SteE enhances the colonization of Salmonella Pullorum in chickens

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Abstract

Salmonella pullorum (S. pullorum) is the causative agent of pullorum disease and results in severe economic losses in poultry, and can long-term survival by colonizing host organs. steE is an effector protein secreted by Salmonella pathogenicity island 2. It is not clear in vivo for the colonization of Salmonella. To investigate the role of steE on the colonization of S. Pullorum in the principal organs of chicken, we used S. pullorum and S. pullorum ΔsteE strains immunized chickens, respectively. The results of the virulence assay showed that the LD50 of S. pullorum ΔsteE was 22.8 times higher than that of S. pullorum in chickens. The colonization experiment of bacteria showed that the overall change trend of the number of S. pullorum and S. pullorum ΔsteE strains were similar in chicken liver, spleen, heart, bursa, and cecum, which increased first and then decreased. However, the deletion of steE caused significantly reduced colonization, pathological change, and virulence of S. pullorum in a chicken infection model. Our findings provide exciting insights into the pathogenic mechanism and live attenuated vaccine associated with steE in S. pullorum.

Keywords: Salmonella pullorum; steE; virulence; chicken; colonization.

1. Introduction

Salmonella enterica serovar Pullorum (S. pullorum) is an intracellular pathogen of host specificity (Li et al., 2018; Chechet et al., 2022). It mainly infects chickens within three weeks of age, resulting in acute systemic diseases and high mortality in poultry. The growth and production performance of young and adult chickens infected with S. pullorum will be badly affected (Li et al., 2019; Foster et al., 2021). S. pullorum can be transmitted vertically, and the hatching rate of contaminated breeding eggs will significantly reduce. Sick chickens and infected chickens are the primary sources of S. pullorum. The cocks carrying S. pullorum can also transmit to the hens through vertical transmission. One of the essential measures to control the occurrence and prevalence of S. pullorum is the purification of S. pullorum in breeding chickens and the disinfection of breeding eggs. At present, S. pullorum is still occurring and prevalent in many countries, especially in developing countries, including Brazil and India, which has caused substantial economic losses to the poultry industry (Geng et al., 2019; Xian et al., 2020). Therefore, further study of the pathogenic mechanism of S. pullorum is necessary.

The survival and proliferation of Salmonella are related to a particular cell membrane region formed in the host cell, namely the Salmonella-containing vacuole (SCV). The formation of SCV is inseparable from type III secretion system 2 (T3SS2) encoded by Salmonella pathogenic island 2 (SPI-2). Therefore, T3SS2 plays an essential role in the pathogenesis of S. pullorum (Figueira et al., 2013; Knuff-Janzen et al., 2021; Cohen et al., 2021). T3SS2 is an injection device that the Salmonella-secreted effector is translocated into the host cells through the device to provide a favorable environment for Salmonella invasion (Greene et al., 2021; Fang & Méresse, 2021). As a novel effector protein of Salmonella T3SS2, steE is encoded in Salmonella prophage gisy-1,
which helps to survive and replicate in macrophages and plays a vital role in the evolution of *Salmonella* and the regulation of host innate immune response (Coombes et al., 2005).

In the present study, little work is reported about the steE of *S. pullorum*. Continuing to explore the relationship between steE and the virulence of *S. pullorum* contributes to revealing the pathogenic mechanism of *S. pullorum*. Therefore, chickens were infected with *S. pullorum* and *S. pullorum* ΔsteE strains as an animal model to analyze their potential role in the virulence and colonization of *S. pullorum*.

2. Materials and methods

2.1 Strains and Animals

*S. pullorum* and *S. pullorum* ΔsteE strains were preserved and constructed in our laboratory. Our laboratory hatched healthy 2-day-old Jinghong laying hens.

2.2 Recovery and Counting of Bacteria

Take out the frozen *S. pullorum* or *S. pullorum* ΔsteE strain glycerol bacteria at -80 °C, rejuvenate it in xylose lysine deoxycholate (XLD, Hopebio Bio-Technology, Qingdao, China) agar plate at 37 °C for 18–24 h. The next day, a single colony of *S. pullorum* was added to 1 ml of Luria-Bertani (LB) broth at 37 °C and 180 rpm for 16–18 h. Overnight cultures of the *S. pullorum* and *S. pullorum* ΔsteE strains with 10-fold serial dilutions (1 × 10⁻⁴, 1 × 10⁻², and 1 × 10⁻⁸) were enumerated by plating on XLD agar. All dilution was repeated three times.

2.3 Analysis of Clinical Symptoms and Autopsy

The clinical symptoms and morbidity of the chicks were observed every day after *S. pullorum* or *S. pullorum* ΔsteE strain infection and recorded and photographed.

2.4 S. Pullorum Virulence Assay

*S. pullorum* and *S. pullorum* ΔsteE strains were inoculated respectively in LB broth for 12 h. The bacteria cultures were washed three times with PBS and suspended to adjust the bacterial concentration. One hundred commercial 2-day-old chickens were randomly divided into ten groups. Each group was infected orally with 10-fold serial dilutions of *S. pullorum* or *S. pullorum* ΔsteE strain (1 × 10⁶, 1 × 10⁷, 1 × 10⁸, 1 × 10⁹ CFU or 1 × 10⁶, 1 × 10⁷, 1 × 10⁸, 1 × 10⁹, 1 × 10¹⁰, 1 × 10¹¹ CFU). Ten chickens received 100 μL of PBS as a control group. Deaths were recorded until 14 days, and the half-lethal dose (LD₅₀) of each strain was calculated to evaluate the virulence of the strain to chickens using Karber’s method.

2.5 Bacterial Colonization in Organs

*S. pullorum* and *S. pullorum* ΔsteE strains were cultured in LB broth for 12 h. The bacteria cultures were washed three times with PBS and suspended to adjust the bacterial concentration. Sixty chickens were randomly divided into three groups (n = 20). Chicken from each group was infected orally with 1 × 10⁸ CFU of *S. pullorum* or *S. pullorum* ΔsteE strain in 100 μL of PBS, according to the LD₅₀ assay as mentioned previously. Twenty chickens received 100 μL of PBS as a control group. Chickens were deprived of food and water for 12 hours before and after chicken immunization. At 12 h, 24 h, 36 h, 2 d, 3 d, 4 d, and 7 d post-challenge, the cecum, liver, spleen, bursa, and heart organs were harvested from each chicken. After weighing, organs from each group were homogenized mechanically, and diluted serially for the subsequent cultivation on XLD agar plates at 37 °C for 12–16 h. The bacterial number was counted and displayed as log10 CFU/g. The dynamic distribution of *S. pullorum* and *S. pullorum* ΔsteE strains in various organs was analyzed by plating on XLD agar. The bacterial number was counted and expressed as log10 CFU/g at 12 h, 24 h, 36 h, 2 d, 3 d, 4 d, and 7 d.

3. Results and discussion

3.1 Results

3.1.1 *S. pullorum* ΔsteE reduces Virulence in Chicken

The virulence of *S. pullorum* and *S. pullorum* ΔsteE strains were analyzed in chicken. As shown in Table 1, the LD₅₀ of *S. pullorum* was 9.14 × 10⁷ CFU, but the LD₅₀ of *S. pullorum* ΔsteE was 2.08 × 10⁹ CFU. The LD₅₀ of *S. pullorum* ΔsteE was 22.8 times higher than that of *S. pullorum*. The result showed that steE could decrease the virulence of *S. pullorum*.

Table 1

<table>
<thead>
<tr>
<th>Strain</th>
<th>Challenge doses</th>
<th>Dead counts/Chicken counts</th>
<th>LD₅₀/ CFU</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Pullorum</td>
<td>1 × 10⁷</td>
<td>10/10</td>
<td>9.14 × 10⁷</td>
</tr>
<tr>
<td>S. Pullorum</td>
<td>1 × 10⁸</td>
<td>9/10</td>
<td>2.08 × 10⁹</td>
</tr>
<tr>
<td>ΔsteE</td>
<td>1 × 10¹</td>
<td>3/10</td>
<td>10⁹</td>
</tr>
</tbody>
</table>

3.1.2 Clinical Symptoms and Changes of Autopsy

One day after the chickens were infected with *S. pullorum* or *S. pullorum* ΔsteE strain, the chicks were depressed and had a poor appetite, accompanied by the phenomenon of gathering together. Some chicks could not stand steadily, discharged white sticky feces, and the feathers around the anus were covered with feces. As shown in Fig. 1, the pathological change of chicken organs infected with *S. pullorum* was more severe than that of the *S. pullorum* ΔsteE and control groups as follows: there were dark red needle tips and large bleeding spots at the edge of the liver; splenomegaly, dark red; bursal enlargement; the cecum has puffed and bled; heart congestion, swelling with blood filaments.

3.1.3 steE Enhances the Colonization of *S. Pullorum* in Chicken Cecum

The results of bacterial colonization showed that the bacterial number of *S. pullorum* and *S. pullorum* ΔsteE strains in the cecum were increased from 12 h to 3 d but decreased from 3 d to 7 d (Fig. 2). At three days post-challenge, the colonization of *S. Pullorum* and *S. pullorum* ΔsteE strains reached the peak in the chicken cecum, and the settlement amount of *S. pullorum* and *S. pullorum* ΔsteE strains reached the lowest at 7 d. The changing trend of *S. Pullorum* and *S. pullorum* ΔsteE strains was increased at first and then decreased in the whole process of the infection. Still, the settled quantity of *S. pullorum* ΔsteE was consistently lower than that of *S. pullorum*.
Fig. 1. The macroscopic image of different organs of chickens.
3.1.4 steE Enhances the colonization of S. pullorum in chicken liver

The results of bacterial colonization showed that the bacterial number of S. Pullorum was increased from 12 h to 4 d in the whole process of the infection and decreased significantly from 4 d to 7 d (Fig. 3). The total amount of S. pullorum ΔsteE was increased from 12 h to 3 d after inoculation, and decreased from 4 d to 7 d. The colonization of S. Pullorum and S. pullorum ΔsteE strains reached the peak about four days after chicken inoculation. The changing trend of S. pullorum and S. pullorum ΔsteE strains increased first and then decreased throughout the experiment. However, the settled quantity of S. pullorum ΔsteE was consistently lower than that of S. pullorum.

3.1.5 steE Enhances the Colonization of S. pullorum in Chicken Spleen

The colonization of S. pullorum and S. pullorum ΔsteE strains increased from 12 h to 3d after chicken inoculation, and decreased significantly from 3 d to 7 d in the chicken spleen (Fig. 4). The colonization of S. pullorum and S. pullorum ΔsteE strains reached the peak about 3 d in the chicken spleen after chicken inoculation. The changing trend of S. pullorum and S. pullorum ΔsteE strains increased initially and then decreased throughout the infection. Still, the settled quantity of S. pullorum ΔsteE was consistently lower than that of S. pullorum.

3.1.6 steE Enhances the Colonization of S. pullorum in Chicken Brusa

The colonization of S. Pullorum was increased from 12 h to 3 d in chicken bursa, increased slowly at 36 h, and decreased from 3 d to 7 d. The results showed that S. pullorum reached the peak of about 3 d in the bursa after chicken inoculation (Fig. 5). The changing trend of S. pullorum ΔsteE in chicken bursa is consistent with that of S. pullorum. At the same time, the settled number of S. pullorum ΔsteE was consistently lower than that of S. pullorum.

3.1.7 steE Enhances the Colonization of S. pullorum in Chicken Heart

The colonization of S. pullorum and S. pullorum ΔsteE strains was increased from 12 h to 4 d in the heart after chicken inoculation and decreased from 4 d to 7 d. As shown in Fig. 6, the bacterial number of S. pullorum and S. pullorum ΔsteE strains peaked about four days in the heart after chicken inoculation. The settlement amount of S. pullorum and S. pullorum ΔsteE strains reached the lowest at 7 d. The changing trend of S. pullorum and S. pullorum ΔsteE strains was increased at first and then decreased in the whole process of the infection, but the settled quantity of S. pullorum ΔsteE was consistently lower than that of S. pullorum in chicken heart.
3.2 Discussion

*Salmonella pullorum* has brought substantial economic losses to the poultry industry. The pathogenic mechanism of *S. Pullorum* needs to be further studied. After the host cell is infected with *Salmonella*, *Salmonella* can form a *Salmonella*-containing vacuole (SCV) in the host cell and survive in it for immune escape (Walch et al., 2021). The maintenance of SCV function is inseparable from the participation of a series of *Salmonella* virulence factors, which play a significant role in the T3SS2 encoded by SPI-2 and its secreted effector protein (Röder et al., 2021; Morrison et al., 2022). SteE is an effector protein deeply involved in regulating the secretion of *Salmonella* pathogenic island two, and its role is significant for *Salmonella* virulence (Gibbs et al., 2020).

This study evaluated the virulence and dynamic distribution of *S. pullorum* and *S. pullorum* ΔsteE strains in chicken organs after chicken immunization. The results of the virulence assay showed that the deletion of steE caused a decrease in the pathogenicity of *S. pullorum* in chickens, which proved the critical role of steE in the virulence of *S. Pullorum* (Fig. 1). At the same time, the colonization of bacteria in chicken organs showed that the overall change trend of *S. Pullorum* was similar to that of *S. pullorum* ΔsteE. The colonization of *S. Pullorum* and *S. pullorum* ΔsteE strains in the chicken cecum, spleen, and bursa peaked at 3d, while the number of bacteria in the liver peaked at 4d. From the whole infection process, the number of bacteria, such as the cleanliness of equipment, reagents, Petri dishes, and so on in the test, and the errors caused by the operation in the test process.

4. Conclusions

In this study, the deletion of steE caused significantly decreased colonization and pathological change of *S. pullorum* in a chicken infection model, and its virulence was also considerably reduced. Altogether, our work shows that steE is closely related to the pathogenicity of *S. pullorum*, which provides exciting insights into the roles of steE in the pathogenic mechanism and the development of the live attenuated vaccine of *S. pullorum*.

Conflict of interest

The authors declare that there is no conflict of interest.

References


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