بسم الله الرحمن الرحيم

MONA MAGHRABY

شبكة المعلومات الجامعية

التوثيق الالكتروني والميكروفيلم

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جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

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Some Studies on Mycoplasma Affections in Sheep and Goats

A thesis submitted by

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For the degree of the Ph.D.

(Infectious Diseases)

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Abstract

Different species of *Mycoplasma* are associated with many pathological problems in small ruminants including respiratory manifestation, infectious keratoconjunctivitis and contagious agalactiae, these problems results in significant losses especially in African countries, our study aimed to isolate and identification of different *Mycoplasma* species from Egyptian sheep and goats with different clinical manifestations and classified them by different genetic analysis methods using PCR (polymerase chain reaction), sequencing and phylogenetic analysis, testing the effect of antimicrobial agents against these isolates in vitro. A total of (437) samples were collected from sheep and goats from Giza governorate in Egypt as (142) nasal swabs from clinically affected animals, (167) pneumonic lungs, (18) samples from tracheal bifurcation, (8) samples by bronchial wash, (76) nasal swabs and (26) milk samples were cultured on PPLO media for cultivation of *Mycoplasma* species. PCR, sequencing and phylogenetic analysis were adopted to identify and classify the isolated *Mycoplasma* species.A total of (33) *Mycoplasma* isolates were isolated on PPLO media, identified by biochemical tests and confirmed and typed by PCR using specific primers. Ten isolates were confirmed as *Mycoplasma arginini*, (4) isolates as *Mycoplasma ovipneumoniae*, (4) *isolates as Mycoplasma conjunctivae* and (2) samples as *Mycoplasma agalactiae* by PCR and 13 isolates as undifferentiated *Mycoplasma* species. A purified isolate of *Mycoplasma arginini* and *Mycoplasma ovipneumoniae* were sequenced and phylogenetic analysis was illustrated.*Mycoplasma arginini, Mycoplasma ovipneumoniae , M. conjunctivae and M. agalactiae* are prevalent in Egyptian sheep and goats. Further studies on *Mycoplasma arginini* are required due to its high frequency of isolation from pneumonic sheep and goats and also from animals suffer from different respiratory manifestations.

Keywords: *Mycoplasma*, sheep, goats, PCR, Sequencing and phylogenetic analysis, MIC

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List of abbreviations

Synonym	Abbreviation
CA	Contagious agalactiae
CCPP	Contagious caprine pleuropneumonia
CFU	colony forming units
DGGE	denaturing gradient gel electrophoresis
H&E	hematoxylin and eosin
IKC	Infectious keratoconjunctivitis
IU	international unit
M. agalactiae	*Mycoplasma agalactiae*
M. arginini	*Mycoplasma arginini*
M. bovirhinis	*Mycoplasma bovirhinis*
bovis	*Mycoplasma bovis*
M. c. capricolum	*Mycoplasma capricolum capricolum*
M. c. capripneumoniae	*Mycoplasma capricolum capripneumoniae*
M. conjunctivae	*Mycoplasma conjunctivae*
M. m. capri	*Mycoplasma mycoides* subsp. *capri*
M. m. mycoides LC	*Mycoplasma mycoides* subsp. *mycoides* large colony
M. ovipneumoniae	*Mycoplasma putrefaciens*
M. putrefaciens	*Mycoplasma ovipneumoniae*
MIC	minimum inhibitory concentration test
MLST	multilocus sequence typing
PCR	polymerase chain reaction
PI3V	Parainfluenza-3 virus
PPLO	Pleuropneuomnia like microorganism
Rpm	revolutions per minute
RSV	Respiratory Syncytial Virus
rt-PCR	real time-PCR
TAE	Tris Acetate EDTA

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Introduction

Sheep and goats have a high economic importance in many countries including Egypt where they are considered as source of meat, wool and milk, more than 10 % of meat production in Egypt is from sheep and goats (**Hakim *et al.* 2014, Abd-Elrahman *et al.*, 2019),** Among different small ruminants diseases; *Mycoplasmas* infection results in significant losses in the African countries, Middle East, European countries, and India leading to high morbidity and mortality rates (**Sandip *et al.*, 2014).**

Mycoplasma is belonging to a group of bacteria named mollicutes which characterized by its minute genome size and persistently devoid of the cell wall. Themollicutes include *Mycoplasmas*, *Acholeplasmas*, *Ureaplasmas*, *Spiroplasmas*, *Entomoplasmas*, *Mesoplasmas* and *Phytoplasma*, recently *Eperythrozoans* and *Haemobartonellas* are also included to mollicutes bacterial group, the mollicutes had derived by a process of degenerative evolution from gram positive bacteria **(Nicholas *et al.*, 2008)**. Different *Mycoplasma* species are accompanied by many diseases and problems in both mammalian and avian species **(Glen *et al.*, 2012).**

Mycoplasma causes various clinical manifestations including pneumonia, conjunctivitis, arthritis and mastitis **(Kumar *et al.*, 2013)**. *Mycoplasma* species commonly associated with pneumonia in small