



Cairo University
Faculty of Veterinary Medicine



**Studies on *Staphylococcus aureus* polyvalent vaccine
to control mastitis among dairy cows in Egypt**

A Thesis submitted by

Ashraf Megahed Abd Elmaksoud El Sheikh Eissa

B.V.Sc. Faculty of Veterinary Medicine - Cairo University 2003
For the degree of M.V.Sc
(Infectious diseases)

UNDER THE SUPERVISION OF

Prof. Dr.

Diea Gamal Eldin Abo Elhassan Elithy

Professor of Infectious diseases
Faculty of Veterinary Medicine
Cairo university.

Dr.

Mohamed Abd El Monaem Salem

Assist. professor of Infectious Diseases
Faculty of Veterinary Medicine
Cairo university.

Dr.

Ahmed Samir Mohamed

Assist. professor of Microbiology
Faculty of Veterinary Medicine
Cairo university.

(2018)



Cairo University
Faculty of Veterinary Medicine
Dep. Internal Medicine And Infectious Diseases

Supervision sheet

Prof. Dr.
Diea Gamal Eldin Abo Elhassan Elithy
Professor of Infectious diseases
Faculty of Veterinary Medicine
Cairo university

Dr.
Mohamed Abd El Monaem Salem
Assistant professor of Infectious diseases
Faculty of Veterinary Medicine
Cairo university

Dr.
Ahmed Samir
Assistant professor of microbiology
Faculty of Veterinary Medicine
Cairo university

(2018)



Cairo University
Faculty of Veterinary Medicine
Department of Veterinary Medicine & Infectious diseases

Name: Ashraf Megahed Abd Elmaksoud El Sheikh Eissa

Birth date: 10 \ 5 \ 1979

Nationality: Egyptian

Degree: Master veterinary science

Specialization: Infectious Diseases

Title of thesis: Studies on *Staphylococcus aureus* polyvalent vaccine to control mastitis among dairy cows in Egypt

Supervision:

Prof. Dr. Diea Gamal Eldin Abo Elhassan Elithy

Professor of Infectious Diseases , Faculty of Veterinary Medicine
Cairo university

Dr. Mohamed Abd El Monaem Salem

Assistant Professor of Infectious diseases, Faculty of Veterinary Medicine
Cairo university

Dr. Ahmed Samir

Assistant Professor of microbiology , Faculty of Veterinary Medicine
Cairo university

Abstract

Staphylococcus aureus is a common udder pathogen in dairy cows, and cause severe mastitis problems in some herds in Egypt. In herds where normal control measures are not successful, vaccination might be an additional tool to use if sufficiently efficient. In the last years the knowledge on cow mastitis is remarkably improving, nevertheless the attention has been never focused on vaccination as preventive strategy for the control of mastitis. A successful *Staphylococcus aureus* vaccine should elicit a long-term antibody response that prevents establishment of the infection. As sera of immunized pregnant heifers with *S. aureus* lysed cells formulated with a classical adjuvant stimulate antibodies production that inhibited internalization in mammary epithelial cells (MEC) and increased phagocytosis by milk macrophages, the aim of the present study was to evaluate the humoral immunological response to a commercially available vaccine (LysiginTM, Boehringer Ingelheim Vetmedica Inc., St. Joseph, MO) and its potential use as a preventive way for *S. aureus* mastitis in Holstein cows Vaccinated (V) and not vaccinated (N-V) groups, of 60 cows, were selected from one herd of the same farm. The herd received a double vaccination (LysiginTM), 30 and 15 days before calving, and 5 months after calving).

In conclusion, The current study on commercially available vaccine (LysiginTM), and its potential use as a preventive way for *Staph. aureus* mastitis in Holstein cows in Egypt showed encouraging results associated with induction of specific and significant antibody responses and significant lower prevalence and incidence of clinical, subclinical and recurrent mastitis. The vaccine is considered an additional tool in the control of *S. aureus* infections on farms and that its use should be always associated with excellent farm management practices to successfully improve the infections control within the herd.

Keywords: Cattle; bacteria; *Staphylococcus aureus*; ELISA; Mastitis; vaccination

Dedication

*First of all,
My gratitude and prayerful to Allah
For giving me the power not only to carry out this work
but also during my whole life.*

Dedication to

My mother &

My two daughters

(Fatma & Amira)

And my professors

And my wife

(Huda)

AKNOWLEDGMENT

*First of all, I would like to thank **Allah** for helping me to carry out this work and all works throughout my life.*

*My deepest gratitude and appreciation to **Prof. Dr. Diea Gamal Eldin Abo Elhassan Elithy** Professor of Infectious Diseases , Faculty of Veterinary Medicine ,Cairo university, for planning the present study, his keen supervision, facilities, helpful guidance, continuous encouragement and science advice from the beginning to the end of this work,*

*I wish to put on recorded my sincere thanks and appreciation **Dr. Mohamed Abd El Monaem Salem** Assistant Professor of Infectious diseases, Faculty of Veterinary Medicine Cairo university , for his supervision of this study.*

*I would like to offer my sincere thanks to **Dr. Ahmed Samir** Assistant Professor of microbiology ,Faculty of Veterinary Medicine Cairo university , for his valuable assistance, remarks and guidance to complete this work,*

*I am greatly indebted to I cannot find enough words to express my deepest feelings towards **Dr. salwa Ahmed Aly** Professor of Food Hygiene, for her continuous kind help for the microbiology in this work,*

*My grateful thanks to all **Team work (doctors, Technicians, and Workers)** in Farm stars company for milk production , for their help and facilities they provided.*

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List of Abbreviations

Abbreviation	Acronym
BMSCC	Bulk milk somatic cell count
BSM	Bovine Subclinical Mastitis
BTB	Bromothymol blue
BTSCC	Bulk Tank Somatic Cell Count
CFU	Colony forming unit
CM	Clinical Mastitis
CMT	California Mastitis Test
CNS	Coagulase Negative Staphylococci
CP	Capsular polysaccharides
CPS	Coagulase Positive Staphylococci
<i>E. coli</i>	<i>Escherichia coli</i>
EC	Electrical conductivity
ELISA	Enzyme Linked Immune Sorbent Assay
GST-mSEC	Glutathione S-transferase -fused SEC
HCM	Heifer Clinical Mastitis
HSCC	High somatic cell count
HSCM	Heifer Sub Clinical Mastitis
IgG	Immunoglobulin G
IMI	Intra mammary Infection
ISCC	Individual Somatic Cell Count
KG	Kilo gram
MCMT	Modified California mastitis test
MEC	Mammary epithelial cells
ML	Mililiter
NMC	National Mastitis Council

List of Abbreviations

No.	Number
N-V	Not vaccinated
O/F	Oxidative/fermentative
P < 0.05	Statistic signeficance
PBS	Phosphate Buffer Saline
PMN	Polymorphnuclear neutrophils
QMS	Quarter milk sample
SAAC	Slime associated antigenic complex
SCC	Somatic Cell Count
SCM	Sub Clinical Mastitis
SD	Standard Deviation
SFMT	Surf Field Mastitis Test
<i>Staph. aureus</i>	<i>Staphylococcus aureus</i>
<i>Str. uberis</i>	<i>Streptococcus uberis</i>
TEL	Teat-End Lesion
TSST	toxic shock syndrome toxin
UK	United Kingdom
USA	United states of America
VTM	Veterinary-treated clinical mastitis
WST	White Side Test

INTRODUCTION

Bovine mastitis, inflammation of the bovine mammary gland, one of the most important worldwide disease in the dairy industry. **(Ruegg, 2003)**. It is known as a functional disorder caused by a wide range of etiological factors causing considerable economical loss. Both clinical and subclinical infections impair animal health and welfare and is accompanied by decreased milk production, increased health care costs, higher culling rates and sometimes even death. The economic losses for dairy farmers are thus considerable and many efforts have been made to reduce the incidence of mastitis in dairy herds. However, despite an impressive reduction in the number of serious mastitis cases, the prevalence of subclinical and chronic mastitis is still high. Epidemiological studies reveal that following treatment with antimicrobial agents bacteriological cure rates can range between 0% and 80%, and seem to depend on age, parity, stage of lactation, position of the infected quarter and somatic cell counts (SCC). The worldwide loss in production is estimated at 900 million liters of milk annually. **(Gruet et al., 2001 and Rainard and Riollet, 2006)**.

Bacteria are by far the most common cause of mastitis. Although mastitis can be caused by 137 different microorganisms **(Ranjan et al., 2006)**, the epidemiology of bovine mastitis has been characterized worldwide by an increase in the prevalence of staphylococci. **(Sommerhausen et al., 2003 and Moret-Stalder et al., 2009)**. Additionally, coagulase-negative staphylococci (CNS), one of the etiological factors of mastitis, are considered in many countries as the main cause of bovine mastitis **(Taponen et al., 2006)**. *Staph. aureus* is the most frequently isolated, coagulase-positive microorganism among the forty-four species belonging to the Staphylococcaceae family, and is considered the main cause of chronic, subclinical bovine mastitis **(Kauf et al., 2007; Saei et al., 2009 and Wang et al., 2009)**. Its particular properties, especially the presence of a wide range of virulence factors, makes this organism one of the most dangerous animal pathogens **(Iwatsuki et al., 2006; Rall et al., 2010 and Seo et al., 2010)**.

Staph. aureus is the most ubiquitous and results in the greatest economic loss **(Foster, 1986)**. *Staph. aureus* mastitis occurs in spite of a series of defense mechanisms that begin with the smooth muscle sphincter surrounding the teat end and