



شبكة المعلومات الجامعية

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ



شبكة المعلومات الجامعية
@ ASUNET



شبكة المعلومات الجامعية التوثيق الالكتروني والميكروفيلم



شبكة المعلومات الجامعية

جامعة عين شمس

التوثيق الالكتروني والميكروفيلم

قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها
علي هذه الأفلام قد أعدت دون أية تغيرات



يجب أن

تحفظ هذه الأفلام بعيدا عن الغبار

في درجة حرارة من ١٥-٢٥ مئوية ورطوبة نسبية من ٢٠-٤٠%

To be Kept away from Dust in Dry Cool place of
15-25- c and relative humidity 20-40%

بعض الوثائق الأصلية تالفة

بالرسالة صفحات لم ترد بالاصل

Cairo University
Faculty of Veterinary Medicine
Department of Microbiology

292.


636,089601

Molecular Typing Of Major Pathogens From Bovine Mastitis

Thesis presented by

Ashgan Mohammed Mostafa Yousef

B.V.Sc. (1997), Cairo University

M.V.Sc. (2001), Cairo University

For the degree of
**Doctor of Philosophy in Veterinary Medical Science,
Microbiology**
(Bacteriology, Immunology and Mycology)

Under the supervision of

Prof. Dr. Kamelia Mahmoud Osman

Professor of Microbiology
Faculty of Veterinary Medicine
Cairo University

Prof. Dr. Jakeen Kamal Abdel Haleem El- Jakee

Professor of Microbiology
Faculty of Veterinary Medicine
Cairo University

(2005)



بسم الله الرحمن الرحيم

﴿قل اللهم مالك الملك تؤتي الملك من تشاء و تنزع
الملك ممن تشاء و تعز من تشاء و تذلل من تشاء
بيدك الخير إنك على كل شيء قدير﴾ * توجّل الليل
في النهار و توجّل النهار في الليل و تخرج الحي من
الميت و تخرج الميت من الحي و ترزق من تشاء

بغير حساب﴾ *

صدق الله العظيم

*Cairo university
Faculty of Veterinary Medicine
Department of Microbiology*

APPROVAL SHEET

This is to certify that the dissertation presented by ***Ashgan Mohamed Mostafa Yousef*** to Cairo University, for the Ph.D. degree in Veterinary Sciences (Microbiology) has been approved by the examining committee:

Prof. Dr. Mohamed Taha Mahmoud El-Said.

*Professor of Microbiology
Faculty of Veterinary Medicine
Zagazig University*



Prof. Dr. Mahmoud Essam Hatem

*Professor and Chairman of Microbiology Department
Faculty of Veterinary Medicine
Cairo University*



Prof. Dr. Kamelia Mahmoud Osman

*Professor of Microbiology
Faculty of Veterinary Medicine
Cairo University
(Supervisor)*



Prof. Dr. Jakeen Kamal Abdel Haleem El-Jakee

*Professor of Microbiology
Faculty of Veterinary Medicine
Cairo University
(Supervisor)*



Date: 7/4/2005

Acknowledgment

I am greatly indebted to gracious **ALLAH** for helping me to carry out this work.

I would like to express my sincere gratitude and thanks to **Prof. Dr. Kamelia Mahmoud Osman**, Professor of Microbiology, Faculty of Veterinary Medicine, Cairo University, for her supervision, endless help, kind and true guidness, constructive criticism , great effort and for the time that she has given up to complete the present investigation in its present state and which will never be forgotten.

I would like to express my heart felt gratitude to **Prof. Dr. Jakeen Kamal Abd El-Halim El-Jakee**, Professor of Microbiology, Faculty of Veterinary Medicine, Cairo University, for her valuable supervision , ideal guidance and continuous advice.

I would like to introduce my great appreciation to **Prof. Dr. Ayman A. El-Ghaysh**, Professor of Parasitology, Faculty of Veterinary Medicine, Cairo University, for helping me in collection of milk samples.

I would like to express my sincere thanks to **Prof. Dr. Magdy Ghoneim**, Professor of Biochemistry and Director of Biotechnology Center of Services and Researches, Faculty of

Veterinary Medicine, Cairo University, for the facilities offered to carry out this work.

Sincere thanks and deep gratitude to **Dr. Ihab Mohamed Ibrahim**, Lecturer of Microbiology, Faculty of Veterinary Medicine, Cairo University, for his help and assistance.

Gratitude is also offered to all my professors and colleagues in the Department of Microbiology, Faculty of Veterinary Medicine, Cairo University, for their help and support.

List of contents

1. Introduction	1
2. Review of Literature	6
2.1. Major pathogens associated with bovine mastitis.....	6
2.2. Genetic identification of the most common pathogens associated with bovine mastitis using polymerase chain reaction(PCR)	17
2.3. Determination of Shiga toxigenic <i>E. coli</i> isolates by using multiplex PCR	26
2.4. Molecular typing of <i>E. coli</i> isolates by random amplified polymorphic DNA-Polymerase chain reaction (RAPD-PCR)	35
3. Materials and Methods	44
3.1. Materials	44
3.2. Methods	62
4. Results	76
4.1. Incidence of mastitis among the examined lactating cows.	76
4.2. Degree of severity of subclinical mastitis using CMT in quarter milk samples.....	78
4.3. Correlation between incidence of mastitis and stage of lactation among the examined lactating cows.	79
4.4. Results of bacteriological examination on milk samples.	80
4.5. Characterization of <i>E. coli</i> isolates recovered from normal and mastitic quarter milk samples	84

4.6. Characterization of shiga toxigenic <i>E. coli</i> (STEC) by multiplex PCR assay for shiga toxin 2 (stx2) and intimin genes (eaeA)	89
4.7. Results of polymerase chain reaction (PCR)	92
4.8. Molecular typing of <i>E. coli</i> strains recovered from milk samples by RAPD-PCR	118
5. Discussion	125
6. Summary	151
7. References	155
8. Arabic Summary	

List of Tables

Table	Title	Page
1	The number of the examined lactating cows and quarter milk samples.	45
2	Standard bacterial strain used for determination of the primers specificity.	53
3	Oligonucleotide primer sequences used for amplification of DNA recovered from milk samples and bacterial isolates.	55
4	Primer conditions during PCR.	56
5	Oligonucleotide primer sequences used for simultaneous detection of stx2 and eae genes by multiplex PCR.	57
6	Identification between <i>S. aureus</i> and <i>S. epidermidis</i> .	64
7	Biochemical reaction for identification of streptococci which cause bovine mastitis.	65
8	Identification of <i>A. pyogenes</i> isolates	66
9	Differentiation of members of Family <i>Enterobacteriaceae</i> .	67
10	Identification of <i>P. aeruginosa</i> isolates	68
11	Incidence of mastitis among the examined milk samples	77
12	The distribution of infected quarter involvement of the examined clinically and subclinically mastitic animals	77
13	The severity of mastitis using the CMT in quarter milk samples collected from apparently normal lactating cows	78

Table	Title	Page
14	Correlation between the incidence of mastitis and the stage of lactation among the examined animals.	79
15	The prevalence rate of bacteriologically positive (single and mixed) quarter milk sample.	81
16	The prevalence of different bacterial isolates recovered from examined quarter milk samples	81
17	Correlation between severity of mastitis and the bacteriological findings (single and mixed) among the examined quarter milk samples	83
18	Serotyping of <i>E. coli</i> recovered from different types of milk samples	85
19	Cytotoxicity of <i>E. coli</i> serovars isolated from normal and mastitic quarter milk samples.	87
20	Characterization of STEC by multiplex PCR assay.	90
21	The specificity of the universal primers.	92
22	Comparison between the bacteriological examination and the PCR using uni678 and uni888 universal primers.	94
23	Comparison between the bacteriological examination and the PCR using uni1870 and uni2308 universal primers.	94
24	Comparison between bacteriological examination and PCR using Eco223-Eco455 primers.	98
25	Comparison between bacteriological examination and PCR using Sau234-Sau1501 primers.	101

Table	Title	Page
26	Comparison between bacteriological examination and PCR using Sau234-Sau1501 primers.	104
27	Comparison between the bacteriological examination and the PCR using Spa2152 forward and Spa2870 reverse primers.	107
28	Comparison between the bacteriological examination and the PCR using Sdy105 forward and Sdy386 reverse primers.	110
29	Comparison between the bacteriological examination and the PCR using Sag40 forward and Sag445 reverse primers.	113
30	Comparison between the bacteriological examination and multiplex PCR for detection of the six major pathogens causing bovine mastitis	116
31	Scoring sheet of 19 <i>E. coli</i> serotypes recovered from milk sample showing the molecular weight and the number of the DNA banding patterns as well as the presence or absence of shared and unique bands	121
32	The molecular weight, the intensity and the number of DNA banding pattern of 19 <i>E. coli</i> serotypes following amplification by RAPD-PCR assay using 5 primers	122
33	Similarity index of RAPD profiles of <i>E. coli</i> recovered from milk samples using Nei and Li's coefficient	123