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Original Article

Immune Response and Histological Changes in Broilers Chickens Vaccinated with *Mycoplasma gallisepticum* Vaccines

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Abstract

Mycoplasma is unique among prokaryotes because of its small size, small genomes, and complete lack of cell walls, which makes them cell wall-less prokaryotes. This study aimed to evaluate the effect of vaccinating one-day-old chicks with inactivated and live vaccines (CRDF) of *Mycoplasma gallisepticum* (MG) on their humoral immune response and immune organs. The Enzyme-Linked Immunosorbent Assay was used to measure Ab titers and investigate histopathological changes. A total of 130 one-day-old broiler chicks were randomly divided into four groups of 30. The groups were treated as follows: G1 included the chicks vaccinated with live F-strain MG vaccine (on eye drop of 0.03ml/dose), G2 included the chicks vaccinated with inactivated MG (0.3 ml s.c) vaccine, G3 included the chicks vaccinated with inactivated and live MG vaccines, and G4 was considered the control group, in which the chicks were not vaccinated. Blood samples were collected on days 21 and 35 of the chick's life to measure the titers of specific antibodies. On day 35, the chicks were dissected, and the bursa of Fabricius, as well as the spleen, were removed for histological evaluations. On day 21, the results showed a significant difference ($P \leq 0.05$) between all vaccinated groups in Ab titers, compared to G4, with the highest mean in G3, followed by G2 and G1, in descending order. On day 35, there was a significant difference ($P \leq 0.05$) between G3 and other vaccinated groups (G2 and G1), as well as G4. In addition, there was a significant increase in all vaccinated groups on day 35, compared to day 21. In G1, histopathological examination results showed a moderate lymphocytic hyperplasia bursal follicle. In G2, varying degrees of lymphoproliferative were observed in the major bursal follicle, and in G3, a marked lymphocytic hyperplasia bursal follicle was observed. In G4, on the other hand, no obvious histopathological findings were recorded. The results of the spleen histopathological evaluation showed various degrees of lymphoproliferative and moderate neutrophilic infiltrate in the red pulp in G1, and mild sinus congestion with scattered lymphocytes was recorded in the lumen in G2. In the spleen of the chicks in G3, reactive lymphoid hyperplasia was observed. In contrast to the groups mentioned above, in G4, the spleen structure showed a typical structure. It was concluded that the chicks vaccinated with inactivated and live MG vaccines experienced increased production of Ab titers and the immune stimulation of immune organs.

Keywords: *Mycoplasma gallisepticum*, Live vaccines, Vaccine

1. Introduction

Mycoplasma is unique among prokaryotes because of its small size, small genomes, and complete lack of cell walls, which makes them cell wall-less prokaryotes (1). A single trilaminar membrane made of protein, glycoprotein, glycolipid, and phospholipid exists in the minuscule bacterial species, *Mycoplasma*, which lacks a cell wall but has the genetic ability to make one (2). *Mycoplasma gallisepticum* (MG) is the most common pathogenic species of avian *Mycoplasma* and is one of the most costly diseases due to a decline in egg production, egg quality, reduced hatchability, poor feed conversion ratio, increased mortality, and carcass condemnations of broilers (3). This respiratory and

reproductive tract infection can cause severe chronic respiratory disease (CRD), whose transmission is speeded up through horizontal and vertical disease transmission (4). During the winter and autumn, the highest prevalence of MG is observed in chickens and turkeys (5, 6). When biosecurity is insufficient to prevent MG-related losses in multi-age farms, vaccination gives a management option for clinical disease prevention (3). The available vaccination options include inactivated, oil emulsion bacterins, live, or recombinant live vaccines.

Oil emulsion bacterins can be used to prevent the entrance of live vaccination strains. Studies have demonstrated that MG bacterin can lessen respiratory symptoms, including airsacculitis, and decrease egg production (7). The contact-challenge model gave only modest protection (3, 8). Serological tests are the preferred method of testing MG antibodies due to the ease of obtaining sera, as well as the sensitivity and reproducibility of the assays (9). Chickens' maternal antibodies gave relatively little protection against the challenge and had no impact on day one of MG immunization with the F-strain or live MGF vaccination (10). The live attenuated vaccine has been widely utilized since it is highly immunogenic and successful at eradicating virulent (field) strains from chicken operations (11). Inactivated MG vaccinations (bacterins) were used in Iraq. The local isolate of MG was found among broilers and layers, which had virulence factors aiding in pathogenicity in the respiratory system and was able to induce inflammation response after one week post-infection (12). Recently, MG vaccines have entered Iraq and have been used in farms. Therefore, this study aimed to assess the impact of vaccinating one-day-old broiler chicks with live and inactivated MG vaccines on their humoral immunity and immune organs.

2. Materials and Methods

2.1. Preparation of Poultry House

The experiment was conducted in an animal house at Baghdad University College of Veterinary Medicine, Baghdad, Iraq. Before initiating the experiments, the experiment house was washed, cleaned, and disinfected with formalin. The same breeding management was applied to all experimental groups. Feeders and watering cans were cleaned and sterilized. As previously mentioned by Burnham, Branton (13), complete rations and tap water were made available, and feed and water were given as needed.

2.2. Study Design

This experiment was conducted to study the efficacy of MG vaccines in 120 chicks. One-day-old broiler chicks (ross308) from (Al-Tajy hatchery) were used to carry out this experiment lasting 1 to 35 days. The animals were divided randomly into four groups (n=30) as follows:

The first group (G1) included the chicks vaccinated with live MG vaccine (CRDF) (one eye drop of 0.03 ml/dose). In the second group (G2), the chicks were vaccinated with inactivated MG vaccine (0.3 ml s.c). In the third group (G3), the chicks were vaccinated with both inactivated and live MG vaccines. The fourth group (G4) was considered the control group, in which the chicks were not vaccinated.

2.3. Immunological Tests

On days 21 and 35 of the chick's life, blood samples were taken from their jugular vein and allowed to clot. Sera were separated and stored at -20°C until they were used to measure specific Ab titers against MG by the Enzyme-Linked Immunosorbent Assay (ELISA). The tests were carried out according to the manufacturer's assay protocol.

2.4. Histopathological Examination

On day 35, the chicks were dissected, and the bursa of Fabricius, as well as the spleen, were removed for histological evaluations. The samples of organs were placed in 10% formalin for fixation, and then, they were prepared for histological examination by passing them with different concentrations of ethyl alcohol, xylene, and paraffin, as well as staining them with hematoxylin and eosin, according to the previously described method (14).

3. Results

The results showed a significant difference ($P \leq 0.05$) between all vaccinated groups in Ab titers, compared to G4 on day 21. The highest mean was observed in G3 (1039.10±40.77), followed by G2 and G1, which were 986.60±16.54 and 980.10±11.42, respectively. The lowest mean was recorded in G4 as 201.70±14.56. On day 35, there was a significant difference ($P \leq 0.05$) between G3 (3115.50±80.19) and other vaccinated groups, including G2 (2774.90±176.78) and G1 (2311.70±111.46), as well as G4 (284.70±18.35). Additionally, there was a significant increase in all vaccinated groups on day 35, compared to day 21 (Table 1). The histopathological examination showed an immune reaction in immune organs (bursa and spleen). In G1, the results showed a moderate lymphocytic hyperplasia bursal follicle and hyperplasia of the epithelial capsule. In G2, varying degrees of lymphoproliferative were observed in the major bursal follicle, while the bursa of Fabricius in G3 showed marked lymphocytic hyperplasia bursal follicle and hyperplasia of the epithelial capsule. On the other hand, the bursa of Fabricius showed no obvious histopathological findings in G4 (Figures 1, 2, 3, and 4). The histopathological evaluation in the spleen also showed an immune reaction expressed by various degrees of lymphoproliferative, fibro hypertrophy of the splenic arterial, and a moderate neutrophilic infiltrate in the red pulp in G1. In G2, mild sinus congestion was observed with the scattered presence of lymphocytes in the lumen. In G3, the animal spleen showed reactive lymphoid hyperplasia. In G4, red and white spleen pulp aspects were observed as typical (Figures 5, 6, 7, 8, and 9).

Table 1. Results of ELISA to measure the specific Ab titer in serum post-vaccination with *Mycoplasma gallisepticum* MG vaccines in broilers chickens (mean±SE)

Groups/Age	21day old	35 day old
G1	980.10±11.42Ba	2311.70±111.46Ac
G2	986.60±16.54Ba	2774.90±176.78Ab
G3	1039.10±40.77Ba	3115.50±80.19Aa
G4control	201.70±14.56Ab	284.70±18.35Ad
LSD	228.86	

Means in the same column with different tiny letters are substantially different (P<0.05).
Means in the same row with different capital letters are substantially different (P<0.05)**. G1**:alive vaccine (eye drop), G2: killed vaccine, G3: a live vaccine and killed vaccine, G4: Not vaccinated.

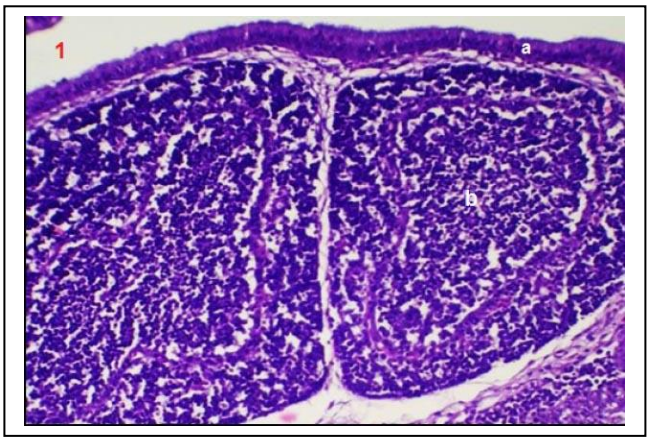

Figure 1. The histopathological section in the bursa of Fabricius (G1) shows moderate lymphocytic hyperplasia in the bursal follicle (b) and hyperplasia of the epithelial capsule (a) (H&E, ×40)

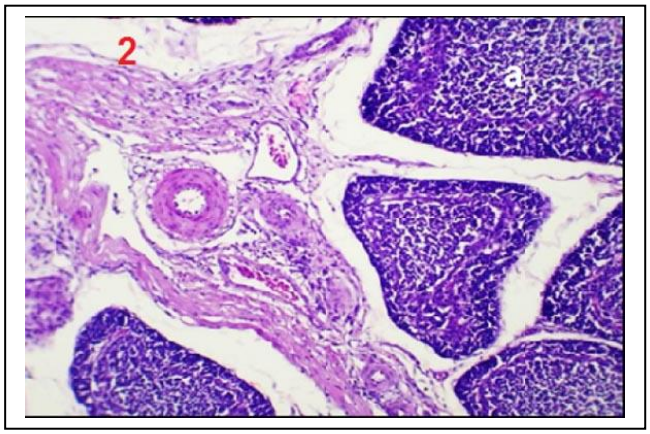

Figure 2. The histopathological section in the bursa of Fabricius (G2) shows a varying degree of lymphoproliferative observed in the primary bursal follicle (a). (H&E**,** ×10)

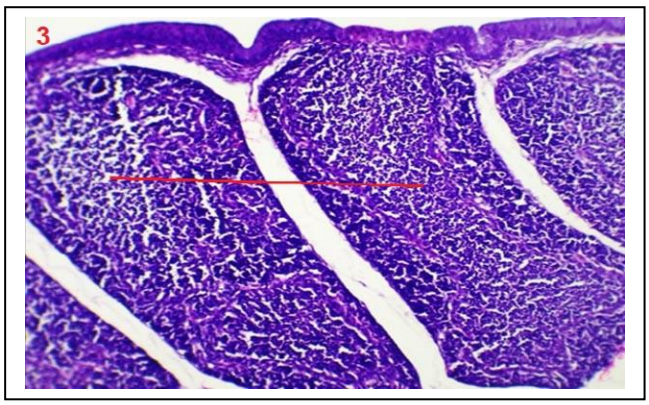

Figure 3. The histopathological section in the bursa of Fabricius (G3) shows marked lymphocytic hyperplasia bursal follicle and hyperplasia epithelial capsule. (H&E, ×40)

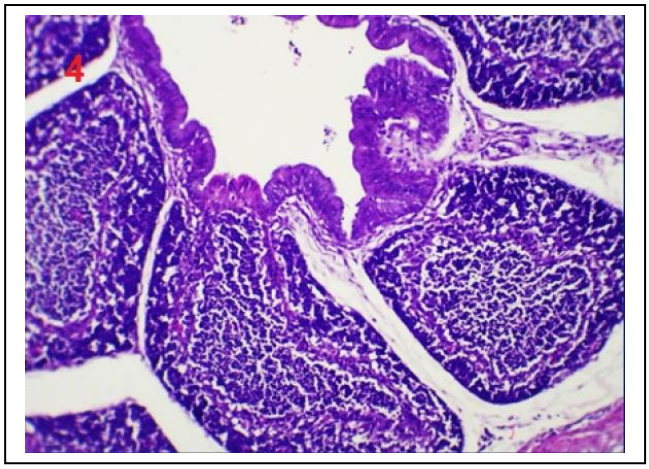

Figure 4. The histopathological section in the bursa of Fabricius (G4) control group shows no obvious histopathological findings. (H&E, ×40)

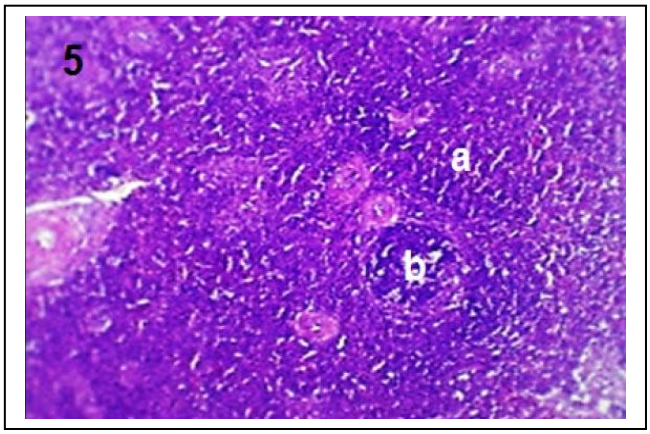

Figure 5. The histopathological section in the spleen (G1) also has various degrees of lymphoproliferative (a&b). H&E, ×10)

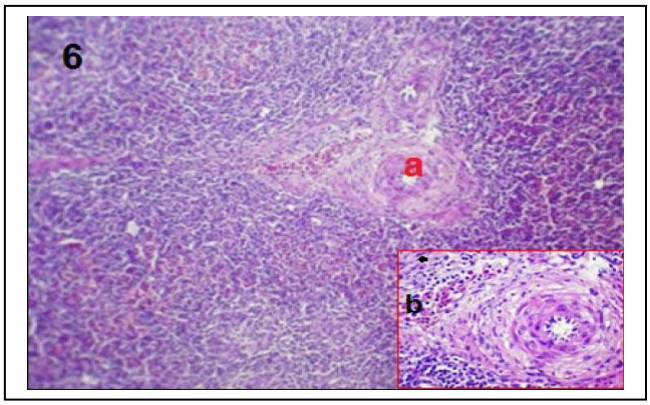

Figure 6. The histopathological section in the spleen (G1) shows fibro hypertrophy of splenic arterial and moderate neutrophilic infiltrate in red pulp (b) tissue. (H&E, ×10 and 40)

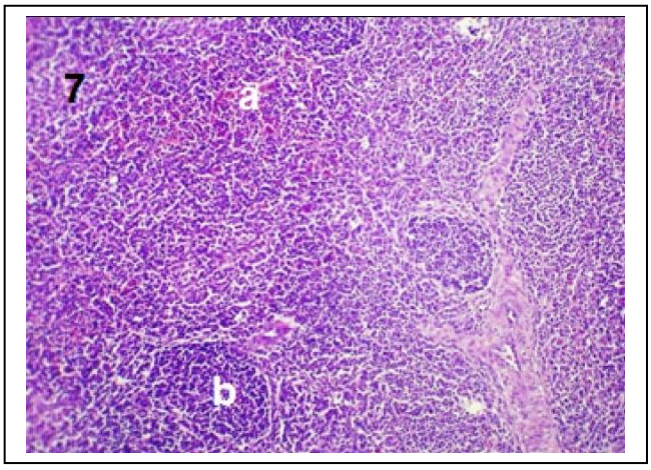

Figure 7. The histopathological section in the spleen (G2) shows mild sinus congestion (a) with the scattered presence of lymphocytes in the lumen (b). (H&E, ×10)

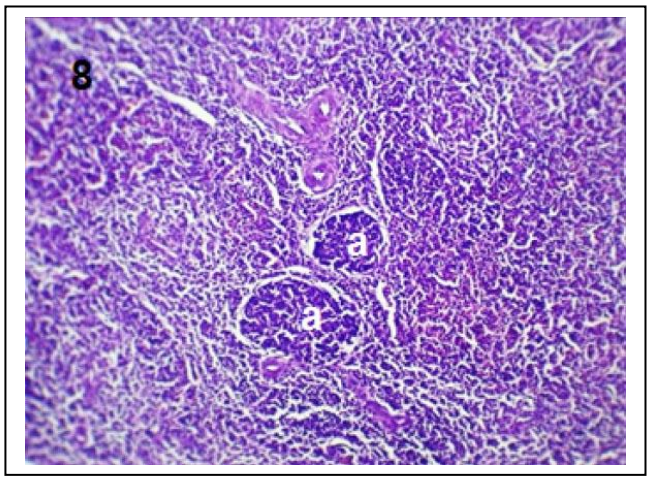

Figure 8. The histopathological section in the spleen (G3) shows reactive lymphoid hyperplasia (a). (H&E, ×10)

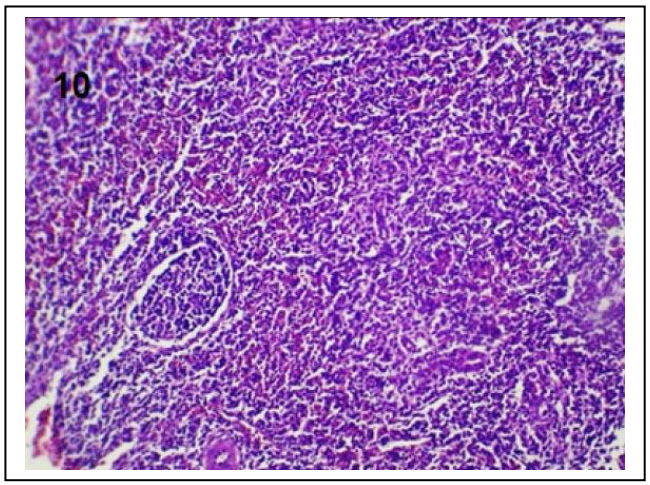

Figure 9. The histopathological section in the spleen (G4) control group shows the presence of a normal aspect of red and white pulp. (H&E, ×10)

4. Discussion

Immune responses to vaccination against MG have been most commonly assessed by detecting serum antibodies using rapid serum agglutination or ELISA (15). In the current study, the immune response of chickens vaccinated with live MGF and inactivated MG vaccines was evaluated by the ELISA, and the results indicated that both types of vaccines induce unique immunological responses through developing certain antibodies in 21- and 35-day-old vaccinated birds. These findings agree with previous studies using the ELISA (9, 16, 17), showing positive titers after receiving live MGF vaccine, which persisted until the experiment's conclusion. Live attenuated vaccine can cause both localized MG-specific immunity and a minimal degree of systemic immune response by colonizing the upper respiratory tract (16). The F-strain is engulfed by macrophages, and the antigen is represented into the T-cell, which is activated and proliferated into the cells. The activated T-cell secretes interleukin (IL), which stimulates the production of inflammatory cells, such as neutrophils, monocytes, and lymphocytes. The activation then leads to the proliferation of B-lymphocytes which are changed to plasma cells to produce immunoglobulin (18). Bacterins from MG vaccinations seem to largely elicit a systemic antibody response. The results of inactivated vaccination agree with other studies (19, 20), which discovered that chickens receiving oil-based MG vaccines develop protective levels of anti-MG antibodies. In addition, Monira Noor (21) found that the "oil-adjuvant MG vaccine" granted anti-MG antibodies in broilers after 15 days of vaccination. Macrophages or antigen-presenting cells (APCs) in the granuloma ingest the microbial antigen from the oily suspension and present the microbial protein antigen on their surface in association with self-MHC II. T helper cells from immunized birds recognize their antigens on the APC surface and undergo blast formation, proliferation, and differentiation into eosinophils. Oil-based bacteria irritate the inoculation site and induce granuloma (22, 23). The effector T-lymphocytes secrete cytokines, such as IL 2, 3, 4, and 5, as well as interferon-gamma, which activate macrophages, cytotoxic T-cells, natural killer cells, and B-lymphocytes that have been triggered by antigens (23). B-lymphocytes from vaccinated birds undertake the processes of blast formation, proliferation, and differentiation into plasma, and memory cells under the influence of cytokine help vaccinated birds recognize their specific antigen from the location of the inoculation. Specific immune globulins, such as IgM and IgG, are secreted by plasma cells and released into

the bloodstream (20). This reflex is the immunological response in the vaccinated groups' spleen and Fabricius bursa (two immune organs), which is more obvious in G3 vaccinated with both vaccines. This finding revealed reactive lymphoid hyperplasia in the spleen and marked lymphocytic hyperplasia bursal follicle because of both vaccines. This study is considered the first to investigate the histopathological effect of live and inactivated MG vaccines on the bursa and spleen. Therefore, the results of this study demonstrate that the vaccination of one-day-old broiler chicks with inactivated and live MGF vaccines is effective in the production of Ab titers and has a positive effect on immune organs.

Authors' Contribution

Study concept and design: S. M. K. and A. J. A.
Acquisition of data: S. M. K.
Analysis and interpretation of data: S. M. K.
Drafting of the manuscript: A. J. A.
Critical revision of the manuscript for important intellectual content: A. J. A.
Statistical analysis: A. J. A.
Administrative, technical, and material support: S. M. K. and A. J. A.

Ethics

The study protocol was approved by the ethics committee of the Veterinary Medicine University of Baghdad, Baghdad, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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