



## SteE regulation of Th1/Th2 cytokines expression in chickens during *S. Pullorum* infection

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**Abstract.** Nowadays, timely monitoring of zoonotic agents, including salmonellosis, which are caused by various serovars of the family Salmonella, is relevant. Attention should be paid to the study of cytokine levels in combination with immunological studies. This helps clarify the pathogenesis of infectious diseases and develop preventive measures. The main purpose of study was to detect the process of regulating Th1/Th2 cytokines expression in chickens infected with salmonellosis. The field strain of *S. Pullorum* CVCC 530 was used in the research. The *steE* deletion mutant ( $\Delta steE$ ) and *steE*-complemented  $\Delta steE:steE$  ( $\Delta steE+steE$ ) strains were constructed in the WT strain using the  $\lambda$ -Red recombination method. Chickens were orally infected with WT,  $\Delta steE$ , and  $\Delta steE+steE$  strains ( $1 \times 10^9$  CFU/individual). The effect of *steE* on the host immune response remains unknown. Compared with the group infected with the WT or  $\Delta steE+steE$  strain, IL-12 and IFN- $\gamma$  mRNA transcript levels were significantly higher, while IL-10 mRNA expression was significantly reduced in the liver and bursa infected with the  $\Delta steE$  strain; IL-4 showed a dramatically reduced transcription level, but IL-18 mRNA expression was significantly increased in the  $\Delta steE$  strain – spleen, cecum, and heart; IL-10 mRNA expression was significantly reduced in the spleen and cecum infected with the  $\Delta steE$  strain. These results suggest that *steE* may regulate the Th1/Th2 cytokine response balance in chickens infected with *S. Pullorum* and provide new insights into the pathogenesis of salmonellosis for the treatment of persistent infection

**Keywords:** poultry; immune system; pathogenesis; immunity; infectious diseases

## Introduction

Poultry breeding is widely developed throughout the world. The concentration of a large number of poultry in a limited area creates favourable conditions for the emergence and spread of infectious diseases among the livestock of poultry farms. Salmonellosis, which is accompanied by damage to the gastrointestinal tract and septicaemia, is one of the poultry diseases that causes concern among veterinary medicine specialists. According to L. Zhike *et al.* (2021), salmonellosis agents pose a threat to human health because salmonellosis is a zoonotic disease.

Infection of poultry with salmonellosis can occur both from the parent flock and during the cultivation of commercial broiler stock. In most cases, given the relatively short life span of broilers, *S. pullorum* infection in most cases occurs vertically from the parent flock. Infection of poultry with the causative agent of salmonellosis can occur through the alimentary route, as well as aerogenic and transovarian (through the

egg). T.I. Fotina & T.V. Sergeychik (2022) proved that an important point for the occurrence of the disease is the presence of sources of infection. Poultry, rodents, other farm animals, feed, wild animals, poor cleaning and disinfection, disposal of poultry, and workers and visitors can be infected.

Researchers substantiated that the key point in preventing the development of salmonellosis is strict compliance with veterinary and sanitary requirements and rules in poultry farms. Researchers also indicate that the disease worsens in the presence of stress factors (Zhike *et al.*, 2021). B. Alosaimi (2020) draws attention to the fact that cytokines, which are produced by almost all cells of the body for the purpose of intercellular interaction and regulation of biochemical processes, are an important factor influencing the immune reactions of poultry. Cytokine imbalance is important in the pathogenesis of infectious diseases.

H.C. Webster (2022) proved that the important properties of cytokines are: pleiotropic action, the presence of overlapping and doubling phenomena in a single regulatory network. Induction conditions are created, that is, when one cytokine acts on others and regulates the expression of cytokine receptors.

Cytokines regulate specific immune reactions and the immune response to the pathogenic properties of pathogens of infectious diseases, including salmonellosis.

In this regard, the development of methods of prevention of poultry salmonellosis is urgent. The purpose of the study is the experimental reproduction of salmonellosis in chickens and the investigation of the process of regulating the expression of Th1/Th2 cytokines under the conditions of infection of chickens with the field strain *S. Pullorum* CVCC 530 (WT) using a deletion mutant. At the second stage, the immune response of chickens was investigated. In connection with the urgency of the problem and the solution of the set goal, the tasks of research were to study the effect of cytokines Th1/Th2 on the development of inflammatory processes in the spleen, liver, tissue bag and small intestine of chickens, under the conditions of their infection with the causative agent of salmonellosis.

### Literature Review

Researchers P. Li *et al.* (2022) reported that *Salmonella Pullorum* (*S. Pullorum*) is an important host-specific pathogen that can cause white diarrhoea with high mortality in young chicks at 2-3 weeks of age. An infected adult chicken does not have a number of clinical symptoms. According to S. Geng *et al.* (2019), *S. Pullorum* causes not only horizontal but also vertical transmission through eggs, which seriously threatens severe economic losses and the healthy development of poultry in some developing countries.

M. Wang *et al.* (2020) concluded that pathogens avoid elimination by the immune system and avoid causing excessive harm to the host, which are two essential elements of bacterial systemic infection. N. Naseer *et al.* (2022) identified the encoded *Salmonella* type III secretion system (T3SS), *Salmonella* pathogenicity islands-1 and -2 (SPI-1 and SPI-2) that deliver effector proteins to host cells. Different effector molecules have different biological activities. N. Foster *et al.* (2021) demonstrated that some effector proteins can manipulate host pathways that promote intracellular survival and replication of *Salmonella* in host cells. For example, researchers from different years (Li *et al.*, 2016; Geng *et al.*, 2019; Zhou *et al.*, 2021) reasoned that spvB and spvC can reduce autophagy or apoptosis of host cells by regulating the expression of inflammatory cytokines, while sseL may enhance intracellular survival induced by other signalling pathways. I.E. Brodsky (2020) proved that *SteE* as an anti-inflammatory effector protein is a gene associated with *Salmonella* virulence and is encoded in *Salmonella* prophage Gifsy-1, which can contribute to persistent *Salmonella* infection by regulating the host's innate immune response in macrophages. And J. Dong *et al.* (2021) determined that nuclear factor  $\kappa$ B (NF- $\kappa$ B) is involved in the inflammatory response and can also promote the development of Th cells. Researchers Q.J. Wu *et al.* (2018), S.L. Clay *et al.* (2020) state that in the normal host immune system, Th1/Th2 cells do not differentiate, but the high expression of inflammatory cytokines causes disorder in case of *Salmonella* infection. X. Kang *et al.* (2022) demonstrated that some host-adapted *Salmonella* serovars can suppress the innate immune response and create an infection-friendly environment for long-term survival in host cells, such as *S. Pullorum*. *SteE* has been reported to be closely associated with colonisation of bacterial invasion in mice (Jaslow *et al.*, 2018). Previous study (Liu *et al.*, 2018)

showed that *steE* was associated with persistent infection and virulence of *S. Pullorum*. Among them, *steE* promotes intracellular replication of *Salmonella* in host cells. However, the role of *steE* prior to *S. Pullorum* infection on host immune responses remained largely unknown. In this regard, *S. Pullorum*-infected chickens were used as an in vivo model to investigate the effect of *steE* on the host's immune response.

## Materials and Methods

The study was carried out during 2022-2023 at the China Veterinary Culture Collection, Beijing, China, and the Laboratory of Innovative Technologies, Safety and Quality of Livestock Products of Sumy National Agrarian University, Ukraine.

### Bacterial strains

The wild-type strain of *S. Pullorum* CVCC 530 (WT) was obtained from the China Veterinary Culture Collection, Beijing, China. The *steE* deletion mutant ( $\Delta steE$ ) and *steE* complemented  $\Delta steE:steE$  ( $\Delta steE+steE$ ) strains were constructed in WT strain by using the  $\lambda$ -Red recombination method, and stored in laboratory (Liu et al., 2018). All strains were grown overnight in Luria-Bertani medium at 37°C with shaking prior to use in chicken infection.

### Chicken infection assay

40 Hy-Line white chicks (2-day-old) were obtained from the Henan Animal Incubation Centre (China). This study was approved by the Animal Care and Ethics Committee of Henan Institute of Science and Technology. All animal studies were conducted in accordance with Directive 2010/63/EU of the European Parliament and of the Council "On the Protection of Animals Used for Scientific Purposes" (2010) approved by the Commission on Ethics and Bioethics of the Faculty of Veterinary Medicine of the Sumy National Agrarian University (protocol No. 3 dated 12.21.2021). The chicks were randomly divided

into four groups (n=10). The chicks in experimental groups were orally infected with WT,  $\Delta steE$  and  $\Delta steE+steE$  strains ( $1 \times 10^9$  CFU/individual), and normal control group were orally infected with PBS as previously method and dose described (Liu et al., 2018). At 3 days post-infection (dpi), the liver, spleen, bursa, cecum, and heart tissues of each chicken from each group was collected and stored at -80°C for further analysis.

### qRT-PCR assay

Total RNA extraction was prepared using TRIzol reagent (Invitrogen, USA) and then reverse transcribed to cDNA synthesis using a PrimeScript RT reagent Kit (TakaRa, Japan). qRT-PCR was performed using SYBR Premix Ex Taq™ II (TakaRa, Japan) according to the manufacturer's instructions. Primer sequences were: IL-12 forward: 5'-TGCCTTACTTTTCATTACTTTTCCTTTG-3' reverse: 5'-TTTAGC TGGTGTCTCATCGTTCC-3'; IL-18 forward: 5'-GTTCCCAAAGACATTCCT GG-3', reverse: 5'-GGCCAAGAACATTCCTTGTT-3'; IFN- $\gamma$  forward: 5'-CTTTG GAGTTGAAGG-CAGTGTGG-3', reverse: 5'-TCTGGGTTGTGGGGTTTGTGAG-3'; IL-4 forward: 5'-AGTGAATGACATCCAGGGAGAGG-3', reverse: 5'-CTGACGCATGTTGAGGAAGAGACTT-3'; IL-10 forward: 5'-CGTGTCACC GCTTCTTCAC-3', reverse: 5'-GGCTCACTTCTCTCCTCATC-3'; and  $\beta$ -actin forward: 5'-TATTGCTGCGCTCGTTGTTGAC-3', reverse: 5'-GATACCTCTTTTG CTCTGGCTTC-3'. The PCR (polymerase chain reaction) programme was set to 95°C for 30s followed by 40 cycles of denaturation at 95°C for 5s, annealing at 59°C for 30s, and extension at 72°C for 30s. Relative mRNA expression level of each gene was determined using the 2- $\Delta\Delta CT$  method, and  $\beta$ -actin was the housekeeping gene. Each experiment was performed in triplicate.

### Statistical analysis

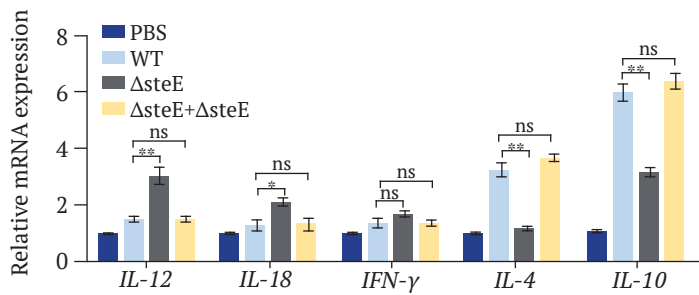
All results are presented as mean  $\pm$  standard deviation (SD) for at least three independent

experiments. Quantitative data was analysed by one-way ANOVA using GraphPad Prism software suite, version 8.0 (Graph Pad Software Inc., San Diego, CA, USA). Statistical significance was expressed as \*P<0.05 and \*\*P<0.01.

**Results and Discussion**

The study has found that *SteE* changes the mRNA of inflammatory cytokines levels in spleen of infected chickens by *S. Pullorum*. At the first stage of research, *SteE* level was evaluated.

It was found to alter mRNA of inflammatory cytokines levels in spleen of chickens during *S. Pullorum* infection. The mRNA levels of IL-18, IL-12 were much higher in spleen of the  $\Delta steE$  group than that in the WT or  $\Delta steE+steE$  strain group at 3 dpi (Fig. 1). Regarding the mRNA anti-inflammatory cytokines levels, the IL-10, IL-4, were substantially decreased in the spleen of  $\Delta steE$  group compared to the WT or  $\Delta steE+steE$  groups, whereas the mRNA IFN- $\gamma$  level was not significantly different.

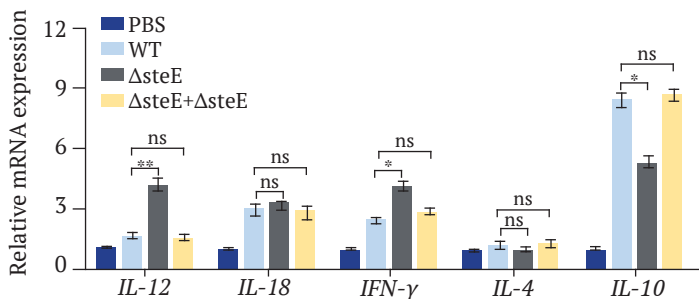


**Figure 1.** *SteE* changes the mRNA inflammatory cytokines levels in spleen of chickens infected with *S. Pullorum*

Source: developed by the authors

*SteE* changes the mRNA inflammatory cytokines levels in liver of chickens infected with *S. Pullorum*. During the second stage of experimental research, it was established that *SteE* changes the mRNA inflammatory cytokines levels in liver of chickens infected with *S. Pullorum*. The mRNA levels of IFN- $\gamma$ , IL-12

were much higher in liver of the  $\Delta steE$  group than that in the WT or  $\Delta steE+steE$  at 3 dpi, but the mRNA level of IL-18 had no significant difference (Fig. 2). The mRNA level of IL-10 was much lower in liver of the  $\Delta steE$  group than in the WT or  $\Delta steE+steE$  groups, whereas the mRNA level of IL-4 had no significant difference.

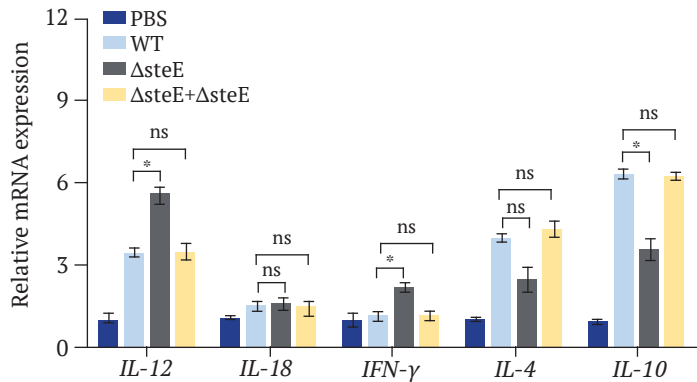


**Figure 2.** *SteE* changes the mRNA inflammatory cytokines levels in liver of chickens infected with *S. Pullorum*

Source: developed by the authors

*SteE* changes the mRNA inflammatory cytokines levels in bursa of chickens infected with *S. Pullorum*. Authors were given the task of investigating *SteE* changes mRNA inflammatory cytokines levels in bursa of chickens infected with *S. Pullorum*. The mRNA levels of

IFN- $\gamma$ , IL-12 were much higher, but no difference was detected in IL-4, IL-18 in bursa of the  $\Delta steE$  group than in the WT group or  $\Delta steE+steE$  at 3 dpi (Fig. 3). The mRNA level of IL-10 was much lower in bursa of the  $\Delta steE$  group than in the WT or  $\Delta steE+steE$  groups.

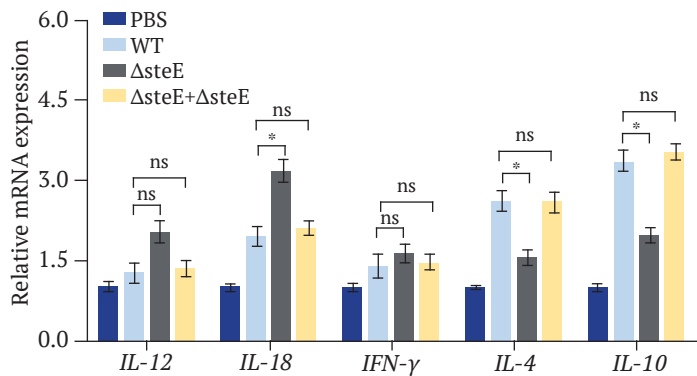


**Figure 3.** *SteE* changes the mRNA of inflammatory cytokines levels in bursa of chickens infected with *S. Pullorum*

**Source:** developed by the authors

*SteE* changes the mRNA of inflammatory cytokines levels in cecum of chickens infected with *S. Pullorum*. Experimental studies of the fourth stage proved that *SteE* changes mRNA of inflammatory cytokines levels in cecum in chickens infected with *S. Pullorum*. The mRNA

level of IL-18 was much higher, but no difference was detected in IFN- $\gamma$ , IL-12 in cecum of the  $\Delta steE$  group than in the WT or  $\Delta steE+steE$  groups at 3 dpi (Fig. 4). The mRNA levels of IL-10, IL-4 were much lower in cecum of the  $\Delta steE$  group than in the WT or  $\Delta steE+steE$  groups.

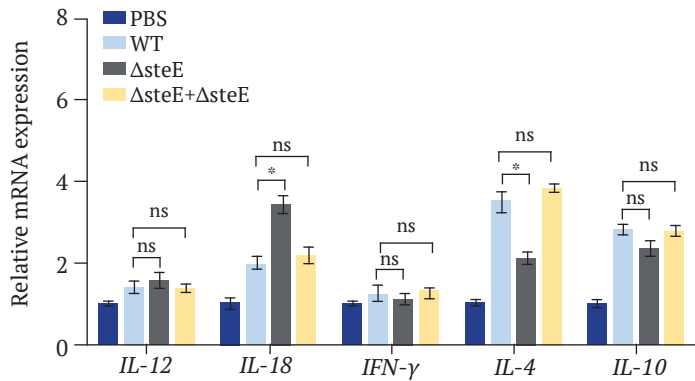


**Figure 4.** *SteE* changes the mRNA of inflammatory cytokines levels in cecum of chickens infected with *S. Pullorum*

**Source:** developed by the authors

SteE changes the mRNA of inflammatory cytokines levels in heart of chickens with *S. Pullorum* infection. The mRNA level of IL-18 was higher, but no difference was detected in IFN- $\gamma$ , IL-12,

IL-10 in heart of the  $\Delta$ steE group than in the WT or  $\Delta$ steE+steE groups at 3 dpi (Fig. 5). The mRNA level of IL-4 was much lower in hearts of the  $\Delta$ steE group than in the WT or  $\Delta$ steE+steE groups.



**Figure 5.** SteE changes the mRNA of inflammatory cytokines levels in heart of chickens infected with *S. Pullorum*

**Source:** developed by the authors

*Salmonella* is an intracellular pathogen that causes great harm to human and livestock health worldwide, with complex and diverse antigenicity and serotypes. Y. Hu *et al.* (2019) demonstrated that among common serotypes, *S. Pullorum* causes systemic lethal disease with high mortality in chickens within 2-3 weeks. An infected adult chicken exhibits abnormalities of the reproductive tract without serious clinical symptoms, leading to chronic or recessive infection. *S. Pullorum* causes significant economic losses to chicken farms worldwide, especially in developing countries. Therefore, T.I. Fotina & T.V. Sergeychik (2022) state that it is very important to control the spread of *S. Pullorum* in poultry farms.

*S. Pullorum* can spread horizontally and vertically to progeny, which is unlikely to be eliminated. Thus, accurate and rapid pathogen diagnosis is important for the control and eradication of *S. Pullorum*. At present, the detection of pathogenic bacteria depends on culture methods and biochemical identification, which

are time-consuming, have a long detection cycle and low sensitivity, and cannot meet the needs of social development. Thus, Zh. Liu *et al.* (2018) suggest the need to develop an accurate and simple detection method for *S. Pullorum* serotype diagnosis.

Studies by Z. Lin *et al.* (2017) found that *Salmonella* can colonise the intestine and spleen and directly accept macrophages as target cells. After infection with *S. Pullorum*, the bacterium can not only escape the destruction of intracellular active substances, but also proliferate and spread within macrophages. SteE is required for *Salmonella* replication and virulence in macrophages. Thus, it was hypothesised that the pathogenic mechanism of *S. Pullorum* infection in chickens may be the same as in HD-11 cells.

In this study, steE was selected as a research gene based on the  $\lambda$ -Red recombination system to construct the *S. Pullorum*  $\Delta$ steE strain. The results showed that the growth and biochemical characteristics of *S. Pullorum* and *S. Pullorum*  $\Delta$ steE strains are similar, which is

consistent with a previous result, but *steE* is not essential for the growth and metabolism of *S. Pullorum* (Liu et al., 2018). The results showed that *steE* would reduce the colonisation ability and virulence of *S. Pullorum* in HD-11 cells. *S. Pullorum*-induced apoptosis of HD-11 cells is a specific virulence mechanism that may facilitate the spread of bacteria between cells. Recent studies have shown that deletion of *S. Pullorum* SPI-2 significantly reduced chicken pathogenicity, which is consistent with *steE* belonging to the SPI-2 effector protein. In addition, T.H.M. Pham et al. (2020) reported that *steE* can drive macrophages to polarise to the M2 type and enhance the ability of the *Salmonella* infection-susceptible state. Therefore, it was suspected that *steE* might be associated with *Salmonella* virulence. The results of *S. Pullorum* infection of HD-11 cells also showed that *steE* enhances the intracellular viability of *S. Pullorum* and promotes HD-11 cell apoptosis.

When determining the virulence, it was found that the deletion of *steE* had an effect on reducing the pathogenicity of *S. Pullorum* in chickens, and this proved the important role of *steE* in the virulence of *S. Pullorum*. In addition, the presence of bacterial colonies in the organs of chickens showed that the general trend of change in *S. Sullorum* is similar to that of the  $\Delta steE$  strain. The presence of colonies of WT strains and  $\Delta steE$  strains in the spleen, caecum, and bursa of chickens reached its maximum peak on day 3, and the number of WT strains and strains  $\Delta steE$  in the liver of chickens reached its maximum peak on day 4. During the process of infection, the number of  $\Delta steE$  in different organs of the initial stage of infection after *Salmonella* infection in chickens that first increased, reached the peak at the middle infection stage, and then decreased at the later infection stage. The amount of  $\Delta steE$  strains in organs of chicken was always much lower than of WT strain. And, the amount of colonisation

of the  $\Delta steE$  strains in organs of chicken was always lower than of the WT strains. The results of the study indicate that *steE* can promote *S. Pullorum* colonisation in organs of chicken and contribute to *S. Pullorum* virulence.

Recent studies have shown that deletion of SPI-2 reduces the virulence of *Salmonella*, which has been attributed to the fact that SPI-2 is a DNA fragment acquired externally during *Salmonella* evolution. I. Panagi et al. (2020) reported that deletion of *steE* significantly attenuated mouse spleen colonisation and reduced *S. Typhimurium* virulence. *SteE* has been considered as an important effector in host organs for persistent *Salmonella* infection. *SteE* was encoded by the *Salmonella* prophage Gifsy-1, which was reported to activate the signal transducer and activator of transcription 3 signalling pathway and then produce the anti-inflammatory cytokine IL-10, thus promoting *Salmonella* replication in cells and increasing bacterial colonisation *in vivo*. S.L. Jaslow et al. (2018) identified many factors that affect the accuracy of the final results. On the one hand, the interval between organ collections was relatively long, and the exact time of penetration of bacteria into different organs cannot be precisely determined. However, only by reducing the interval between organ harvests it is possible to determine the time at which bacteria settle in chicken organs to reach a peak. In addition, there were other factors affecting the amount of *S. Pullorum* in the testing process, such as equipment, reagents, cleanliness of the Petri dish, and errors caused by the operation, etc.

Inflammatory cytokines are one of the most important regulators of host-cell interaction in acute systemic disease (Alosaimi et al., 2020). After *S. Pullorum* infection in chickens, the cells of the host will secrete Th1/Th2 cytokines, which regulate the balance of the internal environment and resist the damage of external harmful substances (Wu et al., 2018). *SteE*

increased the bacterial virulence, translocated into the cytoplasm of the host cell through T3SS-2, and caused the severe disseminated infection of other extra-intestinal tissues, such as the spleen and liver in mouse infected with *Salmonella* (Pham *et al.*, 2020). Here, it was found that *steE* positively regulated the balance of Th1/Th2-related cytokines in tissues of chicken infected with *S. Pullorum*.

*Salmonella* as an intracellular pathogen, can regulate host immune response via T cell activation (Cerny & Holden, 2019). IL-12 is a pro-inflammatory cytokine that plays an important role in the host's defence against intracellular pathogen infection (Yang *et al.*, 2018). IFN- $\gamma$  production by Th1 cells and initiated by IL-12 and IL-18, induced the oxidative effect and improved the ability to suppress intracellular bacteria growth (Withanage *et al.*, 2005). A previous study showed that *sppH2* significantly decreased the mRNA expression of Th1-related cytokines (IL-12 and IFN- $\gamma$ ) in tissues of mice infected with *S. Enteritidis* (Shappo *et al.*, 2020). Furthermore, *S. Pullorum* decreased the mRNA expression levels of pro-inflammation cytokine (IL-12, IL-18, and IFN- $\gamma$ ) compared with *S. Enteritidis* in infected macrophages (Tang *et al.*, 2018).

In the current study, *steE* increased the Th1-related cytokines expression (IL-12, IL-18, and IFN- $\gamma$ ) in the chicken tissues infected with *S. Pullorum*, which was consistent with the previous report. *SteE* might be involved in Th1-mediated tissue injury and elimination of pathogen during *S. Pullorum* infection. Anti-inflammatory cytokines are mainly related to clear pathogens and tissue repair after *Salmonella* infection. IL-10, as an important anti-inflammatory medium, can inhibit the expression of various pro-inflammatory factors induced by Th1 cell, and also participate in Th2 cell-mediated anti-inflammatory response (Rasquinha *et al.*, 2021). Recent studies have found that *steE* can induce the production of anti-inflammatory

cytokine IL-10 in the spleen of mouse infected with *Salmonella*, but it has no relevant data on other tissues (Jaslow *et al.*, 2018). Moreover, the persistent infection of *Mycobacterium tuberculosis* needs the participation of IL-10, which indicates that IL-10 contribute to long-term systemic infection of intracellular bacteria (Park *et al.*, 2021). Another study found that the persistent infection of *Salmonella* needs the support of IL-10, which is associated with the Th1/Th2 balance (Liu *et al.*, 2018). Induced the expression of IL-10 contributed to limit the Th1 cytokines and escape the immune response of host cells (Webster *et al.*, 2022). In the present study, *steE* obviously increased the IL-10 mRNA transcript level during the infection phase. Therefore, *steE* may be more conducive to the repair of damaged tissues by promoting the anti-inflammatory or Th2 cytokines expression during early stages of infection.

The imbalance of Th1/Th2 cytokine is an important indicator of pathogens invasion (Abebe, 2019). Recent studies show that *S. Pullorum* was inclined to regulate host immunity toward a Th2-like immune response in poultry (Tang *et al.*, 2018). In this study, results demonstrated that *steE* inhibited the Th1 immune response, but promoted the Th2 immune response. Furthermore, *steE* activity promoted anti-inflammatory M2 phenotype and facilitated *Salmonella* invasion into host cells, leading to increased pathogen persistence (Jaslow *et al.*, 2018). *SteE* have the function of immune response of transforming different types, which is beneficial to intracellular survival of *Salmonella* (Panagi *et al.*, 2020). The previous study showed that *steE* can also increase the virulence of *S. Pullorum* and aggravate the outcome of host infection (Liu *et al.*, 2018). Thus, researchers speculate that *steE*-inhibited inflammatory response could be related to M2 phenotype, which was beneficial to evade immunity and persistent infection of *S. Pullorum*. However, it needs

to be further confirmed how *steE* regulates the cytokine balance of Th1/Th2 in *S. Pullorum*-infected chicken. It has been established that the results obtained coincide with the findings of other researchers and confirm the influence of *steE* regulation of Th1/Th2 cytokine expression in chickens infected with *S. Pullorum*.

### Conclusions

It was established that IL-12 and IL-18 mRNA levels were much higher in spleen of the  $\Delta steE$  group than in group of WT or  $\Delta steE+steE$  strains at 3 dpi. Regarding the mRNA anti-inflammatory cytokines levels IL-4, IL-10 were strong reduced in spleen of the  $\Delta steE$  compared with the WT or  $\Delta steE+steE$  groups, while the mRNA level of IFN- $\gamma$  had no significant difference.

It was noted that IL-12, IFN- $\gamma$  mRNA levels were much higher in liver of the  $\Delta steE$  group than in the WT or  $\Delta steE+steE$  groups at 3 dpi, but the IL-18 mRNA level had no significant difference. The mRNA level of IL-10 was much lower in liver of  $\Delta steE$  group than of the WT or  $\Delta steE+steE$  groups, and the mRNA level of IL-4 had no significant difference.

It was determined that IL-18 mRNA level was much higher, but no difference was found in IL-12, IFN- $\gamma$  in bursa of  $\Delta steE$  group than in WT or  $\Delta steE+steE$  groups at 3 dpi. IL-10, IL-4 mRNA levels were much lower in bursa of  $\Delta steE$  group

than in WT or  $\Delta steE+steE$  groups. It is argued that IL-18 mRNA level was much higher, but no difference was found in IL-12, IFN- $\gamma$  in cecum of  $\Delta steE$  group than in WT or  $\Delta steE+steE$  groups at 3 dpi. IL-4, IL-10 mRNA levels were much lower in cecum of  $\Delta steE$  group than in WT or  $\Delta steE+steE$  groups. The mRNA IL-18 level was much higher, but no difference was detected in IL-12, IFN- $\gamma$ , IL-10 in hearts of  $\Delta steE$  group than in the WT or  $\Delta steE+steE$  groups at 3 dpi. The mRNA level of IL-4 was much lower in the hearts of  $\Delta steE$  group than in the WT or  $\Delta steE+steE$  groups.

It has been shown that *steE* can modulate the Th1/Th2 immune response in *S. Pullorum*-infected chickens. And *steE* enhanced the anti-inflammatory response induced by a strong Th2 immune response. Overall, this study extends to further elucidating the *S. Pullorum* and the host immune response interaction, providing new insights into *Salmonella* pathogenesis for the treatment of persistent *Salmonella* infection, without the use of antibacterial drugs, which will provide the opportunity to obtain high-quality and safe poultry products.

### Acknowledgements

None.

### Conflict of Interest

None.

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## **SteE регуляція експресії цитокінів TH1/TH2 у курчат за *S. Pullorum* інфекції**

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**Анотація.** На сьогодні актуальним є своєчасне проведення моніторингу збудників зоонозів, у тому числі сальмонельозів, які викликаються різними сероварами родини *Salmonella*. Особливу увагу слід приділити визначенню рівнів цитокінів у комплексі з імунологічними дослідженнями. Це допомагає уточнити патогенез інфекційних захворювань та розробити профілактичні засоби. Метою досліджень було вивчення процесу регуляції експресії

цитокинів Th1/Th2 у курчат за умов зараження сальмонельозом. У дослідженнях використовували польовий штам *S. Pullorum* CVCC 530. Делеційний мутант *steE* ( $\Delta steE$ ) і штами  $\Delta steE:steE$  ( $\Delta steE+steE$ ), доповнені *steE*, були сконструйовані в штамі WT за допомогою методу рекомбінації  $\lambda$ -Red. Курчат перорально інфікували штамми WT,  $\Delta steE$  та  $\Delta steE+steE$  ( $1 \times 10^9$  КУО/особину). Тотальну екстракцію РНК готували за допомогою реагенту TRIzol, синтез кДНК проводили з використанням набору реагентів PrimeScript RT. qRT-PCR проводили за допомогою SYBR Premix Ex Taq™. Вплив *steE* на імунну відповідь господаря залишається невідомим. Порівняно з групою, інфікованою штамом WT або  $\Delta steE+steE$ , рівні транскриптів мРНК IL-12 та IFN- $\gamma$  були значно вищими, тоді як експресія мРНК IL-10 була суттєво знижена в печінці та бурсальній сумці, інфікованій штамом  $\Delta steE$ ; IL-4 показав різко знижений рівень транскрипції, але експресія мРНК IL-18 була значно підвищена в штамі  $\Delta steE$  – селезінка, сліпа кишка та серце; експресія мРНК IL-10 була істотно нижчою в селезінці та сліпій кишці курчат, інфікованих штамом  $\Delta steE$ . Результати свідчать про те, що *steE* може регулювати баланс цитокинової відповіді Th1/Th2 у курчат, інфікованих *S. Pullorum*, та уточнити патогенез збудника сальмонельозу для лікування персистуючої інфекції

**Ключові слова:** птиця; імунна система; патогенез; імунітет; інфекційні захворювання