



The Impact of Two Types of *Mycoplasma gallisepticum* Vaccines on Broiler Chicken Respiratory System.

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Abstract

This study aimed to evaluate the effect of vaccinating 1-day-old broiler chicks with two commercial vaccines such as live chronic respiratory disease F strain (CRDF) and killed *Mycoplasma gallisepticum* (MG) vaccines on the respiratory system. For this purpose, one-day-old broiler chicks (n=120) were randomly divided into four groups; (n=30 birds each). All groups were treated as followed: G1(CRDF): chicks were vaccinated with live CRDF vaccine (eye drop one drop 0.03ml/dose/10⁵), G2 (killed MG): chicks were vaccinated with killed MG vaccine injection with 0.3ml s.c /3 x10⁻¹⁰ C.F.U/ml), G3: chicks were combined vaccinated with killed MG and CRDF live vaccines G4: served as control unvaccinated group. The vaccination was performed at one day old in G1 (CRDF), G2(killed MG), and G3 (killed MG and live CRDF). The histopathological changes of the trachea and air sacs were examined and evaluated at 35 days old. The results of histopathological examination of air sacs in the G1(CRDF) revealed various mild epithelial hyperplasia with papillary-like growth, subepithelial lymphocytic infiltration, in G2 (killed MG), showed hyperplastic epithelial surfaces with moderate lymphocytic infiltration mainly around congestion capillaries. While G3 (killed MG and CRDF) showed focal epithelial hyperplasia (villus-like), and perivascular lymphocytic aggregation in lamina C.T. Whereas G4 (control) showed normal structures without any histopathological lesions. In the trachea the histopathological changes in G1(CRDF) showed focal lymphocytic aggregation with a follicle-like appearance, another section showed focal epithelial and cilia loss with goblet cell hypertrophy of the adjacent gland. G2 (killed MG) showed partial loss of thin mucosa with mild and desquamation lymphocytic infiltration in lamina propria, G3(killed MG and CRDF) showed diffuse mononuclear cells (MNCs) infiltration in tracheal mucous and submucosa with evidence of cystic dilation of adjacent gland together G4 control tracheal tissue showed normal structure without any lesions. Finally, it was determined that vaccination with killed MG and live CRDF vaccines caused a more obvious immune reaction (lymphoproliferative infiltration) in the respiratory system, including the trachea and air sac than the



vaccination with combined killed and live vaccines, while the live *Mycoplasma* vaccines individually caused minor damage, particularly in the trachea.

Key words : *Mycoplasma* ,poultry ,trachea, air sacs ,humoral immunity ,cellular immunity.

I. INTRODUCTION

Mycoplasmas are bacteria that do not have cell walls and are classified as Mollicutes were first discovered in 1935. One of the most important *Mycoplasma* species that affect hens and turkeys known as *Mycoplasma gallisepticum* (MG) is the most serious pathogen of poultry in the commercial poultry industrial world (1;2;3). Extremely pathogenic MG causes severe financial losses in commercial poultry farms around the world, as well as increased feed intake and decreased weight gain in infected birds (4; 5; 6) MG infection causes avian respiratory mycoplasmosis, which can lead to infectious sinusitis in turkeys and chronic respiratory illness in hens, both of which can increase mortality and retard growth (7; 8). Although MG mostly affects chickens and turkeys, other avian species, such as quails, geese, guinea fowls, house finches, starlings, etc., have also been reported to be susceptible (9;10). MG can spread horizontally or vertically through direct or indirect contact (dust, aerosol, etc.) in the egg (3). MG predisposes birds to other infections which cause mortality, poor growth, and condemnation of the carcasses (11;12). Avian mycoplasmosis can be identified by isolation, identification, antibody detection, and polymerase chain reaction (PCR). (13; 14). The antibiotic treatment has been shown to be insufficient for eradicating the pathogen in flocks with a persistent mycoplasma infection since it only works to lessen the clinical symptoms (15; 16). The best way to control the infection and stop the spread of the disease is to maintain MG-free breeder flocks, one such method depends on rigorous biosecurity controls and quick diagnosis of MG infections (17). Another is by immunizing the chickens with MG vaccines (18). Nevertheless, when isolation and biosecurity precautions cannot be implemented, managing MG infection using vaccinations has proven to be successful (19). MG is the most common infection in Iraq, and numerous researchers have successfully isolated it from broiler and layer farms that had respiratory indications of infection (20; 21; 22). There are two types of vaccination: live vaccines like the F-strain, TS-11, and 6/85 strain and killed vaccines (bacterins) (17; 23; 24). F-strain engulfed by macrophage and then represent the antigen into T-cell which activated and proliferated into the cells, activated T-cell secretes interleukin which stimulates the production of inflammatory cells as heterophils, monocytes, and lymphocytes, the activation led to a proliferation of the B-lymphocytes which changed to plasma cells to produce immunoglobulin (12). Inactivated MG vaccines (bacterins), appear to primarily produce a systemic antibody response, (25). No studies or data were available to evaluate the commercial *M. gallisepticum* vaccines introduced into Iraq. Therefore, the present study aimed to evaluate histopathological changes in broiler chickens vaccinated with two commercial MG vaccines.

II. MATERIALS AND METHODS

Preparation of poultry house

The experiment was carried out at the poultry house of the College of Veterinary Medicine, University of Baghdad. Before the experiment started, the poultry house was washed, cleaned, and formalin disinfected. Brooders were used to manage the temperature. 10 cm of wood mince litter was spread throughout the ground. The same breeding management applied to each group. Feeders and watering cans have been cleaned and sterilized. complete rations and tap water were made available, and feed and water were given as needed (26).

The experiment

From 1 to 35 days, 120 1-day-old broiler chicks (ross308) breeds were purchased from the AL-Tajy hatchery and were used in this experiment to evaluate the efficacy of the MG vaccine. On the first day, chicks were randomly divided into equal four groups, each group included 30 chicks, and treated as follows:

The first group (G1) received an eye drop of the live PRO-VAC (CRDF) Strain F vaccines (Komipharm, Korea) (0.03ml/dose/10⁵).

The second group (G2) received MYC-VAC-killed MG R Strain vaccines (Fatro, Italy) (0.3 ml/3x10¹⁰ s.c. in the neck).

The third group (G3) received both killed MG and live CRDF vaccines simultaneously with the same dose above.

The fourth group, G4, is the unvaccinated control group.

Histopathological examination

At 35 days old (end of the experiment) the collected samples (air sac and trachea) from chickens of all groups were placed in formalin 10% (England) for fixation, then the samples were prepared for histological examination by passing them with different concentrations of ethyl alcohol (Baghdad, Iraq) xylene, and paraffin. They worked them into waxy molds and cut them with a shredder of five microns and fixed them on glass slides and dyed them with Hematoxylin and Eosin stain (27).

III. RESULTS AND DISCUSSION

Live vaccine (F-strain) processing by macrophage lead to activated and proliferated of T cells then secretes interleukin which stimulates and production of heterophile, monocyte and the B-lymphocytes, the last which changed to plasma cells and memory cells (12). Inactivated MG vaccines (bacterins), appear to primarily produce a systemic antibody response, (25). The histopathological changes of air sacs in the G1 (vaccinated with a live vaccine (CRDF) revealed various degrees of epithelial hyperplasia, either with papillary-like



growth accompanied by subepithelial lymphocytic infiltration or numerous congestion capillaries (Figure 1). Air sac of G2 (vaccinated with killed MG vaccine) showed numerous cysts formation appeared at hyperplastic epithelial surfaces with moderate lymphocytic infiltration mainly around congestion capillaries (Figure 2). While G3(CRDF and Killed MG) showed focal epithelial hyperplasia with villus feature accompanied by perivascular lymphocytic aggregation in lamina connective tissue (C.T), similar observations may record in other sections with vessel congestion with nodular MNCS infiltration composed mainly of lymphocytes (Figure3). Air sac in G4 (control) showed normal thin-walled structures with poor blood supplies the inner part with ciliated squamous epithelium supported with stroma containing collagen fibers, while the outer part was covered with simple squamous epithelium supported with loose C.T. (Figure 4). In the trachea, the histopathological changes in G1(CRDF) showed focal lymphocytic aggregation with a follicle-like appearance accompanied by submucosal edema rich with congestion capillaries (Figure 5). Also, another section showed focal epithelial and cilia loss with goblet cell hypertrophy of adjacent glands (Figure 6). G2(Killed MG) showed partial loss of thin mucosa with mild lymphocytic infiltration in lamina propria and no signs of inflammatory cell infiltrations in the submucosal layer (Figure 7). G3 (CRDF and Killed MG) showed diffuse MNCs infiltrations in tracheal mucous and submucosa with evidence of cystic dilution of adjacent glands together with mild submucosal congestion and cellular infiltrations (Figure 8). G4 (control) showed normal structures consisting of four layers with normal limits (Figure 9). Several studies have looked at the various lymphocyte subsets invading chickens' respiratory tracts during MG infection or following vaccination and challenge, in research by Javed *et al.* (28) who investigated those immune responses to MG infection in the trachea between vaccinated and uninfected hens, intriguing. Vaccinated chickens formed secondary lymphoid follicle-like aggregates with much fewer lesions than unvaccinated hens. Unvaccinated chickens showed a significant infiltration of B and T cells as well as some plasma cells. Additionally, far more *Mycoplasma*-specific ASC could be found in the tracheal tissue of birds that had received vaccinations than in those who had not received the vaccine. This suggests that many infections can cause lymphoid tissue to develop in the tracheal mucosa. The MG. F-strain immunization displayed a very comparable pattern of infection in which mild lesions in tissues resulted in less tissue damage followed by faster recovery (29; 30; 31;32). The vaccination with killed MG and live CRDF vaccines caused the immune reaction (lymphoproliferative infiltration) in the respiratory (trachea, air sac) and more obvious in vaccination together with killed and live vaccines and alive *Mycoplasma* vaccines caused slight damage, especially in the trachea., This work provides additional evidence for the importance of a locally induced humoral immunological memory response this provide by live vaccine and this lead to improvement of killed vaccine reaction (33) this study considered the first which investigate the histopathological changes effect of live and killed vaccine of MG in respiratory system So the Results of this study demonstrate that vaccination of broilers chicks at one day old with Inactivated MG and MGF live vaccines are effective in production of local immunity by induction of immune reaction by increasing production of B and T lymphocyte cells .



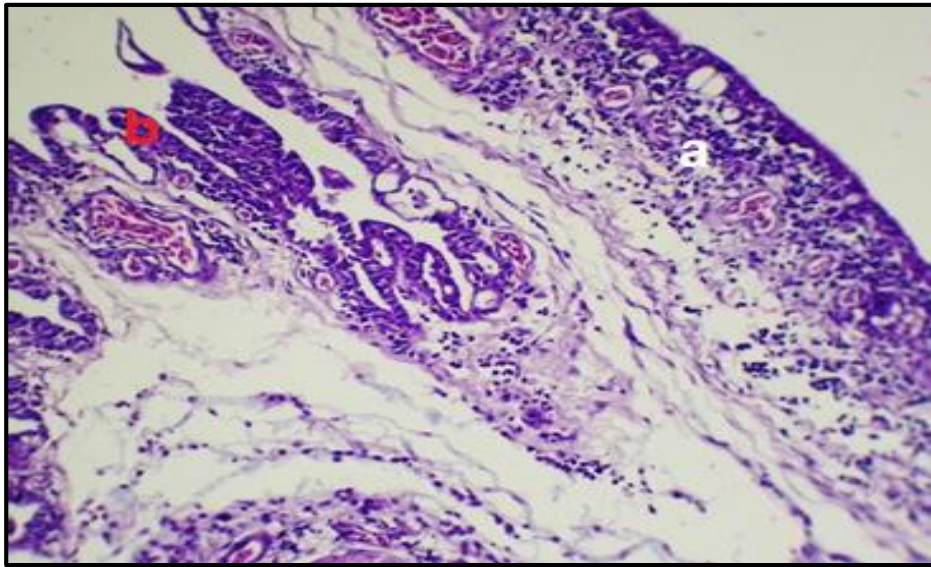


Figure 1. The histopathological section in the air sac of the first group that received an eye drop of live CRDF vaccine showed sub-epithelial lymphocytic infiltration (a) and epithelial hyperplasia papillary-like growth (b) Hematoxylin and eosin staining (H&E) low magnification power (10X)

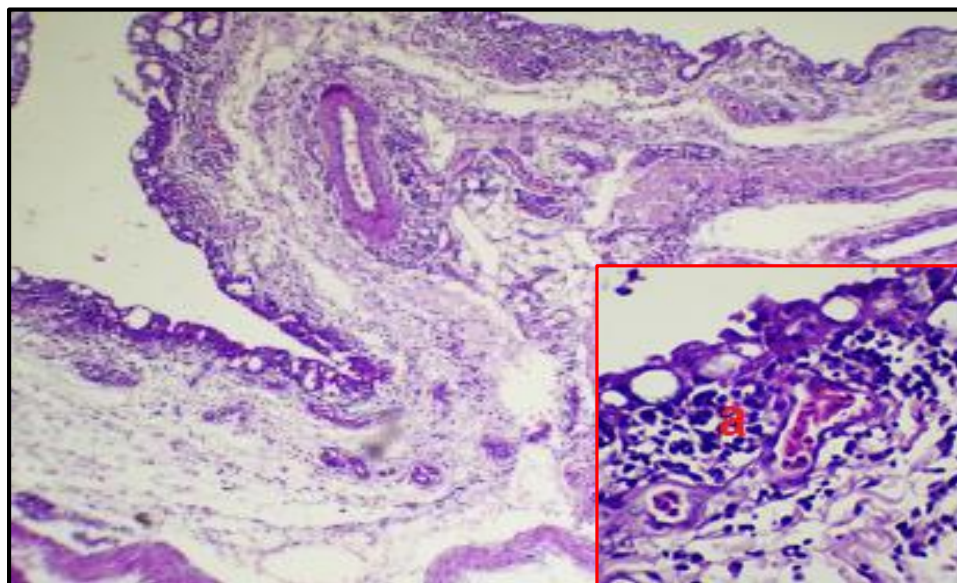


Figure 2. The histopathological section in the air sac of the second group of chicks that received the killed MG vaccine showed moderate lymphocytic infiltration mainly around congestion capillaries' a. (Hematoxylin and eosin staining (H&E) low (10 X) and high (40 X) magnification power.

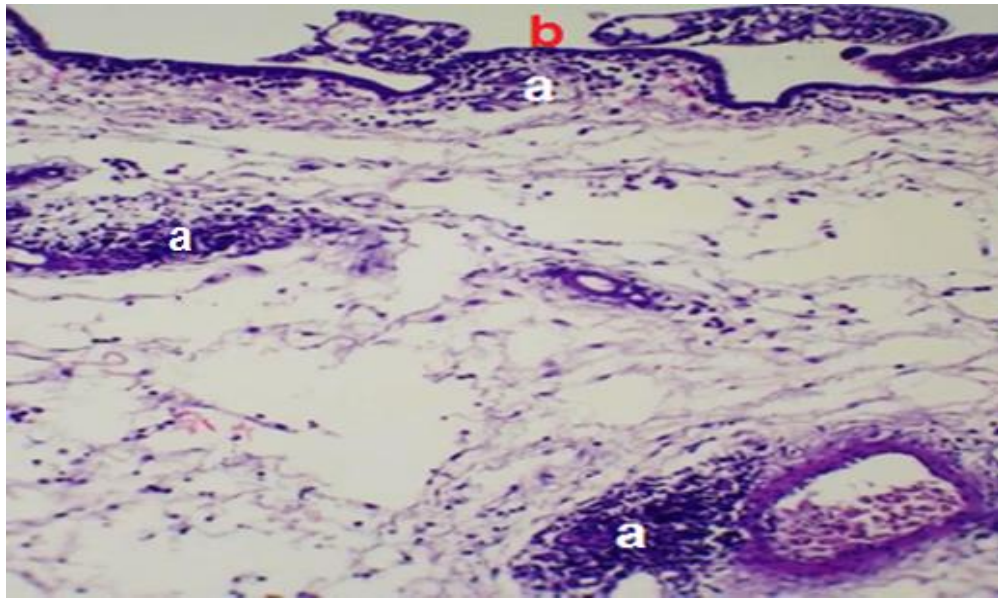


Figure 3. The histopathological section in the air sac of the third group that received both killed and live MG vaccines at the same time shows focal epithelial hyperplasia (b) accompanied by perivascular lymphocytic aggregation in lamina C.T.(a) (Hematoxylin and eosin staining (H&E) low (10 X) magnification power.

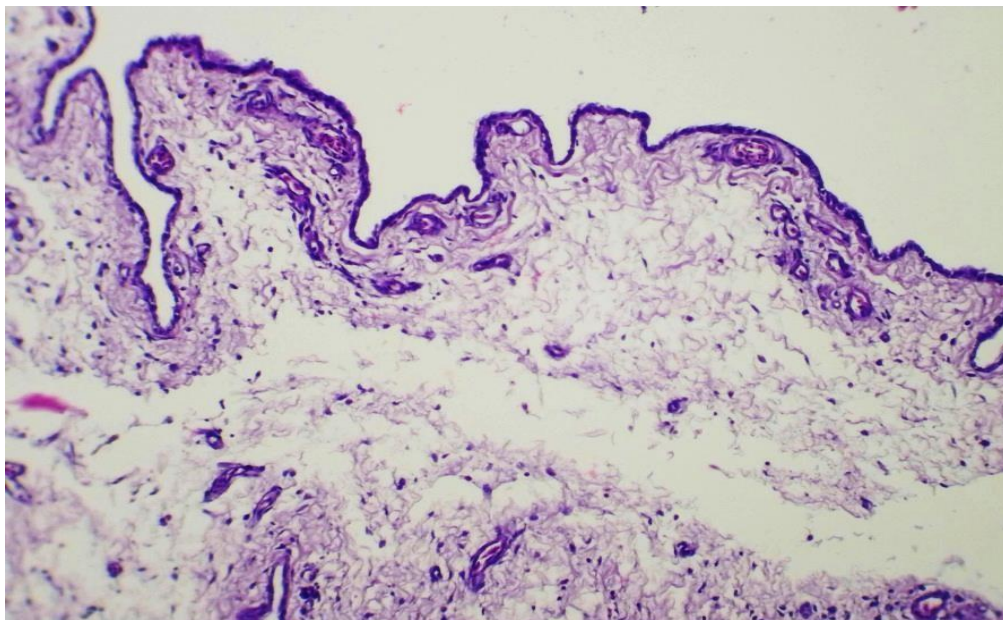


Figure 4. The fourth group is the unvaccinated control group. (Hematoxylin and eosin staining (H&E) low (10X) magnification power.

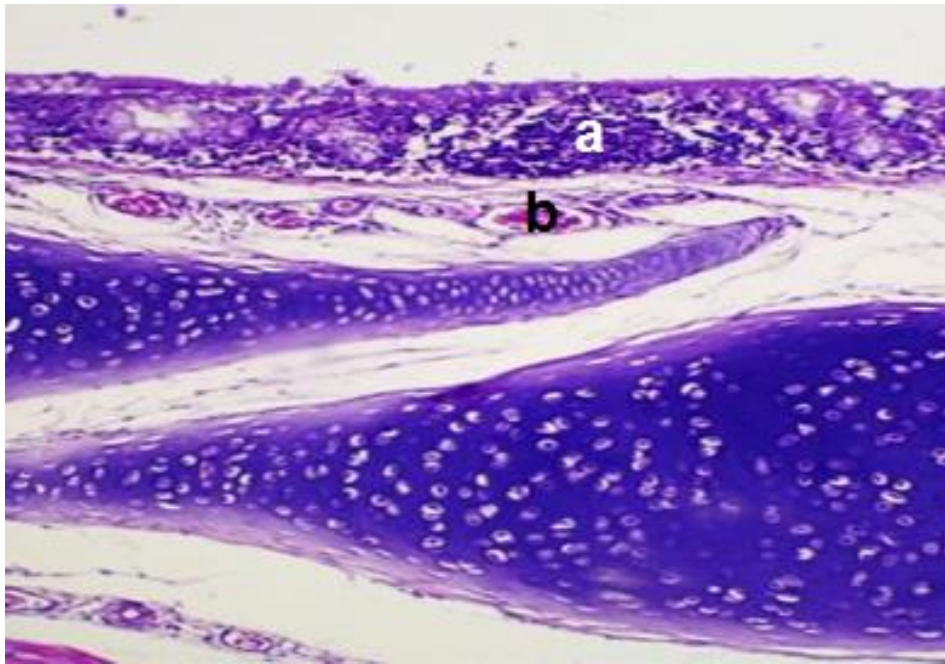


Figure 5. The histopathological section in the trachea of the first group that received an eye drop of the live MG vaccine shows focal lymphocytic aggregation with a follicle-like appearance (a) accompanied by submucosal edema rich with congestion capillaries (b). (Hematoxylin and eosin staining (H&E) low (10X)

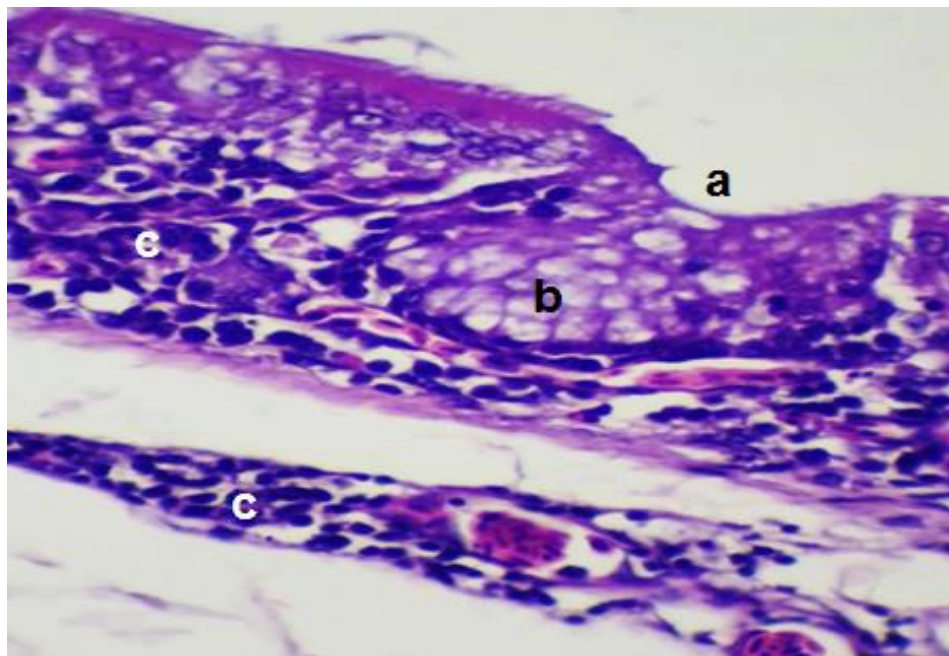


Figure 6. The histopathological section in the trachea of the first group that received an eye drop of the live MG vaccine shows partial loss of epithelium & cilia (a) with slight hyperactivity of adjacent glands (b)

diffuse mucosal & submucosa with MNCs aggregation mainly around congested capillaries(c)
(Hematoxylin and eosin staining (H&E) low (10X) magnification power.

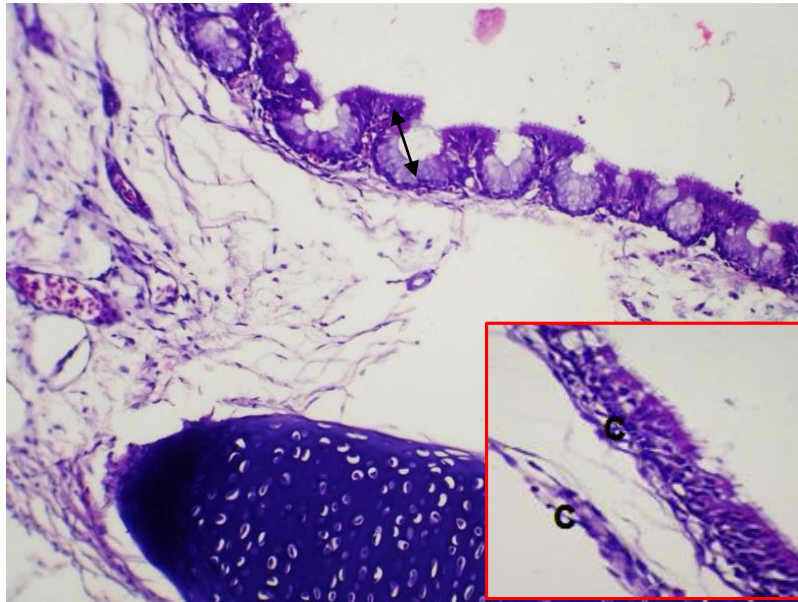


Figure 7. The histopathological section in the trachea (G 2) shows marked mucosal epithelial hyperplasia \longleftrightarrow with intense goblet cell hypertrophy and lymphocytic infiltration. in the mucosa and lamina propria (c) (Hematoxylin and eosin staining (H&E) low (10 X) and high (40 X) magnification power.

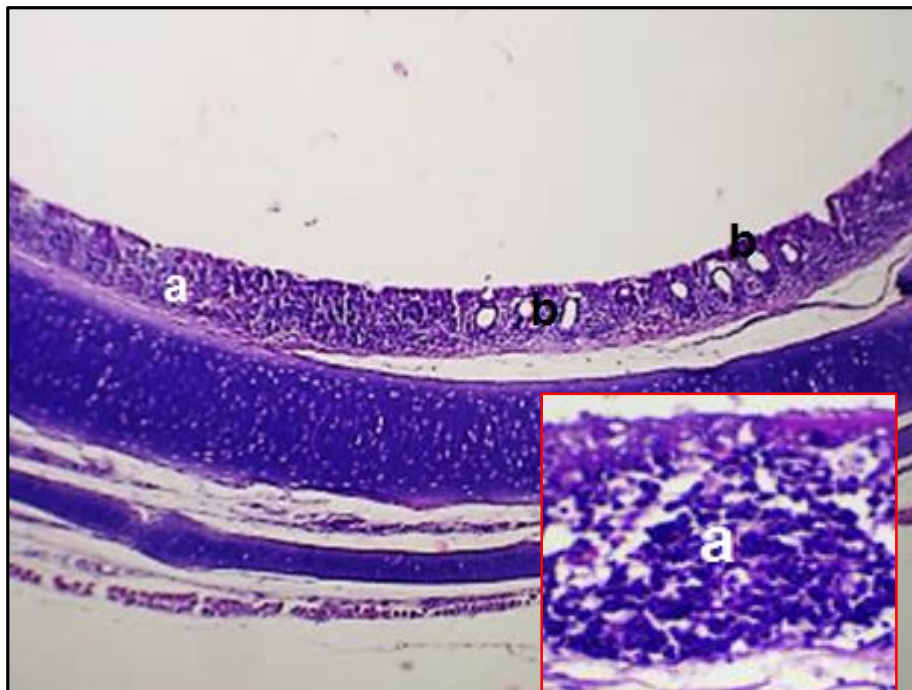


Figure 8. The histopathological section in the trachea (G3) shows diffuse MNCs infiltration in tracheal mucous and submucosa (a) with evidence of cystic dilution of the adjacent gland (b) (Hematoxylin and eosin staining (H&E) low (10X) and high (40 X) magnification power.

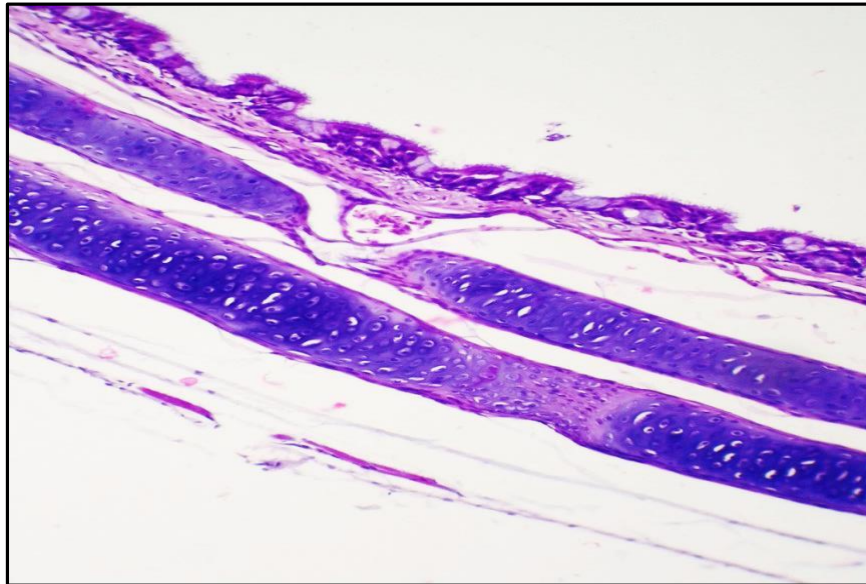


Figure.9 Control tracheal tissue showed normal structures. (Hematoxylin and eosin staining (H&E) low (10X) magnification power.

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