PREVALENCE OF BOVINE INFECTION WITH *MYCOBACTERIUM BOVIS* IN SOME EGYPTIAN GOVERNORATES

BY

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SUMMARY

One hundred and thirty seven (4.6%) out of 3000 cattle at different ages from seven governorates of Egypt (Qualubia, Sharkia; Gharbia, Giza, Behira, Sohag and Ismailia) were found positive for tuberculosis by single inradermal (SID) tuberculin test. Post mortem (PM) examination of slaughtered reactor cattle revealed tuberculous visible lesions (VL) and non-VL (NVL) in 94 (68.6%) and 43 (31.4%) animals, respectively. Tuberculous lesions were observed in 41 (29.9%), 19(13.9%), 20 (15%) and 14 (10.2%) in 137 slaughtered reactor cattle with pulmonary, digestive, mixed and generalized VL, respectively.

Bacteriological examination of tested samples from slaughtered reactor cattle revealed the isolation and identification of Mycobacterium bovis (M. bovis) and Mycobacterium other than tuberculosis (MOTT) in 90 (65.7%) and 7 (5.1%) samples, respectively. One hundred and eight (78.83%) serum samples out of 137 tuberculin reactors were found positive for enzyme linked immunosorbent assay (ELISA) by using M.bovis synthetic early secretory antigenic target 6 peptide antigen (ESAT6-p). Polymerase chain reaction (PCR) amplification assay was performed on selected tissues samples from 10 slaughtered tuberculin reactors with pulmonary, digestive, mixed or generalized VL, or NVL. Eight (80%) out of the 10 samples gave positive results with PCR assay. On the other side, only 6

(60%) and 7 (70%) of these samples were found positive for M.bovis isolation and ELISA, respectively. The results obtained in the present study point to the importance of the periodical tuberculin testing of animals, use of ELISA assays with M. bovis-specific antigens as a complimentary diagnostic toot to tuberculin test and application of PCR test for direct and rapid diagnosis of tuberculosis. Improvement of diagnostic assays for M.bovis may be of value in the effectiveness of test and slaughter eradication programs of bovine tuberculosis in governorates of Egypt.

INTRODUCTION

Bovine tuberculosis caused by M. bovis, characterized by progressive developed granulomatous lesions (tubercles) in any body organ, and affected a large number of species. Tuberculosis is now generally perceived to represent the greatest threat to cattle health and the incidence of bovine tuberculosis is rising, both in number of herd affected and in the number of cases per affected herd (Cobner, 2003). Bovine tuberculosis infected 50 million cattle worldwide resulting in economic losses of approximately $3 billions (Hewinson, 2000). The economic losses by the disease were represented by a reduction of 10-20 % in milk production and weight, to infertility and condemnation of the meat. Without considering the deaths, the loss estimated to be 10-25 % of the productive efficiency (Lilenbaum et al., 1999).Diagnosis of mycobacterial infection is based on traditional method with Ziehl-Neelsen stain, but this method does not allow identification at the species level and of low sensitivity, where it requires a relatively large number of bacteria more than 10,000/ml to be present in the sample (Elisenach et al., 1991). Culturing of organisms has a specificity that approach 100% and permits susceptibility testing of the isolates, but the main disadvantages that growth of the organisms may take 6-8 weeks, culture technique also required viable organisms, and these can be a problem when tissues are inadequately handled (Nolte et al., 1993).Bacteriological, and biological methods, need a longer time in examination of morphology and typing of the organism. Enzyme Linked Immuno Sorbent Assay (ELISA) has been applied for serological diagnosis of bovine tuberculosis (Ayanwale, 1987; Auer, 1987). ELISA test has the highest sensitivity and specificity than the other serological tests for the diagnosis of tuberculosis (Thongkrajai et al., 1989) and the use of ELISA as complementary test to tuberculin test greatly

increased the sensitivity and specificity of the test (Plackett et al., 1989; Duffield, 1990; and Ritacco et al., 1990). In the recent studies, PCR assay showed more promising for mycobacterial species detection in clinical samples (Cobos-Martin et al., 2003; Taylor et al., 2007). PCR was evaluated for the detection of tubercle bacilli from a range of specimens and seemed to have sensitivity equal or greater than that of the culture method (Brisson-Noel et al., 1989; Mishra et al., 2005). PCR technique is much faster than culture and reduces the time for diagnosis from several months to 2 days (Liebana et al., 1995). Also for the detection of *M. bovis*, when rapidly growing mycobaterial species are present in the sample and may was able to detect the presence of M. *bovis* in samples even when organisms have become non-viable (Bal et al., 1994; Romero et al., 1999; Mishra et al., 2005). The present investigation aimed to study the utility of the tuberculin skin test for cattle in some governorates of Egypt, identification of isolated *Mycobacteria* from slaughtered tuberculin reactor cattle, evaluation of ELISA using the ESAT-6P antigen for sero-diagnosis of tuberculosis by using and assessment of PCR amplification assay for rapid and direct detection of tuberculosis in animal samples.

MATERIALS AND METHODS

Animals:

Three thousand cattle at different ages from Qalubia, Sharkia, Gharbia, Giza, Behara, Sohag, and Ismailia governorates were tested with the single intradermal cervical tuberculin (SID) skin test along the period of January 2006 to April 2008 (Table 1).

Table (1): The total number of cattle tested in different governorates.

Governorate	Age				Total
	< 2 years	2-4 years	4-5 years	> 5 years	
Qalubia	25	85	200	140	450
Sharkia	42	160	345	253	800
Gharbia	12	50	110	78	250
Giza	28	100	215	157	500
Behira	15	60	130	95	300
Sohag	6	20	44	30	100
Ismailia	33	122	255	190	600
Total	161	597	1299	943	3000

Tuberculin test:

SID skin test using mammalian PPD tuberculin (2mg/ml; Bacterial diagnostic products, Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo, Egypt) was done according to the methods described by Ovdienko et al. (1987). Blood samples were collected from tuberculin reactor animals prior to slaughter. Sera were obtained, and then stored until further testing.

Bacteriological examination:

Following slaughtering of tuberculin reactor cattle, PM examination was done for tuberculous lesions. Suspected PM lesions of tuberculosis from lymph nodes and samples from all organ tissues ((lung, trachea, lungs, pleural membrane, liver, kidney, intestinal mucosa, ovaries, peritoneal membrane, and mammary glands.) were collected under aseptic conditions and sent to laboratory for bacteriological examination and isolation and identification of mycobacterial species. On arrival to the diagnostic laboratory, samples were processed and cultured onto Lowenstein-Jensen medium slants and then incubated at 37°C for 8 weeks. Suspected colonies were subjected for full morphological, culture and biochemical identification of the acid-fast bacilli according to the procedures previously described by Vestal(1975

ELISA:

ELISA was performed on serum samples from tuberculin test reactor cattle in Flat-bottom 96 well-microtiter plates according to the method of Brandt et al. (2000) by using the synthetic peptide ESAT6-p antigen (School of Molecular Biosciences, Washington State University, Pullman, WA, USA), horseradish peroxidase labeled conjugate and Ortho Phenyl Dalamin substrate. Plates were read for absorbance at 405 nm using an ELISA reader. A serum was considered positive if it yielded a mean OD equal to / or greater than the cut-off value.

PCR:

Lymph nodes (upper respiratory and mesenteric) and lungs of tuberculin positive cattle showed a prominent caseated lesion were subjected to PCR testing. DNA was extracted from tissue samples using EZ-10 spin column genomic DNA isolation kit (Biobasic Inc., Ontario, Canada) according to the manufacture's instructions. To detect presence of the Mycobacterium complex insertion sequence IS6110 gene (Thierry et al., 1990) using the extracted DNA as a template, a pair of primer designed according the Mycobacterium complex published sequence in the gene bank; primer 1 (Sense): 5'- ATG TCA GGT GGT TCA TCG AG-3' and primers 2 (Antisense): 5'- TGG CCG GTC GTG CGA TT-3'. PCR was performed in a T-gradient thermocycler (Biometra biomedizinische Analytik GmbH, Goettingen, Germany) according to procedures described by Glennon et al. (1997). To check for cross-contamination between samples (false positive results), reaction with no DNA template (water instead) was tested in parallel with every PCR testing run.The size of the PCR product DNA (amplicon) was determined by electrophoresis on 1% ethidium bromide-stained agarose gel and visualization under UV transillumination (Sambrook et al., 1989). GeneRuler™ 100 bp DNA Ladder and ΦX174 phage/ Hae III digested DNA were used as a molecular size markers. Amplification of 327 bp-DNA fragment indicated a positive result.

RESULTS AND DISCUSSION

Bovine tuberculosis is a worldwide disease that causes severe economic losses beside its public health importance (Hewinson, 2000; Cobner, 2003). Diagnosis of bovine tuberculosis is principally depends on the tuberculin test which is the golden test for diagnosis of tuberculosis until now. Standardized preparations of tuberculin testing in both veterinary and human medicine for the diagnosis of tuberculosis have been used (Wood and Rothel, 1994; Costello et al., 1997). In the present study as shown in table 2, the

incidence of tuberculin reactor cattle, were 137 These results were comparatively less than that obtained by Lotfy and Guindi (1966) and Guindi et al. (1980) who reported incidence rates of 6.9% and 26.5% , respectively in Egypt,. However, higher incidence rates of 6.6%, 32% and 16% were recorded by Waddington (1965), Lall et al. (1969) and de Olivera et al. (1983) in Kenya, India, and Brazil respectively. One of the main aims of this study was the determination of the prevalence of animal tuberculosis in some out of 3000, in a prevalence rate of 4.6%. Egyptian governorates. As shown in table 2, the highest number of tuberculin skin test reactor cattle was 39 (4.9%) out of 800 cattle tested in Sharkia and the lowest number was 3 (3%) out of 100 animals tested in Sohag. Ten (4%) out of 250, 20 (4.4%), out of 450, 23(4.6%) out of 500, 14 (4.7%) out of 300 and 28(4.7%) out of 600 cattle showed positive reaction to tuberculin skin testing in Gharbia, Qalubia, Giza, Behira and Ismailia governorates, respectively.

Governorate	No. of tested animals	No. of reactors		PM findings												
				VL										NVL		
				Pulmonary		Digestive		Mixed		Generalized		Total				
		No.	%*	No.	%	No.	%	No.	%	No.	%	No.	%**	No.	%
Qalubia	450	20	4.4	5	25.0	4	20.0	3	15.0	2	10.0	14	70.0	6	30.0
Sharkia	800	39	4.9	11	28.2	5	12.8	6	15.4	4	10.3	26	66.7	13	33.3
Gharbia	250	10	4.0	3	30.0	1	10.0	2	20.0	1	10.0	7	70.0	3	30.0
Giza	500	23	4.6	7	30.4	4	17.4	2	8.7	3	13.0	16	69.6	7	30.4
Behira	300	14	4.7	5	35.7	2	14.3	2	14.0	1	7.1	10	71.4	4	28.6
Sohag	100	3	3.0	1	33.3	0	0.0	0	0.0	0	0.0	1	33.3	2	66.7
Ismailia	600	28	4.7	9	32.1	3	10.7	5	18.0	3	10.7	20	71.5	8	28.5
Total	3000	137	4.6	41	29.9	19	13.9	20	15.0	14	10.2	94	68.6	43	31.4

Table (2): Prevalence of tuberculin reactor cattle and PM findings of. Cattle tested in different governorates.

VL : Visible lesions   NVL: Non-Visible lesions   * of tested cattle   ** of slaughtered cattle

The annual report of the General Organization of Veterinary Services (GOVS, 1992) showed that, the prevalence of bovine tuberculosis in cattle was high in certain governorates such as Alexandria (6%), Dakahlia (9.6%) and Behira and (14.06%) during the year 1992, respectively. These results were higher than that recorded in the present study specially those recorded in Behira governorates. On the other hand, our results were coincided with that recorded by Pan America Zoonoses Center (1988) showing that the average of prevalence rate was 4.3% for a tuberculin survey on 20,000 animals in Argentina. Many factors affected the prevalence of the disease such as herd size, density of animals, breeding and management system, uncontrolled animal movement, unhygienic local habits and stress factors due to other diseases and mass vaccination against various diseases (Abu-Eisha et al., 1995). As shown in the same table, 94 (68.6%) and 43 (31.4%) animals out of 137 cattle reactors to skin test, showed VL and NVL, respectively. These results are in agreement with those reported by de Olivera et al. (1983) and Zivkovic et al. (1984). Lower percentages of cattle with VL [52.7%, 46% and 40.8% were

reported by Kuczyuski (1970) in Poland, Yoon et al. (1979) in Korea and Rodriguez et al. (1983) in Spain, respectively]. Regarding to the distribution of lesions in tuberculin cattle reactors, the presence of pulmonary, digestive, mixed , generalized VL and NVL was recorded in 41 (29.9%), 19 (13.9%), 20 (15%), 14 (10.2%) and 43 (31.39%) out of 137 of the slaughtered cattle, respectively. The highest pecentages of cattle with VL were recorded in 20 (71.5%) and 10 (71.4%) out of 28 and 14 tuberculin test reactors in Ismailia and Behira goverorates, respectively. VL was observed in one (33.3%) out of three tuberculin skin test reactors in Sohag governorate. Fourteen (70%), 26(66.7%), 7(70%) and 16(69.6%) out of 20, 39, 10 and 23 tuberculin reactors showed VL in Qalubia, Sharkia , Gharbia and Giza governorates, respectively. On the other hand, the highest percentage of NVL was 66.7% (2 out of 3 tuberculin reactor cattle) in Sohag governorate and the lowest percentages of NVL were 28.5% (8 out of 28 tuberculin reactor cattle) in Ismailia governorate. In other governorates, the percentages of NVL ranged from 28.6 to 33.3%.The distribution of PM lesions in different age groups of slaughtered tuberculin cattle reactors is shown in table 3.

The highest number of VL was noticed in 44 (69.8 %) out of 63 tuberculin reactor cattle at age of 4-5 years, while NVL were in 19 (30.2%). In cattle at age of 2-4 years, VL were observed in 18 (64.3%) out of 28 tuberculin reactor cattle, where NVL were in nine (35.7%). In cattle at age of less than 2 years, VL and NVL were recorded in two (66.7%) and one (33.3%) out of 3 tuberculin test reactor cattle, respectively. In cattle more than 5 years old, 30

(67.4%) out of 43 tuberculin test reactor cattle were with VL, where NVL were observed in 14(32.6%) reactor cattle. These results support the conclusion of Lepper et al. (1977) stating that response to tuberculin test in cattle was associated with generalized tuberculosis. The incidence of infection is directly proportional to the age of the animals (Lotfy et al., 1958 ; Castelnuovo et al., 1964).

Table (3): The distribution of PM lesions in different age groups of slaughtered tuberculin cattle reactors.

Age	No. of tested cattle	Slaughtered reactors		PM finding										
				VL									NVL	
				Pulmonary		Digestive		Mixed		Generalized		Total			
		No.	%*	No.	%	No.	%	No.	%	No.	%	No.	%**	No.	%
<2 years	161	3	1.9	1	33.3	0	0.0	1	33.3	0	0.0	2	66.7	1	33.3
2-4 years	597	28	4.7	8	28.6	6	21.4	3	10.7	1	3.4	18	64.3	9	35.7
4-5 years	1299	63	4.9	22	34.9	7	11.1	10	15.9	5	8.0	44	69.8	19	30.2
>5 years	943	43	4.6	10	23.3	6	14.0	6	14.0	8	18.6	30	67.4	14	32.6
Total	3000	137	4.6	41	29.9	19	13.9	20	14.6	14	18.6	94	68.6	43	31.4

VL : Visible lesions   NVL: Non-Visible lesions   * of tested cattle   ** of slaughtered cattle

The percentage of pulmonary tuberculosis was and 4-5 years age group [one (33.3%) and 22 (34.9%) out of 3 and 63 tuberculin reactor cattle, respectively]. The percentage of pulmonary tuberculosis in cattle of age of 2-4 years was 28.6% (8 out of 28 slaughtered cattle). The lowest

high in cattle of ages of less than 2 years percentage of pulmonary tuberculosis was observed in 10 (23.3%) out of 43 slaughtered cattle of age over 5 years. The relatively high percentages of pulmonary form compared to other VL are in general agreement with the

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findings reported by Zivkovic et al. (1984) and Yassin (2006) who mentioned that tuberculosis lesions in cattle most commonly observed in the lungs and its associated lymph nodes.

Tuberculosis lesions of digestive system were seen in 7 (11.1%) out of 63 ,6 (21.4%) out of 28, and 6 (14%) out of 43 tuberculin test reactor cattle at age of 2-4 years ,4-5 years, over than 5 years, respectively. No digestive lesions were observed in cattle at age less than 2 years. Mixed TB lesions (pulmonary and extra-pulmonary) were found to be highest in cattle at age of less than 2 years [one (33.3%) out of three slaughtered tuberculin test reactors]. Lower percentages of 10.7%, 15.9% and 14% were recorded for slaughtered cattle at age of 2-4, 4-5 and over 5 years, respectively.

The total acid fast bacilli isolated from 137 slaughtered tuberculin cattle reactors were 97 (70.8%) isolates and they were identified according to the morphological characters, growth rate, pigmentation, growth at different temperatures and biochemical tests. Ninety (65.7%) and 7 (5.1%) isolates were identified as M.bovis, and MOTT, respectively (Table 4).

Table (4): Results of Bacteriological examination of tissue samples from slaughtered tuberculin reactor cattle in different governorates:

Governorate	No. of slaughtered animals	Bacteriological examination and isolate identification					
		M.bovis		MOTT		Negative for Mycobacteria	
		No.	%	No.	%	No.	%
Qalubia	20	12	60.0	2	10.0	6	30.0
Sharkia	39	24	61.5	1	2.6	14	35.9
Gharbia	10	7	70.0	2	20.0	1	10.0
Giza	23	15	65.2	0	0.0	8	34.8
Behira	14	9	64.3	1	7.1	4	28.6
Sohag	3	1	33.3	1	33.3	1	33.3
Ismailia	28	22	78.6	0	0.0	6	21.4
total	137	90	65.7	7	5.1	40	29.2

M.bovis : *Mycobacterium bovis*   MOTT   : Mycobacteria Other Than Tuberculosis

The recovery rate of M.bovis figured up to 65.7% was close to that reported by Gouello et al. (1988) which was 69. %. Lower M.bovis recovery rate of 42.9, 35.4, 29.1 and 20.2 % were reported by Abou-Eisha et al. (1995), Osman (1974), Gallo et al., (1983) and Lesslie and Birn (1970), respectively. On the other hand, Choi (1981) reported a much higher isolation rate amounting to 92.1% in Korea. These results depend mainly on the actual disease status present in the tested herds to some extent on the experience of the investigators as well as the technique used for decontamination of tissue specimens. Other authors (Parlas and Rossi, 1964)

and Payeur and Marquardt, 1988) reported much lower M. bovis recovery rate of 5.6 and 14.8%, respectively. In this study, the recovery rate of atypical mycobacteria was 5.1%, however, Choi (1981) reported that 48.7% of the reactors were infected with atypical mycobacteria. As shown in table 5, the overall percentage of bacteriological isolates from the 137 tuberculin cattle reactors showed VL of pulmonary, digestive, mixed and generalized forms and NVL were 90 (65.7%) and 7 (5.1%) for M.bovis and MOTT, respectively. Negative cultures which showed no growth of bacteria were recorded in 40 (29.2%) animals.

Table (5): Results of bacteriological and ELISA testing of tissue and serum samples from tuberculin reactors cattle with various VL and NVL.

PM finding	No. of reactors	Bacteriological Examination and isolate identification						ELISA			
		M.bovis		MOTT		Negative culture		Positive		Negative	
		No.	%	No.	%	No.	%	No.	%	No.	%
Pulmonary form	41	36	87.8	0	0.0	5	12.2	35	85.4	6	14.6
Digestive form	19	13	68.4	2	10.5	4	21.1	13	68.4	6	31.6
Mixed form	20	14	70.0	1	5.0	5	25.0	15	75.0	5	25
Generalized form	14	14	100.0	0	0.0	0	0.0	14	100.0	0	0.0
NVL	43	13	30.2	4	9.3	26	60.5	31	72.1	12	27.9
Total	137	90	65.7	7	5.1	40	29.2	108	78.8	29	21.2

NVL : Non-Visible lesion

M.bovis was isolated from 36 (87.8%), 13 (68.4%), 14 (70%) and 14 (100%) out of 41, 19, 20 and 14 On the other hand, isolates identified as MOTT were 2

slaughtered reactor cattle with visible pulmonary, digestive, mixed and generalized lesions, respectively. (10.5%), one (5%) slaughtered reactor cattle with

visible digestive and mixed lesions, respectively. M.bovis and MOTT were isolated from 13 (30.2%) and 4 (9.3%) out of 43 slaughtered reactor cattle with NVL, respectively. Similar overall results of M.bovis *tuberculosis* . In addition, the results are in general agreement with those reported by El sabban (1980) who isolated M.bovis from 71% of tuberculous tissue samples in Egypt.

Because of the false results of tuberculin test, it is very important to apply another method to confirm the infection with tuberculosis. To overcome this problem and increase the sensitivity and specificity of the test, many authors suggested the use of ELISA in combination of tuberculin test (Plackkett et al., 1989; Duffield et al., 1990 and Ritacco et al., 1990). In this study, serum samples collected from 137 positive reactor cattle were examined by an ELISA using

and MOTT isolation were recorded by Claxton et al. (1979) who found that out of 642 lesions suspected to be tuberculous, 62% yielded M.bovis and 3.6% Mycobacteria other than *M.bovis* ESAT6-p antigen, 108 (78.83%) out samples were found positive, while remaining of samples (21.17%) were found negative. This indicates a higher sensitivity of ELISA in comparison with tuberculin test and M. bovis isolation (Reggiardo et al., 1981; Lilenlbaum et al., 2001; Nasr et al., 2005). The results of bacteriological examination and ELISA testing of tissue and serum samples from tuberculin reactor cattle at different ages are shown in table 6. The results of M.bovis isolation and reactivity of serum samples to ESAT6-p ELISA confirmed PM findings of tuberculosis in slaughtered tuberculin reactor cattle.

Table (6): Results of bacteriological testing and ELISA of tissue and serum samples from tuberculin reactor cattle at different ages.

Age	No. of Tested animal	No. of slaughtered reactors		bacteriological testing of tissue samples and isolate identification						Serum samples positive to ELISA	
				M.bovis		MOTT		*M.bovis*			
		No.	%.	No.	%.	No.	%.	No.	%.	No.	%.
<2 year	161	3	1.9	1	33.3	1	33.3	1	33.3	2	66.7
2-4 year	597	28	4.7	17	60.7	3	10.7	8	28.6	22	78.6
4-5 year	1299	63	4.8	42	66.7	2	3.2	19	30.2	53	84.1
>5 year	943	43	4.6	30	69.8	1	2.3	12	27.9	31	72.1
Total	3000	137	4.6	90	65.7	7	5.1	40	29.2	108	78.8

*M.bovis* : *Mycobacterium bovis*   MOTT: Mycobacterium Other Than Tuberculosis

The PCR has been proved to be a sensitive and specific test for rapid diagnosis M. bovis in naturally-infected animals. The PCR assay allowed for the detection and identification of M. bovis in negative culture specimen (Bal et al., 1994; Glennon et al., 1997; Taylor et al., 2007). The PCR primer set used in this study was designed based on the published sequence of insertion sequence IS6110 of Mycobacterium complex sequence at the gene bank database. The utility of using the sense and antisense primers in the PCR assay was demonstrated by amplification of mycobacterial DNA target sequence in 8 out of 10 tissue samples from tuberculin reactor cattle with VL or NVL of tuberculosis (table 7 and fig.1).

Table (7): Bacteriological isolation, ELISA and PCR testing of 10 tuberculin reactor cattle with different type of lesions.

Sample No.	P/M findings	Bacteriological identification		ELISA		PCR	
1	Digestive	*M.bovis*		Positive		Positive	
2	Generalized	*M.bovis*		Positive		Positive	
3	Pullmonary	*M.bovis*		Positive		Positive	
4	Mixed	*M.bovis*		Positive		Positive	
5	NVL	MOTT		Negative		Negative	
6	Digestive (Calcified)	MOTT		Negative		Negative	
7	Generalized	*M.bovis*		Positive		Positive	
8	Pullmonary	*M.bovis*		Positive		Positive	
9	Mixed (Calcified)	MOTT		Negative		Positive	
10	NVL	-ve culture		Positive		Positive	
Samples positive to the test		No.	%	No.	%	No.	%
		6	60	7	70	8	80

NVL: Non-visible lesions.
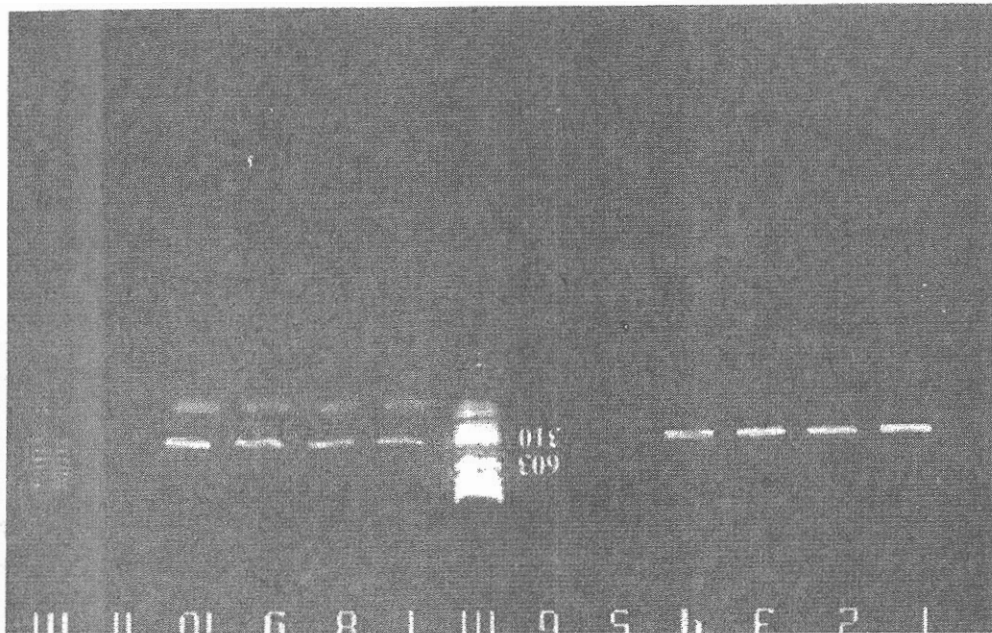
Fig.1: agarose gel electrophoresis of PCR product DNA following amplification of mycobacterial

genomic DNA exracted from tissues of tuberculin reactor cattle with visible lesions (VL) or nonvisible lesions (NVL). Lane 1: digestive VL; lanes 2 and 7: generalized VL; lanes 3 and 8: pulmonary VL; lane 4: mixed VL; lane 5: NVL; lane 6: calcified digestive VL; lane 9: calcified mixed VL; lane 10: NVL, lane 11: negative control reaction (with no template DNA). Lane M (right): ΦX174 phage/ Hae II digested DNA marker [with its fragments in base pairs (bp); 72, 118, 194, 234, 271, 281,310, 603 and 872-1353, from bottom to top]. Lane M (right): 100 bp DNA ladder (with its fragments in bp: 100, 200, 300, 400 and 500/517-1517, from bottom to top). The 327-bp amplified target fragment is indicated on the left on the left of the panel. In conclusion, the PCR amplification assay can be used as an alternative reliable laboratory test for diagnosis and confirming the results obtained by tuberculin test for detection of infection with bovine tuberculosis. The results obtained in the present study also point to the importance of the periodical tuberculin testing of animals, use of ELISA assays with M .bovis-specific antigens as a complimentary diagnostic tool to tuberculin test, and application of PCR test for direct and rapid diagnosis of tuberculosis. Improvement of diagnostic assays for M.bovis may be of value in the effectiveness of test and slaughter eradication programs of bovine tuberculosis in governorates of Egypt.

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الملخص العربي

مدى تواجد الاصابة بميكروب السل البقرى فى بعض محافظات مصر

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قسم البكترولوجى و المناعة و الفطريات ، كلية الطب البيطرى ـ جامعة المنوفية[1] ، قسم الميكروبيولوجيا ، كلية الطب البيطرى ـ جامعة الأسكندرية[2] ، قسم المشخصات الجزيئية ، معهد بحوث الهندسة الوراثية والتكنولوجيا الحيوية ـ جامعة المنوفية[3] ، قسم الميكروبيولوجيا ، كلية الصيدلة ـ جامعة الأزهر[4] ، قسم تشخيص الدرن ، معهد بحوث الأمصال واللقاحات البيطرية ـ مركز البحوث الزراعية[5] ، الهيئة العامة للخدمات البيطرية ـ القاهرة[6].

فى هذه الدراسة تم فحص ٣٠٠٠ رأس ماشية من الأبقار فى أعمار مختلفة من ٧ محافظات مصرية (القليوبية ـ الشرقية ـ الغربية ـ الجيزة ـ البحيرة ـ سوهاج ـ الإسماعيلية) بإستخدام إختبار التيوبركلين المفرد فى الرقبة وتم الحصول على نتائج إيجابية للإختبار فى ١٣٧ رأس بنسبة قدرها ٤,٦%.

وبعد ذبح الحيوانات الإيجابية لإختبار التيوبركلين بمجازر المحافظات المختلفة، تبين وجود آفات تشريحية سلية ظاهرية فى ٩٤ رأس بنسبة ٦٨,٦% بينما لم تظهر آفات تشريحية سلية ظاهرية فى ٤٣ حيوان بنسبة ٣١,٤%. ولقد امكن ملاحظة الآفات التشريحية السلية الظاهرية فى الحيوانات المذبوحة من الانواع الرئوية ، الهضمية، المختلطة و العامة فى ٤١ (٢٩,٩ %) ، ١٩ (١٣,٩ %) ، ٢٠ (١٥ %) ، ١٤(١٠ %) من الحيوانات المذبوحة على الترتيب.

و قد اظهر الفحص البكتيري على عينات من الانسجة من الحيوانات المذبوحة عزل ٩٧ عترة من ميكروبات الميكوبكتيريا وتم تصنيفها بإجراء الإختبارات الكيميائية الحيوية إلى ٩٠ عترة من ميكروب العترة السلية البقرية بنسبة ٦٥,٧% و ٧ عترات سلية اخرى بنسبة ٥,١%. وباجراء إختبار الإليزا على مصل الحيوانات الإيجابية لإختبار التيوبركلين قبل ذبحها بإستخدام أنتيجين ميكروب السل البقري ايساتـ٦ تم الكشف عن ١٠٨ عينة ايجابية بنسبة ٧٨,٨٣%.

كما تم إستخدام إختبار تفاعل انزيم البلمرة المتسلسل مباشرة على ١٠عينات انسجة من الآفات التشريحية المختلفة (الرئوية ، الهضمية ، المختلطة ، و العامة) من الحيوانات الإيجابية لإختبار التيوبركلين ولقد امكن الحصول على نتائج إيجابية في ٨ عينات مختبرة بنسبة ٨٠% . وقد اظهر فحص نفس هذه العينات الحصول على نتائج ايجابية في ٦ (٦٠%) و ٧ (٧٠%) عينات باجراء إختبارات عزل عترة السل البقرية و إختبار الإليزا على الترتيب.

ومن نتائج البحث تبين أهمية إستخدام إختبار التيوبركلين فى الحيوانات بصورة دورية مع إمكانية إستخدام إختبار الإليزا بإستخدام أنتيجين نوعى كإختبار تكميلي للتيوبركلين للكشف على الحيوانات المصابة بالسل النشط وكذلك إستخدام إختبار تفاعل إنزيم البلمرة المتسلسل للكشف السريع عن الإصابة بميكروب السل البقري في العينات المختلفة مباشرة. و يمكن أن يكون تحسين طرق تشخيص الأصابة بميكروب السل البقري ذو قيمة في كفاءة برامج اختبار و ذبح الحيوانات المصابة بالسل البقري في المحافظات المصرية.