

# SPI-1 and SPI-2 defective mutants of *salmonella enterica* serovar enteritidis: promising future *salmonella* vaccine for poultry

**Keywords:** *salmonella* vaccine, poultry, SPI-1 and SPI-2 mutants

**Abbreviations:** SPI, *salmonella* pathogenicity island; T3SS, type iii secretion system; *fliC*, gene encoding for flagella; *lon*, gene coding for lon protease

## Editorial

Non-typhoid *Salmonella enterica* serovars are among the most common causative agents of food-borne diseases in human worldwide.<sup>1</sup> Since poultry is the most frequent reservoir of salmonellosis for humans, vaccination of chickens is considered as an effective measure to decrease *S. enterica* prevalence in poultry as well as to decrease *S. enterica* incidence in humans. Within the last 25 years, many live *S. enterica* vaccines have been described and those with inactivated *aro* or *phoP* genes were amongst the first ones tested.<sup>2,3</sup> Currently, construction of attenuated vaccine strains of *S. enterica* is not an issue and many different mutants have been tested in mice, chickens and even humans.<sup>4-7</sup> However, the main dilemma is which mode of attenuation to choose out of the many possibilities. The major pathogenicity islands of *S. enterica* include SPI-1, SPI-2, SPI-3, SPI-4 and SPI-5. The SPI-1 and SPI-2 genes code for proteins forming the type III secretion system (T3SS) which enable the transport of *S. enterica* proteins from the bacterial cell directly into the cytosol of eukaryotic cells. The SPI-1 encoded T3SS is required for the transport of *S. enterica* proteins across the cytoplasmic membrane of a host cell into its cytosol where they induce cytoskeletal rearrangements resulting in the uptake of *S. enterica* even by non-phagocytic cells.<sup>8</sup> In addition, it has been reported that SPI-1 genes, independent of cell invasion, induce macrophage cytotoxicity.<sup>9</sup> SPI-2 encoded T3SS is required for the transport of *S. enterica* proteins across the phagosomal membrane and increases *S. enterica* survival inside phagocytic cells.<sup>10,11</sup> The function of genes localized on the remaining SPIs is less well characterized and according to recent reports these remaining SPIs individually have no effect on *S. enterica* virulence although collectively they have a low effect on bacterial colonization.<sup>7</sup>

Recently, with an increasing understanding of *S. enterica* pathogenesis, mutants without a functional type III secretion system (T3SS) encoded by either SPI-1 or SPI-2 have been tested for determining their virulence and vaccine potentials. Results of such studies show that whilst SPI2 mutants of *S. enterica* are attenuated in all warm-blooded hosts, SPI1 mutants seem to be attenuated only in hosts for which an enteric type of disease is characteristic and these genes are dispensable when the output of the infection is a typhoid disease.<sup>12-14</sup> In agreement with the previous statement, the removal of SPI1 genes from *S. enterica* or *S. Typhimurium*, i.e. the serovars which cause a mild enteric disease in chickens, results in a decrease in virulence with preserved immunogenicity in these hosts.<sup>7,15,16</sup> Moreover, SPI1 mutants are defective in early interactions with macrophages which may enable the macrophage's proper

antigen processing and presentation<sup>17-19</sup> though the role of SPI1 in the interactions with other antigen presenting cells in the chicken is less clear. When SPI1 and SPI2 mutants of *S. enterica* serovar Enteritidis have been tested for their vaccine potentials in chickens, both the mutants provide protection to chicken against *S. enterica* challenge as documented by findings such as the bacterial counts in tissues, spleen weight, antibody production and cytokine response (namely IL-17 and IL-22). When the 2 mutants are compared, vaccination with the SPI1 mutant proved to be more effective in the protection of chicken against *S. enterica* challenge than the vaccination with the SPI2 mutant. On the other hand, vaccination with the SPI2 mutant stimulates a slightly higher antibody production and such a mutant might therefore be a better choice if *Salmonella* is used as a vector for the delivery of heterologous antigens with a desired stimulation of the humoral part of the immune system.<sup>15</sup> Recently, a triple SPI1-*lon*-*fliC* mutant of *S. enterica* has been constructed and tested for its efficacy as a live attenuated marker vaccine for the oral vaccination of poultry.<sup>20</sup> Deletion of *fliC* gene encoding for flagella in this mutant strain enables serological differentiation of vaccinated and infected chickens which is an increasing demand for the days and is something that the current commercial vaccines cannot provide. *Lon* protease is a negative regulator of SPI1 genes<sup>21</sup> and is required for the resistance to multiple environmental stresses<sup>22</sup> and removal of *lon* reduces the virulence of *S. enterica* even for highly sensitive Balb/C mice.<sup>13</sup> Therefore, the inactivation of gene encoding *Lon* protease results in further independent attenuation of *S. enterica* in virulence with a mucoid colony phenotype due to the overproduction of capsular polysaccharides that enables additional simple differentiation of the vaccine strain from those circulating in the environment. The SPI1-*lon*-*fliC* mutant might therefore be a suitable marker vaccine strain for oral vaccination of poultry in future.

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## Conflict of interest

The author declares no conflict of interest.

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