Replication Kinetics of Salmonella Enteritidis Live Vaccine in the Immune Organs of Chicken after Subcutaneous Immunization

Guang-Zhi He¹ and Shu-Xuan Deng²

¹Guangzhou College of Traditional Chinese Medicine, Guangzhou 510500, Guangzhou Province, China
²Agriculture Office of Dalingshan town, Dongguan 523830, Guangdong Province, China

We used a real-time PCR for Salmonella Enteritidis to detect the genomic DNA of Salmonella Enteritidis live vaccine in the immune organs, including the bursa of Fabricius, thymus, spleen, and Harderian gland, from chicken after subcutaneously vaccinated at different time points. Significant numbers of Salmonella Enteritidis genomes in the immune organs were first detected at 12 hour (post-vaccination) p.v., and subsequently rose to peak levels during 48 h to 72 h p.v. The rapid early increase of vaccine levels in all samples examined followed by a steady decline from 84 h to 15 days p.v. The real-time PCR analysis of a variety of tissues is significant for further investigation of the mechanism of vaccinal protection, and the optimization of vaccination regimes.

Key words: immune organs, Salmonella Enteritidis, vaccine

Introduction

Vaccination has been used as a preventive measure and also for controlling Salmonella Enteritidis outbreaks worldwide (He et al., 2010; Peng et al., 2011). Up to day, few data are present in the literature about the quantitative analysis of live Salmonella Enteritidis live vaccine in chicken at different times following vaccination. The mechanism for the rapid induction of immunity of attenuated Salmonella Enteritidis vaccine remain unclear. The quantification of live vaccine number in vivo may provide a complement for understanding this mechanism. For these reasons, we investigated the dynamic distribution and replication of attenuated Salmonella Enteritidis live vaccine in Immune Organs of chicken after subcutaneously vaccinated using a real-time PCR assay described previously (Deng et al., 2008a).

Materials and Methods

Vaccine

A commercially available Salmonella Enteritidis live vaccine (Avipro Salmonella vac E) was purchased from the National Center for Medical Culture Collection of China.

DNA Extraction from Tissue Samples

Briefly, 0.15-g parenchymatous tissue samples were collected, and cell suspensions prepared by mechanically homogenizing the samples in 600 μL phosphate buffered saline (PBS). The cell suspensions from the various immune organs were frozen and thawed thrice followed by centrifugation at 8000 rpm for 5 min; the cells were then resuspended in 600 μL PBS. DNA was extracted from the suspension using proteinase K and a phenol/chloroform protocol as described previously (Deng et al., 2008a, 2008b). Finally, the purified DNA was resuspended in 30 μL TE buffer pH 8.0 (1 M Tris (pH 8.0) 10 mL, 0.5 M EDTA (pH 8.0) 2 mL, ddH2O added up to 1 L) and stored at −20°C until use.

Fluorescent Quantitative-polymerase Chain Reaction (FQ-PCR)

In our previous study, we established a serovar-specific real-time polymerase chain reaction (PCR) (designed with Salmonella difference fragment I, Sdf I), Genbank Accession No. AF370707.1) (Deng et al., 2008a). In this study, we used a real-time PCR assay described previously to detect the genomic DNA of Salmonella Enteritidis live vaccine in the immune organs.

Differences between the FQ-PCR and Indirect Immunofluorescent Antibody Assay (IFA) Results

To validate the results, we simultaneously performed a quantitative bacteriological test to determine the bacterial burden in the corresponding tissues and compare these data with our PCR data. In our previous study, we also established a specific method of indirect immunofluorescent antibody staining (IFA) for Salmonella Enteritidis (Yan et al., 2008). At present, we relied on the IFA assay to study the distribution pattern and quantity of Salmonella Enteritidis in the immune organs of chicken.
Experiment at present, 102 chicken (White leghorn, age 20 d, negative for Salmonella Enteritidis, not vaccinated against Salmonella Enteritidis) were purchased from the Animal Center of Guiyang College of Traditional Chinese Medicine, China. A group of 68 chicken was vaccinated with one commercial dose of Salmonella Enteritidis live vaccine (0.5 ml, about $3.0 \times 10^5$ CFU per chicken) by subcutaneous. Another group of 34 chicken was treated with an equal volume of water as a control. The bursa of Fabricius, thymus, spleen, and Harderian gland were analyzed by FQ-PCR at different post-vaccination time points: 2, 8, 12, 24, 36, 48, 60, 72, 84, 96, 108, and 120 h; and 6, 7, 9, 12 and 15 d.

Four chicken from the vaccinated group and two from the control group were sacrificed at each time point and their organs were aseptically harvested and immediately placed in 1.5 mL labeled snap-cap tubes and frozen. DNA extraction from tissue samples was described as above, and the purified DNA was resuspended in 30 μL TE buffer. Finally, a 6 μL aliquot of the DNA extract was used as a template in the FQ-PCR assay.

Statistical Analysis

The reaction, data acquisition, and analysis were performed using an iCycler iQ optical detection system software (Bio-Rad, USA, version 3.1). The number of target copies in the reaction was deduced from the threshold cycle (Ct) values corresponding to the fractional cycle number at which the released fluorescence was 15 times higher than the standard deviation of the mean baseline emission. All samples were analyzed 3 times by the FQ-PCR assay, and the data were analyzed using the statistical package for social sciences (SPSS) software version 11 (China software company, China). Comparisons of the means were conducted using Duncan’s multiple range test. A P value of $<0.05$ was considered statistically significant. Finally, the Salmonella Enteritidis DNA copy concentrations were expressed as mean log_{10} genome copy number per 0.15 g.

Results

Distribution of Salmonella Enteritidis in the Immune Organs (FQ-PCR assay)

The distribution of Salmonella Enteritidis within the immune organs after subcutaneously immunized was determined by means of FQ-PCR over a 15 d period at intervals. The results showed that the spleen tested positive for Salmonella Enteritidis at 12 h P.V. Thereafter, Salmonella Enteritidis was consistently detected in all the samples at 24 h P.V; the bursa of Fabricius, and thymus were positive at 24 h P.V. The copy numbers of Salmonella Enteritidis in each tissue reached a peak at 60–72 h P.V. The spleen, and Harderian gland contained high concentrations of Salmonella Enteritidis whereas the bursa of Fabricius and thymus exhibited low concentrations. The numbers of bacteria decreased at 84 h, and by 15 d, the level of Salmonella Enteritidis clearly decreased. The bursa of Fabricius, Harderian gland, and thymus did not show positive results. Salmonella Enteritidis persisted in the spleen of the chicken for up to 12 d. The immune organs of the chicken in the control group did not show any positive results at any time point. The details are provided in Table 1.

Table 1. The distribution of Salmonella Enteritidis within different immune organs

<table>
<thead>
<tr>
<th>Time</th>
<th>2h</th>
<th>8h</th>
<th>12h</th>
<th>24h</th>
<th>36h</th>
<th>48h</th>
<th>60h</th>
<th>72h</th>
<th>84h</th>
<th>96h</th>
<th>108h</th>
<th>120h</th>
<th>6d</th>
<th>7d</th>
<th>9d</th>
<th>12d</th>
<th>15d</th>
</tr>
</thead>
<tbody>
<tr>
<td>BF</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>1.83</td>
<td>2.47</td>
<td>3.23</td>
<td>3.95</td>
<td>4.02</td>
<td>3.77</td>
<td>2.60</td>
<td>2.34</td>
<td>1.97</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Thymus</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>1.88</td>
<td>2.25</td>
<td>2.92</td>
<td>3.89</td>
<td>3.58</td>
<td>3.20</td>
<td>2.33</td>
<td>2.12</td>
<td>1.85</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.00</td>
<td>0.00</td>
<td>2.12</td>
<td>2.92</td>
<td>3.71</td>
<td>5.58</td>
<td>6.60</td>
<td>7.01</td>
<td>5.56</td>
<td>4.51</td>
<td>4.45</td>
<td>3.76</td>
<td>3.02</td>
<td>2.75</td>
<td>2.22</td>
<td>2.35</td>
<td>0.00</td>
</tr>
<tr>
<td>HG</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>1.98</td>
<td>2.94</td>
<td>3.52</td>
<td>4.52</td>
<td>4.87</td>
<td>3.95</td>
<td>3.39</td>
<td>2.89</td>
<td>2.24</td>
<td>1.80</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Note: Unit of the present data is log_{10} CFU per 0.15 g for detected tissue; BF = bursa of Fabricius, HG = Harderian gland

Distribution of Salmonella Enteritidis within different immune organs

The spleen exhibited a positive Salmonella Enteritidis signal by IFA at 12 h P.V. Thereafter, a positive signal was detected in all the samples at 24 h to 120 h P.V; a stronger positive signal was observed in the spleen and Harderian gland compared to the other organs. The positive Salmonella Enteritidis signal clearly decreased at 84 h P.V, and no positive results were detected in the bursa of Fabricius and thymus. However, it was possible to detect a positive signal in the spleen at 12 d P.V. Apparently, the results were similar to the results of FQ-PCR. Therefore, the FQ-PCR assay was considered to be a more sensitive and accurate method for this study. (Fig. 1)

Discussion

A better understanding of the kinetics of Salmonella Enteritidis live vaccine replication, the quantification of live vaccine load will be required as we aim towards more effective vaccines. With this in mind, we examined DNA loads as a measure of the kinetics of attenuated Salmonella Enteritidis vaccine replication in experimentally vaccinated chicken. Various tissues were examined in order to investigate the different stages in the course of vaccine infection and replication.

Long-term immune protection is likely to require periodic stimulation of the immune system by cells expressing Salmonella Enteritidis antigen (Li et al., 2004; He et al., 2011). In this study, most of tissues examined still contained a low-level of Salmonella Enteritidis genome loads at the terminal of experiment, but whether or not vaccine at these tissue sites effectively stimulate the immune system is not known. In this study, the results showed that the Salmonella Enteritidis populations in the bursa of Fabricius and thymus...
were much lower than that in the other organs sampled, with copies of Salmonella Enteritidis being 100–1000 times less than those in other regions. Interestingly, Salmonella Enteritidis organisms have a higher tropism in the Harderian gland than in the other immune organs. The functions of the spleen in filtration, immune responsiveness, and activation of complement have been well documented. The spleen is made up of a lymphocyte-rich white pulp and macrophage-rich red pulp; it is comprised of distinctive B cells and macrophages (Dunlap et al., 1991). Therefore, what we described above may be the reason for why a significant number of Salmonella Enteritidis cells can persist over a long time in the spleen. Thus far, the colonization mechanism of Salmonella Enteritidis in the immune organs is not clear and further studies are required to understand the same.

In summary, we have generated a quantitative set of data for attenuated Salmonella Enteritidis loads and tissue distribution for 15 days after vaccination, giving further insight into the mechanisms of protective immunity induced by this Salmonella Enteritidis vaccine strain. Quantitative real-time PCR detection on multiple samples showed that the attenuated Salmonella Enteritidis vaccine can spread quickly to various tissues and replicate rapidly followed by a steady decline. The next step is to confirm that vaccine load in the vaccinated chicken correlates well with specific immune responses induced by Salmonella Enteritidis vaccine. Once this relationship is understood, the quantitative PCR test could provide data to assist optimization of existing vaccination strategies in terms of delivery route and choice of the route of vaccine administration.

References


Dunlap NE, Benjamin WH, McCall RD, Tilden AB and Briles DE. A safe site for Salmonella Typhimurium is within splenic cells during the early phase of infection in mice. Microbial Pathogenesis, 10: 297–310. 1991.

He GZ, Tian WY, Qian N, Cheng AC and Deng SX. Quantitative studies of the distribution pattern for Salmonella Enteritidis in the internal organs of chicken after oral challenge by a real-time PCR. Veterinary Research Communications, 34: 669–676. 2010.


