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List of Abbreviation

A.O.A.C.	Associtionof Official Agricultural Chemist
A.P.H.A.	American Public Health Association
Ab	Antibody
AF1	African 1
AFB	Acid Fast Bacilli
Ag	Antigen
AHB	Annual Report Animal Health Board
AIDS	Acquired Immuno Deficiency Syndrome
BCG	Bacillus Calmette - Guérin
bp	Base pair
BSA	Bovine serum albumin
BTB	Bovine Tuberculosis
CFU	Colony Forming Unit
CIDT	cervical intradermal tuberculin test
CMT	California Mastitis Test
CTT	comparative tuberculin test
DIVA	Differentiating Infected from Vaccinated Animals
DNA	Deoxyribonucleic acid
DTH	Delayed Type Hyper Sensitivity Reaction
ELISA	Enzyme Linked Immuno Sorbent Assay
ESAT-6	early secretory antigenic target 6
E.S.P.B.H.	European Scientific Panel on Biological Hazards
FAO	Food and Agriculture Organization
Fig	figure
GHQ	General Headquarters
GOVS	General organization for the Veterinary Services

HIV	Human Immuno Deficiency Virus
HTST	High Temperature Short Time
I/D	Interadermal
IGRAs	interferon gamma release assays
IGS	Intergenic Spacer
IRR	Incidence Rate Ratio
KD	Kilodalton
L-J	Lowenstein – Jensen Medium
L.N	Lymph Node
LTBI	Latent Tuberculosis Infection
LTLT	Low Temperature Long Time
M.	Mycobacterium
M. bovis	Mycobacterium bovis
MBSE	M. bovis sonic extracted antigen
MDR	Multi-Drug Resistant
MDRTB	Multi- Drug Resistance Tuberculosis
MF	Macfarland
MIC	Minimal Inhibitory Concentration
Min.	minute
MODS	microscopic observation drug susceptibility test
MOTT	Mycobacterium Other Than Tuberculosis
m-PCR	multiplex-Polymerase Chain Reaction
MTB	Mycobacterium Tuberculosis
MTBC	Mycobacterium tuberculosis Complex
NCCLS	National Committee for Clinical Laboratory Standards
N-PCR	nested PCR
NTB	Non-Tuberculosis

NTM	Non-Tuberculous Mycobacteria
NVL	Non-Visible Lesion
NYC	New York city
OD	Optical density
OIE	Office International de Epizooties
OPD	Orthophenylene-diamine
ORF	open reading frame
OT	Old tuberculin
P.M	Post – Mortem
PBS	Phosphate Buffer Saline
PCR	Polymerase Chain Reaction
PGL	phenolic glycolipids
PGRS	Polymorphic GC- Repeat Sequence.
Ph. Ph.	Phenol Phethaline
PPD	Purified Protein Derivatives
PPD-A	Purified Protein Derivative of M.avium
PPD-B	Purified Protein Derivative of M.bovis
PRA	PCR-Rest Analysis
PRTT	Positive Reactors to Tuberculin Test
PZA	Pyrazinamide
QFT	QuantiFERON®-TB Gold In-Tube
RD	Region of Difference
RE	Restriction Enzyme.
RFLP	Restriction Fragment Length Polymorphism
RNA	Ribonucleic acid.
rpm	Round per minute
RT-PCR	Real Time-PCR

S.	Staphylococcus
SDW	Sterile Distilled Water
SF-PPD	Short- term culture Filtered PPD
SICTT	Single intradermal comparative tuberculin test.
SID	Single Intradermal
SIDT	Single Intradermal Tuberculin
SLNS	supramammary Lymph Nodes
Sol.	Solution
spp.	species
TB	Tuberculosis
T-band	Test band
TBE	Tris Borate EDTA
TCH	Thiophen-2-Carboxylic acid Hydrazide
TE	Tris –EDTA
Temp.	Temperature
TLA	thin layer agar
TST	Tuberculin skin test
UHT	Utra-Heat Treated
UK	United kingdom
USA	United States of America
USP	universal sample processing technology
UV	Ultra Violet
VL	Visible Lesions
VSVRI	Veterinary Serum and Vaccine Research Institute.
WHO	World Health Organization
XDR	Extreme Drug Resistance
ZN	Ziehl –Neelsen

Summary

Tuberculosis is regarded as one of the most widespread diseases in all parts of the world and posed a risk to public health. Bovine TB is considered the most important types of tuberculosis transmitted from animals to humans, where livestock are the main reservoir for this disease.

In the present study three hundred random samples were collected from different localities in Assiut city including 150 raw milk samples including 50 milk samples from positive tuberculin test reactors, 50 milk samples from negative tuberculin reactors in addition to 50 marketable milk samples. As well as 150 samples of some dairy products including locally manufactured yogurt, Kareish cheese and cooking butter (50 samples for each).

The incidence of mastitis using CMT test was 19 (38%), 10 (20%) and 6 (12%) for tuberculin positive reactors, tuberculin negative reactors and marketable milk samples, respectively. Acid Fast Bacilli detected microscopically (by ZN stain) in 7 (14%) positive result for milk samples from tuberculin positive reactors, 3 (6%) for tuberculin negative reactors and 1 (2%) for marketable milk samples. In addition to 4 (8%), 5 (10%) and 2 (4%) positive results for locally manufactured yogurt, Kareish cheese and cooking butter, respectively.

Bacteriological examination of processed milk samples revealed that the incidence of Mycobacteria was 4, 2 and 0% using L-J medium pyruvated and 2, 0 and 0% using L-J medium glycerinated for tuberculin positive reactors, tuberculin negative reactors and marketable milk samples, respectively. In locally manufactured yogurt, Kareish cheese and cooking butter samples the incidence was 4, 2, 2% for pyruvated media and 0, 2, 2% for glycerinated

media, respectively. Correlation between the results of CMT, microscopic and cultural examination of examined milk and milk products samples indicated the highest sensitivity and specificity of cultural method for Mycobacteria.

Concerning the site of infection among tuberculin positive reactors, generalized infection in 2 cases gave 1 (2%) positive result in milk by cultural exam. while, by using ELISA the positive results were 2 (4%). 10 cases of localized lesion gave 2 (4%) positive result in milk and 7 (14%) in serum. 38 cases of tuberculosis (non-visible lesion) did not give any bacteriological result of milk but gave 12 (24%) positive result by using by ELISA bovine PPD. So ELISA technique can be used as a complementary to the skin tuberculin test to determine the disease status of animal or as rapid screening test for herd testing programme.

Identification of isolates indicated *M. bovis* (4%) and MOTT (2%) from tuberculin positive reactors and (2%) from tuberculin negative reactors. *M. bovis* and MOTT could be detected in 1 (2%) of locally manufactured yogurt, Kareish cheese and cooking butter and failed detection in marketable milk samples. These results were confirmed by PCR which considered rapid, accurate, sensitive and specific method for detection of *M. bovis* in regard to conventional, biochemical and serological methods.

There is general agreement that the most critical and most effective control measure to prevent transmission of zoonotic tuberculosis through milk is heat treatment specialy pasteurization prior to human consumption or further processing. This study confirmed that the viability of *M. bovis* in milk during pasteurization at 60 ^{o}C for 30 min. may be occurred specially in high concentration of *M. bovis* (10^{5}cfu) while, at 72 ^{o}C for 15 sec. is prohibited. The use of high temperature with short time (72^{o}C for 15 sec.) impact the highest

and best effect on viability of *M. bovis* than using of lower temperature with the high period of time (60^{o}C for 30 min.) especially in the high concentration of *M. bovis* (10^{5}cfu).

The ability of *M. bovis* to survive during manufacture and storage of kariesh cheese and yoghurt was evaluated. The pathogen was able to survive in stored kariesh cheese up to one month and in yoghurt for a week. So pasteurization of milk before consumption and manufacturing is the most effective methods to avoid the risk from BTB transmission through milk and milk products.

The effect of fennel honey on survival of *M. bovis* was evaluated by adding different concentrations of honey (0, 5, 10 and 20%) to the laboratory pasteurized milk and prepared yoghurt samples infected with *M. bovis* (10^{3}cfu and 10^{5}cfu). The results showed that *M. bovis* could not grow after 1-2 days at 20% fennel honey; lower concentrations nearly had no effect.

The public health significance of the organisms and the precautions which should be taken to control this organism in dairy industry as well as the recommended sanitary measures, were also discussed.