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Comparison between Immunological and Molecular Based Methods for Diagnosis of *Mycobacterium* Infections in Cattle, Buffaloes and Human in Egypt

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Abstract

Pathogenic mycobacteria are notorious for infections in animals and human their diagnosis hampered by atypical types. Animal products consumption is responsible for majority of diseased cases worldwide. Comparison between diagnostic tests appears to be lacking in Egypt. Therefore, this study aimed to evaluate diagnostic values of Flow-cytometry, Immuno-chromatography, Low-cost Density Microarray (LCD) array, High-Performance Liquid Chromatography (HPLC) and multiplex Polymerase Chain Reaction (PCR). Comparative Intradermal Tuberculin Test result was 1.31%. *M. bovis* and *M. kansasii* were high from animal samples while, *M. chelonae* and *M. malmoense*

bu aloes (79 tuberculin positive, 41 tuberculin negative). 10 ml blood in sterile McCartney tubes for serum, 37 ml mixed with 12.5 ml acid citrate dextrose (anticoagulant) for lymphocytes separation. Tuberculin positive slaughtered (according to Egyptian law) then subjected to postmortem examination. Mandibular, mediastinal, le and right bronchial, hepatic and mesenteric lymph nodes were collected. Tissue samples from lungs, liver, spleen and intestine as well. Samples classi ed to the visible lesion (VL) and nonvisible lesion (NVL) then processing for cultivation [16-20] as well as for DNA extraction a er concentration.

H ree rst morning sputum specimens of 5-10 ml a er a deep productive cough for three consecutive days into a sterile cap. e specimens kept at 4°C before processing with N-acetyl-L-cysteine-NaOH (NALC-NaOH).

G Animal samples cultivated onto Modi ed Lowenstein -Jensen pyruvate media (LJ-P) while, human samples cultivated onto Modi ed Lowenstein -Jensen glycerol media (LJ-G) [19-25].

DNA LCD

QIA

	No. of samples	Culture and microscopy	
		No	%
Generalized (VL)	7	7	100%
Localized head lymph nodes (VL)	8	7	87.5%
Pulmonary calcifed (VL)	15	-ve	0.0%
Pulmonary uncalcifed (VL)	8	8	100%
Digestive (VL)	10	-ve	0.0%
Mixed (VL)	12	12	100%
Non Visible Lesion (NVL)	19	7	15.78%
Total	89	51	57.3%
Human	10	10	100%

Table 3:

and multiplex PCR (Table 4), (Figures 1 and 2) Chromatograms (1, 2, 3 and 4).

Untypable (means got mycobacterium DNA but not similar to LCD patterns, HPLC no similarity (means gave curve but not similar to listed species). Perfect uphill (positive) linear relationship (r): +1 between LCD array on tissue and corresponding isolates and between LCD array and HPLC.

C [illegible] B

Chembio DPP VetTB Assay gave the highest sensitivity results with 94.8% while, TB-ST (Tuberkulose Schnelltest) test kit was the lowest with 82%. Furthermore, Acon test gave the highest speci city 96% and Chembio DPP VetTB Assay was the lowest 88.9% (Table 5).

Flow-cytometric evaluation of tuberculin positive and negative animals proved a noticeable increase in tuberculin-positive cattle. CD2+ cells soared over than 3 times, CD4 was twice that of tuberculin-negative cattle. Moreover, from tuberculin positive bu aloes CD2+ and CD8 were twice more the counts of their counterparts from tuberculin negative bu aloes.

Signi cant di erence ($P<0.0001$) present between the counts of CD2+, CD4, CD8 and WC1+δγ of tuberculin-positive from counts of tuberculin- negative animals (Table 6).

D [illegible]

M. bovis is a crucial infectious agent of wide host spectrum besides cattle it a ects other mammal species including humans. It belongs to *Mycobacterium tuberculosis* Complex members [14-16]. Interestingly, the overall incidence level of *Mycobacterium* in Table 2 was 1.31% lower than 2015 records from Egypt [23]. On the contrary, nearly similar to obtained results at Mozambique [27]. is low percentage attributed to regular commitment with test and slaughter program of the authorities to control the spread of infections.

Notably, the obtained VL and NVL results were analogous to that from Ireland 2013 [25]. In comparison with recent records from Egypt the VL was higher than 2015 records [23] while the NVL lower than these records. Signi cant di erence was found between VL and NVL (<0.0001) that considered a feature of *M. bovis* infections that cause variation in the shape and distribution of lesions with respect to the isolation rate 57.3% from Table 3 nearly like that from cattle in Ethiopia a er 2010 data [3]. e obtained isolation result from human sputum 10/10 (100%) was higher than the 2012 records from Egypt [21].

Isolation and identi cation of Mycobacterium species from various lesions in di erent organs

is mainly re ects various routs of infection and even possible secondary spread within the animal body.

Identifying *Mycobacterium* species using traditional methods is time consuming and expensive. For this reason, LCD array, HPLC and multiplex PCR con rmation performed here as attractive alternatives to the most traditional techniques. at's mainly due to the high sensitivity and speci city of these diagnostic techniques:

From the results of molecular identi cation of VL tissue samples and culture (Table 4), Figures 1 and 2 and chromatogram 1, it was

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