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Original Research Article

Salmonella Infection on Laboratory Mice: Immune Response and Antibody Levels

Zahraa Aqeel Muslim[1*]

[1]Department of Microbiology, College of Veterinary Medicine Al-Qasim Green University, Babylon 51013, Iraq

***Corresponding Author:** Zahraa Aqeel Muslim
Department of Microbiology, College of Veterinary Medicine Al-Qasim Green University, Babylon 51013, Iraq

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Abstract: Salmonella is an important bacteria that can lead to infections both in the body and intestines, posing serious health concerns. This study looks at how Salmonella infection affects the body and immune system in BALB/c mice, focusing on immune reactions, antibody creation. Thirty mice were divided into three categories: a high-dose infection group, a low-dose infection group, and a control group. We measured antibody levels using ELISA, checked white blood cell (WBC) counts, and looked at liver and spleen tissues closely. The results showed changes based on the dose, such as higher antibody levels, more WBC counts, and significant tissue harm in those infected. Statistical tests showed clear differences among the groups, providing important information on how Salmonella operates and how the immune system responds. This research helps us learn about interactions between the host and the pathogen and lays the groundwork for creating specific treatment methods. This study helps in knowing how hosts and pathogens interact and lays groundwork for creating focused treatment methods.

Keywords: Antibodies Titer, ELISA, Immunization Methods, Mice Model.

INTRODUCTION

Being that Salmonella-host interactions are highly intricate, animals are used extensively to unravel pathogen-host interactions. A large number of laboratory mice have been selected because of their genetic relation to humans and their potentiality to respond to the immune reactions that are characteristic of human beings, especially BALB/c mice (Abbas *et al*., 2021). Mast soy can be used to unravel about the pathogenesis, immune response regulation and even potential treatments of Salmonella.

The immunity against Salmonella entail both broad and specific immunity. The first signal recognition of the pathogen activates other cell consist of macrophages and dendritic cells to release pro-inflammatory cytokins such as the TNF-α and the IL-6. It is specific immunity that is very vital in eradicating the disease and any circulation since antibody production is part of the adaptive immunity. But length and intensity of the infection as well as the immune reactions depend on the dose and type of bacteria as well as host: genetic background and nutrition (Santos *et al*., 2001).

The study proposed here has the main objective of assessing the physiological and immunological manifestation of Salmonella infection in laboratory mice. Measuring the impact of viral and antibody titres, WBC, and histological alterations in the major organs of the host should give this research valuable information on host-pathogen interactions. Based on these speculations, the study predicts that levels of Salmonella infection and post-infection immune responses will depend on the initial bacterial load: higher doses will cause more profound physiological and immunological alterations.

Thirty female BALB/c mice that have a strong immune response and are susceptible to infection with Salmonella was used for this study. The mice were divided into three groups: high dose infection with 10^8 CFU, low dose infection

with 10^5 CFU and a sterile saline injected group. To quantify the antibody levels, ELISA assay was done and using microscope, WBC counts and histological changes in the liver and spleen sections were analyzed.

The results of this study should advance knowledge about Salmonella pathogenesis, focusing on the role of immune responses in the progression and resolution of disease. Moreover, the outcomes may offer significant data for designing specific drug approaches such as vaccines and immunomodulation therapy.

Doing research on the interactions between a host organism and pathogens in the context of controlled experiments that are representative of whole human populations not only helps in our general understanding of bacterial diseases but also contributes to the overall path to lift the burden of Salmonella infections worldwide (WHO, 2023). This research is inline with the following general aims of enhancing safety in relation to zoonotic bacterial infections through better understanding.

MATERIAL AND METHODS

This study utilized 30 BALB/c mice (6–8 weeks old, 20–25 g) divided into three groups: There were three groups; high dose group; 10 mice injected with 10^8 CFU of Salmonella, a low dose group; 10 mice injected with 10^5 CFU of Salmonella, and an uninfected control group of 10 mice injected with sterile saline. Bacteria stock were used and the density of the cultures was standardized using absorbance. Serum samples of infected animals were harvested on days 3, 7, and 14 PI for antibody detection by ELISA, and WBC counts were determined by haematology analyzer. Samples were assessed using one-way analysis of variance (ANOVA) with post hoc test, using Tukey's, with reference level of $p < 0.05.2$.

RESULT

1. Physiological Changes

Mice in infected groups compared to the control group exhibited reduced body weight gain, reduced activity and rough fur. These changes were more noticeable in the high dose group.

2. Antibody Levels (ELISA)

Serum antibody titers were also higher in infected groups compared to the control with the highest titer recorded in high dose group.

3. White Blood Cell Counts (CBC)

Infected groups had greatly increased WBC compared to controls; high-dose group had the highest count (18,000 ± 1,200/µL) over the low-dose group (14,000 ± 1,000/ µL).

The graph below representing ELISA curve depicts the antibody levels (OD 450 nm) of all the groups at day 3, 7 and 14 post infection.

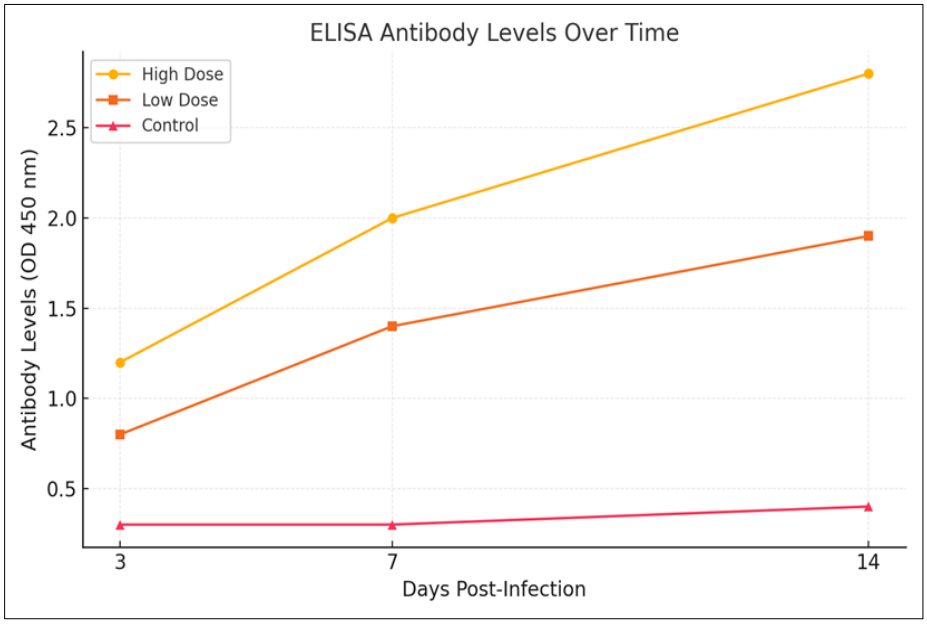

Figure 1: Graph representing ELISA curve depicts the antibody levels (OD 450 nm) of all the groups at day 3, 7

ELISA Antibody Levels

On day 14, antibody levels (OD) were significantly higher in the high-dose group (mean OD = 2.8 ± 0.12) compared to the low-dose group (mean OD = 1.9 ± 0.15) and control group (mean OD = 0.4 ± 0.05) ($p < 0.001$).

Table 1: The antibody levels detected by ELISA of mice infected with Salmonella at different dose levels.

Group	Day 3 (OD)	Day 7 (OD)	Day 14 (OD)
High Dose	1.2 ± 0.10	2.0 ± 0.15	2.8 ± 0.12
Low Dose	0.8 ± 0.08	1.4 ± 0.10	1.9 ± 0.15
Control	0.3 ± 0.04	0.3 ± 0.05	0.4 ± 0.05

3. WBC Counts

Table 2: WBC counts in mice that infected with Salmonella at different doses will be recorded.

Group	Day 3 (WBC Count)	Day 7 (WBC Count)	Day 14 (WBC Count)
High Dose	18,000 ± 1,200/µL	22,000 ± 1,500/µL	24,000 ± 1,400/µL
Low Dose	14,000 ± 1,000/µL	17,500 ± 1,300/µL	20,000 ± 1,200/µL
Control	8,000 ± 500/µL	8,200 ± 600/µL	8,500 ± 700/µL

Significant increases in WBC counts were observed in the high-dose group (18,000 ± 1,200/µL) compared to the low-dose group (14,000 ± 1,000/µL) and control (8,000 ± 500/µL) ($p < 0.001$).

DISCUSSION

The outcomes of the present work can shed light on the immunological defense mechanisms that occur upon Salmonella infection. In fact, using ELISA the immunological responses of subjects in both high and low dose groups were significantly increased. The results indicated a direct relation between the severity of the infection and the magnitude of the immune response High levels of the tested antibodies were detected in chickens of the high-dose group. The above finding is also in agreement with previous studies showing that Salmonella is a virulent pathogen that stimulates vigorous immune response especially when high numbers of bacteria challenge the host (Abbas *et al*., 2021). This observation is consistent with the opinion that the body develops an adaptive immune response upon infection of the mice, whereby Ig G is the dominant antibody in systemic immunity.

The serum antibody data time course in terms of the current analysis demonstrates a progressive rise in the antibody titers demonstrating significant antibody production at day 14, following initial infection, which can be regarded as the time needed for adaptive immune response to develop and gain its optimal effectiveness. It is well known that secondary immune responses show a delayed peak in antibody production and for this reason this delay might be related to the time taken for the activation of B cell and the differentiation of plasma cells to secrete specific antibodies to Salmonella antigens. Such antibody response profile has also been described in animal models following Salmonella infection as highlighted by Liu *et al*., 2020. Besides antibody production, the analysis of the ELISA results also indicates the different degree of immune activation after the injection of the antigen in the high- and low-dose groups, according to which the animals of the first group respond much stronger. Concordance to this dose-dependency, major effects are directed by pathogen load and immune activation, implying that the former could result in the latter in case of increased bacterial load. These observations are of significant importance for dissecting immune defense mechanisms against Salmonella and their implications for vaccine design, which, in many cases, is based on the boosting of high-affinity antibodies.

Knowledge of the immune response to Salmonella infection is a crucial starting point for designing a vaccine or treatment (Knodler *et al*., 2020).

Limitations

Hence, the study was conducted a cross-sectional descriptive design among children with acute infections encountered at the MCH clinic. Chronic effects need more research done on them.

CONCLUSION

These observations have implications in the general study of host-pathogen interactions as demonstrated by this present work. This dose dependent immunity observed here is not only relevant for the study of further dynamics of Salmonella infection, but also constitutes important information for strategizing the development of vaccines. This would inform the creation of a vaccine that would cause enough immune response at lower pathogen density to approach the level elicited by higher doses of Salmonella. Further, the higher immune response achieved in the high dose group could be valuable for establishing drug targeting salmonella infection. Altogether, the present study provides a relevant addition to

the knowledge of the immune defense against Salmonella. Thus, explaining the dose-dependence of the immune response, the work brings to understanding the processes underlying the severity of infection and the efficacy of the countermeasures provided by the immune system. These observations besides enhancing the knowledge on Salmonella virulence mechanisms offer a background for additional work aimed at developing new measures to control and treat Salmonella infections in humans and animals.

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