Cairo University.

Faculty of Veterinary Medicine

Department of Microbiology



# Immunological, molecular and pathological studies on respiratory mycoplasmosis

A thesis

Presented by

#### Mohammed Sayed Abd El Rahman Soliman

(B.V.Sc. 1998, Cairo University)

For the degree of M.V.Sc.

In Microbiology

(Bacteriology, Immunology & Mycology)

Under supervision of

#### Prof. Dr. Saad Ahmed Attia Said

Professor of Microbiology, Faculty of Veterinary Medicine
Cairo University

#### Prof.Dr. Mahmoud Ali Mahmoud

Professor of pathology, Faculty of Veterinary Medicine
Cairo University

#### Dr. Ahmed Adel Seida

Lecturer of Microbiology, Faculty of Veterinary Medicine
Cairo University
(2015)

### Cairo University





#### Department of Microbiology

#### Approval sheet

This is to approve that the dissertation presented by

#### Mohammed Sayed Abd El Rahman Soliman

To Cairo University has been approved for the Master Degree in Veterinary Medicine Science–Microbiology (Bacteriology, Immunology & Mycology) by the examination committee:

#### Prof. Dr. Khalid Mohammed Hasanain

Professor and Head of Medical Microbiology and Immunology Department, Faculty of Medicine,

Assut University

#### Prof. Dr. Jakeen Kamal Abd El Haleem El Jakee

Professor of Microbiology, Faculty of Veterinary Medicine, Cairo University

#### Prof. Dr. Saad Ahmed Attia Said

Professor of Microbiology, Faculty of Veterinary Medicine, Cairo University

#### Prof. Dr. Mahmoud Ali Mahmoud

Professor of Pathology Faculty of Veterinary Medicine, Cairo University

Date: 21 / 10/2015

**University:** Cairo

**Faculty:** Veterinary Medicine

**Department:** Microbiology

Name: Mohammed Saied Abd El Rahman Soliman

**Nationality:** Egyptian

**Date of birth:** 25 / 7 / 1973

Place of birth: Minima, Egypt

**Degree:** Masters in Veterinary Sciences

**Specification:** Microbiology (Bacteriology, Immunology and Mycology)

Thesis title: Immunological, molecular and pathological studies on

respiratory mycoplasmosis

#### **Supervisors:**

Prof.Dr. / Saad Ahmed Attia Ahmed Saied

Prof.Dr. / Mahmoud Ali Mahmoud

Dr. / Ahmed Adel Seida

#### abstract

One hundred and fifty positive serum samples for mycoplasma using serum plate agglutination test (SPA) were collected .The included samples were one day old chicken (36 samples), broilers (40 samples), layers (38 samples), balady chicken (14 samples) and turkeys (22 samples). The samples were collected from different farms in El-Kaliobia, Eldakahlia, El behera and Beni-seuif governorates and examined To study the incidence of MG in the respiratory tract. Samples from nasal cavity, ifra-orbital sinus, trachea, air-sacs and lung were collected from the examined birds. were cultivated in mycoplasma media containing sterile filtrated swine serum and using also horse serum to detect *Mycoplasma gallisepticum*. All suspected colonies were identified by conventional method, growth inhibition test and *PCR*.

The results of isolation (fried egg colony apearance) were recovered 23.33 % from the total number of tested samples was positive, whereas the rate of positive samples was 22.22% for one day old chick ,broiler samples showed 32.5%, layer samples showed 18.42%, balady samples showed 14.28% and turkey samples showed 22.72% from specific total samples for each species. Interestingly, samples from the diseased flocks, 23.33% were only positive by isolation.

In this studies culture methods detect MG from 150 samples was 23.33 % from the suspected samples and Polymerase chain reaction confirmed the *nucleic acid* of the *Mycoplasma gallisepticum* using universal and MG specific primers. Cumulatively PCR confirmed *MG* positive in (42.85%) Of samples from the same field samples collected during the study.

Tissue samples from the positive MG serological cases were examined histo-pathologically to detect lesions in the tissues. The clinical signs and pathologic lesions were much more significant in the diseased bird by *Mycoplasma gallisepticum*. The pathological changes in upper respiratory tract and lung showed large masses of caseous exudate in the air sacs and predominantly in the abdominal ones. Peritonitis was observed in combined infection with CB. Thoracic air-sacs. Bilateral thoracic as well as abdominal air saculitis with caseous exudate were observed in severely affected combined cases. Histo-pathological examination of hepatic tissue, heart, trachea and lungs of broiler chicken showed Severe pathological changes varied from mild degenerative changes to severe inflammation with different types of inflammatory cells infiltration and /or necrosis in the affected organs.

.

I dedicate this work to Spirit my father My mother My wife My daughter Malak My son Malik All my family

بسنم اللهِ الرَّحْمنِ الرَّحِيمِ

( وَأَنْزَلَ اللَّهُ عَلَيْكَ الْكِتَابَ

وَالْحِكْمَةُ وَعَلَّمَكَ مَا لَمْ تَكُنْ

تَعْلَمُ وَكَانَ فَضْلُ اللّهِ عَلَيْكَ

عَظِيمًا)

صدق الله العظيم

من الآية (١١٣) سورة النساء

## Acknowledgment

First of all thanks Alla that gave me power, patience and ambition to begin and finish this work, without your blessing and mercy nothing would be done.

I would like to express deep gratitude to my supervisor **Prof. Dr. Saad Ahmed Attia** professor of Microbiology, Faculty of Veterinary Medicine, Cairo University, for his unlimited help, planning, his kind supervision, constant encouragement, trust, provision of so much of his time to follow me and to ensure that this work ends in the best way it can happen.

I would like to express my deep gratitude to my supervisor **Prof.Dr. Mahmoud Aly** professor of pathology department, faculty of Veterinary medicine, Cairo university for his endless knowledge that brightened my mind in too many aspects of both science and life, I was honoured by his supervision on this work.

I would like to express my deep gratitude to my supervisor **Dr. Ahmed Adel** Lecturer of Microbiology, for his kind help and support with all the resources they could provide.

I would like to express my deep gratitude to **Prof.Dr. Jakeen Kamal Abd El –Haleem El-Jakee,** professor of Microbiology, Faculty of Veterinary Medicine, Cairo University, for here careful guidance, advice, help, infinite support, here continuous encouragement all over the work and for here valuable advices in work and life that put me on the right track and helped me to finish this work.

I would like to show gratefulness and appreciation to **Prof.Dr.Heidy Mohammed Shawkey**, Professor of Microbiology Department, Faculty of Veterinary Medicine, Cairo University, for her unlimited help and advices.

I would like to express my deep gratitude to **Dr. Ahmed Samir Assistant professor** of Microbiology, Microbiology Department,, faculty of Veterinary medicine, Cairo university for his endless knowledge that brightened my mind in too many aspects of both science and life, I was honoured by his kind help on this work.

I would like to show gratefulness and appreciation to **Prof.Dr. Mostafa Ahmed Bastamy**, Professor of Poultry diseases, Faculty of Veterinary Medicine, Cairo University, for his kind help.

I would like to show great thanks to **Dr. Mahmoud El-Hariri** Assistant professor Microbiology Department,, faculty of Veterinary medicine, Cairo university and **Dr. Sherif Marouf** Lecturer of Microbiology Department,, faculty of Veterinary medicine, Cairo university for his kind help and support with all the resources they could provide.

From all my heart I want to deeply thank my friends **Architect**Mohammed shalaby, for their great support and impressive help.

To all Staff members of the Department of Microbiology, Faculty of Veterinary Medicine, At last but not least I would like to thank all personnel by their names (**Doctors, Technicians Saber , Abd Ealeem and workers**), who giving a hand whenever needed.

Last, but not least, all thanks are to my family.

I want to deeply thank for **Magdy Ghonaim** (Sama Libarery)



### **List of contents**

Subject	Page
List of content	I
List of tables.	IV
List of figures.	V
List of abbreviations.	VII
1. Introduction	1
2. Review of literature	4
2.1. Characters and genomic structure of the genus mycoplasma	4
2.2. Pathogenesis of mycoplasma infection.	10
2.3. Signs and postmortem examination of mycoplasma infection	13
2.4. Detection of <i>Mycoplasma gallisepticum</i> by conventional PCR technique	16
3. Material and methods	22
3.1. Materials	22
3.1.1. Samples	22
3.1.2. Media used.	22
3.1.3. Enrichments	25
3.1.4. Inhibitors.	25
3.1.6. Mycoplasma strains and antisera	26
3.1.8. Equipments and apparatuses.	28
3.2. METHODS.	30
3.2.1. Sampling.	30
3.2.2. Preparation of the media	31