Cairo University

Faculty of Veterinary Medicine

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Molecular Characterization of *Mycoplasma gallisepticum* in Chicken.

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Abstract:

Mycoplasma gallisepticum (MG) is an important avian pathogen causes significant economic losses within the poultry industry. One of the options of the controlling MG infection is the isolation and identification of MG. Two hundreds and fifteen samples collected from the lungs, tracheal bifurcation, tracheal swabs and frontal sinus aspirates and 80 blood samples were collected from broiler and layer flocks in Damietta governorate. Organ and tracheal swab samples were cultivated on Frey's media giving 142 samples positive as (Order. *Mycoplasmatales)* as 66%. The 142 samples was used for digitonin sensitivity test and the results were 98 samples as digitonin positive (F. *Mycoplasmataceae*) 69% and 44 samples was digitonin negative (F. *Acholeplasmataceae*) 31%.One hundred and ninety six samples were characterized by PCR by using MG primer. The samples had amplified fragments at 300 bp against MG. Four samples were used for gene targeted sequencing.

Keywords: *Mycoplasma gallisepticum* , Chicken, Isolation, Digitonin, PCR, Sequencing

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Abd El-mohsen Ahmed Rabiee

Dedication

To My Mother, My Wife, My Lovely Daughter And Son, My Sister And My Brothers….

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Abbreviations

AFLP	Amplified Fragment Length Polymorphism
AP-PCR	Arbitrarily PCR
CFU	Colony forming unit
CRD	Chronic respiratory disease
CCRD	Complicated chronic respiratory disease
DNA	Deoxyribonucleic Acid
ELISA	Enzyme-linked immunosorbent assay
GIT	Growth inhibition test
GTS	Gene-targeted sequencing analysis
HA	Haemagglutination
HI	Haemagglutination inhibition
iELISA	Indirect Enzyme-linked immunosorbent assay
IFT	Immunofluorescence test
IP	Immunoperoxidase
IU	International unit
MAbs	Monoclonal antibodies
MG	*Mycoplasma gallisepticum*
MI	*Mycoplasma iowae*
MICs	Minimum inhibitory concentrations
MM	*Mycoplasma meleagridis*
MS	*Mycoplasma synoviae*
NAD	Nicotinamide adenine dinucleotide
NPIP	National Poultry Improvement Plan
OIE	Office international des Epizootics
PBS	Phosphate buffer saline
PCR	Polymerase chain reaction
PPLO	Pleuropnemonia like organism
RAPD	Random amplified polymorphic DNA
RBCs	Red blood cells
SPA	Serum plate agglutination test
SPF	Specific pathogen free
USA	United States of America

1- Introduction

Some of mycoplasmas are of economic importance to the poultry industry through their causation of disease either as sole pathogen or, more frequently in association with other pathogens. There are 22 named species recovered from avian sources but only four of them are the established pathogens for domestic poultry that are *Mycoplasma gallisepticum* (MG), *Mycoplasma synoviae* (MS), *Mycoplasma meleagridis* (MM) and *Mycoplasma iowae* (MI) **(Jordan and Horrocks, 1996)**. MG and MS are pathogenic species for chickens and turkeys, whereas, MM and MI are the important pathogens for turkeys **(Ley and Yoder, 1997)**.

MG is a major problem in poultry industry worldwide and the infection is commonly known as CRD of chickens and infectious sinusitis in turkeys. Losses attributed to mycoplasmosis, mainly through MG infection, are due to decrease in egg production and egg quality, poor hatchability (high rate of embryonic mortality and culling of day-old birds), poor feed efficiency, increase in mortality and carcass condemnations, besides medication costs. MG is the most pathogenic and economically significant bacterial respiratory pathogen of poultry. It is considered to be the most important of the pathogenic Mycoplasmas and OIE has declared the disease caused by MG as notifiable. MG causes considerable economical losses to the poultry industry, especially in chickens and turkeys all over the world, associated with respiratory diseases, poor performance, and embryo mortality **(Ley and Yoder, 1997, Levisohn and Kleven, 2000, OIE, 2004 and Eissa *et al.*, 2014).**

Mycoplasmas are the smallest known bacteria that are capable of replicating outside the cells occur in humans and animals and have usual optimum growth temperature of 37^{o}C. Mycoplasmas are small prokaryotes, 300-800 nm in diameter.

They lack in cell wall but are bounded by a triple layered plasma membrane **(Razin *et al.*, 1998 and Brown *et al.*, 2007)**. Transmission of MG in-ovo from infected breeder birds to progeny is the major route for spread of infection, and is the major consideration for international trade. In most part of world, the control programs for MG are based on maintaining commercial breeding stock free of infection **(Levisohn and Kleven, 2000)**.

Three main approaches are used for the diagnosis of avian mycoplasmosis, isolation and identification, detection of antibodies and molecular detection of organism nucleic acid by polymerase chain reaction (PCR). Isolation and identification of pathogenic avian mycoplasmas organisms are difficult, slow growing, relatively fastidious organisms and might require up to 3 weeks for detectable growth. Therefore, serological assays such as the rapid serum plate agglutination test (SPA) and haemagglutination inhibition test (HI) tests have been used routinely. Because the SPA test is quick, relatively inexpensive, and sensitive, it has been widely used as an initial screening test for flock monitoring and serodiagnosis. Problems of low sensitivity, cross reactions, and non- specific reaction were encountered with rapid SPA and HI tests **(Ewing *et al.*, 1996, Kleven *et al.*, 1998 and OIE, 2008)**.

Several workers have described the use of conventional PCR in detecting avian mycoplasmas directly from clinical samples such as nasal swab, trachea and air sac. PCR has some advantage over cultural detection as it can detect the pathogens in dead organism also and also rapid, inexpensive and sensitive method. Molecular biology techniques such as PCR and RAPD or the AP-PCR have been applied for detection and identification of mycoplasmas. Recently, the use of gene targeted sequencing GTS analysis of MG surface-protein genes as a typing method, have been used to identify and differentiate MG strains. PCR assays successfully targeted more species specific

genes; independence of the sequencing method on the isolation of MG in pure culture is a significant advantage. The *mgc2* gene which encodes a second cytadhesin protein known to play a role in MG antigenicity and virulence and identified as genome CDS MGA -0932 **(Fan *et al.*, 1995, Hnatow *et al.*, 1998, Papazisi *et al.*, 2002, Ferguson *et al.*, 2005, Garcia *et al.*, 2005, Ramadass *et al.*, 2006 and Bibank *et al.*, 2013)**.

Aim of the work:

Survey on the incidence of MG in chicken farms of Damietta Governorate through:

1) Isolation of MG from chicken farms at different age and different seasons in Damietta Governorate.
2) Serodiagnosis of MG isolates by SPA, GIT and ELISA.
3) Genotyping of MG isolates by PCR.
4) Sequencing of MG isolates.

2. Review of literature

2.1 Historical overview of mycoplasmas:

The first accurate description of the disease in 1935, Nelson described coccobacilli form bodies associated with an infectious coryza of chickens. Later, he associated the organisms with a coryza of slow onset and long duration and eventually was able to grow the coccobacilli form bodies in embryonating eggs, tissue culture, and cell-free medium **(Nelson, 1935)**.

Dickinson and Hinshaw, (1938) named the disease "infectious sinusitis" of turkeys. **Markham and Wong, (1952)** reported the expertise and effort of Yoder in the successful cultivation of the organisms from chickens and turkeys and suggested they were both members of the pleuropneumonia group (*Mycoplasma* spp.).

MG was first classified and differentiated from other avian Mycoplasmas by serotyping **(Adler *et al.*, 1957 and Yamamoto and Adler, 1958)** and commonly was designated serotype A **(Kleckner, 1960 and Yoder, 1964)**.

The term *Mycoplasma gallisepticum* is only proposed in 1960 by Edward and Kanack to call the agent responsible for chronic respiratory disease and infectious sinusitis (**Edward and Kanarek, 1960**).

The term ''chronic respiratory disease'' (CRD) was first given by Delaplane and Stuart (1943) for the clinical manifestations of the disease in chicken **(Lancaster and Fabricant, 1988).**

In 1993, mycoplasmas with phenotypic and antigenic similarities to MG were differentiated by molecular techniques and designated MI **(Bradbury *et al.*, 1993).**

Kempf *et al.* (1997) reported that mild or subclinical cases of MG, called (atypical) infections, have been observed naturally and experimentally in chicken and turkey. They stated that these atypical infections are often difficult to diagnose.

The microorganisms of the class Mollicutes (Mycoplasma) were first identified in 1898 as the etiologic agent of the bovine contagious pleuropneumonia (BCPP) and thereafter, all similar agents were named pleuropneumonia-like (PPLO-like) organisms (**Pourbakhsh, 2014)**.

2.2 Taxonomy of mycoplasmas:

The genus *Mycoplasma* belongs to the class Mollicutes (in Latin, mollis, soft; cutis, skin). The name ‘’Mollicutes’’ refers to the nature of these organisms that lack the bacterial cell wall known in other bacteria. Mycoplasmas are surrounded by just a trilaminar membrane **(Razin et al., 1998).** Mycoplasmas are the smallest free living eubacteria possessing the smallest genome (0.58 – 2.2 Mbp) with low G+C content (23 – 40 mol %). The genome of *Mycoplasma*s lacks even the genetic data required for a cell wall synthesis. Lack of cell wall renders *Mycoplasma* resistant to antibiotics affecting cell wall synthesis like penicillin which is usually incorporated in cultivation medium to control other bacterial growth **(Razin and Herrmann, 2002).** Phylogenetic analysis of the 16S ribosomal RNA gene has been used to analyze genetic relationships among Mycoplasmas (**Gasparich *et al.*, 2004**).