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Prevalence and Molecular Characterization of *Mycobacterium avium* subsp. *Paratuberculosis* in Raw milk of dairy herds.

A Thesis submitted by

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(Hygiene and control of milk and its products)

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Abstract

The main objective of the study is to investigate the existence of different members of Non-Tuberculous Mycobacteria (NTM) with special reference to *Mycobacterium avium* subspecies *paratuberculosis* (MAP) in raw milk at the farm level and to address a mean of control on *M. paratuberculosis* in milk and dairy product that is safe for consumer. Interestingly, 5 different NTM species were identified and confirmed genotypically including, *M. fortuitum*, *M. avium* ssp. *hominissuis*,*M. abscessus*, *M. simiae* and *M. avium* ssp. *Paratuberculosis.* In tank milk, *M. fortuitum* was the predominant one with 48% predominance compared to *M. simiae* which detected in only 4% of the samples. On the other hand, *M. abscessus* and *M. fortuitum* were obtained from 77% and 23% of the examined milk filters, respectively. Surprisingly, *M. avium* subsp. *hominissuis*, *Mycobacterium avium* subsp. *paratuberculosis* (*M. paratuberculosis*) and *M. simiae* were isolated from 16.7%, 10.4% and 4% of the examined tank milk samples, respectively, but not from milk filters. To investigate the impact of natural bacteriocins on the survival of different strains of *M. paratuberculosis* in milk, nisin was tested. Surprisingly, nisin had a minimum inhibitory concentration (MIC) that was higher against the laboratory strain (*M. paratuberculosis* K10) with 500 U/ml compared to other field isolates (e.g. *M. paratuberculosis* 4B and JTC 1281) with 15 U/ml respectively. In milk, the *M. paratuberculosis* cultures were inhibited after treatment with permissible levels of nisin at 4^oC and 37^oC. Using both fluorescent and scanning electron microscopy (SEM), we were able to identify clear defects on bacterial cell walls of treated cultures.

Key words: Non-Tuberculous Mycobacteria, *M. paratuberculosis*, Raw milk, Milk filters Nisin, Johne's disease.

Dedication

To my lovely husband, Emad, for your patience, love, friendship.

To my pretty princess Rahma and Nour for letting me experience the kind of love that people freely die for.

And also, to my Beloved Parents, my sisters Latafa, Aida and Hoda and my brothers Hamdy, Sayed, Mohamed and Eid for your endless, unconditional love and support.

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CONTENTS

List of Abbreviations

ATP = Adenosine triphosphate

BTM = Bulk Tank Milk

CD = Crohn’s disease

CFU = Colony Forming Unit

CTAB = Cetyl Trimethyl Ammonium Bromide

DNA = Deoxyribonecleic acid

FDA = Food and Drug Administration

gDNA = genomic DNA

GMPs = Good Manufacturing Practices

GRAS = Generally Regarded As Safe

HACCP = Hazard Analysis and Critical Control Point

HEYM = Herrold’s Egg Yolk Medium

HIV = Human immunodeficiency Virus

HPC = 1- Hexadecylpyridinium Chloride

HTST = High Temperature Short Time

JD = Johne’s disease

LAB = Lactic Acid Bacteria

LJ = Lowenstein Johnson

MABA = Microplate Alamar Blue Assay

MAC = Mycobacterium avium complex

MAP = *Mycobacterium avium* subsp. *Paratuberculosis*

MIC = Minimum Inhibitory Concentration

MJ = Mycobactin J

MTC = Mycobacterium tuberculosis complex

NTM = Non-Tuberculous Mycobacteria

OIE = Office International des Epizooties

PBS-T = phosphate-buffered saline containing Tween 20

PCR = Polymerase Chain Reaction

PI = Propidium Iodide

SDS = Sodium Dodecyl Sulfate

SEM = Scanning Electron Microscopy

U/ml = International Unit/milliliter

WHO = World Health Organization

ZN = Ziehl-Neelsen

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Chapter (1)

INTRODUCTION

INTRODUCTION

Milk is a highly nutritious food that can be obtained from a variety of animal sources such as cows, goats, sheep and buffalo. However, the high nutrient content of these milks, including proteins, fats, carbohydrates, vitamins, minerals and essential amino acids, all at a near neutral pH with high water activity, provides an ideal environment for the growth of many microorganisms **(Kable *et al.*, 2016).**

Being a rich and nutritious fluid, milk supports the growth of many microorganisms and host a complex and varied community of bacteria. Therefore, in addition to its endogenous microbiota, once milked it is rapidly colonized by a variety of other microbes coming from the teat canal, udder skin, milking machines, containers and tanks used for its storage, reflecting the farm and the pasture environment as well. Furthermore, contamination with potentially pathogenic bacteria or their toxins in milk can affect the human health. **(Quigley *et al.*, 2013).**

Milk is safe to consume after pasteurization but is still susceptible to microbe-induced spoilage and quality defects. In contrast, some of the surviving microorganisms in milk contribute beneficially to the organoleptic qualities of fermented dairy products **(Kable *et al.*, 2016)**.

Lactic acid bacteria (LAB) are particularly important because of their positive and negative impacts on fresh and fermented dairy foods **(Ledenbach and Marshall, 2009).** A wide variety of bacterial species have been detected in raw and minimally processed milk **(Quigley *et al.*, 2012)**.

Non-tuberculous mycobacteria (NTM) are ubiquitous in the environment and are responsible for several diseases in humans and/or animals known as mycobacterioses **(Shitaye *et al.*, 2009)**. More than 140 mycobacterial species are currently described and the number is increasing **(Tortoli *et al.*, 2000)**. NTM infections have been increasing in number over the past decades, especially in immunocompromised and HIV/AIDS patients **(Tortoli *et al.*, 2000)**. A globally disseminated species, contaminating milk, is *Mycobacterium avium* subsp. *paratuberculosis*. Many other mycobacteria are present in the environment and in food and potable water, and high numbers of cells are often consumed daily over an extended period of time. Generally, although highly resistant to higher temperatures and some disinfectants, mycobacteria are not yet considered to pose a risk as food pathogens. Nevertheless, Non-tuberculous mycobacterioses represent serious infections for immunodeficient persons and food or water can be a vector for their transmission **(Hruska and Kaevska, 2012)**.

Johne's disease, is a chronic granulomatous infection of the intestines of wild and domestic ruminants caused by *Mycobacterium avium* subspecies *paratuberculosis* (*M. paratuberculosis*) **(Collins, 1997)**. Johne's disease can be transmitted from cows to calves through ingestion of *M. paratuberculosis*-infected colostrum or milk that can be contaminated with *M. paratuberculosis* **(Grant *et al.*, 2002a)**. Milk obtained under aseptic clean conditions from asymptomatic cow infected with Johne's disease was found to contain less than 10 CFU/50