

COMPARISON OF TUBERCULIN SKIN TEST AND LATERAL FLOW RAPID TEST FOR DETECTION OF BOVINE TUBERCULOSIS IN DAIRY CATTLE

Nasr E.A.*, Marwah M.*, ** Lilian F.S. MelikaAbeer A. Tammam, ****Seham F. Gorge.**

***Department of Bacterial Diagnostic Products (Tuberculosis), Veterinary Serum and Vaccine Research Institute, Abassia, Cairo, Egypt.**

****Parasitological Vaccine Research Department, Veterinary Serum and Vaccine Research Institute, Abassia, Cairo, Egypt.**

*****Department of Rinder Pest, Veterinary Serum and Vaccine Research Institute, Abassia, Cairo, Egypt.**

****** Animal Healthy Institute Beheira Branch**

ABSTRACT

Tuberculosis is the most important zoonotic bacterial disease that is hazardous to both man and animals. A huge economic loss which could be direct or indirect is associated with the disease, so rapid diagnostic tests for tuberculosis are needed to facilitate early detection and prevention of disease transmission. The aim of this work is the detection of bovine tuberculosis by application of different serological tests. Tuberculin skin test applied on 1900 cattle, only 50 (2.6%) showed positive results, and then slaughtered. Forty five (90%) of slaughtered animals showed visible lesions on post mortem examination, while the other five (10%) showed non visible lesions. The bacteriological examination of the 50 samples revealed *Mycobacterium bovis* form 40 processed samples (80%). Results of Anigen Rapid Bovine TB Ab test and ELISA test had detected 42% and 48% of tuberculin positive cattle respectively. It was concluded that the Anigen Rapid Bovine TB Ab kit test is rapid, safe, simple and easy to perform and provide yes or no results within 15 to 20 minutes but it is not efficient for detection of bovine tuberculosis in cattle and could be useful as a complementary for tuberculin test.

Key words: Anigen Rapid Bovine TB Kit - bovine tuberculosis - ELISA - *M. bovis* -

Corresponding author

Email: essamnasr@yahoo.com

Mobile: 0100575061

1. INTRODUCTION

Bovine tuberculosis is a worldwide disease that causes a great harm on dairy farms and poses health risks to the population that consumes products of animal origin. It is still a problem with public health and economic importance in large areas of the world (**Ritacco *et al.*, 1987**). The economic losses caused by the disease are not only a reduction of 10-20% in milk production and weight, but also due to infertility and condemnation of meat. The loss is estimated to be 10-25% of the reproductive efficiency, excluding the losses from mortality (**Lilenbaum *et al.*, 2001**). The disease has been difficult to control in livestock because of the lack of an effective vaccine and the presence of wildlife reservoirs. Currently, the primary methods used for the detection of TB in humans and ruminants include the measurement of a delayed type hypersensitivity (skin test) to purified protein derivative (PPD) and an indirect in vitro assay that measures the concentration of gamma interferon (IFN- γ) produced in response to stimulation with PPD (**Monaghan *et al.*, 1994, Wood *et al.*, 1992, Wood and Jones 2001**).

Some infected animals may have antibody response in absence of cell – mediated response, particularly when the bacterial load is high. A number of Enzyme Linked Immunosorbent Assays (ELISA) have been described based on complex *M. bovis* antigens, such as Purified Protein Derivatives (PPD) and phosphatide antigens. All of these assays were successful in detecting circulating antibodies to mycobacteria but have been considered to lack specificity (**Mcnair *et al.*, 2001**).

Serological assays are generally simple, rapid and inexpensive, but the development of improved serodiagnostic assays also require understanding the bovine tuberculosis humeral immune mechanisms which is characterized by heterogeneous antigen recognition (**Lyashchenko *et al.*, 1998**). Advances in humeral based responses tests

have led to the recently development of two membrane-based antibody detection methods, Multi Antigen Print Immuno Assay (MAPIA) and a lateral-flow test. Ab Test Kit is a solid phase chromatographic immunoassay for the qualitative detection of *Mycobacterium bovis* antibody in serum or plasma (Greenwood *et al.*, 2003). The purpose for conducting this study was to compare between the sensitivity of recent lateral flow rapid test and ELISA for diagnosis of bovine tuberculosis of tuberculin skin test reactor cattle.

2. MATERIAL AND METHODS:

I - Tuberculin skin test: A total number of (1900) cross- breed dairy cattle from different farms were tested by Single Intradermal Cervical (SIC) tuberculin test as performed by OIE (2009).

II- Serum samples: From the positive tuberculin reactors cattle, blood samples were collected and serum samples were separated and stored at - 20°C till used in serological test.

III- Post – mortem examination: After slaughtering of tuberculin positive reactors, Post – mortem examination was done to detect the presence of any suspected tuberculous lesions such as caseation, calcification, or congestion that might be present in any lymph node (head, bronchial, hepatic, mesenteric, prescapular, popliteal and internal iliac lymph nodes). Moreover, specimens were collected from the lung, liver, kidney, diaphragm and peritoneum which showed congestion or suspected tuberculous lesions.

IV – Tissue samples: The internal organs (livers, spleens, and lungs lymph nodes) and lymph nodes showing tuberculous-like lesions were collected using aseptic techniques, placed in an ice box and submitted as soon as possible to the laboratory where they were processed for isolation and identification of the organism.

V- Bacteriological examination: The organs, lymph nodes and/or tissues showing gross lesions were prepared for bacteriological examination. Prepared sections were stained with Ziehl – Neelsen’s stain. Samples were cultured on four tubes of Lowenstein-Jensen slants after being decontaminated with 4% H₂SO₄. Obtained isolates were identified by conventional methods (rate of growth, colonial morphology, pigmentation, and biochemical properties) according to **Brasil (1994)**.

VI - Enzyme Linked Immunosorbent Assay (ELISA): The Enzyme Linked Immunosorbent Assay (ELISA) was applied in sera of tuberculin positive cattle according to **Collee *et al.* (1996)** using Bovine Purified Protein Derivatives (B – PPD). The optical density was measured at 405 nm using spectra III ELISA reader. Sample was considered positive if it yield a mean OD of each group equal to / or greater than the cut off value {Cut off value was calculated according to **Nassau *et al.* (1976)** which equal to the mean OD of negative serum plus 2 standard deviation}.

VII- Lateral flow test Kit: The testing of sera was carried out according to the manufacturer's instructions using the Anigen Rapid Bovine TB Ab test kit as follows:

1. The foil pouch of test kit was removed and placed on a flat, dry surface.
2. Test units were labeled with samples names.
3. Four drops of serum were added slowly to sample well with the specimen dropper (if the migration has not appeared after one minute, one more drop of the specimen was added to the sample well).
4. A test result will be seen as a band in the result window of the kit.
5. The test results were interpreted within 20 minutes (no interpretation after 20 minutes).

Interpretation of the test:

- Negative result: The presence of only one color band within the result window.
- Positive result: The presence of two color bands (T band and C band) within the result window. (Even if the intensity of the band color is faint it should be considered as positive).
- Invalid: If the color band was not visible within the result window after performing the test, the result was considered invalid and the specimen was re-tested.

3. RESULTS :

3.1. Results of tuberculin skin test and post mortem finding of slaughtered tuberculin positive cattle.

The results in table (1) illustrated the prevalence of tuberculin reactors in dairy cattle from different farms and PM findings of slaughtered tuberculin reactor cattle. From total 1900 tuberculin tested cattle, 50 were found to be reactors with a prevalence rate of 2.6%. On other hand, the number of non – visible lesion (NVL) reactors amounted to 5 animals (10%), while the number of visible lesions found to be 45 animals with an overall percentage of 90% as shown in the same table.

3.2. Results of postmortem finding in slaughtered tuberculin reactor cattle according to the site of lesion.

Out of 50 tuberculin reactor animals, 45(90%) showed visible and 5(10%) had non visible lesions, on the same time the visible lesions showing 6(12%) head, 24(48%) pulmonary, 10(20%) digestive and 5(10%) generalized as shown in the same table (Table-2).

3.3. Results of Anigen Rapid Bovine TB Ab test kit from tuberculin reactor cattle in comparison to the type of lesions.

It is cleared from table (3) that 21(42%) of tuberculin reactor cattle were positive with Ani- gen Rapid Bovine TB kit. While, 29 (58%) were negative with Ani- gen Rapid Bovine TB kit.

3.4. Comparison between the results of bacteriological isolation, ELISA and Anigen Rapid Bovine TB Ab test kit on samples obtained from tuberculin positive animals.

The obtained results in table (4) showed that, from 50 carcasses, 40 cultures positive for *M. bovis* were recovered with an isolation rate of 80 %. While, Anigen Rapid Bovine TB AB Test Kit has detected 42% of tuberculin positive cattle and the ELISA with B-PPD antigen has detected 48 % of tuberculin positive reactor cattle as shown in the table.

4. DISCUSSION:

Bovine tuberculosis is an important zoonotic disease transmitted by direct contact, respiratory pathway, ingestion of unpasteurized milk and milk product, raw or uncooked meat. Tuberculosis can be difficult to diagnose based only on the clinical signs. Tuberculosis is usually diagnosed in the field with the tuberculin skin test, sputum and other body fluids may be collected for microbiological examination (**Kaya *et al.*, 2015**). The tuberculin skin test was found to be the test of choice. Although the tuberculin test is a very reliable diagnostic method, yet as in all biological test, difficulties have been encountered, the greatest problem is the occurrence of so – called false negative responses, which sometimes seen soon after infection, in the late stages of the disease (anergic cattle), in animals with poor immune responses and in those that have recently calved. To obviate this problem, an extensive effort has been under way to identify and characterize antigens unique to *M. bovis* that could be used in diagnostic assay (**El- Mahrouk and El- Balawy, 2010**).The objective of this study was to compare the sensitivity of two serological test for diagnosis of bovine tuberculosis in cattle.

The results in table (1) illustrated the prevalence of tuberculin reactors in dairy cattle from different farms and PM findings of slaughtered tuberculin reactor cattle. From total 1900 tuberculin tested cattle, 50 were found to be reactors with a prevalence rate of 2.6%. The prevalence rate recorded in the present study is comparatively lower than that given by other investigators in Egypt (**Lotfy *et al.*, 1960, 6.9% ; Guindi *et al.*, 1965, 26.5% ; El- Sabban *et al.*,1992, 24% and El battawy 2008, 4.6**

% and in other countries of the world **Oliveira *et al.* 1983, 3.2% in Brazil ; Ameni and Erkihun, 2007 in Ethiopia 11.6 % ; Borna *et al.*, 2009, 8% in Chad.**

In the present study, the low incidence of infection could be attributed to many factors such as herd size, density of animals, breeding and management system, uncontrolled animal movements, unhygienic local habits and stress factors due to other diseases and mass vaccination against various diseases (**Abuo – Eisha *et al.*, 1995**). At the same time, the prevalence recorded in the present study is comparatively higher than that given by other investigators (**Shirma *et al.*, 2003**) and (**Cleaveland *et al.*, 2007**) in Tanzania, as it was 1.3 % and 0.9%, respectively. The high prevalence rate of tuberculin reactors, in general, is a function of different factors such as the past history of tuberculosis in the dairy herd (**Thornton and Gracey, 1976**), method of breeding and housing besides the susceptibility and age of the animals (**Guinidi *et al.*, 1980; Sharama *et al.*, 1985**).

On other hand, the number of non – visible lesion (NVL) reactors as shown in table (1) amounted to 5 animals (10%), a finding which may be attributed to the non-specific reaction to the tuberculin test which may be due to sensitization by other mycobacteria rather than *M. bovis* or even closely related microorganisms especially of the genus *Nocardia* or a combination of liver fluke infestation with saprophytic mycobacteria (**Karlson, 1962, Cortina and Vera, 1986**). Moreover, **O'Reilly (1992) and Huitema (1994)** ascribed the cause of non-specific reaction to the assumption that reactors may be slaughtered at stage of the disease where the tuberculous lesions are invisible or the lesions may be found in parts of carcass such as bone or skin.

As shown in table (1) the visible lesions found with an overall percentage of 90% which are higher than that reported by **Oliviera *et al.***

(1983), 75% ; **Zivkovic *et al.*(1984)**,75.2% and **Nasr (1997)**,73.4 %. On the other hand, other authors claimed a much higher percentage (**Kilian, 1982**, 96.3% in Germany and **El- Sabban, 1992**, 100% in Egypt).

Table (2) showed that the higher lesion severity was observed in the pulmonary lymph nodes (48%), this may be due to the intensive husbandry systems which make the respiratory excretion the main route by which animal – to – animal transmission occurs (**Smyth *et al.*, 2001**).

The total isolation rates of *M. bovis* from carcasses of tuberculin reactors with and without lesions demonstrated in table (4). From a total of 50 carcasses, 40 positive culture were recovered with an isolation rate of 80 %. The obtained results were near to that mentioned by **Tammemagi *et al.* (1973)** (89.1 %) and **Naglaa (2008)** (70.59 %). Other investigators reported lower *M. bovis* recovery rate, by **Adawy (1986)** (17.5 %) and **Zschoc *et al.* (1990)** (1.6 %). These results depend mainly on the actual disease status present in the tested herd and to some extent on the experience of the investigators as well as the technique used for decontamination of tissue specimens. Additionally the harsh decontamination which was used to destroy contaminating bacteria other than mycobacteria in a sample may also have a harmful effect on the *M. bovis* causative organism (**Victor *et al.* 1992** and **Quinn *et al.*, 1994**).

It is cleared from table (3) that 21(42%) of tuberculin reactor cattle were positive with Ani- gen Rapid Bovine TB kit. While, 29 (58%) were negative with Ani- gen Rapid Bovine TB kit. The obtained results indicated the difference between the tuberculin test and Anigen Rapid Bovine TB kit for detection of tuberculosis in cattle. The Negative Anigen Rapid Bovine TB kit explained by the fact that low titer of antibodies to mycobacterial antigens which may be associated with heavy infection and that antigens may be released into the blood circulation and cause temporary suppression of antibody formation **Krambovitis (1986)**

and that agree with **Thorns and Morris (1983)** who cleared the level of specific anti- bodies in many *M. bovis* infected cattle may be low or undetectable. Again this is supported with **Amadori *et al.* (1998)** who pointed that antibodies to mycobacterial antigens were investigated with various rates of success since the humeral immune response to *M. bovis* is late and irregular during the course of the disease.

Table (4) showed that the Anigen Rapid Bovine TB AB Test Kit has detected 42% of tuberculin positive cattle. While the ELISA with B-PPD antigen has detected 48 % of tuberculin positive reactor cattle. The results agreed with those of **Danbirni *et al.* (2013)** who mentioned that the antigen rapid bovine TB Ab test alone is not efficient in diagnosis of TB and that the serological tests like ELISA must be used to validate results and disagreed with **Danbirni *et al.* (2009)** who found that (62%) of cows gave positive in antigen rapid bovine test TB Ab test. Moreover, **Kalaf *et al.* (2014)** showed that out of (28) cows examined with comparative tuberculin test, (21) cows showed positive tuberculin reactions (75%) and twenty two cow with a percentage of (78.57%) showed positive results for antigen rapid bovine TB.

The differences may be attributed to that the humoral immune test using the Enzme Linked Immunosorbent Assay (ELISA), immunochromatography (lateral flow) assay and other serologic based test may complement test of cellular immunity in anergic hosts (**Awah-Ndukum, 2010**).

Conclusion: It could be concluded that the Anigen Rapid Bovine TB Ab kit test is rapid, safe, simple and easy to perform and provide yes or no results within 15 to 20 minutes but alone it is not efficient for detection of bovine tuberculosis in cattle and may be useful as a complementary test for tuberculin test in some cases as in the late stages of the disease (anergic cattle).

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Table (1): Results of tuberculin skin test and post mortem finding of slaughtered tuberculin positive cattle.

No. of tested cattle	Reactor cattle		PM finding			
			Visible Lesion		Non Visible Lesion	
	No.	%	No.	%	No.	%
1900	50	2.6	45	90	5	10

PM: Postmortem.

No.: Number.

Table (2) Results of postmortem finding in 50 slaughtered tuberculin reactor cattle according to the site of lesion.

Reactor cattle		PM finding									
		VL (45)								NVL(5)	
		Head		Pulmonary		Digestive		Generalized		No	%
No.	%	No.	%	No.	%	No.	%	No.	%		
50	2.6	6	12	24	48	10	20	5	10	5	10

VL: Visible Lesion

NVL: Non Visible Lesion

PM: Postmortem.

Table (3): Results of Anigen Rapid Bovine TB Ab test kit from tuberculin reactor cattle in comparison to the type of lesions.

PM finding	Number of positive tuberculin reactor	Anigen Rapid Bovine TB kit			
		Positive		Negative	
		No.	%	No.	%
Visible	45	20	44.4	25	55.6
Non visible	5	1	20	4	80
Total	50	21	42	29	58

PM: Postmortem. TB: Tuberculosis.

Table (4) Comparison between the results of bacteriological isolation, ELISA and Anigen Rapid Bovine TB Ab test kit on samples obtained from tuberculin positive animals.

PM finding	Sites of lesions	N0	<i>M. bovis</i> isolates		ELISA		Ani gen Rapid Bovine TB kit	
			No.	%	No.	%	No.	%
I. Visible lesions	1. Local							
	a. Head	6	4	66.7	2	33.3	2	33.3
	b. Pulmonary	24	22	91.7	12	50	10	41.7
	c. Digestive	10	7	70	4	40	3	30
	2. Generalized	5	5	100	5	100	5	100
Sub total		45	38	84.4	23	51.1	20	44.4
II. Non visible lesions	Congestion in L.N.	5	2	40	1	20	1	20
Total		50	40	80	24	48	21	42

B-PPD: Bovine Protein Purified Derivatives. TB: Tuberculosis.

مقارنة اختبار التيوبركلين فى الجلد والأختبار السريع للكشف عن السل البقرى فى ماشية الألبان.

أ.د/ عصام أمين نصر* ، د/ مروة محمد عبد المنعم* د. ليليان فرج سعد مليكة* ، د/ عبير عطية تمام* ،

د/ سهام فؤاد جورجى**

*معهد بحوث الامصال واللقاحات البيطرية ** معهد بحوث صحة الحيوان (فرع بنها)

يعتبر مرض السل من أهم الأمراض البكتيرية المشتركة بين الانسان والحيوان. وعلى الرغم من الجهود المبذولة للقضاء علىه فانه مازال متسببا فى خسائر للثروة الحيوانية و لذلك فانه من المهم سرعة ودقة الكشف عنه لمنع إنتشار المرض وفى هذه الدراسة تم أختبار عدد (١٩٠٠ حيوان) باستخدام اختبار التيوبركلين المفرد فى الجلد وقد أسفرت النتائج عن وجود ٥٠ بقرة ايجابية بنسبة ٢,٦ % وقد تم ذبحهم وعند إجراء الصفة التشريحية لهم كانت الحيوانات التى بها إصابة سلبية ظاهرية ٤٥ بنسبة ٩٠ % بينما الخمسة الباقية بنسبة ١٠ % لم يظهر بها اصابة سلبية ظاهرية.وبالفحص البكتريولوجى لهم تم عزل وتصنيف عدد ٤٠ بنسبة ٨٠ % عترة للميكوبكتريم بوفس. وكان من نتائج أستخدام إختبار Anigen Rapid Bovine TB Ab الكشف عن ٢١ من الحالات الإيجابية للتيوبركلين بنسبة ٤٢ % بينما عند استخدام اختبار الإليزا تم الكشف عن ٢٤ بنسبة ٤٨ %. ونستنتج من النتائج السابقة أن أختبار اللاتيرال فلو سريع وأمن وبسيط وسهل التطبيق ويعطى نتائج إيجابية او سلبية خلال ١٥ - ٢٠ دقيقة ولكنة ليس كافيا ولايعتمد علىه بمفرده للكشف عن السل البقرى فى الحيوانات ولكنة يمكن أستخدامه كإختبار مكمل لإختبار التيوبركلين فى المراحل المتأخرة للمرض.