Vet.Med.J.,Giza. Vol. 49, No.3. (2001): 355-369.

COMPARISON BETWEEN TUBERCULIN TEST AND ENZYME LINKED IMMUNOSORBANT ASSAY FOR DIAGNOSIS OF TUBERCULOSIS IN CATTLE AND BUFFALOES

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Received: 4. 10. 2000
Accepted: 17. 03. 2001

SUMMARY

Through this study a total of 190 cattle at Sharkia governorate were tuberculin tested by single intradermal cervical tuberculin test using human and bovine PPD. Out of this number, 124 cattle were tested by single intradermal cervical tuberculin test using human PPD and 36 of them were positive (29%). The positive reactors were retested two months later by an American bovine PPD and 33 (91.66%) out of 36 the positive animals were positive. The other 66 mixed bread dairy cattle were tested simultaneously by three types of PPD and the positive reactors for the American bovine, local bovine and local human PPD were 43 (65.15%), 40 (60.6%) and 28 (42.42%), respectively, So the overall tuberculin positivity was 64 (33.68%) cases when using human PPD . However, the positive tuberculin reactors of buffaloes were almost 1% using human PPD. 55 tuberculin positive cattle for human PPD were slaughtered and subjected to P. M. Examination and visible lesions were observed in 26 cases (47.27%). While the visible lesions seen in 39 tuberculin positive buffaloes were 18 (46.15%) cases. The bacteriological examination of the samples collected from all slaughtered cattle yielded 18 *M. bovis* (32.73%), while *M. bovis* was not isolated from any tuberculine positive buffaloe. ELISA was done on the serum samples collected from those 55 slaughtered cattle and results were commpared with tuberculin test and P.M. findings. It was found that 38 out of 55 slaughtered cattle were positive by ELISA (69.1%) at the same time. ELISA could be detected 20 out of 30 cases bacteriologically negative (36.36). However, 16 (41.02%) positive ELISA were obtained in tuberculin positive bufaloes. By comparing the results of ELISA and the yielded isolated by bacteriological examination of 55 slaughtered cattle it was found that 12 out of 18 bacteriologically positive cases to *M. bovis* were ELISA positive

(21.82%). At the same time 6 cases of Mycobacterium other than *M. bovis* (MOTT) were positive for ELISA (10.92). In conclusion, ELISA as well as bacteriological examination are a complementary tests for tuberculin test for diagnosis of bovine tuberculosis, and bovine PPD expressed higher sensitivity than human PPD

INTRODUCTION

Tuberculosis is one of the most important bacterial world wide disease of all species of animals as well as human being specially in the third world countries. The diagnosis of bovine tuberculosis is still uptill now depends upon the use of tuberculin test and the purified protein derivatives (PPD) is still the most widely used antigen, however it contains many antigenic determinants of broad specificity which lead to appearance of non-specific false positive reactions. The false positive reactors is not only the main problem of the tuberculin test as there is also false negative animals which give negative tuberculin tests although it is actually infected with tuberculosis (Castro and Nemoto, 1972; Cortina and Vera, 1986 and O Reilly, 1989). To increase the sensitivity and decrease the non-specificity of tuberculin test the antigen used for the tuberculin test as well as the route and type of tuberculin used in investigation of cattle were studied and standardized in different countries. The success of using the tuberculin test and diagnosis of tuberculosis differe from country to anotyher. Therefore, it is essential that isn each country or geographical area to prepare well designed fiedl trials to establish a standard strict interpretation key under the local conditions for the different national breeds of animals as concluded by O Reilly, (1992), who indicatted that no single test and interpretation key is suitable for all environment. The test and interpretation keys chosen should be based on epidemiological considereation, the management practices in regions and also the results of well designed local field trials to deremine test specificity and sensitivity. To overcome the non-specific problems of tuberculin test in diagnosis of bovine tuberculosis, various procedures have been used to measure humoral and cell mediated immune response to ***M. tuberculosis*** infection. As the common future of mycobacterium infection is the induction of a wide spectrum of immune response and at the same time inverse relationship between T-cell and antibody response usually response usually observed, many serological tests as complement fixation, agar gel immunodifusion, passive heamaglutination and Enzyme Linked Immuno Sorbant Assay (ELISA) were used. ELISA is general has the highest sensitivity and specificity than other serological tests for diagnosis of bovine tuberculosis (Tongkrajal et al., 1989) and the use of ELISA as a complement test to the tuberculin test well greatly increased the sensitivity and specificity of the diagnosis as reported by Plackett et al., (1989); Duffield (1990) and Ritacco et al., (1990). The present investigation is planned to study the possibility of the substitution of mammalian PPD

with locally prepared bovine PPD in skin testing of cattle and buffaloes under our environmental conditions. As well as the using of ELISA in diagnosis of tuberculosis in cattle and buffaloes and at the same time evaluation of test sensitivity and specificity by comparing the results with those of tuberculin test, necropsy and bacteriological examination.

MATERIALS AND METHODS

Materials:

Cattle: A total of 190 cross bread dairy cattle at Sharkia govemorate were concerned in this investigation. Water buffaloes (Bubalus bubolis): 135 tuberculine positive buffaloes were also concerned in this study and were obtained from Menofia governorate.

Collected samples: Lymph nodes specially bronchial, prescapulare, prefemoral, hepatic and mesenteric lymph nodes were collected from 55 cattle and 39 buffaloes, whether showing visible lesion or not. Samples were also collected from lung, liver, kidney and diaphragm of the slaughtered animals specially in case of generalized tuberculosis.

Serum samples: Serum samples were collected from all animals either positive or negative to the tuberculin test to be tested by ELISA .

Purified protein derivatives (PPD): American bovine PPD: was supplied by the National Veterinary Service Laboratory (NVSL), Ames, Iowa, USA. The product was dispensed in 10 ml amounts, in a concentration of 50000 T.U/ml (1 mg/ml). **Local bovine PPD:** was available in two concentrations, the usual concentration 50000 T.U/ml (1 mg/ml) and the double concentration 100000 T.U/ml (2 mg/1ml). - **Local human PPD:** was available in a concentration of 2 mg/ml and supplied by the Veterinary Serum and Vaccine Research Institute Abassia, Cairo.

Bacteriological Medium:- Modified Lowenstein-Jensen medium; Modified Lowenstein-Jensen glycerol medium and Blood agar medium: (Mackie and McCartney, 1989).

ELISA buffers and reagents: Coating antigen : Human PPD at 0.005% and bovine PPD at 0.005% were obtained from Abassia Research Institute; **Coating buffer** [Carbonate bicarbonate buffer (pH 9.6)]; **Blocking buffers:** 3% bovine serum albumin (BSA) and 7% non fat dry milk; **Washing buffer:** (0.05% Tween 20 in PBS) ; **Diluting buffers:** 0.5% non fat dry milk in PBS containing 0.05% Tween: **Citric acid buffer:** Stock solution A: 0.1 M citric acid; Stock solution B: 0.1 M sodium citrate; 33 ml of A + 17 ml of B, diluted to a total of 100 ml ; **Conjugate :** Anti-

bovine horseradish conjugated with peroxidase against whole IgG molecule (Sigma); **Substrate solution:** 5 mg of 22-azino-bis (3-ethyl-benzthiazoline-6-sulphonic acid (ABTS) (Sigma); 10 ml citric acid buffer; 3 ul H2O2 (30%) (MERCK); **Stopping buffer:** 5% sodium dodecyle sulphate in phosphate buffer saline.

Methods

Tuberculin test: It was carried out according to Ovdienko et al., (1987). A narrow zone at the middle third of the neck of the tested animals (cattle and buffaloes) was marked by clipping the hair. The skin thickness was measured using the clipper and by using an automatic syringe adjusted to inject intradermally 0.1 ml per one jet of either human or bovine PPD. The skin thickness was measured 72 hours post injection and the differences in mm were recorded. According to the General Organization of Veterinary Services (GOVS), an increase in the skin thickness of 4 mm or more was considered positive, less than 2 mm was considered as negative and from 2-4 mm was considered as doughtful.

Tuberclin testing of cattle and buffaloes: Firstly 124 cattle were tested by local human PPD and two months latter the positive reactors were re-tested by the American bovine PPD. Another group of 66 cattle were injected simultaneously with 0.1 ml of American bovine PPD and with 0.2 mg/0.1 ml of local human PPD; both were injected in the right side of the neck in addition to local bovine PPD in its normal concentration, 0.1 mg/ 0.1 ml in the left side of the neck. Buffaloes were tested by human and bovine PPD in its recommended concentration in the middle third of the left side of the neck.

Bacteriological examination: The positive tuberculin reactors were slaughtered and subjected to P. M. examination to detect the presence of any tuberculous lesions and at the same time different samples were collected for bacteriological examination (Rattedge and Stanford, 1982).

Microscopical examination: Smears were prepared from the suspected colonies and stained with Ziehl-Neelsen stain for morphological identification.

Rate of growth: It was detected as described by Mackle and McCartney (1989).

Effect of light on pigment production: It was carried out according to Mackie and McCarteny (1989).

Biochemical tests: Niacin test (Gangadhram and Droubi, 1971).

Growth inhibition by Thiophene-2-carboxylic acid hydrazide (THE): (Mackie and McCartney, 1989).

Enzyme Linked Immunosorbant Assay (ELISA): This assay was carried out according to Narayanan et al., (1983), with some modification, Briefly ELISA plates were coated with 100 ul per well of 0.005% of bovine or human PPD in coating buffer. The plates were incubated at 37°C for 1 hour then overnight at 42°C followed by washing once. Blocking was done by adding 200 ul/well of 7% non fat dry milk and incubated for 2 hours at 37°C followed by three times washing. Serum samples were added (100 ul/well) in a dilution of 1:100 and incubated at 37°C for one hour. The plates were then washed three times and the conjugate (antibovine serum conjugated with peroxidase in a dilution of 1:3000) were added and incubated at 37°C for one hour then washed three times. The substrate indicator mixture was then added (100 ul/well) and incubated in a dark place for 25 minutes. The reaction was stopped using 50 ul/well stopping buffer and the optical densities (absorbances) were recoreded using an automated ELISA reader. The optical density (OD) that equal to or higher than the double figure of the OD of the negative control was considered positive, as reported by Bassiri et al., (1993), Preliminary work was carried out for standardization of the test and selection of the negative control sera which were represented by a pooled sera of 10 newly born calves that could be considered free from antibodies against PPD. Therefore the control negative sample was inoculated in each plate and represented by pooled calve sera.

RESULTS

Results of tuberculin test in cattle: 124 mixed dairy cattle breed from Sharkia governorate by single intradermal cervical tuberculin test using human PPD. 36 (29.03%) out of 124 tested animals proved to be positive tuberculin reactors. Two months later all 36 positive reactors were retested by American bovine PPD. 33 animals (91.66%) out of those 36 positive reactors were positive to American bovine PPD. Two animals had suspicious reaction (5.56%) and one animal was negative (2.78%) as shown in Table (1). Another group of 66 cattle from the same governorate were examined simultaneously by cervical intradermal tuberculin test using an American bovine PPD, subgroup (A), local human PPD, subgroup (H), both at the right side, and local bovine PPD in its normal concentration (0.1 mg/0.1ml) subgroup (B) on the left side of the neck. Out of 66 tested animals, 28 (42.42%) were positive and 14 animals (21.21%) were suspicious for human PPD while the positive reactors and suspicious animals for American bovine PPD were 43

(65.15%) and 13 (19.69%) respectively. At the same time out of those 66 animals tested by local bovine PPD at a concentration of 0.1mg/0.1ml 40 (60.6%) were positive and 13 (19.69) were suspicious, as shown in Table (2). The over all results of those 190 examined cattle using human PPD were 64 (33.68%) animals.

Results of postmortem findings and bacteriological examination in cattle: The 55 which gave positive reaction to the intradermal tuberculin test using human and bovine PPD (36 from first group and 19 from the second group) were slaughtered and subjected to postmortem examination. 26 cases (47.27%) out of those 55 tuberculin positive reactors showed visible lesion in bronchial lymph node alone and other cases showed visible lesions in the mesentric lymph nodes. 29 animals showed non-visible lesions. Bacteriological examination yielded 18 *M. bovis* and 7 MOTT but no isolates could be obtained from the other 30 reactors as shown in Table (3) . At the same time most of MOTT isolated from a cases with non visible lesions (NVL) while most of *M. bovis* isolated from cases with VL and only 6 isolates were obtained form NVL positive reactor as shown in Table (3).

Table (1): Results of tuberculin test of cattle tested by human PPD and positive reactors retested by American bovine PPD.

Types of PPD / Tuberculin Reactivity	Human PPD		American bovine PPD	
	No.	%	No.	%
Positive reactors	36	29.03	33	91.66
Suspicious cases	00	00.00	2.0	5.56
Negative reactors	88	70.97	1.0	2.73
Total No.	124	100	36	100

Table (2): Results of tuberculin test of examined cattle using human and bovine PPD.

Reactivity / Types of PPD	Positive tuberculin		Suspicious		Negative tuberculin		Total
	No.	%	No.	%	No.	%	No.
American bovine PPD (A)	43	65.15	13	19.70	10	15.15	66
Local bovine PPD (B)	28	42.43	14	21.21	24	36.36	66
Local human PPD (H)	40	60.60	13	19.70	13	19.70	66

A: American bovine PPD at concentration (0.1 mg/0.1 ml).
B: Local bovine PPD at concentration (0.1 mg/1 ml).
H: Local human PPD at concentration (0.2 mg/0.1 ml).

Comparison between results of ELISA and bacteriological examination of tuberculin positive Cattle : The results in Table (4) express the results of 55 slaughtered tuberculin positive cattle and show 12 (21.82%) out of 18 (32.73%) bacteriologically positive cases to *M. bovis* were ELISA positive However, the other 6 (10.9%) *M. bovis* positive cattle were ELISA negative Furthermore, 20 (36.36%) bacteriologically negative cases were ELISA positive.

Results of ELISA, Postmortem Findings and Bacteriological examination of tuberculin positive Buffaloes: 135 tuberculin positive buffaloes were obtained from 13500 examined animals (1%) in Menofia governorate by using human PPD. 39 buffaloes out of those 135 tuberculin positive animals were randomly selected, slaughtered and subjected to P. M. examination as well as blood samples were collected for separation of serum for ELISA. The results in (Table 5) show that 18 cases out of 39 slaughtered buffaloes showed visible lesions, 11 cases showed parasitic infestation (e.g. *S. fusiforms* and Fasclolasis) and 10 NVL. While the bacteriological examination yielded 9 isolates, 6 MOTT and 3 Corynbacterium spp. The results of ELISA showed 16 (41.02%) positive cases out of those 39 tested serum samples as shown in Table (5).

Table (3): Comparison between postmortum findings and bacteriological examination of tuberculin positive cattle to human and bovine PPD.

Findings / Yielded Isolates	Visible lesions		Non-visible lesions		Total	
	No.	%	No.	%	No.	%
M. bovis	12	21.82	6	10.91	18	32.73
MOTT	1	1.82	6	10.91	7	12.73
Negative bacteriology	13	23.63	17	30.91	30	54.54
Total	26	47.27	29	52.73	55	100

MOTT= Mycobacteria other than *M. bovis*

Table (4): Collective table showing the correlation between results of ELISA and bacteriological findings of tuberculin positive cattle to human and bovine PPD.

Results / Bacteriological findings	ELISA positive		ELISA negative		Total	
	No.	%	No.	%	No.	%
M. bovis	12	21.82	6	10.92	18	32.73
MOTT	6	10.92	1	1.18	7	12.73
Negative bacteriology	20	36.36	10	18.18	30	54.54
Total	38	69.1	17	30.90	55	100

MOTT= Mycobacteria other than *M. bovis*

Table (5): Comparison between results of ELISA, Bacteriological and postmortem findings of tuberculin positive buffaloes to human PPD.

Results / Isolates PM findings	ELISA positive		ELISA negative		Total	
	No.	%	No.	%	No.	%
M. bovis	0	0:0	0	0.0	0	0.0
MOTT	4	10.26	2	5.13	6	15.88
Corynebact. spp	0	0.0	3	7.69	3	7.69
Negative bacteriology	12	3.77	18	46.15	30	76.92
Total	16	41.03	23	58.97	39	100
MOTT	13	33.33	5	12.82	18	46.15
Corynebact. spp	0	00.00	11	28.21	11	28.21
Negative bacteriology	3	7.69	7	17.95	10	25.64
Total	16	41.02	23	58.98	39	100

NVL = Non-visible lesions; MOTT= *Mycobacteria* other than *M. bovis*.

DISCUSSION

In Egypt, diagnosis of bovine tuberculosis is still undertaken by the tuberculin test and PPD prepared from *M. tuberculosis* (human PPD) remains the most widely used antigen, however, many countries substitute the human PPD by the bovine PPD as many authors (Francis et al., 1973; Lesslie et al., 1975 a & b; O Reilly and MacClacy 1975; Cotrina et al., 1977; Macllory et al., 1986 and O Reilly 1992) suggested the use of bovine PPD instead of human PPD for testing animals for diagnosis of bovine tuberculosis. These authors concluded that bovine PPD has significant higher sensitivity and specificity than the huma PPD and this agreed with the obtained results. Moreover, it can help in the detection of some of the anergic animals which does not react with the human PPD. In our trial, 124 mixed dairy cattle breed were tested by local human PPD and the positive reactors were retested two months later by American bovine PPD. Results showed that 36 out of 124 tested animals by human PPD were positive (29.03%) which is higher than the percentage of positive reactors to the tuberculin test observed by Lotfy et al., (1960) 6.9%; Guindi et al., (1965) 26.5% and El-Sabban et al., (1992) in Egypt 24%. The obtained results coincide with Francis et al., (1973); Lesslie et al., (1975a & b); O Reily and MacClancy, (1975); Cotrina et al., (1977); Macllory et al., (1986) and O Reilly (1992), who mentioned that bovine PPD had higher specificity than human PPD for testing animals for diagnosis of bovine tuberculosis. At the same time, the skin reactions observed by using the bovine PPD revealed more intensive reaction than using human PPD. The present results, in this respect coincide with the results of Lotfy and Guindi (1966) who mentioned that the Egyptian bovine PPD tuberculin showed much higher intensive skin reaction in positive cows if compared with Egyptian human tuberculin. Moreover, the P. M. examination of all slaughtered 55 cattle revealed the presence of visible lesions in 26 out of 55 cattle (47.27%) with tuberculous lesions in the bronchial lymph nodes, in addition to, the mesenteric lymph nodes and retropharnegeal lymph node. These results agree with the conclusion of Pritchart et al., (1975); Cheneau and Bloancou (1976); Yoon et al., (1979); Guindi et al., (1980) and Zivkovic et al., (1984) who mentioned that tuberculous lesions in cattle were most commonly observed in the lungs and its associated lymph nodes in a percentage of 80%, followed by retropharyngal and mesenteric lymph nodes.

The bacteriological examination of the 55 positive reactors yielded 18 *M. bovis* (32.73%), all of them were isolated from cases with positive tuberculin test for both human and bovine PPD. Moreover 6 cases with NVL yielded *M. bovis*. This result coincides with the results of Rogers et al., (1980) who isolated *M. boivi* from NVL reactors. At the

same time, MOTT mostly isolated from cases with NVL. The obtained results confirmed the results of Gallo et al., (1983) who isolated other mycobacteria (OM) from 23.6% positive reactors, all of them were with NVL. Also, with the results of Sanchez and Rosell (1983) who isolated atypical mycobacterium from lymph nodes free from any gross lesions.

From the obstained results we can say that the false positive reactors to the tuberculin test most commonly observed as a result of sensitization with atypical type of mycobacteria other than *M. bovis*. The result coincide with the conclusion of Nyiredy et al., (1966); Lesslie (1967); El-Ahwal (1970); Corner and pearson (1979); Tkackenk (1985); Cotrina and Vera (1986); Chavez et al., (1987) and Vargas et al., (1988), who mentioned that non specific reaction to the tuberculin test may be attributed to the sensitization of the animals with atypical type of mycobacteria other than *M. bovis*, this result confirmed the disadvantage of using tuberculin test as a sole test for diagnosis of bovine tuberculosis.

The results in (Table 2) indicate that bovine PPD has significantly higher sensitivity than the human PPD for testing cattle for diagnosis of bovine tuberculosis which confirm the results of the above mentioned authors who proved that bovine PPD had higher sensitivity than the human PPD. At the same time from the obtained data we can say that the sensitivity and the potency of the local bovine PPD resemble that of American bovine PPD.

In Egypt, tuberculin test uptill now when used for diagnosis of tuberculosis in buffaloes depends upon the interpretation key which is used in evaluation of tuberculin reaction in cattle. Therefore, the evaluation of the tuberculin test in buffaloes and at the same time evaluation of the locally produced bovine and human PPDs for diagnosis of bovine tuberculosis in buffaloes is essential. Moreover, O Reilly, (1992) concluded that tuberculin test must be standatdized for every species of animal under the local environmental condition. During the PM examination, the liver fluke was observed in two cases with the false positive reactions, this results coincide with the results of Gonthier, (1962) who stated that false positive rectors to the tuberculin test may be attributed to liver fluke infestation as after anthelmentic treatment reactions became negative. In the mean time the results of Hejj et al., (1968) recorded that the sensitizing effect of *F. hepatica* to the tuberculin test is not due to the sensitizing effect of its own body proteins but it is due to the saprophytic mycobacteria which inter the body through the injuries caused by the paraiste migration. Also the pyometra observed in the other case attribute the cause of false positive reaction in such case as mentioned by Vargas et al., (1988) who stated that non-specific reaction to tuberculin test may

be attributed to (OM), *Corynebacterium*, fascioliasis and abscess, while, the animals with positive tuberculin and NVL, with isolation of *Corynebacterulin* spp, which may be the cause of skin reaction observed in such case as mentioned by Vargas et al., (1988) or it may be due to the old lesions which was observed in the mesenteric lymph nodes. Bacteriological examination revealed MOTT from the NVL positive reactors which support the above results. It was concluded that MOTT mostly isolated from NVL positive rectors at the same time indicate that MOTT play an important role in causing false positive reactions.

As a whole all buffaloes investigated in this study and proved to be tuberculin positive reactors wither to human or American bovine or local bovine PPD when slaughtered and examined revealed no *M. bovis*. This confirm the idea that tuberculin test in buffaloes evaluated by the present interpretation key is not valid and it is necessary to design suitable investigation to find out the interpretation key which can be used for evaluation of tuberculin test in buffaloes under the local environmental condition.

To overcome the problems of tuberculin test and at the same time increase the sensitivity and specificity of the test many authors suggested the use of ELISA in conjunction with the tuberculin test (Plackett et al., 1989, Duffield et al., 1990) who stated that better sensitivity and specificity could be obtained when ELISA is used in conjunction with the tuberculin test. For such purpose the present study aimed to determine the usefulness of ELISA as a complementary test to the tuberculin test. The serum samples collected from the 55 tuberculin positive cattle reactors when examined by ELISA using bovine PPD as coating antigen showed that 38 out of 55 tested serum samples were positive (69.1%). This indicated the lower sensitivity of ELISA in comparison with the sensitivity of tuberculin test. The obtained results coincide with the conclusions of Plackett et al., (1989) and Ritacco et al., (1990) who concluded that the lower sensitivity of ELISA compared with that of tuberculin test make it of low value as an alternative to the tuberculin test. But by comparing the results of such negative cases with the results of P. M. and bacteriological examination as shown in (Tables 3 & 4), 20 positive ELISA were negative with bacteriological examinations (36.36%), while the other cases (6) showed *M. bovis* but ELISA negative, this may be attributed to the lower sensitivity of ELISA as mentioned before or to inactive tuberculosis as concluded by Chau et al., (1987) who mentioned that positive ELISA results were obtained only when the disease is relatively long standing or extensive and also the results of Narayanan et al., (1983) who stated that the antibody titers were greatly elevated in the patient with active pulmonary T. B. than

of inactive cases.

For studying the usefulness of ELISA as a complementary test to the tuberculin test in buffaloes the serum samples were collected from all slaughtered 39 tuberculin positive bufflaoes examined at Menofya governorate and were tested by ELISA. By comparing the results of ELISA and the results of P. M. finding as well as the bacteriological examination of slaughtered buffaloes (Table 5), it could be concluded that ELISA could detect 3 out of 10 positive reactors with NVL (7.8%). At the same time all cases with parasitic infestation were negative by ELISA i. e. the false positive result due to parasitic infestation could be detected y ELISA. Moreover, it could detect 13 out of 18 cases with VL (72.22%) resembling the results of Ritacco et al., (1990) who recorded that sensitivity and specificity of ELISA for the detection of bovine IgG anti- *M. bovis* antibodies were (73.1%) and (94.1%) . At the same time all cases yielded *Corynebacterium* spp. were negative by ELISA also 2 out of 6 cases with MOTT (5.13%) were negative by ELISA i. e. ELISA could detect some of the false positive reactors occurring as a result of sensitization with atypical type of mycobacteria and *Corynebacterium spp.* This confirmed the results of Duffield (1990) who mentioned that ELISA could detect the false positive reactors due to sensitization with OM or *Rhodococcus equi* or *E. coli. Moreover*, ELISA could detect 12 out of 30 bacteriologically negative cases which confirm the above mentioned result which concluded that positive ELISA results was obtained only when the disease was relatively long standing or extensive. From the obtained data we can conclude that tuberculin test by the interpretation key currently used in Egypt for evaluation of tuberculin test in cattle is not suitable for diagnosis of tuberculosis in buffaloes and the test requires more standardization.

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