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The *Salmonella* Pathogenicity Island 2 regulator ssrA promotes reproductive tract but not intestinal colonization in chickens

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Abstract

Using a deletion mutant in the regulator of SPI-2, ssrA, we investigated the role of SPI-2 in invasion, intestinal colonization and reproductive tract infection of chickens by *Salmonella* Enteritidis. The *ssrA* mutant was fully invasive in phagocytic and non-phagocytic cells but failed to persist within chicken macrophages. The ability of *Salmonella* Enteritidis to cause disease in orally infected 1-day-old chicks was not altered when *ssrA* was deleted. Furthermore, caecal colonization was not affected, while spleen and liver showed reduced colonization. Following intra-peritoneal and intravenous infection of 1-day-old chicks, internal organ colonization was strongly reduced. After intravenous inoculation in adult laying hens bacterial numbers of the *ssrA* mutant were significantly lower in oviducts and ovaries as compared to the wild type strain. The chickens showed less reproductive tract lesions and the recovery of egg production were faster compared to the wild type strain infected chickens. These findings indicate that the SPI-2 regulator ssrA promotes reproductive tract colonization, but is not essential for intestinal colonization of chickens with the host non-specific serotype Enteritidis.

Keywords: *Salmonella* Enteritidis; *Salmonella* Pathogenicity Island 2 regulator ssrA; Colonization; Poultry

1. Introduction

Salmonella enterica serovar Enteritidis (*Salmonella* Enteritidis) is a facultative intracellular bacterium with a broad host range. It can cause foodborne illness in humans, following the consumption of chicken products (Guard-Petter, 2001).

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Poultry products become contaminated as asymptomatic carrier birds enter the slaughterhouse and asymptomatic carrier hens lay contaminated eggs. Infection of 1-day-old chickens with high doses of *Salmonella* Enteritidis can cause severe clinical salmonellosis with a high rate of mortality, while this serovar produces a chronic asymptomatic carrier state in low-dosed infected young chickens and infected adult birds (Gast and Benson, 1995; Desmidt et al., 1997; Van Immerseel et al., 2004). These carrier birds may excrete the bacteria intermittently until slaughter age (6 weeks) in broilers until the end of the reproduction cycle in layer type chickens and thus constitute an important source of *Salmonella* infection (Gast and Benson, 1995; Desmidt et al., 1997; Humphrey et al., 1991). *Salmonella* Enteritidis specifically interacts with the reproductive organs of hens leading to egg contamination (De Buck et al., 2004). Following oral ingestion, *Salmonella* colonizes the chicken gut, especially the caeca, where it penetrates the mucosal epithelium (Desmidt et al., 1997). Interaction between *Salmonella* and the epithelium triggers infiltration of phagocytic cells to the infected site, where these cells take up the bacteria. *Salmonella* is capable to survive and replicate within bacterially modified macrophage spacious phagosomes, which can be attributed to the type III secretion system (TTSS) encoded by *Salmonella* pathogenicity Island 2 (SPI-2) (Knodler and Steele-Mortimer, 2003). The infected phagocytes disseminate to the internal organs, such as liver, spleen and the reproductive tissues and thus spread the bacteria within the host (Turnbull and Snoeyenbos, 1974; Barrow, 1999).

SPI-2 is an essential virulence locus for systemic disease in host-specific *Salmonella* serotypes including *Salmonella* Pullorum in poultry, and SPI-2 mutants fail to induce systemic disease and clinical signs in the infected animals (Jones et al., 2001; Wigley et al., 2002; Bispham et al., 2001).

The exact role of SPI-2 in the colonization and systemic spread of host non-specific serotypes and in the subsequent development of asymptomatic carriers in chickens however is currently not clear. Therefore, in this study, the role of ssrA in shedding, gut and internal organ colonization was investigated after oral inoculation of 1-day-old chickens with *Salmonella* Enteritidis. Organ colonization was evaluated after intra-peritoneal inoculation of 5-day-old chickens and intravenous inoculation of adult laying hens to assess the role of ssrA in organ colonization, including oviduct and ovaria colonization and egg contamination, after passing the mucosal barrier of the gut.

2. Materials and methods

2.1. Bacterial strain

Salmonella Enteritidis phage type 4, strain 76Sa88 Nal^r was used in the experiments. This strain was isolated from an outbreak of salmonellosis on a poultry farm. The nalidixic acid resistant strain and its 76Sa88 wild type progenitor strain are well characterized (Bohez et al., 2006).

2.2. Construction of defined mutant

A deletion mutant in the *Salmonella* Pathogenicity Island 2 (SPI-2) encoded regulator ssrA was constructed according to the one-step inactivation method, first described by Datsenko and Wanner (2000). The targeted gene, *ssrA* was completely deleted from the start codon till the stop codon, as confirmed by sequencing.

2.3. In vitro experiments

2.3.1. Invasion and survival in T84 and HD11 cell lines

Invasion in T84 human colon carcinoma cell line was performed according to the setup of Bohez et al. (2006). Invasion and proliferation in HD11 chicken macrophage cells were determined by seeding the HD11-cells in a 24-well cell culture plates (Greiner, Frickenhausen, Germany) at a density of 5×10^5 cells/well in culture medium. The bacteria were grown to stationary phase (20 h) at 37 °C with shaking and added to the cells at a multiplicity of infection of 10 CFU/cell. Invasion and proliferation were determined after 1 and 6 h of incubation, respectively. Experiments were performed in triplicate with three repeats in each experiment. Statistical analysis was performed by analysis of variance methods using SPSS 12.0 software.