Chicks Infected by Salmonella enteritidis

Subjects: Virology
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Chicks showed heterogeneous responses to S. enteritidis infection. Enhanced intestinal barrier function and cecal microbiota structure, especially a higher abundance of Desulfovibrio_piger, may help chicks resist S. enteritidis invasion.

Keywords: Salmonella enteritidis ; heterogeneity ; cecal microbiome ; intestinal barrier ; Desulfovibrio_piger

1. Introduction

Salmonella is a major foodborne pathogen of global importance, which has led to large numbers of deaths in humans and caused economic losses in animal husbandry [1]. Among the more than 2500 identified *Salmonella enterica* serotypes, *Salmonella enteritidis* (*S. enteritidis*) is the most frequently spread from animals to humans globally [2]. *S. enteritidis* has caused occasional epidemic outbreaks around the world, such as in China [3], South Africa [4] and the United States [5]. Poultry are the primary *S. enteritidis* host, and the percent prevalence of *S. enteritidis* in chicken meat is strongly positively correlated ($r = 0.804$, $p \leq 0.01$) with the incidence of human illnesses caused by this serotype [6]. These observations highlight the importance of studying *S. enteritidis* infection in poultry for reasons associated with both public health and poultry production.

Host susceptibility to pathogen infection is frequently heterogeneous [7], as demonstrated by the phenomenon of the median lethal dose (lethal dose 50 [LD_{50}]), which describes the microbe dose that will kill only 50% of a test population [8]. Poultry infected with *S. enteritidis* may suffer systemic infection that can potentially lead to death, or may evolve into a long-term asymptomatic carrier-state [9]. Several studies have confirmed that heterogeneous responses to *Salmonella* infection can be partly explained by the genetic background and immune function status of the host [10][11]. However, numerous studies have also reported the phenomenon of heterogeneous bacterial shedding (super-shedders and low-shedders) in genetically homogeneous host populations [12][13], suggestive of the existence of additional factors that can influence the susceptibility and resistance of individuals to *Salmonella* colonization. Over recent years, the composition of the intestinal microbiota has been increasingly associated with heterogeneous host responses to pathogen infection [14][15][16].

The intestinal microbiota comprises a complex bacterial community and maintaining a mutually beneficial balance between the host and the gut microflora is very important for human health [17][18]. Intestinal dysbiosis can promote or even directly lead to a variety of conditions, including inflammatory diseases, colon cancer, and autoimmune disorders [19]. Pathogen infection is also closely related to the intestinal microbiota. Pathogen infection can lead to an imbalance in the intestinal environment, where pathogen growth is favored over that of probiotics [20][21][22]. Conversely, the gut microbiota can help inhibit pathogen colonization [23][24]. Although various mechanisms through which gut microbiota can protect the host against intestinal infection have been described, it remains unclear whether the heterogeneous responses of poultry to *S. enteritidis* infection are related to subtle changes in gut microbiota composition. In this study, we investigated the infection of *S. enteritidis*-susceptible and -resistant chicks from the aspects of tissue lesions, intestinal health and inflammatory response, and analyzed their cecal microbiota differences.

2. Discussion

Salmonella can be transmitted horizontally to chickens from contaminated environmental vectors and vertically from infected hens to offspring. In this study, 1-d-old female AA female chicks, with *Salmonella* infection excluded by cloacal swab testing, were reared and challenged under the same conditions, therefore eliminating the influence of genetic background and environment on the experimental results to the greatest extent, and ensured that all the phenotypic results obtained in this study were due to individual differences. *S. enteritidis* mainly colonizes the liver, spleen, and intestine of poultry after infection [25][26], leading to intestinal damage, a decline in growth performance, and even death. Growth performance, pathological changes in organs, *Salmonella* loads, and intestinal morphology are important indicators of the severity of *S. enteritidis* infection. In this study, compared with *S. enteritidis*-resistant chicks, the livers of

S. enteritidis-susceptible chicks became swollen (**Table 3**) and displayed salient lesions (**Figure 1**b). In addition, *Salmonella* loads in the liver and spleen of *S. enteritidis*-susceptible chicks were significantly higher than those of *S. enteritidis*-resistant chicks (**Table 1**). The VCR showed an increasing trend in chicks of group R than in chicks of group S (**Table 2**). These results indicated that our grouping scheme, i.e., selecting chicks with differential *S. enteritidis* susceptibility, was appropriate, and confirmed the heterogeneous nature of the response of the birds to *S. enteritidis* infection.

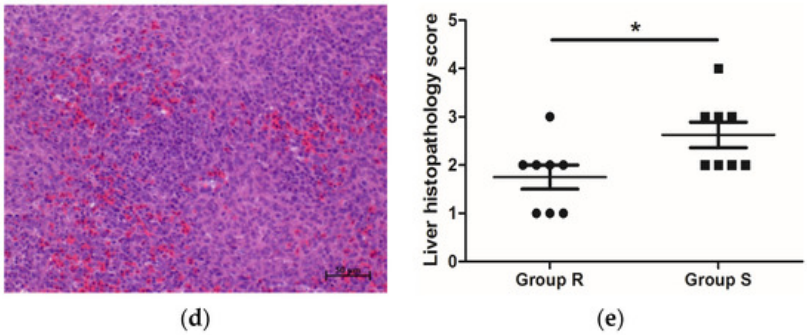

Figure 1. Histopathology of *S. enteritidis*-resistant and *S. enteritidis*-susceptible chicks. (**a**) Representative liver histopathology of *S. enteritidis*-resistant chicks (HE staining); (**b**) Representative liver histopathology of *S. enteritidis*-susceptible chicks (HE staining); (**c**) Representative spleen histopathology of *S. enteritidis*-resistant chicks (HE staining); (**d**) Representative spleen histopathology of *S. enteritidis*-susceptible chicks (HE staining). Original magnification, ×200. Black arrows indicate the lymphocytes, yellow arrows indicate the heterophilic cells, and red arrows indicate the lymphocyte nodules in liver tissue. Scale bar = 50 μm. (**e**) Liver histopathology score of *S. enteritidis*-resistant and -susceptible chicks, n = 8 per group, Result of significance test was $p < 0.05$ when marked *.

Table 1. Body weight, tissue index [1], and *Salmonella* loads of *S. enteritidis*-susceptible and -resistant chicks [2].

Items	Group S [3]	Group R [3]	*p*-Value
BW (g)	229.40 ± 7.47	230.88 ± 5.47	0.875
Liver Index (%)	0.043 ± 0.001 [a]	0.038 ± 0.001 [b]	0.006
Spleen Index (%)	0.024 ± 0.002	0.020 ± 0.001	0.158
Liver *Salmonella* loads (log_{10}CFU/g)	2.750 ± 0.405 [a]	1.152 ± 0.435 [b]	0.018
Spleen *Salmonella* loads (log_{10}CFU/g)	4.784 ± 0.100 [a]	2.491 ± 0.055 [b]	<0.001

[1] Tissue index: Percent of tissue weight relative to body weight. [2] Results are expressed as means ± SEM, with n = 8 per group.[3] Group S = selected *S. enteritidis*-susceptible chicks; Group R = selected *S. enteritidis*-resistant chicks. [a,b] In the same row, values with different letters are significantly different between two groups ($p < 0.05$).

Table 2. Jejunum morphology of *S. enteritidis*-susceptible and -resistant chicks [1].

Items	Group S [2]	Group R [2]	*p*-Value
Villus height (μm)	1084.62 ± 35.20	1125.93 ± 90.23	0.683
Crypt depth (μm)	149.56 ± 7.48	131.55 ± 16.28	0.348
Ratio of villus height-to-crypt depth	7.32 ± 0.34	8.94 ± 0.75	0.090
Muscle thickness (μm)	117.86 ± 7.09	118.02 ± 14.37	0.992

[1] Results are expressed as means ± SEM, with n = 8 per group. [2] Group S = selected *S. enteritidis*-susceptible chicks; Group R = selected *S. enteritidis*-resistant chicks.

The intestinal mucosal barrier serves as the first line of defense between the host and the luminal environment. Composed of epithelial cells and tight junctions, this barrier can prevent the entry of harmful substances, such as pathogens and toxins, into host tissues, organs, and circulating blood [27]. The intestinal epithelium is involved in the formation of the intestinal mucosal barrier by continuously secreting MUC2 to renew the intestinal mucosal layer. Impaired intestinal mucosal barrier function is a key determinant of the pathogenicity of some intestinal bacteria. Studies have

shown that *Salmonella* infection can disrupt the intestinal barrier of broilers, and promoting the expression of tight junction proteins through L-arginine supplementation can alleviate *Salmonella* infection, indicating that there is a negative correlation between intestinal barrier function and the severity of *Salmonella* infection [28]. In this study, we compared the expression of genes encoding tight junction proteins and *MUC2* in *S. enteritidis*-susceptible and *S. enteritidis*-resistant chicks. The results showed that the mRNA expression of *occludin* and *MUC2* in the jejunum of *S. enteritidis*-resistant chicks was significantly higher than that of *S. enteritidis*-susceptible chicks, further supporting that a negative correlation exists between intestinal mucosal barrier function and *S. enteritidis* susceptibility.

Because proinflammatory cytokines are essential for initiating immune responses and eliminating pathogens in the host, we hypothesized that chicks in group R would exhibit higher levels of inflammation than those of group S, therefore explaining the greater resistance of the birds in group R to *S. enteritidis* infection at the same dose of *S. enteritidis* challenge. However, our results showed that there was no significant difference in the expression of most proinflammatory factor-related genes between the two groups. Furthermore, the gene expression of *iNOS* and *IL6* showed the opposite trend to what would be expected, i.e., the expression of both genes was significantly higher in group S than in group R, whereas that of *IL10*, coding for an anti-inflammatory factor, was significantly lower. These results suggested that inflammatory cytokines may play a role in the heterogeneous responses in an unexpected way. Or the higher expression levels of proinflammatory cytokine-related genes may also be considered to be a phenotype of *S. enteritidis*-susceptible chicks, which is consistent with the results of the histopathological analysis of liver tissue. In addition, although *iNOS* is believed to help cells resist bacterial invasion through the production of a large amount of NO, which serves as an antibacterial [29], it is notable that the relationship between NO and *Salmonella* in the host may not be merely antagonistic. It has been reported that *Salmonella* needs NO as a nitrogen source for nitrate respiration, and a low NO concentration is indispensable for promoting *Salmonella* growth [30]. This may also explain why the invasion of *S. enteritidis* in birds of group S was more severe, but their expression of the *iNOS* gene were higher in our research.

In the chicken, the intestinal microbiota is composed of complex microbial communities that are involved in digestion and metabolism, the regulation of intestinal cells, vitamin synthesis, and the development and regulation of the host immune system [31]. There is also accumulating evidence indicating that the intestinal microbiota profoundly influences the pathogenicity of *S. enteritidis* [24]. Because the cecum is the most densely colonized microbial habitat in the chicken [32], we systematically compared the cecal microbial composition of chicks from the different *S. enteritidis* susceptibility groups. Alpha diversity refers to the richness and diversity within a microbial community in individual samples [33], whereas beta diversity is a comparative analysis of microbial community composition in different samples. Although no significant difference was recorded for alpha diversity, significant differences in beta diversity were observed between the cecal samples of the two groups, which agreed with previous results showing that *Salmonella* infection can lead to changes in cecal microbiota [21].

The cecal microbial composition of the two groups at both the phylum and genus levels was analyzed using the Wilcoxon test. The results showed that at the phylum level, the relative abundance of *Acidobacteria*, *Campilobacterota*, and *Fusobacteriota* were enriched in group S. The same results were obtained using LEfSe. At the genus level, 18 genera were identified as significantly differential microorganisms by the Wilcoxon test. Among them, *Fusobacterium*, *Helicobacter*, and *Butycicoccus* were identified as marker microorganisms in group S using LEfSe. As we know, *Fusobacterium* has been associated with gastric ulcers in pigs [34] and colon carcinoma in humans [35][36], and may represent a kind of new opportunistic pathogens of chickens worthy of further investigation [37]. In addition, in the species level, *Helicobacter_pullorum* has also been identified as a marker microorganism of group S, which is member of *Campilobacterota* and a well-known zoonotic pathogen [38]. These results revealed that chicks showing higher *S. enteritidis* resistance has lower abundance of pathogenic bacteria in their cecal.

Furthermore, we identified a marker microorganism, *Desulfovibrio_piger*, which was enriched in chicks of group R. *Desulfovibrio_piger*, belonging to *Desulfovibrio spp.*, is a kind of sulfate reducing bacteria, which can functional reducing sulfate to hydrogen sulfide (H_2S) and plays an important role in intestinal hydrogen and sulfur metabolism. Although H_2S has been found to have dichotomous effects (stimulatory and inhibitory) on several gastrointestinal processes, it seems to be hazardous at high concentrations but favorable at low concentrations, and the overarching effect of H_2S appears to be beneficial. For example, H_2S can attenuate DSS-induced colitis, lessen the shortening of the colon lengths and colonic pathological damages, showing an overall protective effect in colitis via its anti-inflammatory properties [39]. In addition, ATB-429, an H_2S releasing derivative of mesalamine, exhibits a marked increase in anti-inflammatory activity and potency in a murine model of colitis, as compared to mesalamine, seems promising in the treatment of inflammatory bowel disease [40]. Our results were consistent with these above reports, as our chicks in group R showed higher abundance of *Desulfovibrio_piger* and lower inflammation response at the same time. However, whether *Desulfovibrio_piger* can really help chicks to resist the infection of *S. enteritidis* by producing H_2S still need to be verified.

3. Conclusions

In conclusion, our results confirmed that chicks showed heterogeneous responses to *S. enteritidis* infection, including different degrees of *Salmonella* loads in tissues, different tissue lesion severity, and distinct inflammatory responses. Our findings suggested that enhanced intestinal barrier function and cecal microbiota structure, especially a higher abundance of *Desulfovibrio_piger*, may help chicks resist *S. enteritidis* invasion.

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