MAJOR ARTICLE

Host-pathogen Interaction at the Intestinal Mucosa Correlates With Zoonotic Potential of *Streptococcus suis*

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Background. *Streptococcus suis* has emerged as an important cause of bacterial meningitis in adults. The ingestion of undercooked pork is a risk factor for human *S. suis* serotype 2 (SS2) infection. Here we provide experimental evidence indicating that the gastrointestinal tract is an entry site of SS2 infection.

Methods. We developed a noninvasive in vivo model to study oral SS2 infection in piglets. We compared in vitro interaction of *S. suis* with human and porcine intestinal epithelial cells (IEC).

Results. Two out of 15 piglets showed clinical symptoms compatible with *S. suis* infection 24–48 hours after ingestion of SS2. SS2 was detected in mesenteric lymph nodes of 40% of challenged piglets. SS2 strains isolated from patients showed significantly higher adhesion to human IEC compared to invasive strains isolated from pigs. In contrast, invasive SS9 strains showed significantly higher adhesion to porcine IEC. Translocation across human IEC, which occurred predominately via a paracellular route, was significantly associated with clonal complex 1, the predominant zoonotic genotype. Adhesion and translocation were dependent on capsular polysaccharide production.

Conclusions. SS2 should be considered a food-borne pathogen. *S. suis* interaction with human and pig IEC correlates with *S. suis* serotype and genotype, which can explain the zoonotic potential of SS2.

Keywords. *Streptococcus suis*; intestinal translocation; zoonotic infections; serotype; clonal complex; tight junctions; piglets.

Streptococcus suis is an emerging zoonotic pathogen, which causes meningitis and septicaemia in humans and pigs [1, 2]. Ingestion of *S. suis* serotype 2 (SS2) contaminated food was identified as a risk factor for meningitis caused by SS2 in adult patients in Vietnam and Thailand [2], indicating that the gastrointestinal tract (GIT) might serve as an entry site of SS2 infection in humans [3, 4].

The upper respiratory tract (URT) is considered the main entry site of *S. suis* infection in pigs [5]. However, recent studies showed that the porcine GIT is rapidly colonized by *S. suis* after weaning [6] and that bacterial entry through the GIT occurs in piglets after mild stress induction [7], indicating that also the porcine GIT is an entry site of *S. suis* infection.

The capsular polysaccharide (CPS), which determines the serotype, appears to play an essential role during the interaction with the URT epithelium [8]. Suilysin (Sly), a hemolytic pore-forming toxin, may also play a role facilitating translocation across the mucosal epithelium [9].

SS2 is responsible for most reported infections in humans and pigs worldwide [1, 10]. However, *S. suis*

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serotype 9 (SS9) is the most common serotype isolated from diseased pigs in Northern Europe [11] and is also prevalent in China [12] but has never been isolated from human patients [1, 10]. It is not known why isolates with serotypes that are highly prevalent among diseased pigs, such as SS9, have never been reported from human cases of SS infections.

In addition to serotype, the genotype contributes to virulence of *S. suis*. Invasive *S. suis* strains are limited to certain sequence types (ST) and multi locus sequence typing (MLST) clonal complexes (CC) [13]. The majority of the SS2 strains isolated from human patients belong to CC1. However, we recently identified SS2 isolates from human patients in The Netherlands belonging to CC20, which is unrelated to CC1, indicating that multiple zoonotic clones are circulating worldwide [11]. In contrast, Dutch SS9 strains isolated from diseased pigs mainly belong to CC16, which is unrelated to both CC1 and CC20 [11, 14].

We aimed to provide experimental proof of concept that *S. suis* is capable of translocation in the GIT, in the absence of any invasive procedures. We then studied strain- and host-specific factors that may modulate *S. suis* infectivity at the intestinal mucosal surface, potentially providing clues to the zoonotic potential of SS2.

METHODS

Animal Studies

The Danish Animal Experiments Inspectorate reviewed and approved the animal study protocol (2012-15-2934-00289). Twenty specific pathogen free male piglets weaned at 5 weeks of age, were transferred to the ABSL3 facilities at National Veterinary Institute (DK), 2 weeks before challenge at 7 weeks of age. All animals were SS2 free as determined by culture and real-time polymerase chain reaction (PCR) of tonsil swabs taken upon arrival. During the experimental period, 4 control and 16 intervention piglets were housed in 2 separate animal boxes each, with double HEPA filtered air. The pigs were fed with a controlled feed (9033, Altromin, Germany) twice daily. The day of the experiment, piglets were transported for 1 hour over a winding road immediately after feeding, to induce a mild stress [15]. After the transport, the piglets were fed a gastric-acid resistant capsule (23.3 × 8.53 mm, volume 0.95 mL) (Spruyt-Hillen, NL) containing 1.2×10^9 CFUs/mL SS2 strain 3 (tetracycline resistant) grown in exponential phase [16], or milk powder (controls). Feces was collected in stoma bags. Piglets were monitored every 2 hours for clinical symptoms; body weight and temperature were measured daily. Daily blood samples were collected for culture and white blood cell (WBC) counts and differentiation [17–19]. Rectal and tonsil swab samples (Puritan, USA) were collected daily for bacteriological examination. The animals were killed 72 hours postinoculation or at occurrence of clinical signs, followed by necropsy. Tissue specimens from tonsils, ileum, and mesenteric lymph nodes were collected for examination.

Microbiology

Rectal and tonsil swabs were cultured overnight in selective Todd-Hewitt broth (sTHB) supplemented with Streptococcus Selective Supplement (Oxoid), tetracycline (10 µg/mL) and 0.01% crystal violet (2 mg/mL) (Sigma-Aldrich), and stored at −80°C in 15% of glycerol.

Tissue swab samples were plated on Columbia agar plates containing 6% horse blood (Oxoid) and tetracycline. Ten grams of tissue were thoroughly crushed and stored in sTHB-15% glycerol at −80°C. SS2 was identified by colony morphology, and agglutination with specific antisera (SSI Diagnostica, DK), as well as by PCR, as described previously (Supplementary Table 1)[18, 19].

Histology

Tissue sections, embedded in paraffin, were stained with haematoxylin–eosin for histological evaluation. For immunohistochemistry, tissue sections were stained for 2 hours with rabbit monoclonal anti-SS2 antibody (SSI Diagnostica, DK), followed by BrightVision Alkaline Phosphatase (AP)-conjugated anti-rabbit immunoglobulin G (IgG; Immunologic) and Vector Red (Vector Labs). Slides were counter stained with haematoxylin, evaluated by light microscopy (Leica DMRE), and images were captured using Nuance software.

Bacterial Strains and Intestinal Cell Lines

Strains studied are listed in Supplementary Table 1 [20, 21]. All strains were grown in THB or on Columbia blood agar in presence of 5% CO_2. Caco-2 cells (HTB-37, ATCC) and IPEC-J2 cells (ACC 701, DSMZ) were grown as described [22, 23]. Both cell lines were seeded at 1×10^5 cells/cm^2 and incubated up to 15–20 days.

Construction of a *S. suis* Serotype 9 Capsule Mutant

An unencapsulated mutant (8067*cps*ΔE) was constructed in the wild-type (WT) SS9 strain 8067 containing the intact *cps*9E gene (AF155805_2) by insertion of spectinomycin (*spc*) resistance cassette (Supplementary Table 1). The resulting fragment *cps*9E-*spc* was amplified and ligated to the thermo-sensitive vector pSET5 carrying chloramphenicol resistance [24]. The pSET5-*cps*9E-*spc* plasmid was then introduced into SS9 8067 by electroporation, and transformants were selected as described [24]. The construct was verified by PCR and Southern blotting.

Adherence and Invasion Assays Using Caco-2 and IPEC-J2 Cells

Bacteria were added to 24-well plates containing confluent cells at a multiplicity of infection (MOI) of approximately 10 bacteria/cell. The MOI was calculated based on the number of cells/

well at confluent state (Caco-2 approximately 5.0×10^5, IPEC-J2 appproximately 2.2×10^5). Adhesion and invasion assays were performed as previously described [25, 26]. The total number of cell-associated bacteria (extracellular and intracellular) recovered after 2 hours of incubation, was expressed as a percentage of the original inoculum. The percentage of bacteria that invaded the cells, was calculated by dividing the number of intracellular bacteria (gentamicin/penicillin treatment survivors) by the number of bacteria in the start inoculum. The percentage of adherent bacteria was calculated by subtracting the percentage intracellular bacteria from the percentage of cell-associated bacteria. Adhesion and invasion tests were performed in duplicate or triplicate as indicated, by 2 different operators working in parallel. SS2 strain BM407 was included in each experiment to control for inter-experiment variation. Low, medium, and high adhesion were defined as adhesion of 0%–10%, 10%–20%, and ≥20% of starting inoculum, respectively.

Translocation Assay

Cells were cultured on 12-Transwell permeable polycarbonate filters (3-μm pore size, Corning). The Trans Epithelial Electric Resistant (TEER) across the monolayer was measured using an epithelial tissue Volt-Ohmmeter (Millipore) every 3–4 days, and the cells were used when a plateau was reached (approximately 500 Ω/cm^2 Caco-2, approximately 300 Ω/cm^2 IPEC-J2). Bacteria were added to the upper chamber at MOI 50 and coincubated with EICs for 6 hours. At each 2-hourly sampling, medium in the lower chamber was replaced to prevent growth of translocated bacteria. Samples of the upper and lower chamber were taken every 2 hours and plated. We calculated the percentage bacterial translocation by dividing the CFUs number in the lower chamber by the CFUs number in the upper chamber. Data are given as mean ± SEM of at least 3 independent experiments, performed in triplicates by 2 different operators. SS2 BM407 was included in each experiment. Low, medium, and high translocation were defined as 0%–0.2%, 0.2%–1.0%, and ≥1.0% of starting inoculum, respectively.

Confocal Immuno-fluorescence and Transmission Electron (TEM) Microscopy

Differentiated cells were grown on sterile glass slides pretreated with Poly-L-lysine (Sigma-Aldrich) and infected with *S. suis* at MOI 50 for 4 hours. Mouse-anti-occludin antibody (Jackson ImmunoResearch) and rabbit anti-CPS-SS2 antibody were incubated at room temperature (RT) for 2 hours followed by anti-Mouse-Alexa 488 or anti-Rabbit-Cy3 (Jackson ImmunoResearch) to visualize occludin and bacteria, respectively. Nuclei were stained using DAPI. The slides were analyzed with laser scanning confocal microscopy (Leica TCS SP8X).

For TEM, Caco-2 cells were cultured in PET transwells and infected with SS2 with MOI 100 for 4 hours. The MOI was enhanced to increase the number of events for TEM analysis. TEM images were produced as described elsewhere [27] using FEI Tecnai T12 microscope.

Statistical Analysis

Data were analyzed using GraphPad Prism version 6.0 (GraphPad Software, USA). Data were expressed as means with standard errors (SEM). Adhesion, invasion, translocation, and TEER values were compared by 1-way analysis of variance (ANOVA) followed by a multiple pair-wise comparison of sample means of ranked data. Kruskal–Wallis nonparametric test was used to determine the significance of the differences in adhesion, invasion, and translocation between *S. suis* strains of different serotypes and genotypes.

RESULTS

S. suis Translocates the Porcine Intestinal Mucosa in Vivo

Two out of 16 orally challenged piglets showed signs and symptoms of systemic infection (Supplementary Table 2). Piglet 11 developed arthritis, and SS2 was cultured from its joints at 48 hours postinoculation (Supplementary Table 2). In addition, SS2 DNA was detected in blood and cerebellum by SS2 specific PCR (Supplementary Table 3). Piglet 10 developed signs of sepsis within 12 hours postinoculation, and a severe peri-esophageal inflammation in the anterior neck region was found during post-mortem analysis. SS2 was cultured from blood and multiple organs (Supplementary Table 2). The capsule was found in the laryngeal mucosa during post-mortem analysis of piglet 7 suggesting that the capsule had not reached the stomach. This piglet was excluded from further analysis. Two piglets (6 and 8) showed mild nonspecific symptoms (Supplementary Table 3). One piglet in the control group had fever detected at planned necropsy 72 hours postinoculation. The remaining piglets did not exhibit any clinical signs of SS infection until euthanasia, and infection related gross pathological changes or changes at the inoculation site were not observed at post-mortem analysis. However, culture of joint fluid of piglet 12 yielded SS2.

In 6 out of 15 challenged piglets, intestinal mesenteric lymph nodes (IMLNs) were positive in PCR (Supplementary Table 3). For all positive IMLNs, with the exception of IMLNs from piglet 14, the presence of viable SS2 was confirmed by culture. Intestinal scrapings were positive by PCR in 2 of the 6 piglets with positive IMLNs and in 1 additional piglet. All tonsils swabs were negative at $t = 0$ before challenge, whereas 7 piglets (4 of which with positive IMLNs), had SS2 positive tonsil swabs after 48 hours (Supplementary Table 3). In none of the control piglets and the remaining 6 challenged piglets, SS2 was detected in any of the sampled sites and organs by PCR.

Histological analysis of the intestinal mucosa did not reveal pathological changes in the epithelium. In addition, we did not observe influx of granulocytes in the lamina propria of the intestinal mucosa or into the sinuses of the IMLNs (data not shown).

Immunohistochemistry of jejunum and colon sections of piglet 11 indicated the presence of SS2 in the apical part of the mucosa (Figure 1). SS2 was detected by immunohistochemistry in tonsil sections of 6 infected piglets, all of which were positive in PCR.

Adhesion to Human and Porcine IEC is Associated With Serotype and Genotype

We included 48 *S. suis* strains isolated from human patients with meningitis, diseased and carrier pigs, and originating from The Netherlands and Vietnam in a period from 1982 to 2008 (Supplementary Table 1).

Serotype was significantly associated with adhesion to host cells of different origin (Figure 2*A* and 2*B*). SS2 strains showed significantly more adhesion to human Caco-2 cells compared with SS1 and SS9 strains (Figure 2*A*). In contrast, SS9 strains adhered more efficiently to the porcine IPEC-J2 cells compared with SS2 and SS1 strains (Figure 2*A*). In a genotype comparison, strains of CC20 and CC1 showed significantly more adhesion to Caco-2 cells than the strains of CC13 and CC16 (Figure 2*B*). In contrast, strains of CC16 adhered significantly better to IPEC-J2 cells than strains representing all the other genotypes (Figure 2*B*). Thus, we observed differential adhesion of *S. suis* to IECs of human or porcine origin, which correlated with the serotype of the strains.

All strains tested showed poor invasion of Caco-2 cells, with the exception of a subset of five SS1 strains of CC13 (Figure 2*C*). Invasion of IPEC-J2 cells was virtually absent, in particular for SS1 and SS9 strains (Figure 2*D*).

We observed a significant increase in adhesion to Caco-2 cells for both unencapsulated mutant strains compared to their parent strains (Supplementary Table 1; Figure 3*A*). The unencapsulated SS9 showed higher adhesion capacity to porcine IPEC-J2 compared to its parent, whereas the unencapsulated SS2 had a similar adhesion capacity compared to its parent.

Both unencapsulated mutants were able to invade both human and porcine IECs more efficiently than their parent strains. However, the unencapsulated SS2 strain appeared to have a higher invasion capacity compared to the SS9 mutant strain for both cell lines ($P < .05$; Figure 3*B*).

Translocation Capacity Across Human Polarized IEC is Associated With Genotype

We did not observe significant differences in translocation capacity between strains of different serotypes (Figure 4*A*). In contrast, when comparing translocation across CCs, significantly higher mean translocation percentages for CC1 strains (0.95%) compared to CC20 strains (0.30%) and CC16 strains (0.31%) were observed ($P < .05$). Although the small number

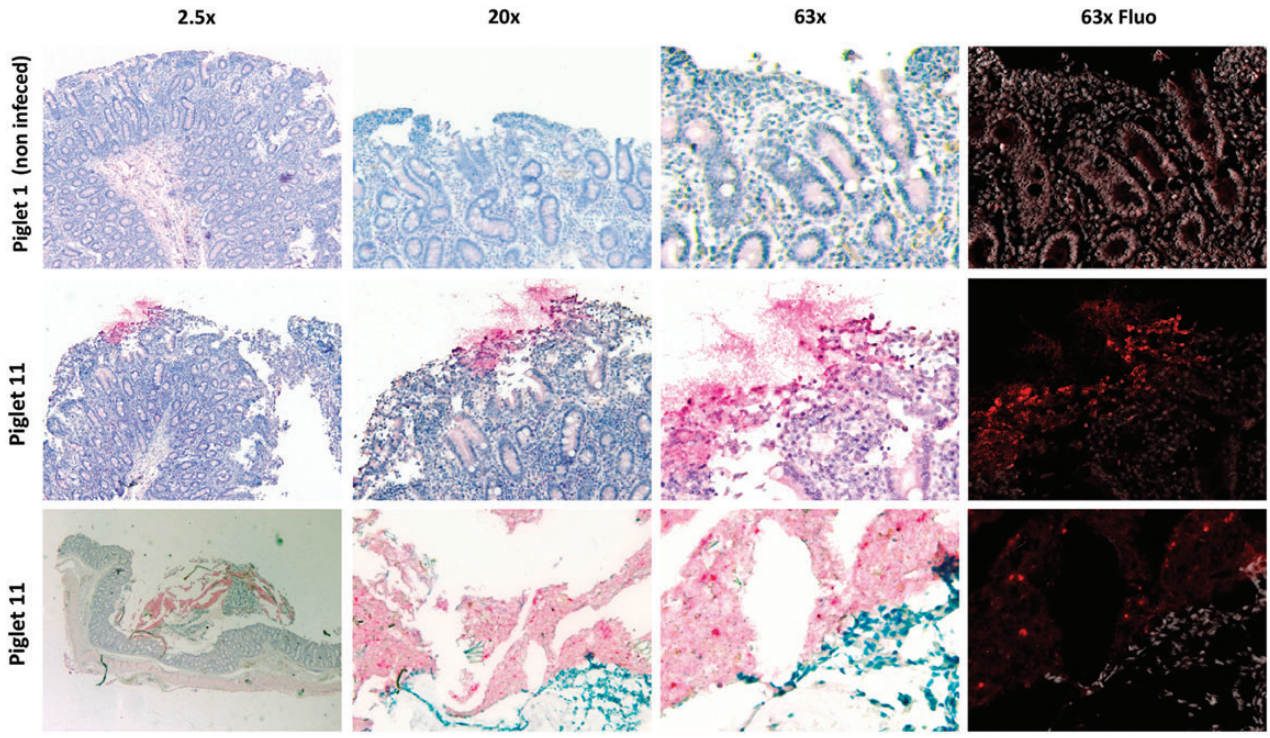

Figure 1. *S. suis* serotype 2 (SS2) in intestinal mucosa tissue derived from SS2 free piglets after oral SS2 challenge. Multi-spectral imaging of immunohistochemistry staining of intestinal tissue of piglet 1 (not infected, jejunum) and piglet 11 (jejunum and colon). Sections were stained with hematoxylin 1×. SS2 was detected using rabbit monoclonal anti-SS2 antibody. Slides were evaluated by light microscopy using 2.5×, 20× and 63× immersion oil objectives in digital bright field image and digital fluorescence (Nuance software).

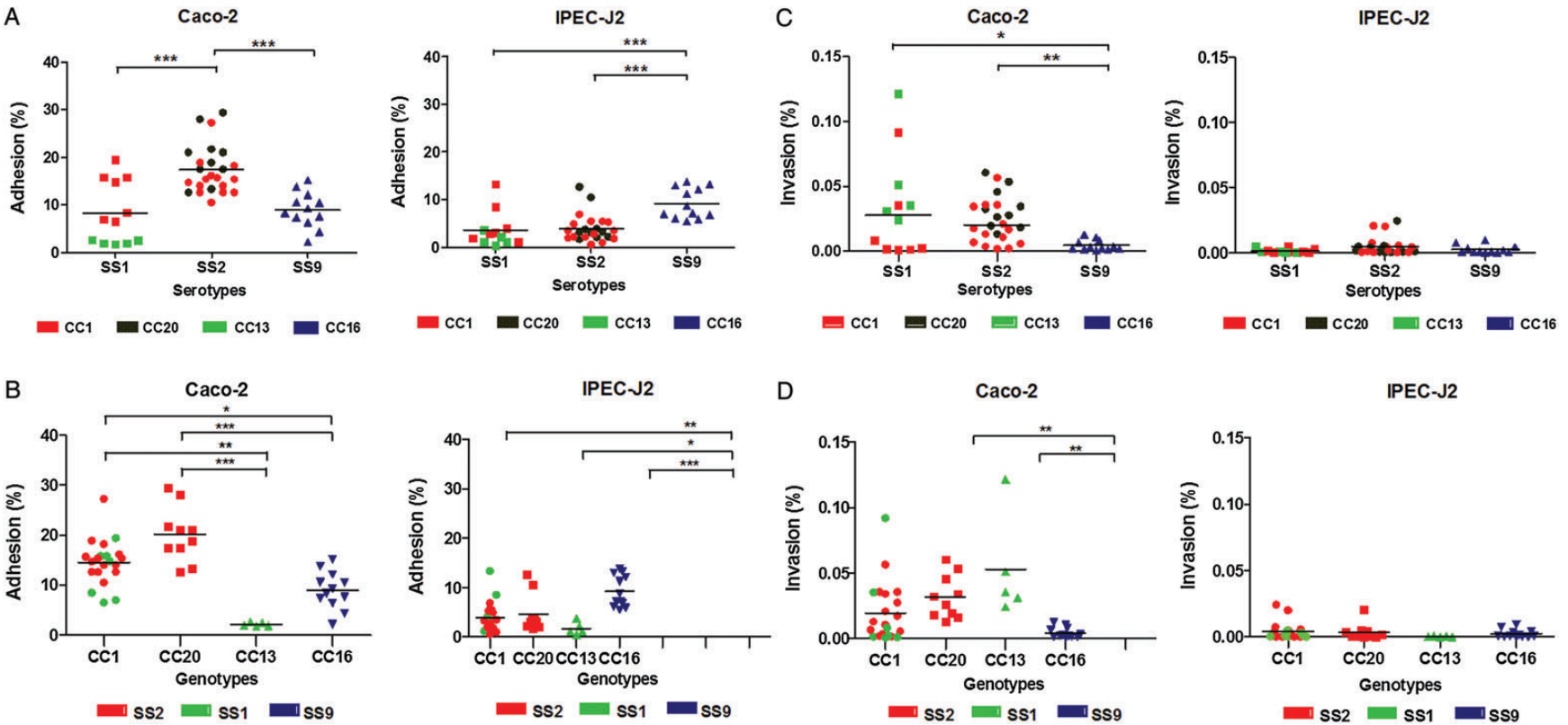

Figure 2. *S. suis* strains associated with different ability to adhere to and invade the human and porcine IEC. *A* and *B*, Multiple comparisons of *S. suis* strains with different serotypes (SS) (*A*) and genotypes (CC) (*B*) for their adhesion to human (Caco-2) and porcine (IPEC-J2) EIC. *C* and *D*, Multiple comparisons of *S. suis* strains with different serotypes (SS) (*C*) and genotypes (CC) (*D*) for their invasion capacity to Caco-2 and IPEC-J2 cells. Serotypes analyzed: SS1 ■, SS2 • and SS9 ▲. MLST Clonal Complex (CC) CC1, CC20, CC13 and CC16 strains are indicated in red, grey, green, and blue respectively. Genotypes analyzed: CC1 •, CC20 ■, CC13 ▲, and CC16 ▼. SS2, SS9 and SS1 strains are indicated in red, blue and green, respectively. Lines denote the median percentage of adhesion for each group of strains. *$P < .05$; **$P < .01$; ***$P < .001$ (Kruskal–Wallis test).

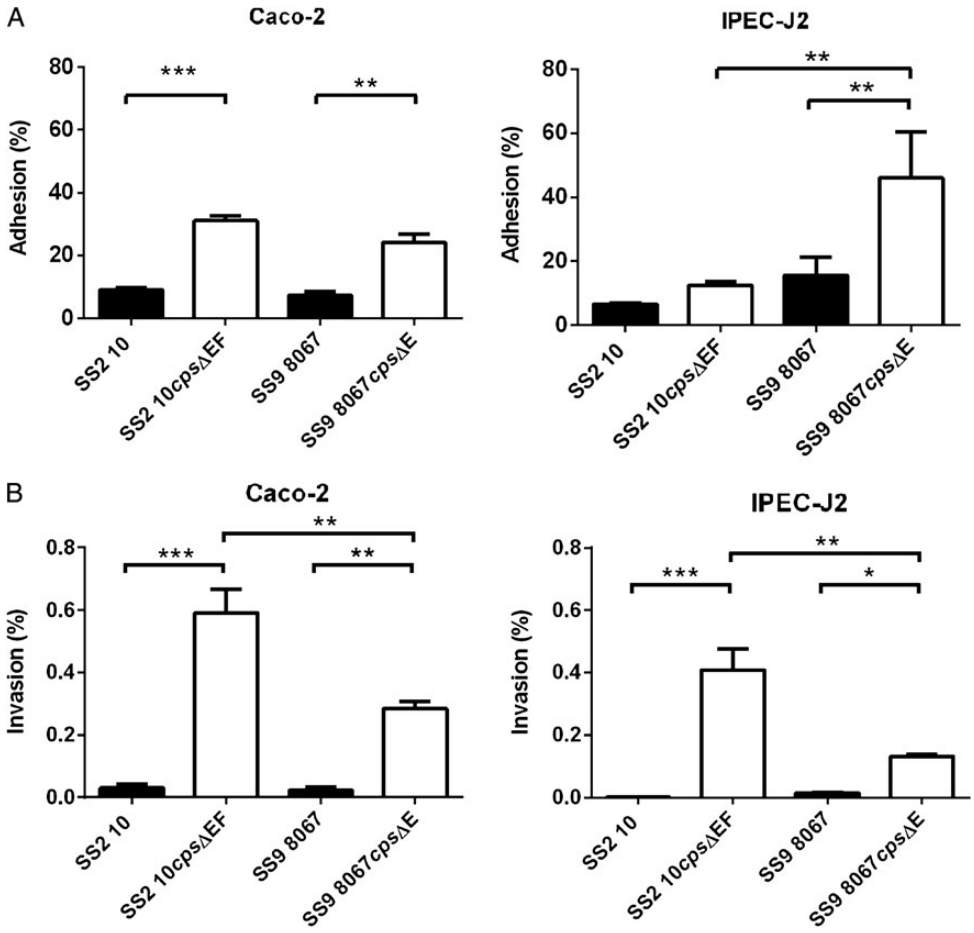

Figure 3. The polysaccharide capsule hampers *S. suis* adhesion to and invasion of human and porcine IEC. *A*, Adhesion and (*B*) invasion of Caco-2 and IPEC-J2 cells displayed by the wild type (WT) strains SS2 strain 10 and SS9 strain 8067 and their isogenic unencapsulated mutants 10*cps*ΔEF and 8067Δ*cps*ΔE respectively. Data are given as means ± standard error of the mean (SEM) of at least 2 separate experiments performed in triplicate and analyzed by 1-way analysis of variance (ANOVA). **P* < .05; ***P* < .001.

of CC13 strains precluded statistical analysis, results suggest a low translocation capacity. Strains of CC20 did not translocate efficiently across the epithelial monolayer, with the exception of three strains (Figure 4*B*).

For a subset of strains, which demonstrated high or low translocation capacity across Caco-2 cells, we analyzed the translocation across IPEC-J2 cells. We observed similar translocation capacity across both IECs (Supplementary Figure 1*A*).

Cell monolayers were still viable after 6 hours of bacterial coculture as determined with propidium iodide [28] (data not shown). We measured the change in TEER induced by strains of each serotype and genotype under study, which showed high and low translocation (HT and LT, respectively) (Supplementary Figure 1*B*). TEER values decreased by 20% after 2 hours of infection but returned to baseline levels after 6 hours for HT strains, indicating that monolayer integrity was maintained for the duration of the 6-hour assay. For LT strains, an increase in TEER of approximately 30% from baseline after 6 hours of infection was observed.

The 2 unencapsulated mutant strains showed 2-fold increased translocation capacity compared to the capsulated WTs over all time points tested (data for 6-hour infection shown in Figure 4*C*).

SS2 strain P1/7 strain translocated across Caco-2 cells slightly better than its isogenic P1/7Δ*sly* suilysin mutant [20] after 6 hours of co-incubation. In contrast, the P1/7Δ*sly* mutant strain exhibited a significantly reduced translocation across IPEC-J2 cells after 6 hours of coincubation (Figure 4*D*).

S. suis can Damage the Tight Junctions (TJ) of IEC Monolayers

SS2 strain BM407 did not cause macroscopic damage to the monolayer after 2 hours of coincubation, but we observed disruption of cell borders with reduced staining of TJ (Figure 5). Bacteria clustered at the junctions between Caco-2 cells. Confocal microscopy of Caco-2 and IPEC-J2 cells, incubated with strains with high, medium and low adhesion/translocation capacity (Figure 5), showed a strong correlation between TJ damage and the adhesion/translocation capacity of the strains.

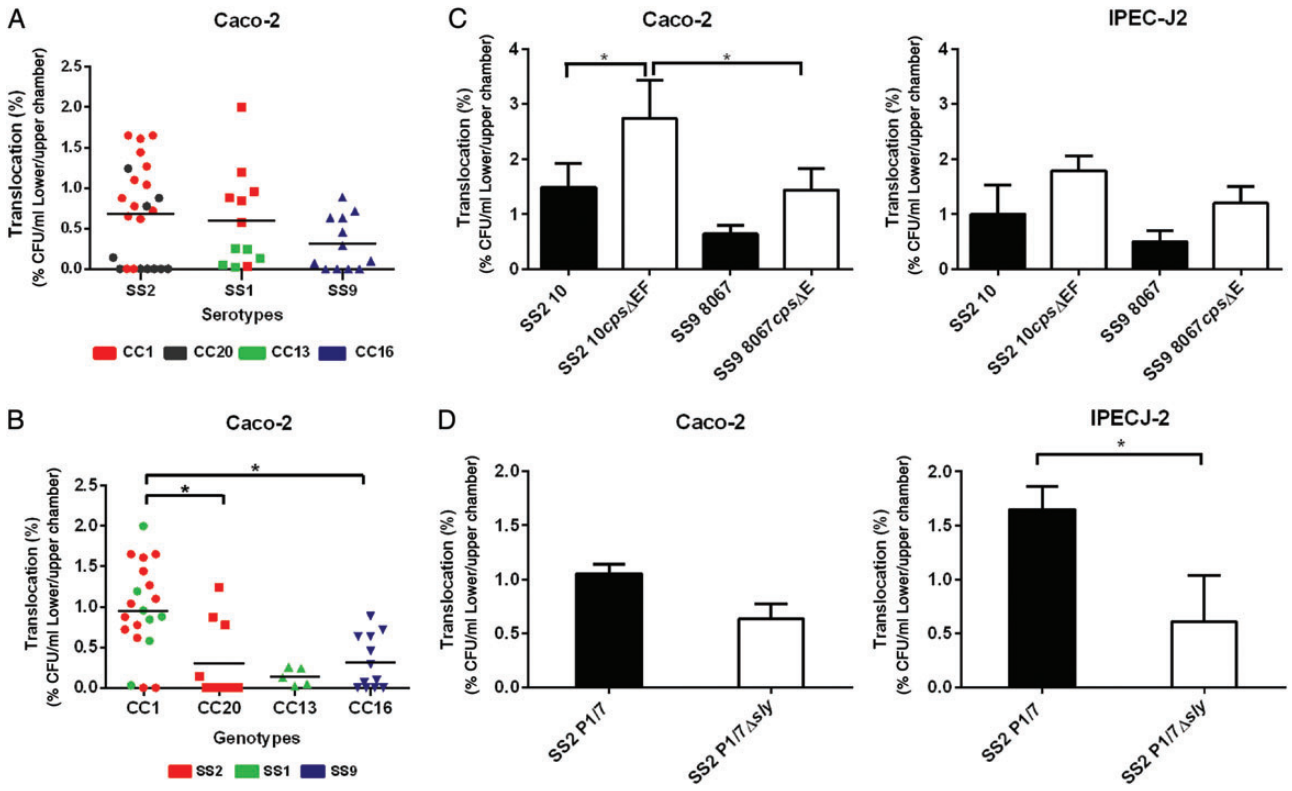

Figure 4. Genetic background of *S. suis* determines the translocation across human IEC. *A*, Multiple comparisons of *S. suis* translocation across differentiated Caco-2 monolayers after 6 h of co-incubation, analyzed by *S. suis* serotype (SS): SS1 ■, SS2 • and SS9 ▲. MLST Clonal Complex (CC) CC1, CC20, CC13 and CC16 strains are indicated in red, grey, green, and blue respectively. *B*, Multiple comparisons of *S. suis* translocation across differentiated Caco-2 monolayers after 6 h of co-incubation, analyzed by *S. suis* genotype: MLST clonal complex (CC) CC1 •, CC20 ■, CC13 ▲, and CC16 ▼. SS2, SS9 and SS1 strains are indicated in red, blue, and green, respectively. Lines denote the median percentage of translocation for each group of strains, analyzed by Kruskal–Wallis test. *C*, Comparison of bacterial translocation efficiency of unencapsulated (10*cps*ΔEF and 8067Δ*cps*ΔE) mutant strains and their wild-type parent strains across differentiated human (Caco-2) and porcine (IPEC-J2) IEC. *D*, Comparison of bacterial translocation efficiency of a suilysin mutant (P1/7Δ*sly*) and its wild-type parent strain across differentiated human (Caco-2) and porcine (IPEC-J2) IEC. Data are given as means ± standard error of the mean (SEM) of at least 2 independent experiments, each performed in triplicate and analyzed by 1-way analysis of variance (ANOVA). **P* < .05; ***P* < .01; ****P* < .001.

Three-dimensional confocal imaging showed clusters of fluorescent bacteria, some of which co-localized with TJ (Figure 6*A*). Z-stack images from the apical (0 µm) to the basolateral (180 µm) side confirmed that SS2 BM407 bacteria could be found in close contact with the TJ and at the basolateral side of the monolayer (Figure 6*A*). Caco-2 cells infected with SS2 strain BM407 were characterized by a deteriorated microvilli brush border when compared to uninfected Caco-2 cells (Figure 6*B*). Adherent bacteria were observed in close contact with the apical microvilli of the intestinal cells. Translocating bacteria were found in close vicinity of TJ as well as in intracellular vacuole (Figure 6*B*).

DISCUSSION

We have provided experimental evidence that the GIT represents an entry site of *S. suis* infection. Two out of 15 evaluable piglets developed clinical symptoms after oral challenge with SS2. Our results are in accordance with a previous study on *S. suis* intestinal translocation, in which *S. suis* were recovered from the organs of 3 out of 11 piglets infected through a catheter placed surgically in the duodenum [7]. The relatively small number of diseased animals after oral infection may be explained by a slow translocation process of *S. suis* through the intestinal mucosa, which is dependent on multiple factors, including host intestinal homeostasis, immune clearance mechanisms, and bacterial growth rate [22, 29]. A longer observation period may have resulted in more piglets with clinical symptoms of infection. One line of defense in intestinal homeostasis includes the IMLNs [29, 30]. Viable *S. suis* were detected in the IMLNs of 6 infected piglets (40%) indicating bacterial intestinal translocation (Supplementary Table 3). *Listeria monocytogenes* was shown to migrate extracellularly to IMLNs or to be carried inside migratory phagocytes such as dendritic cells (DCs) [30]. *S. suis* was also shown to bind and survive inside DCs for

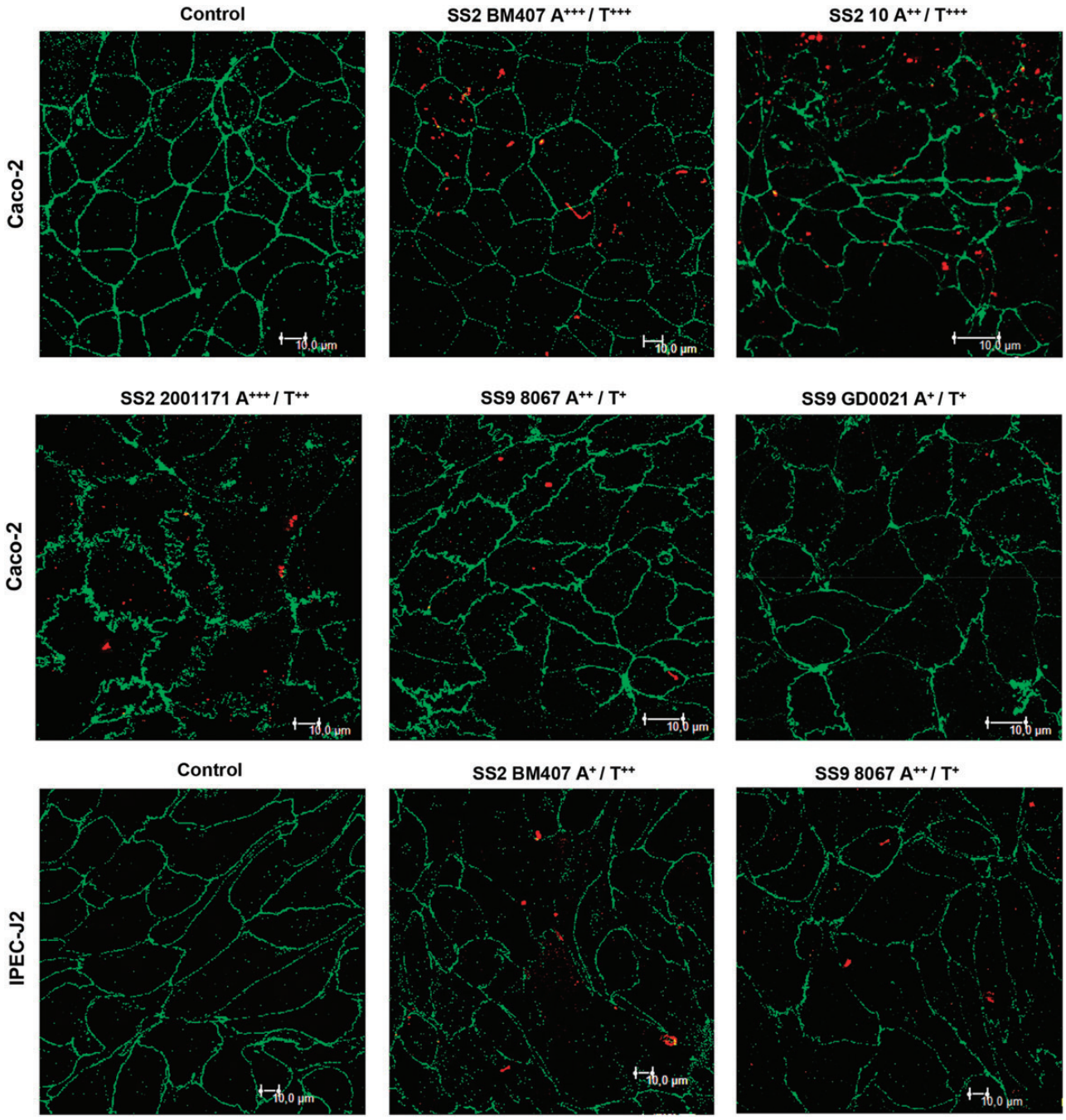

Figure 5. *S. suis* strains with high translocation capacity induce re-arrangement of Tight Junction (TJ) of Caco-2 and IPEC-J2 cells. Confocal imaging of *A*, Caco-2 and *B*, IPEC-J2 intestinal epithelial cells stained for TJ protein occludin (green) in the absence (control) and in the presence of *S. suis* strains with low ($^{+}$) medium ($^{++}$), high ($^{+++}$),adhesion (A) and translocation (T) ability, after 4 h of co-incubation (see "Materials and Methods" section for definitions of low, medium, and high). Bacteria (red) were stained with rabbit monoclonal antisera raised against capsular polysaccharides of serotype 2 and were counterstained with Alexa Fluor 488–conjugated anti-rabbit antibodies. Co-localization of bacteria and TJ (yellow). Bar 10 µm.

24 hours [31]. Bacterial spread via the lymphatic system may explain how not only IMLNs but also tonsils became SS2 positive. However, we cannot rule out that tonsils were colonized shortly after inoculation, for example, due to gastro-esophageal reflux after the ingested capsule had dissolved.

In order to gain insight into how *S. suis* interacts with intestinal epithelial cells, we established an in vitro model using human (Caco-2) and porcine (IPEC-J2) intestinal epithelial cells. SS2 strains, including strain 3 used for oral challenge of piglets, adhered significantly better to human Caco-2 cells,

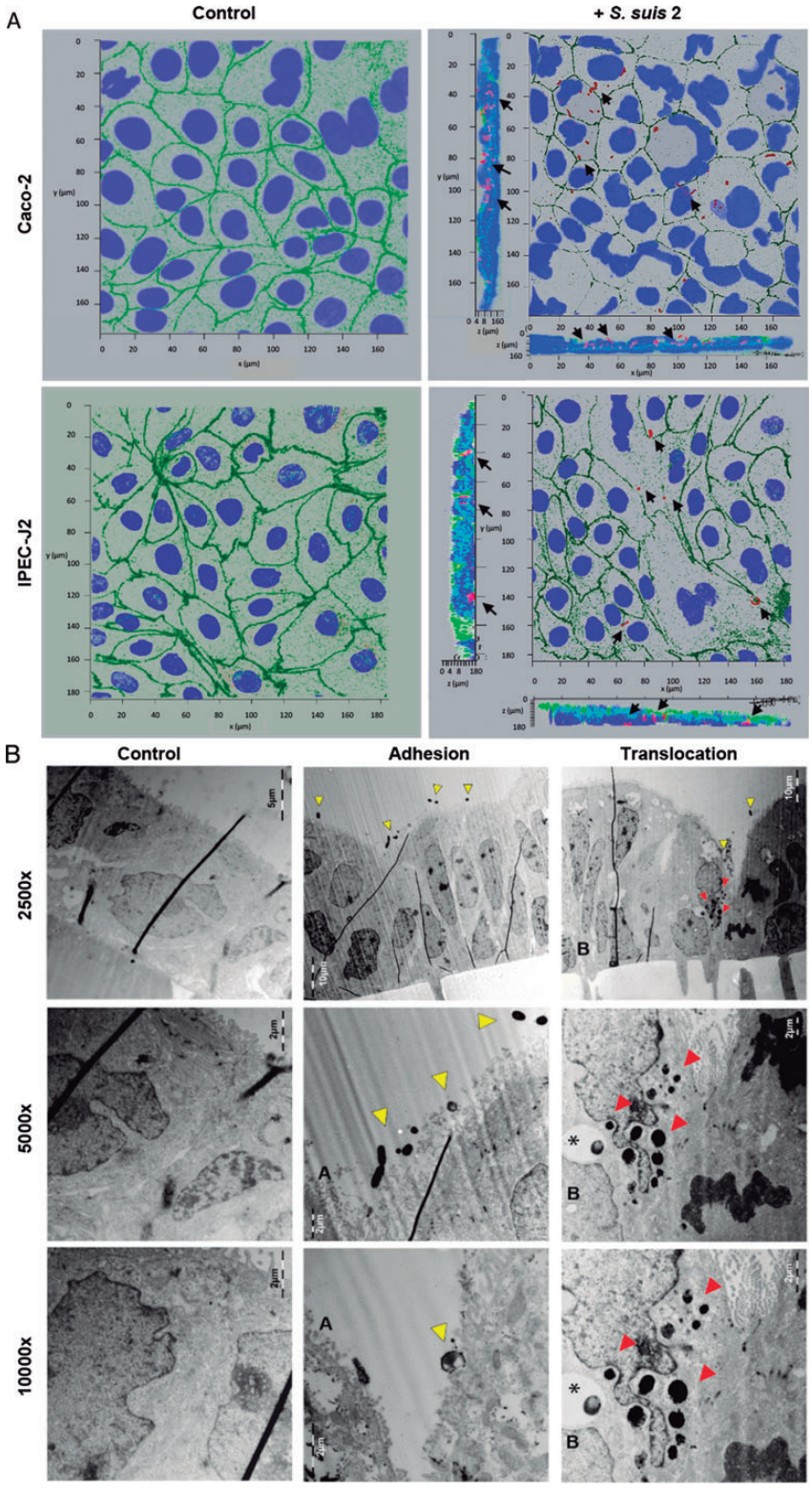

Figure 6. *S. suis* co-localizes with cellular junctions and translocates from the apical to the basolateral side of polarized IEC. *A*, 3D confocal images of Caco-2 and IPEC-J2 cell layers stained for the TJ protein occludin (green) in the absence (control) and in the presence of *S. suis* 2 BM407 (red) after 4 h incubation. The nuclei of intestinal cells are stained with DAPI (blue). Lower and side strips are relative to x and y sections through the monolayer where Z-stack images are made from the apical (0 µm) to the basolateral (180 µm) side. Bacteria stained in light pink indicate co-localization with occludin (black arrow). *B*, TEM of differentiated Caco-2 cells fixed at 4 h after infection with *S. suis* serotype 2 strain BM407. SS2 strain BM407 adheres to the apical microvilli after apical infection (yellow arrows). Translocating bacteria (red arrows) are found in close vicinity to tight junctions and within a vacuole (*). Images were made at magnifications of 2500×, 5000× and 10 000×. Bar: 10, 5 or 2 µm as indicated.

whereas SS9 strains showed higher adhesion to porcine IPEC-J2 cells. Adhesion increased significantly in the absence of the polysaccharide capsule of serotypes 2 and 9. The *S. suis* capsule thus appears to influence bacterial adhesion through its primary structure as well as by partially masking of bacterial adhesins, reducing the binding of bacteria to the host cells. Differences in primary structure of capsule, such as differences in sialic acid content, may contribute to the differences in cell-microbe-interaction during adhesion between SS2 and SS9 strains. Several adhesins of *S. suis* have been described [32], including the Streptococcal Adhesin P (SadP) [33], which was shown to bind to galactosyl-(α1,4)-galactose-containing glycolipids present in the cell membranes of human and porcine IEC, such as Caco-2 and IPEC-J2 cells [34, 35]. Analysis of available protein sequences of SadP, reveals substantial variation at amino acid level across different serotypes and genotypes (data not shown). Differences in adhesin structure and/or in receptor affinity may provide a clue to the observed differences in adhesion to IEC between strains and hosts.

Several enteric pathogens can migrate across a polarized epithelium using a transcellular and/or paracellular route [36, 37]. In addition, other streptococci were shown to translocate across Caco-2 cells using a paracellular route [27, 38, 39]. Thus, paracellular passage of epithelial cells is a common mechanism applied by bacterial pathogens to penetrate the intestinal mucosa. For *S. suis*, the production of suilysin is not essential, whereas the capsule appears to interfere with this passage. Expression of virulence features, including capsule and suilysin, are regulated by environmental pressures such as nutrient availability [25]. Changes in these environmental conditions during passage or colonization of the human or porcine GIT may induce changes in virulence factors expression such as capsule, which favor subsequent bacterial translocation.

SS2 is the most common cause of zoonotic *S. suis* infection [1, 40]. Human carriage of SS2 is probably very rare [3] and human-to-human transmission has never been documented. It is striking how the interaction of *S. suis* of different serotypes with different host cells correlates with zoonotic potential; invasive strains isolated from human patients (SS2; CC1, or CC20) showed significantly higher adhesion to Caco-2 cells of human origin compared to a representative selection of invasive strains isolated from pigs (predominantly SS9; CC16). In contrast, these same strains isolated from pigs showed significantly higher adhesion to IPEC-J2 cells of porcine origin.

In addition, translocation across human EICs was associated with CC1, which is the genotype most commonly found in human patients, even if other genotypes are more prevalent in porcine disease, as was demonstrated in The Netherlands [11]. However, the majority of human isolates belonging to CC20 showed a low translocation capacity across Caco-2 cells. We hypothesize that virulent *S. suis* strains, including CC20, can use multiple mechanisms of infection and spread into the host, after initial adhesion to host epithelial cells. The intestinal route of infection is less likely to occur in The Netherlands because high-risk *S. suis* contaminated food, such as raw pig blood or fermented raw pork, is rarely on the menu. Direct passage of *S. suis* into the blood stream, after exposure to slaughtered pigs in the presence of skin injuries, is the most common route of infection and CC20 strains may be well equipped to achieve this.

CONCLUSIONS

SS2 infection is a food-borne disease and interventions to reduce colonization of piglets with highly virulent *S. suis*, in the benefit of both veterinary and human health, should include interventions to reduce gastro-intestinal colonization.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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