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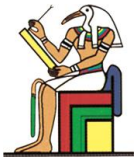

Molecular studies on Mycobacteria

A thesis presented by:

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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

صَدقَ اللهُ العَظِيم

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-Monieb-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

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List of Contents