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DETECTION OF MYCOPLASMA INFECTIONS USING IMMUNOLOGICAL MARKERS IN BROILER CHICKENS INFECTED WITH CHRONIC RESPIRATORY DISEASE IN DIYALA GOVERNORATE

A Thesis
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By

Noor Hussein Abdul-Rahman

Supervised by

Prof.

Ghassan Hamdan Jameel

Prof. Dr.

Talib Jawad Kadhim

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بِسْمِ ٱللَّهِ ٱلرَّحْمَٰنِ ٱلرَّحِيمِ

﴿ يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ ﴾

صدق الله العلي العظيم

سورة المجادلة ﴿11﴾

Dedication

To all the meanings of hope,

all the manifestations of divine justice and all the manifestations of truth

(Sir,the owner of the epoch)

To the owner of a fragrant biography, and an enlightened thought;

He was the first to be credited with my attaining higher education

(My dear father)

To whom I prefer it to myself, and why not; You sacrificed for me

You have always spared no effort to make me happy

(My beloved mother).

To the support, the humerus and the forearm

(my brothers and sisters)

I give you gifts with love, honor and dignity

To everyone who literally taught me

To everyone who supported me, even with a smile..

Noor

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I thank God Almighty for His bounty as He allowed me to accomplish this work by His grace, to Him be praised first and foremost.

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ABSTRACT

The present study aimed to detect the *Mycoplasma gallisepticum* Bacteria in 67 broiler chickens with respiratory signs by using various diagnostic methods, and 15 healthy considered as control group in different age groups. over the six-month period between October 2021 and March 2022. Sample were collected from broiler chicken farms with respiratory diseases includes Chronic Respiratory Disease (CRD) appeared after post mortem technique. Blood samples from all groups were collected in order to perform an enzyme-linked immunosorbent assay to look for antibodies to *Mycoplasma gallisepticum* in the serum (ELISA). Diagnosis is also supported by using culture media in order to detect the presence of other bacterial infections.

The sample collected including lung, liver and trachea and have been taken under aseptic condition. Infected chickens were show many clinical signs summarized by frothiness about the eyes and congested of mucous membranes (32.8%), rapid and difficult respiration (41.7%) in addition to other chicken were suffering from lethargy (71.6%), weight loss (62.6%) and nasal discharge (91%).

Infected birds with *Mycoplasma gallisepticum* alone reached 47.76% and infections with another bacteria were reach to 52.238% , but in some time MG appeared mixed with other bacteria such as *Escherichia coli* which is consider as the main bacteria associated with *Mycoplasma* infection in prevalence rate of up to 83.58% followed by *Salmonella* spp.

The results of the present study showed that tracheal swabs are the most accessible site for bacterial infection 29.8 % , liver show a 14.9% while air sac swabs show 10.4%, and the most cases of infection in *Mycoplasma* bacteria is at the age of 25-27 days and contain secondary infection . The infection rate was 65.62% of the total 32 chickens, followed by 28-30 days 18.75% .

Regarding to the immunological tests to detect cytokines, it was found that out of a total of 32 chickens infected with mycoplasma, It had a substantial rise in IFN-γ concentration (102.34 31.37 pg/ml) and a significant increase in IL-10 concentration (33.18 8.28 pg/ml) as compared to the control group (both $P \leq 0.05$).

The histopathological results of the infected trachea revealed clear hyperactivity of the mucous glands, which was shown to be the underlying cause of the thickening of the tracheal mucous membrane, lung tissues of chickens that had been infected with avian mycoplasma indicated that there were obvious pathological changes in the bronchial tree and the parenchymal cells and the liver tissue revealed that there was mild to moderate inflammation in the hepatic tissue along with multifocal necrosis and degeneration of the hepatocytes

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List of Abbreviation

abbreviations	Meaning
ATP	Adenosine Triphosphate
E.coli	Escherichia Coli
ELISA	Enzyme-Linked Immunosorbent Assay
GapA	Glyceraldehyde-3-Phosphate Dehydrogenase A
GTS	Gene-Targeted Sequencing
H and E	Hematoxylin and Eosin
IFN-γ	Interferon-Gamma
IL-10	Interleukin-10
M.G	*Mycoplasma gallisepticum*
Mgc2	*M. gallisepticum* Cytadhesin 2
PvpA	phase-variable protein A
RAPD	Random Amplified Polymorphic DNA
RSA	Rapid Slide Agglutination Test
rtPCR	Real-Time Polymerase Chain Reaction
TNF	Tumor Necrosis Factor

Chapter One

Introduction

1.1 Introduction

Mycoplasma gallisepticum is the causative agent of chronic respiratory 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). Without a cell wall, proteins in the plasma membrane of *Mycoplasma gallisepticum* make up nearly two-thirds of its mass; membrane lipids make up the remainder (Qi *et al* ., 2018).

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 Rovid, 2016).Infected birds may both spread the disease and harbor it dormantly. 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 Rovid , 2016). Chickens, turkeys, pheasants, and chukar partridges all served as the first hosts for MG's isolation (Nadeem *et al* ., 2014). Isolated from Bobwhite quail, Japanese quail, ducks, geese, and house finches simultaneously (Sawicka-Durkalec *et al*., 2021). Numerous veterinary diagnostic facilities provide multiple standard laboratory tests for the detection of *M. gallisepticum* in chickens. The Real-time polymerase chain reaction (rtPCR) technique, which is performed on tracheal and/or oropharyngeal swabs of clinically unwell birds, is one of the most used diagnostic methods. Thus, the presence of the pathogen *M. gallisepticum* is verified in a definitive manner (Butche, 2012).

Mycoplasmas may also be diagnosed by the use of serological assays; these samples can be collected from both living and recently dead birds, and a

number of effective culture media have been developed. In order to prevent bacterial growth, MG media often include serum or a serum fraction, yeast factors, glucose, and bacterial inhibitors in addition to a protein digest and a meat-infusion basis (Abdelrahman *et al* ., 2021).

Serum samples were analyzed using the enzyme-linked immunosorbent assay (ELISA) method from both sick and healthy birds (Butch *et al* ., 2012).

The Gross examination of the lungs showed develop a white film, the air sacs become murky, and the lungs' thick coverings color from yellow to cream. When the outer layers were peeled back, the dark crimson lungs underneath were exposed. Tracheal hemorrhages were also seen in a handful of the instances. The histological examination of the lung under the microscope showed congestion and hemorrhages (Casagrande *et al* ., 2014).

1.2 Aims of the Study:

The study aimed to identify the *Mycoplasma gallisepticum* in broiler chickens in diyala province using the following diagnostic means :

1- Detection of *Anti-M. gallisepticum* antibodies In Chickens by Using ELISA Technique**.**

2-Detection of IFN- γ and IL 10 In Serum Of Chickens Using ELISA Technique**.**

3-Identification of co-infection microorganism associated with *Mycoplasma gallisepticum*.

4-Study of the histopathological changes in some organs in infected and healthy chickens groups.