RELATIONSHIP BETWEEN MASTITIS AND *MYCOPLASMA BOVIS*

By

Ebtesam Shebika Osman Mohammed
B. Sc. Agric. Sci. (Biochemistry), Fac. Agric. ,Cairo Univ.,2010

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APPROVAL SHEET

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Title of Thesis: Relationship Between Mastitis and *Mycoplasma Bovis*
Supervisors: Dr. Mohammed Zakaria Sedik
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ABSTRACT

Mycoplasma species were the simplest form of self-replicating organism. *Mycoplasma* is one of the causative agents causing mastitis in cattle, buffaloes which alone or together with viruses and/or bacteria cause important specific disorders. The present study aims to Isolation and serological identification of *Mycoplasma* strains from milk samples. During this study 350 milk samples collected from some private farms from El-fayoum governate of dairy cows and buffaloes (50ml each) collected aseptically in sterile McCartney bottles . The results of *Mycoplasma* species isolation in cattle from clinical mastitic samples (58%) , followed by Subclinical mastitis samples (16%) and finally bulk milk tank samples (12%). in buffaloes, clinical mastitic samples (36%) ,Subclinical mastitis samples (10%) , and finally bulk milk tank samples (8%). In this study we used digitonin sensitivity test to differantiate between *Mycoplasma* and *Acholeplasma* genera, In cattle from clinical mastitic 89.7% were *Mycoplasma* and 10.3% were *Acholeplasma*. In Subclinical mastitis samples 93.8% were *Mycoplasma* and 6.2% were *Acholeplasma*, in bulk milk tank samples 100% were *Mycoplasma* and 0% were *Acholeplasma* In the buffaloes from clinical mastitic samples 94.4% were *Mycoplasma* and 5.6% were *Acholeplasma*. In subclinical mastitis samples 90% were *Mycoplasma* and 10% were *Acholeplasma*, finally in bulk milk tank samples 100% were *Mycoplasma* and 0% were *Acholeplasma*. The morphological, biochemical and genetically tests were applied on pure isolates. The pure isolates (*Mycoplasma* spp.) were applied with MIC technique such as (Spiramycin and Tilmicosin) and immunosorbent assay (ELISA) was used for the detection of *M. bovis* antibodies in milk samples of cattle and buffaloes. The examined polymerase chain reaction (VspA gene of *Mycoplasma bovis*) for clinical mastitic milk (15 positive isolates), Subclinical mastitic milk (19 positive isolates), bulk milk tank (5 positive isolates) were amplified PCR fragment size 342 bp. *M. bovirhinis* was isolated by 29 % and *M. bovigenitallium* was 11.63% . It was concluded that PCR was a sensitive and specific methods for diagnosis of mycoplasma mastitis. It is therefore crucial to ensure that diagnostic PCR technique are available, which can perform rapid detection of *Mycoplasma* at acceptable costs.

Keywords: *Mycoplasma* spp.; mastitis; PCR; cows and buffloes and bulk milk; ELISA; MIC technique.

DEDICATION

*In the Name of **Allah**, the most gracious, the Most Merciful, all praise be to **Allah**, the Lord of the worlds; and prayers and peace be upon **Prophet Mohammed** His servant and messenger . I dedicate this work to my dear and beloved **father** and **mother**, my dear **brothers** , my fiancé " **Mahmoud** " for all the support they lovely offered during my post-graduate studies and all my life. I will never let them down. I will always appreciate all they have done for me.*

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CONTENT

INTRODUCTION

Bovine mastitis represents a disease of high incidence in dairy cattle herds worldwide. It causes considerable economic losses due to decreases in the quality and quantity of milk production, as well as increases in the cost of treatment, veterinary services and waste (Dego *et al*., 2002).

The bacterial causes of bovine mastitis can be classified as environmental (*Escherichia coli, Streptococcus dysgalactiae, Streptococcus parauberis* and *Streptococcus uberis*) or contagious (*Staphylococcus aureus, Streptococcus agalactiae* and some *Mycoplasma* species) depending on their primary reservoir (Smith, 1996).

Mycoplasmal mastitis is classified as a contagious mastitis pathogen because the infection can be spread from cow to cow during milking. The reservoirs for the infection are the udder and lungs of other infected cattle. Unlike other forms of contagious mastitis, *Mycoplasma* infection can spread from the respiratory system via the blood or lymph system to the udder (Mike Maroney, 2005).

Genus *Mycoplasma* belongs to the class Mollicutes to the order *Mycoplasmatales* and family Mycoplasmataceae (Razin *et al*., 1998).

The name *Mycoplasma*, from the Greek mykes (fungus) and plasma (formed), was first used by Frank in 1889. He

thought it was a fungus, due to fungus-like characteristics (Krass and Gardner, 1973).

An older name for *Mycoplasma* was PleuroPneumonia-Like Organisms (PPLO), referring to organisms similar to the causative agent of Contagious Bovine Pleuro Pneumonia (CBPP) (Edward and Freundt, 1967). *Mycoplasma bovis* is a tiny bacterium; it is even smaller than some viruses. This species of organisms is common in cattle. A unique feature is that it lacks a cell wall, this is important as many of our currently marketed antibiotics attack the cell wall of organisms, that makes *Mycoplasma* difficult to be treated and hinders the ability to develop an effective vaccine against it. It is frequently found in the nose and upper throat of cattle of all ages and in the reproductive tracts of both cows and bulls (Jasper, 1987).

Mycoplasma only contains three layers of plasma membrane (no cell wall); it allows them to do a wide range of things with their specific structural shape (Taylor-Robinson, 2001). This allows *Mycoplasma* to alter easily its shape to optimize its efficiency within the host. Some shapes that *Mycoplasma* can be found in include: pear shape, filamentous, or a "fried egg" appearance (Cree, 2002).

Because *Mycoplasmas* are very small, only 0.2 to 0.8 micrometers in size, therefore, they are difficult to detect with a conventional microscope.

Mycoplasmal mastitis is distinguished by its persistent course, lack of response to antibiotic treatment and high contagiousness (Pfützner and Sachse, 1996). Apart from *Mycoplasma bovis (M. bovis)* as the major aetiological agent, several other species, such as *M. californicum, M. canadense, M. bovigenitalium, M. alkalescens, M. arginini, M. bovirhinis, M. dispar* and *M. leachii (*formerly M. bovine group 7*)* were reported to be associated with mastitis in dairy cows (Jasper, 1981; Alexander *et al*., 1985 and Kumar and Garg, 1996). More than one bovine *Mycoplasma* species could be detected simultaneously using PCR tests based on the 16S rRNA gene sequences (Cardoso *et al*., 2000 and Hirose *et al*., 2001).

To eradicate the disease and eliminate *M. bovis*, there is no other effective measure than culling all infected animals. Pfützner *et al*. (1979) described a successful sanitation programme in a herd of 1230 heads, in which 165 (13.4%) cattle suffered from Mycoplasma mastitis. Systematic testing facilitated the identification of all infected animals, which were separated and finally culled. The whole procedure was repeated twice within six months.

Because *Mycoplasmas* are very small, therefore they are difficult to detect with a conventional microscope. PCR hold much promise in *Mycoplasma* mastitis detection. They are rapid and opposed to reduce time to hours as opposed to days and weeks with conventional culture.

M. bovis infection can be controlled effectively only if appropriate measures are implemented at the earliest possible stage since immuno-prophylaxis and antibiotic treatment are known to be ineffective.

Consequently this work is planned for:

1. Collection of milk sample from bulk tank milk and clinical mastitic cattle and sub clinical mastitic cattle.
2. Isolation and serological identification of *Mycoplasma* strains from milk samples.
3. Applying of Polymerase chain reaction (PCR) technique to confirm the identification of some isolated strains of *Mycoplasma* (*M. bovis*).
4. Applying antibiotic sensitivity test by the broth indicator microdilution technique on some selected field *Mycoplasma* isolates which isolated from cattle and baffaloes.
5. Using of ELISA technique to detect antibodies of *M. bovis* in milk samples of cattle and buffaloes.
6. Discussion the results included in this study.

   In general the aim of this study is to evaluate the *Mycoplasmal* biomolecular studies in mastitic milk.

REVIEW OF LITERATURE

1. History and General Properties of class Mollicutes

Mollicutes are a class of bacteria distinguished by the absence of a cell wall. The word "Mollicutes" is derived from the Latin *mollis* (meaning "soft" or "pliable"), and *cutis* (meaning "skin"). Individuals are very small, typically only 0.2–0.3 μm (200-300 nm) in size and have a very small genome size. They vary in form, although most have sterols that make the cell membrane somewhat more rigid. Many are able to move about through gliding, but members of the genus *Spiroplasma* are helical and move by twisting. The best-known genus in Mollicutes is *Mycoplasma*. This feature explains many of the unique properties of *Mycoplasmas* as, their typical morphology and plasticity; their sensitivity to lyses by osmotic shock, alcohols, organic solvents, detergents, antibody and complement; their filterability through 0.45-μm pore diameter filters; the fried-egg shape of their colonies on agar; and their total resistance to penicillin and other antibacterial substances which degrade or inhibit peptidoglycan synthesis. They are the smallest self-replicating prokaryotes. Members of this class are capable of reproduction. Contractile movement was seen in some species. Most species are pathogenic but few species are commensally (Razin, 1983). Budding and filamentous forms were seen due to the genome replication preceded cell division (Edward and Freundt, 1967).

The classification of the Mollicutes has always been difficult. The individuals are tiny and, being parasites, they have to be cultivated on special media. Until now many species could not be isolated at all. In the beginning it was not clear whether they were fungi, viruses or bacteria.

Also, the resemblance to L forms was confusing. At first all members of the class Mollicutes were generally named "*mycoplasma*" or "Pleuro Pneumonia-Like Organism" (PPLO). Mollicutes other than some members of genus *Mycoplasma* were still unidentified. The first species of *Mycoplasma*, that could be isolated was *Mycoplasma mycoides*. (Nocard and Roux, 1998)

The *M. pneumoniae* genome sequence was published soon after and was the first genome sequence determined by primer walking of a cosmid library instead of the wholegenome shotgun method (Himmelreich *et al*., 1996). *Mycoplasma* genomics and proteomics continue in efforts to understand the so-called minimal cell (Hutchison and Montague, 2002), catalog the entire protein content of a cell (Regula *et al*., 2000), and generally continue to take advantage of the small genome of these organisms to understand broad biological concepts.

2. Taxonomy of Class Mollicutes

Freundt (1983) tabulated the subcommittee taxonomy of Mollicutes according to (1) sterol requirement (2) location of NADH oxidase (3) and Genome size as in Table (1).

Razin (1988) proposed new classification of Mollicutes according to (1) DNA content (2) nutritional requirements (3) Antigenic composition, (4) host specificity and (5) pathogenicity.

Table1.Taxonomy of the class Mollicutes according to subcommittee (Freundt, 1983).

Class Mollicutes	Order: Mycoplasmatales
Family 1:	***Mycoplasmataceae***
	1- Sterol required for growth
	2- Genome size approximately $5.0x10^9$ Dalton
	3- NADH oxidase localized in cytoplasm
Genus I:	***Mycoplasma*** (64 species current) don't hydrolyze urea
Genus II:	***Ureaplasma*** (1 species with serotypes)
Family II:	***Acholeplasmataceae***
	1- Sterol not required for growth
	2- Genome size $1.0x10^9$ Daltons approx.
	3- NADH oxidase localized in membrane
Genus 1:	***Acholeplasma*** (8 species current)
Family III:	***Spiroplasmataceae***
	1- Helical organisms during some phase of growth
	2- Sterole required for growth
	3- Genome size $1.0x10^9$ Daltons
	4- NADH oxidase localized in cytoplasm
Genus 1 :	***Spiroplasma*** (3 species current)

GENERA OF UNCERTAIN TAXONOMIC POSITION.

Thermoplasma (1 species, apparently belong to Archaebacteria. *Anaeroplasma* (2 species).

(Tully *et al.*, 1993) proposed the recent taxonomy and characterization of members of class Mollicutes as shown in Table (2).

Tully and Bradbury (2003) listed the Subcommittee taxonomy of Mollicutes. These strains lists include *Candidatus* as a new species belonging to genus *Mycoplasma* (Table3).