



Molecular studies on Mycobacteria

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Master degree in Veterinary Medical Sciences (Microbiology), 2014

Bachelor Degree of Veterinary Sciences, 2009

In the partial fulfillment of the requirements for the:

Degree of Doctor of Philosophy in Veterinary Medical Sciences

Microbiology

(Bacteriology, Immunology and Mycology)

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا
عَلَّمْتَنَا إِنَّكَ أَنْتَ الْعَلِيمُ الْحَكِيمُ

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سورة البقرة - الآية 32

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Key words: *M. bovis* – *M. tuberculosis* – MTBC – BTB – Isolation – Real-time PCR – Multiplex real-time PCR

Abstract: *Mycobacterium bovis* is the major causative agent of bovine tuberculosis (BTB) and part of the *Mycobacterium tuberculosis* complex (MTBC). BTB has an impact on the national and international economy, affects the ecosystem via transmission to wildlife and is of public health concern due to its zoonotic potential. Although still present in some industrialized countries, BTB today mostly affects developing countries lacking the resources to apply expensive test and slaughter schemes. Tuberculosis (TB) remains a global health problem despite near eradication in some developed countries. This study was conducted from early winter of 2015 to spring of 2017 to compare between conventional and molecular techniques for detection of *Mycobacterium bovis* (*M. bovis*) in Egypt.

A total of 49 specimens were collected from four major abattoirs (El-Basateen- El-Monieeb- Beni-Suef- Al-fayoum) to be analyzed bacteriologically and biochemically for: isolation, identification and confirmation of *M. bovis* with molecular methods. Only 19 isolates were found to be positive slow-growers *Mycobacterium* species by conventional cultivation method on solid medium (LJ medium and Stone brink) and identified biochemically to 17 *M. bovis* isolates and 2 isolates *M. tuberculosis*. Genotyping detection of *Mycobacterium tuberculosis* complex by amplification of ext-RD9 region by real-time PCR was carried out on positive cultures and directly on specimens. Out of 49 DNA templates extracted directly from specimens, 31 specimens were confirmed to be infected by *Mycobacterium tuberculosis* complex by amplification of ext-RD9 region by real-time PCR. This study reports the development and evaluation of a single-tube, two-targets, real-time PCR assay which can differentiate between *M. bovis* and *M. tuberculosis*. The multiplex real-time PCR target RD1 and RD4 using 2 sets of primers-probes. A 31 MTBC positive DNA from clinical specimens were identified by this assay as, 27 *M. bovis* isolates and 4 *M. tuberculosis* isolates. A 19 positive cultures were confirmed to be *Mycobacterium tuberculosis* complex were identified by this assay as, 17 *M. bovis* isolates and 2 *M. tuberculosis* isolates.

Dedication to

my mother,

my father,

*my beloved husband
Ahmed*

*my daughters
Joury and Kady
&
my sisters*

Acknowledgement

First of all, I would like to express my all-embracing gratitude and praise to **ALLAH**, Glorified be He, for his unmitigated support and graceful benevolence in carrying out this humble thesis.

I would like to seize the opportunity to express my greatest indebtedness to **Prof. Dr. Khaled Farouk Al-Amry**, Professor of Microbiology, Microbiology Department, Faculty of Veterinary Medicine, Cairo University for his initiating power, effective scientific supervision.

I shall never forget the highly appreciated advices and most esteemed constructive criticism of **Prof. Dr. Nashwa Mohamed Helmy**, Chief researcher of Biotechnology, Biotechnology Department, Animal Health Research Institute, ARC, Doki, Giza.

I am obliged to offer the utmost respect and thanks for the generous help of **Prof. Dr. Salah El-Din Abd ElKarim Selim**, Professor of Microbiology, Microbiology Department, Faculty of Veterinary Medicine, Cairo University.

I would like also to express my gratitude to **Dr. Sherif Abd El Monam Omar**, Assistant professor of Microbiology, Microbiology Department, Faculty of Veterinary Medicine, Cairo University, for his sincere efforts and help.

Countless thanks and filial gratitude, which engulf my heart are kept for my most beloved parents for their caring soul and for their continuous inspiration for me.

All my feelings and love are kept for my husband, **Ahmed Mohamed**, who has always given me all power and support to complete this work. He is the source of enthusiasm, energy and hope that God bestowed me with.

My daughter's sweet roses (**Joury and Kady**) were affected by my preoccupation and be patient with me for the end. Give me the enthusiasm with their laughter and the look of love that is with their eyes. God save my little daughters.

My sisters (**Walaa and Rana**) have always given me all support and encouragement.

I want to thank **Dr. Mostafa Ramadan Abd-El Samie salem**, General Manager of Automatic Slaughterhouse, with all love, appreciation and respect for his sincere efforts, help and fatigue with me in this work.

All love and respect to the respectable professors, members especially **Chem. Dalia Mostafa** and workers in the Biotechnology Department, Animal Health Research Institute, ARC, Doki, Giza for their continuous support.

List of Contents

	Contents	Page No.
1.	Introduction	1
2.	Review of Literature	5
2.1	Overview	5
2.2	Etiology	7
2.2.1	Taxonomy of Mycobacteria	7
2.2.2	Mycobacteria structure	8
2.2.3	Physical and biochemical characteristics	11
2.2.3.1	Morphology and staining	11
2.2.3.2	Growth requirement and culture characteristics	13
2.3	Pathogenesis	13
2.3.1	Infection	13
2.3.2	Lesion	13
2.3.3	Virulence	14
2.4	Immunity against Mycobacterial infection	14
2.5	Epidemiology of <i>Mycobacterium bovis</i> infections	16
2.5.1	Source of infection and mode of transmission	16
2.5.2	Risk factors: Animal population	17
2.5.2.1	Environment	18
2.5.2.2	Agent	18
2.5.2.3	Host range	18
2.5.3	Risk factors: Human population	20
2.5.3.1	Close physical contact	20
2.5.3.2	The increase in the demand for milk	20

2.5.3.3	Feeding habit	20
2.5.3.4	HIV infection	21
2.5.3.5	Absence of control mechanism	21
2.5.4	Distribution	22
2.6	Bovine TB	23
2.6.1	<i>M. bovis</i> in cattle	23
2.6.2	<i>M. bovis</i> in humans	25
2.6.3	<i>M. bovis</i> in wildlife	27
2.7	Diagnosis	29
2.7.1	Clinical examination	30
2.7.2	Tuberculin skin test	31
2.7.3	Postmortem examination	31
2.7.4	Laboratory Detection of <i>M. bovis</i> infection	32
2.7.4.1	Microscopic examination	32
2.7.4.1.1	Histology	34
2.7.4.1.2	Differential staining	35
2.7.4.2	Culture	36
2.7.4.2.1	Culture Media	40
2.7.4.2.2	Culture Isolate Identification	43
2.7.4.3	Immunological/serological diagnostic methods	44
2.7.4.3.1	Gamma interferon assay/Bovigam	45
2.7.4.3.2	Enzyme Linked Immunosorbent Assay	45
2.7.4.4	Molecular Methods	46
2.8	TB treatment Overview	51
2.9	TB Vaccine	54
2.10	Bovine TB Eradication Scheme	55
2.11	Zoonotic Importance of Bovine Tuberculosis	59
3.	Materials and Methods	63

3.1	Materials	63
3.1.1	Samples	63
3.1.2	Media used for phenotypic detection of <i>Mycobacterium tuberculosis</i> complex	64
3.1.2.1	Solid Media	64
3.1.2.1.1	Löwenstein–Jensen (LJ) media	65
3.1.2.1.2	L.J Media containing PNBA	65
3.1.2.1.3	L.J Media containing TCH	65
3.1.2.1.4	Stone brink medium	66
3.1.3	Chemicals	66
3.1.3.1	Chemicals used for phenotypic detection	66
3.1.3.1.1	Chemicals used for decontamination	66
3.1.3.1.2	Chemicals used for LJ culturing	67
3.1.3.1.3	Chemicals used for biochemical differentiation	67
3.1.4	Buffers and solutions	68
3.1.4.1	Buffers and solutions used for phenotypic detection	68
3.1.4.1.1	Buffers and solutions used for decontamination	68
3.1.4.1.2	Buffers and solutions used for LJ culturing	69
3.1.4.1.3	Buffers and solutions used for biochemical differentiation	69
3.1.4.1.3	Catalase test	69
3.1.5	Stains	70
3.1.5.1	Ziehl-Neelsen stain	70
3.1.6	Biologicals	71
3.1.6.1	Biologicals used for phenotypic detection	71