Chromosomal and plasmidic virulence determinants of *Salmonella Infantis* in broiler chicks

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INTRODUCTION

*Salmonella Infantis* has been reported to be predominant among broiler chicks in Hungary and in several other countries in and outside Europe. Recently the chicken clone B2, carrying a large multiresistant (MDR) plasmid became also prevalent in the human population in some of these countries (1, 2, 3). The plasmid pSI 54/04 represents the prime difference when compared genomes of the recent and earlier clones B2 and A1 (4). It is however uncertain whether and how much of this spreading can be related to virulence determinants residing on *Salmonella* pathogenicity islands (SPIs) or to the MDR plasmid.

HYPOTHESIS

The SPI1 and SPI2 or the large MDR plasmid pSI 54/04 may influence the colonization and spread of the *S. Infantis* clone B2 in broiler chicks.

OBJECTIVES

• to test the *in vitro* and *in vivo* pathogenicity of chromosomal mutants (∆SPI) on chicken embryo fibroblast (CEF) cells and on orally infected day old chicks
• to test the impact of pSI 54/04 plasmid carriage on the *in vitro* and *in vivo* pathogenicity of the strains
• to provide the molecular characterization of the MDR plasmid pSI 54/04

MATERIALS AND METHODS

RESULTS ON PLASMID PATIOGENICITY

1. *In vitro* and *in vivo* pathogenic potential of MDR plasmid pSI 54/04

• The plasmidic strain SI 54/04 wt (clone B2) proved to be significantly less invasive than SI 69/94 wt (clone A1) for CEF cells.
• The pSI 54/04 plasmid transformant of SI 69/94 was also less invasive *in vitro* and showed reduced colonization of the chicken caeca as well, however *in vivo* differences were not significant (Fig. 2).
• Invasion of the spleen was detected in only one animal/group, therefore can not be considered as characteristic to strains SI 54/04 wt and plasmidic SI 69/94. *Salmonella* was not detectable in the liver of infected chicks.

RESULTS ON CHROMOSOMAL VIRULENCE REGIONS (SPIs)

Deletion of *Salmonella* pathogenicity islands SPI1 and SPI2

• The deletion of SPIs resulted in a significantly reduced invasiveness for CEFs, with a more pronounced effect for SPI1 (Fig 1).
• Chicken infection resulted in no significant difference between the wild type strain SI 69/94 and its SPI1 and SPI2 mutants regarding caecum and spleen (Fig 1).
• Histopathology of the infected caecum has only shown an increased lymphoid infiltration and thickening of the caecal submucosa.

CONCLUSIONS

In harmony with our earlier results, it seems that *S. Infantis* is poorly invasive for broiler chicks but it is colonizing well in their intestine. These traits of *S. Infantis* are not influenced by SPI1 or SPI2.

It seems that the MDR plasmid pSI 54/04 represents a disadvantage in the interaction with the host cells. Therefore the spread of plasmidic clone B2 is more likely due to its multiresistance, conferring survival in the poultry food.

REFERENCES


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**TABLE 1.**

<table>
<thead>
<tr>
<th>Chicken embryo fibroblasts (CEF)</th>
<th>Day old chicks</th>
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<tbody>
<tr>
<td><strong>Cell invasion</strong></td>
<td><strong>Colonization and invasion</strong></td>
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<tr>
<td>Injection of tryptically prepared CEFs</td>
<td>One injection and 4 day invasion</td>
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<tr>
<td>Incubation for 2h + 1h in media with tetracycline (to kill enterobacterial bacteria)</td>
<td>Organ samples</td>
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<tr>
<td>Lysis for 30 min and detection of intracellular <em>Salmonella</em> (dye counting)</td>
<td>Liver, spleen</td>
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**Figure 1.** The impact of SPI-deletion to CEF invasion and colonization of the chicken caecum (C) and spleen (S)

**Figure 2.** The impact of pSI 54/04 carriage to CEF invasion and colonization of the chicken caecum (C) and spleen (S)

**Figure 3.** Physical map of the large MDR plasmid pSI 54/04