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Concentration of Interferon - gamma in Respiratory Disease Infected Chickens Caused by *Mycoplasma gallisepticum*

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ABSTRACT

Mycoplasma gallisepticum is commonly involved in the polymicrobial "chronic respiratory disease" in broiler chickens, leading to increased condemnations in the processing plant. In layers and breeders, it is usually subclinical, but causes a reduction in the number of eggs laid per hen over the production cycle. The present study aim to sero-identification of the *Mycoplasma gallisepticum* and study the concentration of interferon gamma. Sixty seven broiler chickens with respiratory signs (infected group), and fifteen healthy chickens considered as control group were used in this study. Blood samples were collected from all groups to perform in enzyme-linked immunosorbent test to identification antibodies for *Mycoplasma gallisepticum* and the level of interferon gamma. The result was shown (32/67) sample from infected chicken was positive for *Mycoplasma gallisepticum* and the level of INF-Gamma is significantly higher in the positive chicken for *Mycoplasma gallisepticum* infection at (102.34 ± 31.37) pg/ml when compared to control group at found (74.68 ± 12.75) pg/ml. The conclusion that increasing the level of INF-Gamma was affected in the increasing the susceptible to *Mycoplasma gallisepticum* infection and caused chronic respiratory disease infected to chickens in Iraq.

KEY WORDS:

CRD, Immunological marker , ELISA , Mycoplasmosis

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تركيز الانترفيرون- جاما في الدواجن المصابة بالأمراض التنفسية التي تسببها ال *Mycoplasma gallisepticum*

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الخلاصة

تشارك *Mycoplasma gallisepticum* بشكل شائع في "مرض الجهاز التنفسي المزمن" متعدد الميكروبات في دجاج التسمين ، مما يؤدي إلى زيادة الإذانات في مصنع المعالجة. في البياض ودجاج التسمين، عادة ما تكون تحت السريري ، ولكنها

تسبب انخفاضًا في عدد البيض الذي يتم وضعه لكل دجاجة خلال دورة الإنتاج. تهدف الدراسة الحالية إلى التعرف المصلي على *Mycoplasma gallisepticum* ودراسة تركيز الإنترفيرون جاما. استخدمت في هذه الدراسة 67 دجاجة لاجم تحمل علامات تنفسية (مجموعة مصابة) و 15 دجاجة سليمة تعتبر مجموعة ضابطة. تم جمع عينات الدم من جميع المجموعات لإجراء اختبار الامتصاص المناعي المرتبط بالإنزيم لتحديد الأجسام المضادة لـ *Mycoplasma gallisepticum* ومستوى interferon gamma. أظهرت النتائج (67/32) عينة من الدجاج المصاب كانت موجبة للميكوبلازما *Mycoplasma gallisepticum* ومستوى INF-Gamma أعلى بشكل ملحوظ في الدجاج الموجب لعدوى *Mycoplasma gallisepticum* عند (31.37 ± 102.34) بيكوغرام / مل بالمقارنة مع المجموعة الضابطة التي وجدت (12.75 ± 74.68) بيكوغرام / مل. الاستنتاج بأن زيادة مستوى انزيم INF-Gamma قد تؤثر في زيادة القابلية للإصابة بعدوى *Mycoplasma gallisepticum* وتسبب مرض تنفسي مزمن يصيب الدجاج في العراق.

الكلمات المفتاحية: مرض الجهاز التنفسي المزمن, علامة مناعية, الاليزا, داء المايكوبلازما

INTRODUCTION

Mycoplasma gallisepticum is the causative agent of chronic obstructive pulmonary disease. Chicken keepers throughout the globe are experiencing an epidemic of a fatal sickness that is affecting their flocks. Infections are often worse in young birds (less than 4 months old) and roosters than in older birds (Ficken, 2019). The characteristic signs that include tracheal rales (or gurgling noises), nasal discharge, sneezing, gasping, and coughing, are classic symptoms of this illness. One or both eyes may show symptoms of conjunctivitis, including discharge. Facial swelling and trembling of the head are possible, although uncommon (Spickler and Anna Rovid, 2016). Infected birds may both spread the disease and dormant. It may be transmitted from parent to offspring (vertical transmission) and from bird to bird (horizontal transmission), both directly and indirectly via the use of live and inanimate vectors. It takes time for a disease to propagate across a flock (Spickler and Anna Rovid, 2016).

Chickens, turkeys, pheasants, and Chukar partridges all served as the first hosts for MG's isolation (Nadeem et al., 2014). The *Mycoplasma gallisepticum* was Isolated from Bobwhite quail, Japanese quail, ducks, geese, and house Finches simultaneously (Sawicka-Durkalec et al., 2021). The thymus as well as Bursa of Fabricius are the principal lymphoid organs in birds, and they are responsible for producing immunological T and B cells, respectively. (Ciriaco et al., 2003). Interferons (IFNs) are important organizers of the immune response; they are pleiotropic cytokines having antiviral, anticancer, and immunomodulatory characteristics. "interferons" refers to molecules that block viral infection in their cells (Castro et al., 2018).

MATERIAL AND METHODS

Sample collection

This study was conducted during a period of 6 months from 1 October 2021 to 31 March 2022, sample were collected from 10 chicken farms diagnosed infected with Chronic respiratory disease (CRD). Sixty seven broiler chickens with respiratory signs (infected group), and fifteen healthy chickens considered as control group were used in this study. Blood samples were collected from all groups to perform in enzyme-linked immunosorbent test to identification antibodies for *Mycoplasma gallisepticum* and the level of interferon gamma. The blood sample were put in the vacuum gel tube , centrifugation 20 min at the speed 4000 r.p.m. the collected serum was kept in sterile tube and preserved in -20 for ELISA test.

Serological Detection of *Mycoplasma gallisepticum*

The ELISA test(Mornmed,CAT#: MB-19669A, Chaina) was sued in the present study, which is based on the the qualitative enzyme immunoassay technique. An antibody specific to *Mycoplasma gallisepticum* (MG) has been pre-coated on the microplate included in this kit, turning

it into a solid-phase antibody. Samples are incorporated with the particular antigen in the microplate wells. The antibody-antigen-enzyme labeled antibody complex then forms after each microplate well is treated with a Horseradish Peroxidase (HRP)-conjugated antigen specific for MG. Each well is then filled with the TMB substrate solution after being washed to get rid of any unbound reagent. Only the wells that include MG and HRP conjugated MG antibodies will first look blue before changing to yellow after the stop solution is added. Spectrophotometric measurements of the optical density (OD) are made at 450 nm in wavelength. By comparing with the cut of value, the qualitative determination of MG is determined.

Estimation the level of IFN-gamma

The particular ELISA kit (Mornmed, CAT#: MB-2660A, China) the Sandwich -ELISA technique was used in this study. The kit contains a micro ELISA plate that has previously coated with an anti-chicken IFN-gamma antibody. In a micro ELISA plate, a particular antibody is mixed with a sample (or standard) in each well. Each microplate well is then treated with a biotinylated detection antibody specific for chicken IFN-gamma and Avidin-Horseradish Peroxidase (HRP) conjugate. Substances that aren't bound by anything are rinsed away. Each well is thereafter conjugated with the substrate solution. Only wells with chicken IFN-gamma, Biotinylated antibodies are used for the detection of low-abundance proteins, and Avidin-HRP conjugate showed as blue. With the addition of stop solution, the color changed to a bright yellow. At a wavelength of 450 nm, optical density (OD) is measured spectrophotometrically. The amount of chicken IFN-gamma in the samples can be calculated by comparing their OD to the optical density of the standard curve.

Statistical analysis

All the data were analyze in by using IPM, SPSS Statistic 20. The results were analyzed by using T test and presented as mean ± SD significantly at P value ≤ 0.05.

RESULTS AND DISCUSSION

Serological Detection of *Mycoplasma gallisepticum*

The preliminary serological diagnosis for *Mycoplasma gallisepticum* exhibited that all the thirty two chickens infected with *M. gallisepticum* only and showed positive with Anti-*M. gallisepticum* antibodies, while there were 35 chickens non infected with mycoplasma (Table1). The results of clinical signs of infected chicken of the present study showed that out of 82 suspected chicken infected with *M. gallisepticum* 67 chickens (81.7%) have respiratory clinical signs. Whereas 15 chickens (18.3%) as apparently health chicken, so it was represented as control chicken.

Table 1: Serological Detection of *Mycoplasma gallisepticum* in the study groups

| | | Groups | | Total |
|------------|----------|----------------------|-----------|-------|
| | | Control non infected | Infection | |
| Mycoplasma | Positive | 0 | 32 | 32 |
| | Negative | 15 | 35 | 50 |
| Total | | 15 | 67 | 82 |

Estimation the level of IFN-gamma

The results of the current study showed that out of a total of 32 chickens infected with *Mycoplasma gallisepticum*, it had a significant increasing (P< 0.05) in INF-gamma level at (102.34 ± 31.37) pg/ml compared to control (non-infected with mycoplasmas) 15 chickens, where a decreasing in the level of INF-gamma at (74.68 ± 12.75) pg/ml Figure (1).

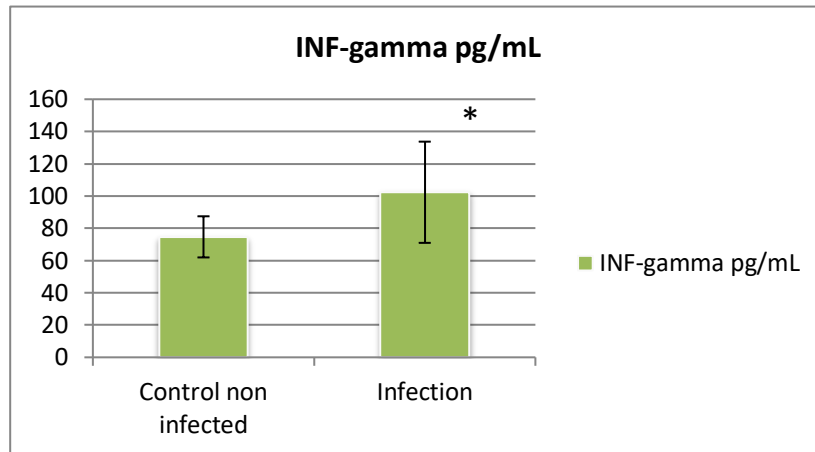


Figure 3: Level of INF-gamma compared between infected and non-infected chicken with *Mycoplasma gallisepticum* groups. *Significant at $P < 0.05$

In attempt to determine the frequency of mycoplasmas infection in and out of the poultry environment, the data obtained in this study were analyzed. This analysis may indicate the rate of mycoplasmas distribution within their environments. Table (1) showed the rate of infection with *Mycoplasma gallisepticum*. Infected chicken accounted for 32 (39%) versus 50 (61 %) in non infected (Negative to *Mycoplasma gallisepticum* infection). The results were in agreement with almost all results being reported worldwide (Bagal *et al.*, 2019 ; Awad *et al.*, 2019 ; Wu *et al.*, 2020). The poor environment in addition to the use of random antibiotics treatment perhaps constitutes a habitat for microorganisms and a focus for subsequent infections or what they called (Mycoplasma coinfection). Furthermore the attention should be drawn to this phenomenon, the bacterium *Mycoplasma gallisepticum* (MG) is a global threat, affecting numerous bird species and leading to substantial economic losses around the world. Therefore, some of studies were identify the prevalence of MG in commercial broiler, layer chicken, and turkey farms, as well as in ambient litter samples throughout various governorates in Iraq (Ali and Ali, 2019 ; Jafar and Noomi, 2019 ; Al-Mahmoudi *et al.*, 2020).

Interferon Gamma (IFN- γ), Interleukin-4 (IL-4), tumor necrosis factor alpha (TNF) , interleukin-1 (IL-1), and macrophage inflammatory protein 1 (MIP-1) have all been found to be expressed and released upon direct interaction between mycoplasmas and macrophages and monocytes in many in vitro studies. A strong correlation exists between this pattern of expression and the presence of overt inflammatory lesions (Majumder and Silbart, 2016). *M. gallisepticum* attaches and colonizes the respiratory epithelium, with little or no classical invasion. Mycoplasma causes a robust inflammatory response that includes heterophils, macrophages, B and T lymphocytes. Macrophages play a major role in mycoplasma clearance by phagocytosis, while polymorphonuclear leukocytes may contribute in mycoplasma dissemination (Hickman, 2002). B and T lymphocytes help remove mycoplasma and inhibit its spread. Due to the absence of a cell wall and based on sequence homology with recognized toxin genes, mycoplasmas lack endotoxin (Medina *et al.*, 2012). There are no known exotoxins produced by *M. gallisepticum*. This contains the well-characterized CARDS toxin of *Mycoplasma pneumoniae* . We are yet unsure of how *M. gallisepticum* triggers the severe inflammatory response that is said to give an abundant supply of nutrients and an ecological niche that is conducive to persistent infection. (Kannan and Baseman, 2006).IFN-gamma is an important cytokine in the body's immune response to viruses . It plays essential functions in the enhancement of the development of cellular immunity in animals, particularly in the formation of CD8+ T cell responses, Th1 cells, and cell cytotoxicity of natural killer cells (Mah and Cooper, 2016). IFN also plays essential functions in mucosal immune responses. Recent research has found that cellular immune responses in the tracheal mucosa, such as cytotoxic T cell and natural killer responses, are key contributors to the protective effects of the

immune system (Guo *et al.*, 2018). One of the most important causes of cytokine elevation in an infected chickens in mycoplasma is outside the cell and not inside it, IFN- γ is a major cytokine that is critical for host resistance to a broad range of intracellular pathogens (Rottem *et al.*, 2012). Production of IFN- γ by natural killer and T cells is initiated by the recognition of pathogens by Toll-like receptors (TLRs) (Sturge *et al.*, 2013). Interferon (IFN), a critical cytokine for the management of infectious illnesses, is produced in huge amounts by antigen-presenting cells (APC), such as dendritic cells and macrophages. (Suzue *et al.*, 2003).

One of the most crucial cytokines for preventing the spread of infectious diseases is IFN-. Compared to SCID or wild-type (WT) mice, IFN- gene knockout (IFN $^{-/-}$) mice, IFN- receptor gene knockout (IFN-R $^{-/-}$) mice, and other IFN-related gene knockout mice are highly susceptible to intracellular infections. We and others have demonstrated that antigen-presenting cells (APC), such as dendritic cells (DC) and macrophages, produce significant amounts of IFN- in response to interleukin (IL)-12 that is produced by APC upon microbial infection. NK cells among lymphoid cells had previously been thought to be a major source of IFN- during the early phase of infection. Interferon gamma (IFN-) synthesis by antigen-presenting cells (APCs) is contentious because it contradicts the original paradigm, according to which IFN- production was only possible in lymphoid cells. Recent discoveries of high-level IFN- production and intracellular expression by interleukin-12 (IL-12)-stimulated macrophages and dendritic cells have, however, provided some answers to this skepticism (Frucht *et al.*, 2001). Mycoplasma adherence to host cells is a first and crucial step in tissue colonization, so mycoplasmas have evolved complex molecular mechanisms to enable their prolonged adhesion. Many mycoplasmas exhibit the typical polymorphism of mycoplasmas, with the most common filamentous, flask shapes, or ovoid structures (Kornspan *et al.*, 2010). Adherence is linked to adhesins and host cell receptors, which facilitate communication between the bacterium and the host cells (Razin and Jacobs, 1992).

CONCLUSION

The conclusion that increasing the level of INF-Gamma was affected in the increasing the susceptible to *Mycoplasma gallisepticum* infection and caused pulmonary disease infected to chickens in Iraq.

CONFLICT OF INTEREST

The authors declare no conflicts of interest associated with this manuscript.

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