

Studies on Tuberculosis in Slaughtered Animals at Menufia Governorate

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Abstract

Bovine tuberculosis is highly infectious zoonotic disease of economic and public health importance. This study aimed to detection the prevalence of bovine tuberculosis by bacteriological examination and Real time PCR. A total of 75 animals (61 cattle and 14 buffaloes) were routinely examined in the slaughterhouses of Menufia governorate, Egypt during the period of 2015 and 2016 for detection of tuberculosis. The suspected tuberculous lesions collected from cattle were from respiratory, from digestive, from head, mixed lesions and generalized lesions. While in buffaloes, the tuberculous lesions were from respiratory, from digestive, mixed lesions and from head lesions. The bacteriological examination revealed that the isolation rate of *Mycobacterium bovis* (*M. bovis*) was 86.9% and 57.1% in cattle and buffaloes, respectively. The real time PCR technique was applied to confirm the results of bacteriological examination and revealed that 90.2% and 85.7% *M. bovis* isolates in cattle and buffaloes respectively were positive with high sensitivity and specificity more than culture.

Keywords: Bovine tuberculosis, *Mycobacterium bovis*, Menufia, PCR.

1. Introduction

Bovine tuberculosis is a chronic debilitating disease which affects cattle, other domesticated animals and characterized by the formation of nodular granulomas known as tubercles [1]. Bovine tuberculosis is an important zoonosis which poses a significant threat to humans cause approximately 2000 human deaths per annum (6%) worldwide. It can be transmitted through consumption of contaminated milk and close contact with infected cattle [2]. Early and accurate diagnosis of BTB in cattle is very important, since the disease can become chronic with prolonged incubation periods [3].

The intradermal tuberculin test is the standard method for the diagnosis of tuberculosis in live cattle on the basis of measurement of a delayed hypersensitivity reaction to bovine tuberculin. In case of positive reactors, the test is followed by three traditional diagnostic technique; post-mortem examination, Ziehl Neelsen stain and bacteriological identification [4]. Additionally 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 bovis* in clinical specimens requiring 2-3 days [5].

Therefore 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 real time PCR test in diagnosis of tuberculosis.

2. Materials and methods

2.1 Sample collection

A total number of 75 Animals are random slaughtered, were routinely postmortem examined

in the slaughterhouses of Menufia governorate. The samples included (61) cattle and (14) buffaloes. The collected samples were lymph nodes samples showing tuberculous like lesions include head LNS, retropharyngeal, submaxillary, parotid, bronchial, mediastinal, hepatic, Intestinal, kidney prescapular and prefemoral LN were collected during routine meat inspection. Organs sample all organs tissues were inspected for tuberculous lesions all samples were collected in ice box and sent as quickly as possible to the laboratory for bacterial isolation, and PCR.

2.2 Isolation of *Mycobacterium* species on culture medium [6]

Tissues of organs and lymph nodes showed the gross lesions were chopped into small pieces under aseptic condition in sterile mortar containing sterile sand. The tissues were crushed by the sand by sterile mortar's hand until they become pasty. Two ml of sterile distilled water was then added. After that, added 2 ml of 4% H₂SO₄ were added and incubated for 30 m. then diluted in 16 ml of sterile distilled water was added and centrifuged at 3000 rpm for 20 minutes. The supernatant was decanted into 5% phenol and the sediment was used to make direct smear and inoculated into two L-J medium slant one with 4% sodium pyruvate and the other with 5% glycerol then incubated at 37C for 3 weeks. Cultures were examined daily for one week and then once weekly for 6-8 weeks.

2.3 Extraction of mycobacterial DNA From infected tissues [7]

The extraction was carried out in briefly as follow: lysis and digestion, A total of 20mg of grinded tissue + 180ul digestion sol. + 20 ul proteinase K + mix and incubate at 56 °c for 3hr. Fixation, The lysate was transferred to purification column, centrifuge for 1min./ 8000 rpm, discard the collection tube then place column into new

collection tube. Washing, 500 ul of wash buffer 1 was added, centrifuged for 1 min./ 10000 rpm then the flow was discard, 500 ul wash buffer 11 was added and centrifuged at 4 min./ 14000 rpm then the collection tube was discarded. Elution, the column put in new microfuge tube, the elution buffer was added, incubated for 2 min. at room temperature and centrifuge for 1 min. / 10000 rpm.

2.4 Detection of *M. tuberculosis* complex [8]

By using MTplex dtec-RT-qPCR Test (Edifici-Quórum3, Spain) that comprises a series of species-specific targeted reagents designed for detection of all species contained in the *Mycobacterium tuberculosis* complex. Sequence INS1 (5'CGTGAGGGCATCGAGGTGGC 3') INS2 (5' GCGTAGGCGTCGGTGACAAA3'). The primers and TaqMan probe target a sequence conserved for all strains belonging to *Mycobacterium tuberculosis* complex. The reaction of 20 µl final volume consisted of 10 µl Hot Start-Mix qPCR 2x, 1 µl MTplex dtec-qPCR-mix, 4 µl DNase/RNase free water and 5 µl DNA sample., the reaction conditions consisted of one cycle of 95°C for 5 min followed by 45 cycles of 95°C for 0.5 m' and 60°C for 1m' for hybridization, extension and data collection.

3. Results

Table (1) showed that the incidence of tuberculosis was more in females 72% than in males 28% in cattle but in buffaloes incidence was more in male 71% than in females 29%. Incidence in old animals (86.9% and 85.7% in cattle and buffaloes) more than in young animals (13.1% and 14.3% in cattle and buffaloes).

Table (2) illustrate results of bacteriological examination of suspected randomly slaughtered animals it revealed that the isolation rate of *M. bovis* was 86.9% and 57.1% in cattle and buffaloes. Also table (2) shows the results of real time PCR 90.2% and 85.7% in cattle and buffaloes.

Table (3) illustrated the relationship between the site of suspected tuberculosis lesions and mycobacterial isolates. In cattle recovery rate of *M. bovis* was 90.9% pulmonary TB, 85% digestive TB, 90% head TB, 66.7% mixed TB and 100% generalized TB. In buffaloes recovery rate of *M. bovis* was 100% pulmonary, 40% digestive, 60% head, 50% mixed and 0% generalized.

4. Discussion

Bovine tuberculosis remains a major problem throughout the world [9]. It caused by *Mycobacterium bovis* is an animal health problem throughout the world and also constitutes a major threat to human public health [10]. Table (1) show the incidence of tuberculosis was more in females 64% than in males 36% this result agree with [11] who found that most affected animals were female. Incidence according to age increase with progress of age this results agree with [12].

Table (2) illustrate results of bacteriological examination of suspected randomly slaughtered animals it revealed that the isolation rate of *M. bovis* was 86.9% in cattle this results almost agree with that reported by Seham [13] 82.6% and [14] 77.78%. Lower than that reported by [15] 100%. Higher than that obtained by [16] 29% and [17] 5.5%. In buffaloes 57.1% isolates of *M. bovis*. This results nearly similar to results obtained by [18] 58.3%. Higher than that reported by [19] 4.49%. Lower than [20] 94.6%. Results of PCR in cattle 90.2% and 85.7% in buffaloes.

Table (3) illustrate the relationship between the site of suspected tuberculosis lesions and mycobacterial isolates. In cattle recovery rate of *M. bovis* was 90.9% pulmonary TB, 85% digestive TB, 90% head TB, 66.7% mixed TB and 100% generalized TB. In buffaloes recovery rate of *M. bovis* was 100% pulmonary, 40% digestive, 60% head, 50% mixed and 0% generalized. This result showed that isolation rate of *M. bovis* in pulmonary lesions is higher than other sites.

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

Examined animals	No	Sex				Age			
		Male		Female		young		old	
		No	%	No	%	No	%	No	%
Cattle	61	17	28	44	72	8	13.1	53	86.9
Buffaloes	14	10	71	4	29	2	14.3	12	85.7
Total	75	27	36	48	64	10	13.3	65	86.7

Table (2) Results of bacteriological examination of suspected randomly slaughtered animals.

Examined carcasses	Total No.	<i>M. bovis</i>		MOTT		PCR			
		No.	%	No.	%	+ve	%	-ve	%
Cattle	61	53	86.9	8	13.1	55	90.2	6	9.8
Buffaloes	14	8	57.1	6	42.9	12	85.7	2	14.3

Table (3) Relationship between the site of suspected tuberculosis lesions

Type of infection	Examined carcasses		Bacteriological examination							
	Cattle	Buffalo	<i>M. bovis</i>				MOTT			
			cattle		buffalo		cattle		buffalo	
			No.	%	No.	%	No.	%	No.	%
Respiratory lesions	22	2	20	90.9	2	100	2	9.1	0	0
Digestive lesions	20	5	17	85	2	40	3	15	3	60
Head lesions	10	5	9	90	3	60	1	10	2	40
Mixed lesions	6	2	4	66.7	1	50	2	33.3	1	50
Generalized lesions	3	0	3	100	0	0	0	0	0	0
Total	61	14	53	86.9	8	57.1	8	13.1	6	42.9

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