Detection of Tuberculosis in slaughtered food animals by using recent technique.

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A B S T R A C T

Bovine tuberculosis is still a major infectious disease among animal population and its transmission to human constitutes a public health problem. This study aimed to application of recent technique such as PCR for detection of tuberculosis among cattle and buffaloes carcasses. A grand total of 52 animals (40 cattle and 12 buffaloes) were routinely examined in the slaughterhouses of Dakahlia governorate, Egypt during the year 2014 for detection of tuberculosis. The suspected tuberculous lesions collected from cattle were 19 from respiratory, 8 from digestive, 12 from head and 1 generalized lesion. While in buffaloes, the tuberculous lesions were 3 from respiratory, 5 from digestive and 4 from head lesions. The bacteriological examination revealed that the isolation rate of *Mycobacterium bovis* (*M.bovis*) was 75% and 58.3% in cattle and buffaloes, respectively. The PCR technique was applied to confirm the results of bacteriological examination on 10 isolates of different mycobacteria and revealed 6 *M.bovis* isolates were positive with high sensitivity and specificity (100%).

Keywords: Bovine tuberculosis, PCR, Cattle carcass, *Mycobacterium bovis*.

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1. INTRODUCTION

Bovine tuberculosis (BTB) is a chronic infectious disease characterized by the formation of granulomatous lesions in organs, mainly lungs and lymph node (Yingyu et al., 2009). The cause of bovine tuberculosis in cattle mainly is *Mycobacterium bovis (M.bovis)* which is pathogenic for a large number of other animals, and its transmission to human constitutes a public health problem (Ameni et al., 2007). Bovine TB is responsible for condemnation of significant amount of inspected meat and viscera during 1991 in Egypt that estimated at value nearly US $ 5 million (El-Taweel, 1992). Control of bovine tuberculosis in Egypt is mainly depending on test and slaughter policy which consume the economy and yet there is no evidence of reducing the prevalence of tuberculosis (Soliman et al., 2004). The diagnosis of bovine tuberculosis in live animals mainly depends on clinical manifestations of the disease, skin testing, staining with Ziehl-Neelsen (ZN) stain and more recently by molecular methods. Subsequent identification of the pathogen has been made by culturing and biochemical tests (Mishra *et al.,* 2005). An accurate diagnosis plays an important role in the control of tuberculosis, the development of cost effective, specific diagnostic assays to allow improved detection of disease is urgently needed. Several forms of new technology were brought into the diagnostic approach to mycobacterial infection. Polymerase Chain Reaction (PCR) is the most promising technique for rapid and specific detection of *Mycobacterium* in clinical specimens requiring 2-3 days (Therese et al., 2005). It has strong impaction on epidemiology, treatment and prevention of diseases in veterinary practice. In addition, PCR is considered as a good test for detecting of Mycobacterial DNA even when small numbers of bacteria are present in the

clinical samples (Cajal and Pharma, 1994). There for the present study aimed to estimate the prevalence of bovine tuberculosis through post-mortem examination in slaughterhouse during routine meat inspection, isolation and identification of mycobacteria using conventional method, and application of PCR test in diagnosis of tuberculosis.

2. MATERIAL AND METHODS

2.1. Sample collection

A total number of 52 animals (40 cattle and 12 buffaloes) were routinely examined in the slaughterhouses of Dakahlia governorate, Egypt for detection of tuberculosis. The Suspected tissue samples (lung, pleural membrane, liver,intestinal mucosa, peritoneum membrane) and lymph nodes (submaxillary, mediastinal , Mesentric, Prescapular and Prefemoral L.N.) showing tuberculous like lesions were collected during routine meat inspection under aseptic technique and preserved in ice box and sent as quickly as possible to the laboratory (Vet. Serum and Vaccine Research Institute) for bacterial isolation and PCR.

2.2. Isolation and Identification of Mycobacteria (Marks, 1972)

All bovine samples were processed for isolation and cultivation of mycobacteria using Lowenstein- Jensen media (LJ). The obtained sediment was inoculated into Lowenstein-Jensen slants and incubated at 37 ºC in inclined position for overnight then vertically for at least 6-8 weeks with weekly examination starting from three days post inoculation. Suspected colonies were subjected for full morphological and biochemical identification of the acid-fact bacilli.

2.3. DNA Extraction (Darwish et al., 2013)

Briefly, 1 to 3 loop full of bacterial isolates were well mixed in 200 µl TE buffer. The suspended bacterial isolates were centrifuged at 12000 xg for 5 min. The supernatant was decanted and the resultant pellet was washed twice using TE buffer till be clean. Then, the washed pellets were suspended in 200 µl chelex 100 (10% in water) and vortexed. The mixture was boiled for 20 min and then centrifuged for 5 min at 10000 rpm. The supernatant was carefully aspirated to clean tube to be used as source for template DNA in PCR reactions.

2.4. PCR Amplification (Romero et al., 1999)

The PCR primers' oligonucleotide sequences were deduced for *M. bovis* DNA fragment were named LI (5'CCCGCTGATGCAA GTGCC3') and L2 (5 'CCCGCACATC CCAACACC 3'). Ten microliters suspended DNA was used for PCR amplification under standard conditions. All reactions were taken to a final volume of 50 uL containing: 10 ul of DNA, Taq polymerase lx buffer (10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.3 mM MgCl2, and 0.001% of gelatine), 2.5 U Taq polymerase, 0.2 mM of each triphosphate nucleotide, and 20 pmol of each primer. All reactions were performed in a Perkin Elmer thermocycler (Perkin Elmer Cetus) programmed for 25 amplification cycles as follows: initial denaturation at 95 °C for3 min. followed by 40 cycles consisting of 95ºC/30sec, 56 ºC/30 second and 72 ºC/30 sec. Final extension was given at 72 °C /5min. Specific *M. bovis* DNA detection for each sample was identified by specific 470 bp DNA bands on 1% agarose, stained with ethidium bromide and evaluated under UV transilluminator.

3. RESULTS

Table (1) showed that the incidence of tuberculosis was more in females (60% and 75% in cattle and buffaloes) than in males (40% and 25%in cattle and buffaloes) and in old animals (85% and 83.3% in cattle and buffaloes) than in young animals (15% and 16.7% in cattle and buffaloes).

Table (2) illustrated the relationship between the site of suspected tuberculosis lesions and mycobacterial isolates, it revealed that the isolation rate of *M.bovis* was 75% and 58.3% in cattle and buffaloes.

Table (3) showed that the PCR technique was applied on isolates of different mycobacteria and revealed 6 *M.bovis* were positive with high sensitivity and specificity (100%).

Table (1) Incidence of tuberculosis lesions in routinely examined slaughtered animals in relation to age and sex.

animals	No.	Sex				Age			
		Male		Female		1-5 years		> 5 years	
		No.	%	No.	%	No.	%	No.	%
Cattle	40	16	40	24	60	6	15	34	85
Buffalos	12	3	25	9	75	2	16.7	10	83.3

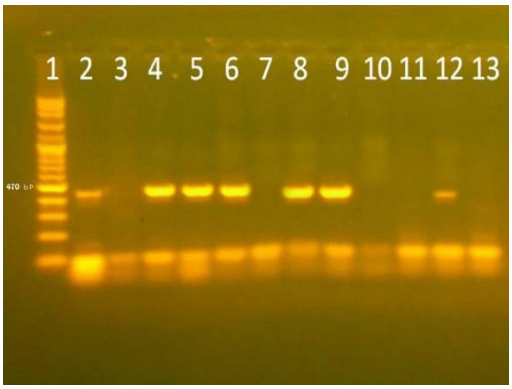

Photo (1): Lane 1: 100 bp ladder DNA marker, Lane 2: positive control (standard M. bovis), Lane 3: negative control, Lane 4, 5, 6, 8, 9, and 12: M. bovis isolate, Lane 7, 10, 11, 13: negative.

Table (2) Relationship between mycobacterial isolates and site of suspected tuberculosis lesions

Site of suspected lesions	Type of slaughtered animal		Incidence of M.bovis			
	cattle	buffaloes	cattle		buffaloes	
			No.	%	No.	%
Respiratory lesions	19	3	15	79	2	66.7
Digestive lesions	8	5	5	62.5	3	60
Head lesions	12	4	9	75	2	50
Generalized lesions	1	0	1	100	0	0
Total	40	12	30	75	7	58.3

Table (3) Correlation between PCR results, mycobacterial isolates and site of infection.

No. of Samples	Site of infection	M. bovis isolates	PCR result
1	Respiratory lesion	+ve M.bovis	+ve
2	Respiratory lesion	+ve M.bovis	+ve
3	Mixed lesion	+ve M.bovis	+ve
4	Mixed lesion	+ve M.bovis	+ve
5	Generalized lesion	+ve M.bovis	+ve
6	Head lesion	+ve M.bovis	+ve
Total	6	6	6

4. DISCUSSION

For Bovine tuberculosis control, infected cattle need to be identified accurately and in the early stages of the disease. The failure in achieving this will allow continuing transmission of the disease with subsequent public health hazards due to transmission of *M. bovis* from animals to human. Abattoir inspection through macroscopic examination of tuberculosis lesion is important in the context of tuberculosis surveillance and disease monitoring (Liebana et al., 2008). Table (1) showed that the incidence of tuberculosis was more in females than in males and in old animals than in young animals. Such findings come in accordance with Seham (2009) and El-Hadad (2014).

Table (2) illustrated the relationship between mycobacterial isolates and site of suspected tuberculous lesions. It revealed that the isolation rate of *M.bovis* in cattle was 75%, and this result was almost agree with that reported by Rivas et al. (1985), 70.7% and Saleh (1990), 70%. Higher results were reported by Grange et al. (1990), 100% and Seham (2009), 82.6%. Lower results were reported by Kassich et al. (1990) 50.5% and Ramadan et al. (2012), 66.7%. While in buffaloes the isolation rate of *M.bovis was* 58.3%. This result was lower than that reported by Seham (2009) 68.2% and higher than that reported by Mikhail (1985) 4.49%.

Results of the conventional methods were confirmed by PCR according to table (3). PCR technique was applied on 10 isolates of different mycobacteria, and it revealed that (6) *M.bovis* were positive with high sensitivity and specificity (100%).These results were in consistent with results of Moussa et al. (2000), Shaimaa. (2009), Sabry and Elkerdasy (2014). PCR has been evaluated for the detection of *M. bovis* from a range of specimens and seems to have sensitivity equal to or greater than that of the culture method, but in short time (Beige et al. 1995). For the time consuming point of view, PCR technique in the present study is a rapid and accurate method for diagnosis of tuberculosis within 3 days but this take several weeks (8 weeks) by using culture methods.

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