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Short communication

The fibronectin binding protein ShdA is not a prerequisite for long term faecal shedding of *Salmonella typhimurium* in pigs

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Abstract

Porcine carcasses contaminated with *Salmonella typhimurium* pose significant public health problems. Prolonged faecal shedding of *Salmonella* in pigs contributes to the contamination level of carcasses. Although the mechanism of prolonged faecal shedding is not yet clarified, the CS54 Island, and more specifically the *shdA* gene encoding a fibronectin binding autotransporter protein, was identified as an important locus for intestinal colonization and persistence of *Salmonella typhimurium* in mice. The aim of this study was to assess the contribution of ShdA in faecal shedding of *Salmonella typhimurium* in pigs. Pigs were orally inoculated with a *Salmonella typhimurium* wild type field strain or its isogenic *shdA* mutant strain. For the first few days after inoculation, the *shdA* mutant strain was excreted more, the diarrhoea was more pronounced and higher numbers of internal organs were infected. No effect on long-term shedding was found. In a porcine ileal loop model, the wild type strain and *shdA* mutant strain did not show any differences in the induction of neutrophil influx into the intestinal wall and lumen. In conclusion, we have shown that a *Salmonella typhimurium* deletion mutant in *shdA* is more virulent during the first days after inoculation and is not significantly impaired in persistence or prolonged shedding in pigs.

Keywords: *Salmonella typhimurium*; Pig; shdA; Fibronectin binding protein

1. Introduction

Salmonella enterica subspecies *enterica* serovar *typhimurium* (*Salmonella typhimurium*) is an important zoonotic agent. The prolonged excretion of *Salmonella typhimurium* in pig faeces is a major risk factor both for human and animal health (Berends et al., 1997; 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

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the carrier state or to prolonged faecal shedding in pigs are unknown.

The CS54 Island has been characterized as an important locus for intestinal colonization and prolonged shedding in mice (Kingsley et al., 2000, 2003). The most important component of this island is ShdA, an outer membrane protein of the autotransporter family, which is expressed solely in the intestine. Its passenger domain mediates adhesion to fibronectin possibly through a heparin-mimicking binding (Kingsley et al., 2002, 2004). Fibronectin binding proteins are common in bacterial pathogens and mainly in Gram positive bacteria. Although their specific role in pathogenesis is not always known, they frequently mediate adherence and entry into mammalian cells (Joh et al., 1999; Schwarz-Linek et al., 2004). A sandwich model has been proposed in which fibronectin acts as a molecular bridge between the fibronectin binding proteins on the pathogen and the integrins on the host cells (Joh et al., 1999; Menzies, 2003).

Kingsley et al. (2002) demonstrated that a *Salmonella typhimurium* strain harbouring a mutation in *shdA* was shed in reduced numbers and for a shorter period of time in the faeces of mice compared to its isogenic parent strain. 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 was not investigated. It was, therefore, the purpose of the present studies to determine the role of this fibronectin binding protein in the persistence and shedding of *Salmonella typhimurium* in the pig.

2. Materials and methods

All experiments were approved by the ethical committee of the Faculty of Veterinary Medicine, Ghent University.

2.1. Bacterial strains and growth conditions

Salmonella enterica subspecies *enterica* serovar *typhimurium* (*Salmonella typhimurium*) strain 112910a phage type 120/ad, was isolated from a pig stool sample and was used as the wild type strain. The *shdA* deletion mutant was constructed in this strain.

The inocula for the intestinal loop model were prepared as follows. Cultures in LB medium were shaken at 130 rpm for 18 h at 25 °C. After diluting two-fold with fresh LB, the cells were incubated for 2 h at 37 °C, while shaking at 130 rpm. Afterwards, three syringes of 5 ml were filled with cultures of each strain. The actual number of bacteria ml^{-1} was assessed by plating serial dilutions on McConkey agar (Oxoid, Basingstoke, UK) plates.

For the oral inoculation of pigs, the bacteria were grown in brain heart infusion broth (BHI; Oxoid, Basingstoke, UK) for 6 h at 37 °C. The bacteria were washed twice in phosphate buffered saline (PBS, 2000 × *g* for 10 min at 4 °C). After a third wash with sterile Hanks' balanced salt solution (HBSS) with Ca^{2+}/Mg^{2+} (Gibco, Life Technologies, Paisley, Scotland), the bacteria were resuspended in 10 ml HBSS with Ca^{2+}/Mg^{2+}. The number of viable *Salmonella* bacteria ml^{-1} was determined by plating 10-fold dilutions on Brilliant Green Agar (BGA; Oxoid, Basingstoke, UK).

2.2. Construction of a non-polar shdA deletion mutant

The deletion mutant in *shdA* was constructed according to the one-step inactivation method first described by Datsenko and Wanner (2000) and slightly modified for use in *Salmonella* as described before (Boyen et al., 2006). Primers were designed using the nucleotide sequence with accession number AE008813. The primers *shdA*-P1 5′-aaagggaaatttaaaaattgaacaggacttacagtattgtctggagcgcctgtgtaggctggagctgcttc-3′ (primer homologous with sequence upstream of ATG start codon) and *shdA*-P2 5′-tgtcattcgcctcaaaacgggcagggaacacccgcccggttttgtctaaccatatgaatatcctccttag-3′ (primer homologous with sequence downstream of stop codon) were constructed. These primers and the plasmid pKD4 DNA were utilized to amplify the linear fragment, containing the antibiotic resistance gene *kan*, that was used for the substitution of the gene. The helper plasmid pKD46, encoding the λ Red recombinase, was introduced into *Salmonella* by electroporation, followed by selection on LB agar supplemented with 100 mg carbenicillin l^{-1} (Duchefa Biochemie, Haarlem, The Netherlands). Substitutions of the genes by a kanamycin resistance gene were obtained by electro-