 pg/dl), TNF-α, (mean, 178 pg/dl; SD, 185 pg/dl), and MIP-1α, (mean, 204 pg/dl; SD, 180 pg/dl). In general, the levels of these proinflammatory cytokines were rather low in blood samples incubated with any of the German zoonotic strains. Significant differences among the German zoonotic strains in cytokine induction were not observed with the exception of a significantly higher level of TNF-α in strain 224-infected than in strain 199-infected blood samples. None of the other *S. suis* strains tested, including the isolates from wild boars and the two *cps7* and two *cps9* strains, induced IL-1β, IL-6, and MIP-1α concentrations significantly different from those for the German zoonotic strains with the clear exception of the CC27 strain B2441/96 inducing IL-1β, IL-6, TNF-α, and MIP-1α levels similar to those for the Chinese STSLS

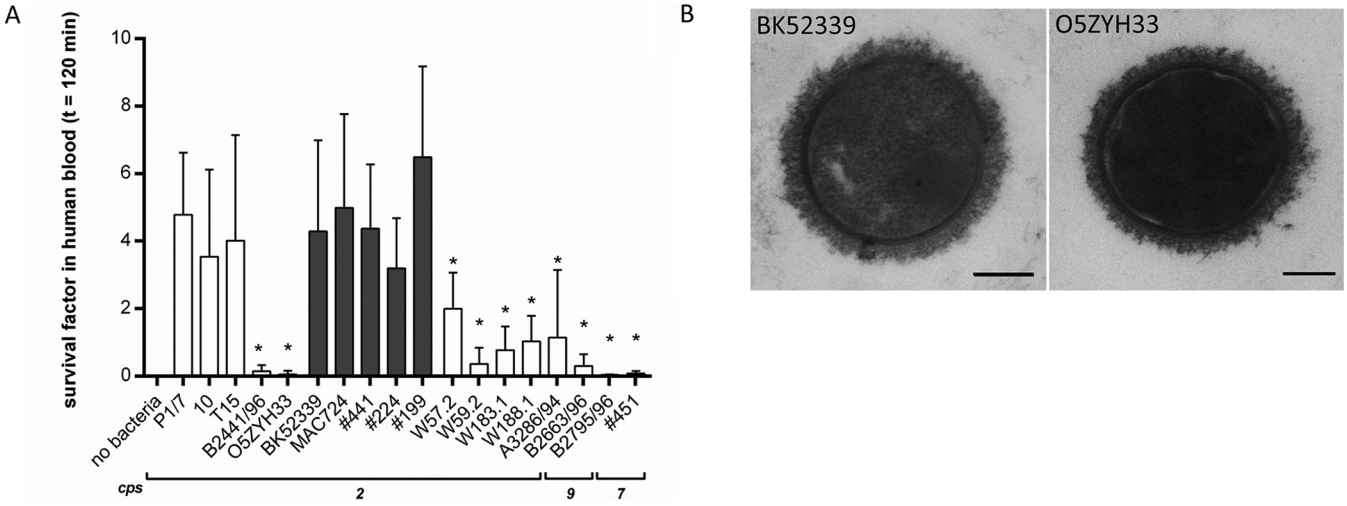

FIG 2 Comparative analysis of survival of *S. suis* strains isolated from humans in Germany (■) to other *cps2*, *cps9*, and *cps7* strains in human blood samples *ex vivo* (A) and confirmation of capsule expression by LRR staining and transmission electron microscopy in the strains indicated (B). The German STSLS isolate BK52339 showed a high survival factor in human blood samples *ex vivo* comparable to the survival factors of other *cps2* strains of CC1 but significantly higher than the survival factors of the STSLS Chinese isolate O5ZYH33 and the CC27 strain B2441/96. The survival factor of each strain was determined 7 times in independent experiments with blood samples from different German volunteers not exposed to pigs occupationally. Strains 10, MAC724, W183.1, W188.1, A3286/94, B2663/93, B2795/96, and 451 were not included in every experiment but at least in 5 of the 7. The specific bacterial contents (CFU/ml) were determined through serial platings after 0 min and 120 min of incubation at 37°C. The survival factor represents the ratio of the CFU after 2 h to the CFU at time zero. Bars and error bars represent mean values and standard deviations, respectively. Analysis of variance (ANOVA) was conducted for statistics. Survival factors significantly different from those of strain BK52339 are indicated by asterisks ($P < 0.05$). Bars in panel B, 200 nm.

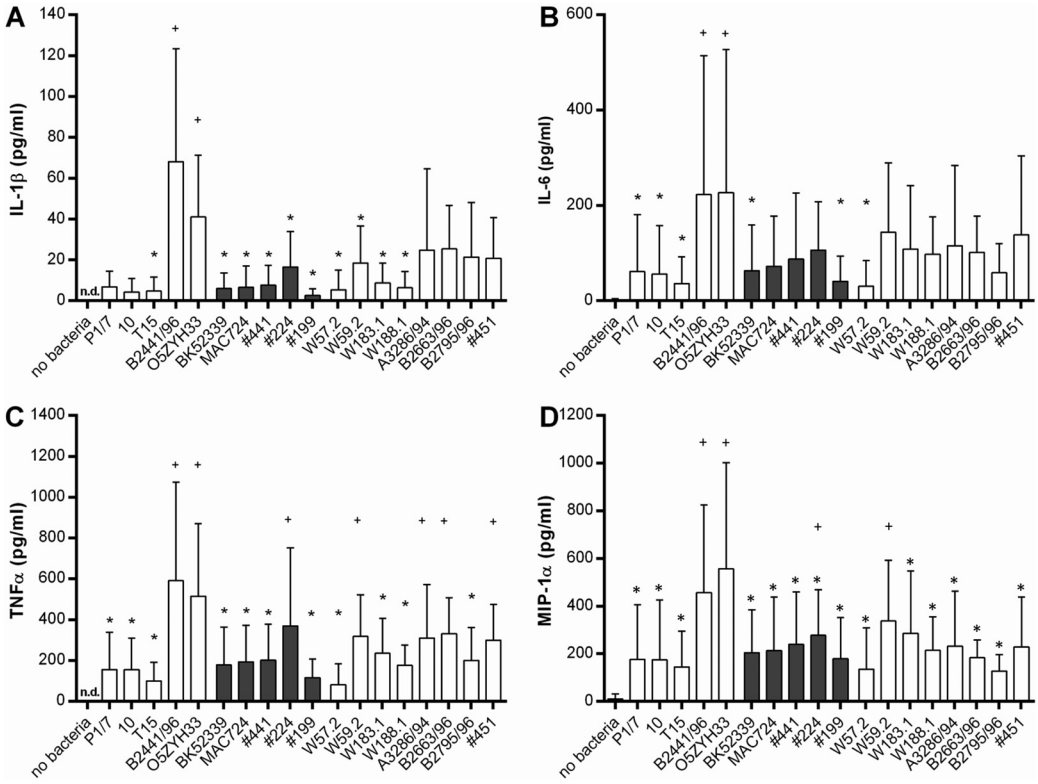

FIG 3 The Chinese *S. suis* STSLS strain O5ZYH33 and the CC27 strain B2441/96 elicited significantly higher titers of proinflammatory cytokines in 2 h of infection of human blood samples *ex vivo* than the STSLS isolate BK52399 of the hunter and other zoonotic strains from Germany (■). Bars show the mean concentrations of IL-1β (A), IL-6 (B), TNF-α (C), and MIP-1α (D) in heparin plasma collected after 2 h of *ex vivo* infection with the indicated *S. suis* strains of 7 independent experiments (see Fig. 2; no bacteria refers to the mock control; n.d., not detectable). Strains 10, MAC724, W183.1, W188.1, A3286/94, B2663/96, B2795/96, and 451 were not included in every experiment but at least in 5 of the 7. Significant differences from the values for strain O5ZYH33 are indicated by asterisks ($P < 0.05$). The mean values of strain B2441/96 were also significantly increased from the values marked with asterisks in addition to the TNF-α concentrations of strains 451, A3286/94, and W59.2. Significant differences among the German zoonotic strains were not recorded, except for a significant higher induction of TNF-α in strain 224-infected in comparison to 199-infected blood samples. Significant differences from the control lacking bacteria are indicated by plus signs ($P < 0.05$).

strain O5ZYH33 (Fig. 3). In conclusion, the German zoonotic strains exhibited prominent growth in human blood samples but, in comparison to the STSLS Chinese strain O5ZYH33 and the CC27 strain B2441/96, only low induction of proinflammatory cytokines such as IL-1β, IL-6, TNF-α, and MIP-1α within 2 h of *ex vivo* infection of human blood samples.

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