THE ROLE OF SHDA IN FAECAL SHEDDING OF SALMONELLA TYPHIMURIUM IN PIGS

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Abstract Prolonged faecal shedding of Salmonella in pigs contributes to contamination of carcasses. The shdA gene has been characterized as an important locus for persistency of Salmonella Typhimurium in mice. The aim of this study was to assess the contribution of ShdA in faecal shedding of S. Typhimurium in pigs.

Pigs were orally inoculated with a S. Typhimurium strain or its isogenic shdA mutant strain. For the first few days after inoculation, the shdA mutant strain was more virulent than the wild type strain, as indicated by higher excretion levels, more pronounced diarrhea and higher numbers of infected organs. No effect on long-term shedding was found. Increased in vitro invasion levels of the shdA mutant strain were noticed in intestinal epithelial cells.

In conclusion, a shdA mutant strain of S. Typhimurium is more virulent during the first days after inoculation and is not impaired in persistency or prolonged shedding in pigs.

Introduction S. Typhimurium is an important zoonotic agent. The prolonged excretion of S. Typhimurium in pig faeces is a major risk factor both for human and animal health (Berends et al., 1997; Poppe et al., 1998; Beloeil et al., 2004). It has been estimated that 5-30% of finisher pigs originally infected may still excrete Salmonella at the end of the finishing period, and this percentage can double in periods of stress, for example during transport and lairage (Berends et al., 1996). The mechanisms leading to the carrier state or to prolonged faecal shedding in pigs are unknown.

The CS54 Island of S. Typhimurium has been characterized as an important locus for intestinal colonization and prolonged shedding in mice (Kingsley et al., 2000; Kingsley et al., 2003). The most important component of this island is ShdA, an outer membrane protein which is expressed solely in the intestine and mediates adhesion to fibronectin (Kingsley et al., 2002). A S. Typhimurium strain harbouring a mutation in shdA is shed in reduced numbers and for a shorter period of time in the faeces of mice compared to its isogenic parent strain (Kingsley et al., 2002).

Although these studies are of great value, they were exclusively conducted in mice and the role of ShdA in the pathogenesis of salmonellosis in other animal species is not clear. It was, therefore, the purpose of the present studies to examine whether ShdA contributes to the persistence and shedding of S. Typhimurium in the pig.

Materials and Methods The shdA mutant strain was constructed in the S. Typhimurium MB2486 background. This strain was isolated from a pig stool sample and from carcasses at the slaughterhouse. The deletion mutant was constructed by a one-step inactivation method, using a linear PCR product, mediated by the Red recombinase of bacteriophage lambda (Datsenko and Wanner 2000).

Experimental infections were performed in 4-week-old piglets. The piglets were divided at random into 3 groups: 2 groups of 10 piglets and one negative control group of 6 piglets. They were penned in pairs for the first 5 days and individually for the remainder of the experiment. Group 1 was orally inoculated with 10⁷ cfu S. Typhimurium MB 2486, group 2 was orally inoculated with 10⁷ cfu S. Typhimurium MB 2486 ΔshdA and group 3, the negative control group, was sham-inoculated with 2 ml PBS. At regular time points the rectal temperature was measured and fresh faecal samples were taken from each pig for bacteriological analysis. On day 5 and day 28 post inoculation (pi), 5 pigs of each Salmonella inoculated group and 3 control pigs were euthanized. Samples of various internal organs were taken for bacteriological analysis.

The IPI-2I cell line, derived from the ileum of a boar (Kaeffer et al., 1993), was chosen for invasion assays. IPI-2I cells were seeded in 24 well plates and were allowed to attach and grow until monolayers were obtained. The number of invaded bacteria was assessed using a standard gentamicin protection assay.
**Results** During the first days after oral inoculation of the piglets, the shdA mutant strain was shed in higher numbers than the wild type strain (Figure 1). On days 1 and 2 post inoculation, the difference between the wild type strain and the shdA mutant strain was significant (p≤0.05), on days 5 and 6, there was a trend (p≤0.1) to higher excretion numbers of the shdA mutant strain (Figure 1). In accordance with the excretion data, diarrhea in the group of piglets inoculated with the shdA mutant strain was more pronounced. At day 5, the number of infected internal organs of the piglets inoculated with the shdA mutant strain (21/30) was higher than the number of infected internal organs of the piglets inoculated with the wild type strain (10/30). One piglet inoculated with the shdA mutant strain died suddenly at day 4 pi. The internal organs were massively infected (>106 cfu/gram tissue) with *Salmonella*.

From day 8 until day 28, no significant differences in mean faecal shedding were noticed between piglets inoculated with the mutant and wild type strain (Figure 1). From day 18 on, all animals of both groups shed *Salmonella* intermittently at enrichment levels. At day 28, all animals of both groups were positive for *Salmonella* in the ileum and caecum and only the tonsils were colonized in significantly higher numbers in the piglets inoculated with the shdA mutant strain. The negative control pigs remained negative for *Salmonella* throughout the experiment.

The shdA mutant strain invaded in significantly (p≤0.05) higher numbers in the porcine intestinal epithelial cell line IPI-2I, compared to the wild type strain. The wild type strain showed a mean percentage (± standard error of the mean) of gentamicin protected bacteria of 1.02% (±0.16%) of the inoculum. The shdA mutant strain showed a mean percentage (± standard error of the mean) of gentamicin protected bacteria of 1.37% (±0.09%) of the inoculum.

**Discussion** For the first few days following oral inoculation of the piglets, the shdA mutant strain was found to be more virulent than the wild type strain. This resulted in higher faecal excretion levels, more pronounced diarrhea and higher numbers of infected internal organs. Also 1 piglet inoculated with the shdA mutant strain died. These findings are consistent with some of the results of Kingsley et al. (2003), reporting a threefold increase in excretion at day 5 after inoculation of CBA/J mice with the shdA mutant strain. The *in vitro* results suggest that increased invasion in the intestinal epithelial cells was responsible for both higher colonization levels and more pronounced diarrhea the first days after inoculation.

Although Kingsley et al. (2000, 2003) found a reduction in excretion of the shdA mutant strain in comparison with its isogenic wild type strain by 15 days after inoculation of CBA/J mice, we did not find any signs of decreased shedding of the shdA mutant strain until the end of the experiment (28 days pi). At this time point, the excretion had already reached a very low level (periodically positive after enrichment) in both groups. It is becoming more and more obvious that the pathogenesis of *Salmonella*-infections is strongly host-related (Tsolis et al., 1999; Morgan et al., 2004). Therefore, it might not be surprising to find some bacterial proteins to be of great importance in the colonization of one host, but not in another.
In conclusion, we have shown that ShdA does not contribute to persistence and prolonged shedding in pigs. A deletion mutant in shdA causes more severe clinical symptoms and is excreted in higher numbers during the first days after inoculation, probably because of higher invasion rates in the intestinal epithelial cells.

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References