

**ASSESSMENT OF APPARENTLY NORMAL MILK  
THROUGH FIELD AND LABORATORY EXAMINATIONS**

A Thesis

Presented to the Graduate School  
Faculty of Veterinary Medicine, Alexandria University

In Partial fulfillment of the  
Requirements for the Degree

Of

**Ph.D. of Veterinary Sciences**

In

**Milk Hygiene**

By

**Hamdy Abdel Baeth Abdel Ghany Hegazy**

2009

# تقييم اللبن الطبيعي ظاهرياً خلال الفحوص الحقلية والمعملية

رسالة علمية

مقدمة إلى الدراسات العليا بكلية الطب البيطرى – جامعة الأسكندرية  
إستيفاء للدراسات المقررة للحصول على درجة

دكتور الفلسفة فى العلوم الطبية البيطرية

فى

الرقابة الصحية على الألبان و منتجاتها

مقدمة من

ط.ب./ حمدى عبد الباعث عبد الغنى حجازى

٢٠٠٩

## **Advisors' Committee**

### ***Prof. Dr. Ashraf Mohamed Nazem***

Professor of Milk Hygiene

Vice Dean for Community Development  
and Environment Affairs

Faculty of Veterinary Medicine

Alexandria University

### ***Dr. Amr Abdel-Moemen Amer***

Assistant Professor of Milk Hygiene

Faculty of Veterinary Medicine

Alexandria University

## لجنة الإشراف

### **الأستاذ الدكتور / أشرف محمد ناظم**

أستاذ الرقابة الصحية على الألبان ومنتجاتها  
وكيل الكلية لشؤون خدمة المجتمع وتنمية البيئة  
كلية الطب البيطرى  
جامعة الأسكندرية

### **الدكتور / عمرو عبد المؤمن عامر**

أستاذ مساعد الرقابة الصحية على الألبان ومنتجاتها  
كلية الطب البيطرى  
جامعة الأسكندرية

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

# وما أوتيتم من العلم إلا قليلاً

صدق الله العظيم

سورة الإسراء الآية رقم ٨٥

**Dedicated To  
My Family**



## ACKNOWLEDGMENT

*First of all, my deepest thanks to our merciful **GOD**, who gives me the power and chance to fulfill this work*

*My deepest gratitude and heartily thanks to **Prof. Dr. Ashraf M. Nazem**, Professor of Milk Hygiene, Vice Dean of Faculty of Veterinary Medicine for Community Development and Environmental Affairs, Alexandria University for his stimulating supervision, valuable and continuous share, interest and encouragement.*

*Special thanks to **Dr. Amr Abdel-Moemen Amer**, Assistant Professor of Milk Hygiene, Faculty of Veterinary Medicine, Alexandria University for his help, support and cooperation during the fulfillment of this study.*

*My appreciation goes to all members of the Department of Food Hygiene, Faculty of Veterinary Medicine, Alexandria University for their unfailing help and the facilities they provided during the study.*

## **TABLE OF CONTENTS**

	<b>PAGE</b>
<b>ACKNOWLEDGEMENT</b>	<b>I</b>
<b>TABLE OF CONTENTS</b>	<b>II</b>
<b>LIST OF TABLES</b>	<b>IV</b>
<b>LIST OF ABBREVIATIONS</b>	<b>VI</b>
<b>1. INTRODUCTION</b>	<b>1</b>
<b>2. REVIEW OF LITERATURE</b>	<b>4</b>
2.1. Microbiological examination of milk	4
2.2. California mastitis test	13
2.3. Measurement of electrical conductivity	18
2.4. Somatic cell count and chemical changes	21
<b>3. MATERIAL AND METHODS</b>	<b>28</b>
3.1. Collection of the samples	28
3.2. Microbiological examinations	28
3.2.1. Examination of milk sediment	28
3.2.2. Identification of isolates	28
3.2.2.1. Identification of streptococci	28
3.2.2.2. Identification of staphylococci	29
3.2.2.3. Identification of isolated coliform organisms	30
3.3. Field tests	31
3.3.1. Measurement of electrical conductivity	31
3.3.2. Qualitative chloride test	31
3.3.3. Gel test	31
3.3.3.1 Modified Whiteside test	31
3.3.3.2. California mastitis test	32
3.4. Laboratory measurement of milk somatic cells and chemical parameters by means of milkscan	32
3.5. Statistical analysis	32



<b>4. RESULTS</b>	33
<b>5. DISCUSSION</b>	67
5.1. Microbiological examination	67
5.2. California mastitis test	69
5.3. Modified Whiteside test	70
5.4. Qualitative chloride test	71
5.5. Somatic cell count	73
5.6. Electrical conductivity test	75
5.7. Chemical parameters	76
<b>6. CONCLUSION AND RECOMMENDATIONS</b>	78
<b>7. SUMMARY</b>	79
<b>8. REFERENCES</b>	81
<b>ARABIC SUMMARY</b>	

## LIST OF TABLES

	PAGE
Table (1) Incidence of subclinical mastitis in examined milk samples according to culture results	33
Table (2) Scoring of subclinical mastitis milk collected from cow's according to California mastitis test	34
Table (3) Scoring of subclinical mastitis milk collected from buffaloes according to California mastitis test	35
Table (4) Evaluation of California mastitis test in relation to culture results on cow's milk samples	36
Table (5) Evaluation of California mastitis test in relation to culture results on buffalo's milk samples	37
Table (6) Scoring subclinical mastitis milk collected from dairy animals according to modified Whiteside test in relation to California mastitis test (CMT) scores	38
Table (7) Evaluation of modified Whiteside test in relation to culture result on cow's milk samples	39
Table (8) Evaluation of modified Whiteside test in relation to culture results on buffalo's milk	40
Table (9) Relationship between qualitative chloride test and California mastitis test scores in examined milk samples	41
Table (10) Evaluation of qualitative chloride test in relation to culture results on cow's milk samples	42
Table (11) Evaluation of qualitative chloride test in relation to culture results on buffalo's milk samples	43
Table (12) Relationship between somatic cell counts (SCC) and California mastitis test (CMT) scores in examined milk samples	44
Table (13) Evaluation of somatic cell count in relation to culture results on cow's milk samples	45
Table (14) Evaluation of somatic cell count in relation to culture results on buffalo's milk samples	46
Table (15) Relationship between electrical conductivity (EC) test and California mastitis test (CMT) scores in examined milk samples	47
Table (16) Evaluation of electrical conductivity test in relation to culture results on cow's milk samples	48
Table (17) Evaluation of electrical conductivity test in relation to culture results in buffalo's milk samples	49

Table (18) Relationship between <i>Streptococcus agalactiae</i> and California mastitis test (CMT) scores in examined milk samples	50
Table (19) Relationship between <i>Staphylococcus aureus</i> and California mastitis test (CMT) scores in examined milk samples	51
Table (20) Relationship between <i>Escherichia coli</i> and California mastitis test (CMT) scores in examined milk samples	52
Table (21) Evaluation of indirect mastitis diagnostic tests in relation to culture results on cow's milk samples obtained by hand milking	53
Table (22) Evaluation of indirect mastitis diagnostic tests in relation to culture results on cow's milk samples obtained by machine milking	54
Table (23) Evaluation of indirect mastitis diagnostic tests in relation to culture results on cow's milk samples obtained by hand milking	55
Table (24) Evaluation of indirect mastitis diagnostic tests in relation to culture results on cow's milk samples obtained by machine milking	56
Table (25) Evaluation of indirect mastitis diagnostic tests in relation to culture results on cow's milk samples obtained by hand milking	57
Table (26) Evaluation of indirect mastitis diagnostic tests in relation to culture results on cow's milk samples obtained by machine milking	58
Table (27) Evaluation of indirect mastitis diagnostic tests in relation to culture results on buffalo's milk samples obtained by hand milking	59
Table (28) Evaluation of indirect mastitis diagnostic tests in relation to culture results on buffalo's milk samples obtained by machine milking	60
Table (29) Evaluation of indirect mastitis diagnostic tests in relation to culture results on buffalo's milk samples obtained by hand milking	61
Table (30) Evaluation of indirect mastitis diagnostic tests in relation to culture results on buffalo's milk samples obtained by machine milking	62
Table (31) Evaluation of indirect mastitis diagnostic tests in relation to culture results on buffalo's milk samples obtained by hand milking	63
Table (32) Evaluation of indirect mastitis diagnostic tests in relation to culture results on buffalo's milk samples obtained by machine milking	64
Table (33) Impact of subclinical mastitis on some chemical parameters of cow's milk obtained by hand milking	65
Table (34) Impact of subclinical mastitis on some chemical parameters of cow's milk obtained by machine milking	65
Table (35) Impact of subclinical mastitis on some chemical parameters of buffalo's milk obtained by hand milking	66
Table (36) Impact of subclinical mastitis on some chemical parameters of buffalo's milk obtained by machine milking	66

## LIST OF ABBREVIATIONS

BMSCC	Bulk milk somatic cell count
CMT	California mastitis test
CNS	Coagulase negative staphylococci
IMI	Intramammary infection
MWT	Modified Whiteside test
<i>S. aureus</i>	<i>Staphylococcus aureus</i>
SCC	Somatic cell count
SNF	Solids not fat
<i>Str. agalactiae</i>	<i>Streptococcus agalactiae</i>

## INTRODUCTION

Milk used for human consumption should be delivered from healthy udders to be a valuable food for consumers as it contains all nutrients (Proteins, fats, vitamins and minerals) required for both infants and adults.

Mastitis stands out as the most widespread and destructive dairy disease. Despite the immense amount of research conducted by various investigators in this field, it continues to be the most expensive and most trying problem which confronts dairymen and practitioners in all countries. Due to its insidious nature, mastitis may exist in a herd for comparatively long durations without being recognized by the dairymen. The advance of the infection may be so profound as to involve a large proportion of the dairy cattle in the community and thus unfortunately causes tremendous economic losses.

From the public health point of view, mastitis is considered of paramount importance due to its association with infections that are communicable from animals to man through the intermediation of milk. Tubercle bacilli, the paratyphoid enteritidis group, Streptococcus epidemics and foot and mouth disease virus are regarded amongst the etiological factors which induce mastitis in dairy cattle and consumption of such a quality of milk undoubtedly causes the prevalence of these diseases amongst human consumers.

Bovine mastitis is one of the most important problems in our dairy farms especially in small private farms where hygienic measures and milking sanitation are often insufficient (*Zatoun and Manaa, 1992*).

Subclinical mastitis is universally present in dairy farms in one farm or other, and around 40% and above of cows were reported to be suffering from subclinical mastitis (*Ramachandrainh et al., 1990*).

The serious effects extended by mastitis are mostly due to its subclinical form, during which the causative microorganisms act as invisible potential source for spreading the infection among the herd without the owners being aware of it. Mastitis will continue as a problem in animal health as well as the causative microorganisms may be eliminated in milk which may be harmful to consumers with special reference to children.

Early detection of mastitis especially in case of subclinical form where there is no obvious symptoms and secreted milk apparently normal is very important for most dairy farms to reduce the production losses and to enhance prospects recovery. Many efforts have been expended to provide veterinarians and farmers with efficient tool for mastitis detection (*Emanuelson et al., 1987*). Several methods for diagnosis of mastitis (especially subclinical form) have been reported. Bacteriological methods are expensive and time consuming but they are still the most accurate methods. The disadvantage of this method hence the need for simple sensitive and reliable method sufficient to be applied on large scale herd testing. Many tests based on the detection of pathological changes, often associated with inflammation, have been proposed while others are microscopic for detection of abnormal cellular material in milk (*Moursy and Zakarya, 1972*).

Several methods have been reported for detection of subclinical mastitis. Isolation of the causative microorganisms is the most accurate one, whereas it is expensive and time

consuming. The need for a simple quite sensitive, rapid and reliable test sufficient to be applied on large scale of animals is therefore required.

The California Mastitis Test (CMT) has a useful role in the dairy herd monitoring as a simple, inexpensive and rapid screening test to detect fresh cows with intramammary infections (IMI) caused by major pathogens (*Sargeant et al., 2001*). When CMT is regularly performed as a control measure, significantly lower risks of subclinical mastitis are observed (*Busato et al., 2000*).

Somatic Cell Counts (SCCs) are accepted as the international standard measurement of milk quality. Milk somatic cells are primarily leukocytes (white blood cells) which include phagocytes and lymphocytes. During mastitis the major increase in SCC is due to the influx neutrophils to the milk to fight infection (*Harmon, 1994*).

Electrical conductivity of milk has been introduced as an indicator trait for mastitis. The electrical conductivity is determined by the concentration of anions and cations. If the cow suffers from mastitis, the concentration of  $\text{Na}^+$  and  $\text{Cl}^-$  in the milk increase, which leads to an increase in the electrical conductivity of milk from infected quarter (*Norberg et al., 2004*).

Regarding public health, mastitis is considered as vital importance due to its association with many zoonotic diseases in milk which act as a source of infection (*APHA, 1993*).

The bacteriological analysis provides information about the primary organisms present in the herd which may cause mastitis, especially that linked between human disease and consumption of bovine products (unpasteurized milk and cheese) and indicated that cattle are primary reservoirs for some pathogens which may cause illness to man as well as mastitis in dairy animals, such as *Str. agalactiae*, *S. aureus* and *E. coli*.

*S. aureus* is among the most prevalent causative agents of food poisoning and it is a common cause of mastitis in dairy cattle. The intramammary colonization by *S. aureus* provokes mastitis in the cow, once established, the infection is difficult to eradicate with available therapies and may become chronic (*Brouillette and Malounin, 2005*).

*E. coli* also has become of major concern in the industry since its association with human disease. In developing countries, *E. coli* being one of the major causative agents for diarrhoea which is one of the most common causes of morbidity and mortality among infants and young children. The main source of the organism in the environment is probably the faeces of infected humans, but there may also be animal reservoirs (*Adrian et al., 2002*).

Owing to the epidemiological link between human diseases and consumption of bovine products, and at the same time due to continuous demand for the milk and its products, so it is essential to examine milk microbiologically to ensure its freedom from health hazards.

The aim of the present study is to estimate the effect of mastitis on milk constituents and to evaluate the different rapid screening tests for the diagnosis of subclinical mastitis in comparison with bacteriological examination; to spot out the most efficient and simple tests to be used under field conditions, so the correlations among the

used methods and occurrence of mastitis were studied to verify the relation between every method and presence of subclinical mastitis.

Several lines of treatments and numerous remedies including the use of antibiotics have throughout the world been utilized to combat this highly contagious and destructive disease. Despite all the varied and strenuous efforts and strict sanitary measures which have been adopted in its combat, mastitis still prevails and is considered the major problem of dairy production all over the world.

The highly contagious character and insidious nature of this infection make it highly desirable and essential that investigations in this field should be directed towards the establishment of a practical and accurate method for its early recognition and thorough detection. The bacteriological examination of suspected mastitic milk is a time consuming procedure not disregarding the big expense that its application incurs particularly when undertaken on a large scale.

To have at hand an accurate uncostly technique for the early detection and recognition of this widespread disease and which can be executed in a comparatively short time is obviously of high scientific and economic value. Whatever method is aimed at or adopted, the standard of its accuracy should compete with that of the bacteriological methods.

With this particular interest of establishing such a technique in view, the present investigation has been undertaken in which an endeavor has been made to bring forth a sound method for an early diagnosis of mastitis which may then facilitate its combating on a large scale.

The work topics include

1. California Mastitis Test (CMT)
2. Measurement of Electrical Conductivity (EC) using milk checker.
3. Laboratory measurement of somatic cells by means milk scan apparatus (Milk Soma Counter).
4. Investigation of the effect of subclinical mastitis on milk constituents.
5. Bacteriological examination of milk samples for identification of:
  - a. *Staphylococcus aureus*.
  - b. *Streptococcus agalactiae*.
  - c. *Escherichia coli*.
6. Isolation and biochemical identification of the above mentioned microbial agents.