

Evaluation of blood based tests for detection of bovine tuberculosis in Suez and Ismailia Governorates

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Abstract

In the present study, a total of 1933 dairy cattle (1340 from Ismailia and 593 from Suez governorate) were subjected to tuberculin test. Sixty five serum samples as well as whole blood samples were collected without anticoagulant and with anticoagulant (heparine) from positive reactors before being slaughtered for ELISA technique and Gamma interferon assay respectively. By using ELISA technique, the sensitivity of PPD-B antigen was 91.4% and 86.6% at Ismailia Governorate and Suez Governorate respectively, while the sensitivity of the commercial mixture antigen was 94.3% and 90% at Ismailia and Suez respectively. On the other hand, the sensitivity of gamma interferon assay was 97.1% and 96.6% at Ismailia and Suez governorates respectively and the interpretation of the result of different techniques was discussed.

Keywords: Bovine tuberculosis, ELISA, Gamma Interferon.

Introduction

Bovine tuberculosis caused by *Mycobacterium bovis* (*M. bovis*) which belongs to Mycobacterium tuberculosis complex (MTC), is a chronic debilitating disease of animals characterized by formation of nodular granulomas in different body tissue (Thoen and Barletta, 2006).

The disease poses a threat to animals as well as humans either by close contact to infected animals or consumes infected products. It is recorded that about 85% of the animal population and 82% of the human population in areas where bovine tuberculosis control are inadequate or unavailable (WHO, 2002).

The tuberculin skin test (OIE, official test) is the oldest immunological test and still used for diagnosis of tuberculosis among animals and humans.

Despite the test has a lot of problems as the tuberculin test gave positive reactions in animals infected by non- tuberculous mycobacteria. This non-specificity is due to the presence of common antigens in all mycobacteria and

these antigens are skin reactive. In addition to the problem of false positive reactions, there are also false negative reactions. Due to these limitations various alternative tests should be used for diagnosis of bovine tuberculosis (Bezoz *et al.*, 2014).

An accurate serological test required to determine the presence of antibodies against different antigens by ELISA technique (Diaz-Otero *et al.*, 2003).

The Interferon- gamma release assay (IFN- γ) is advised to replace the tuberculin test for diagnosis of tuberculosis as IFN- γ assay can overcome many drawbacks of tuberculin skin test such as application obstacles and false reading (Abd ellrazeq *et al.*, 2014).

So, This study was aimed to follow up tuberculin positive cattle and making comparison between the interpretation of the ELISA technique using different antigens and standardize the IFN- γ assay as a complementary test for diagnosis of bovine tuberculosis.

Materials and Methods

Samples:

Animals: A total of 1933 dairy cattle (1340 from Ismailia governorate and 593 from Suez governorate).

Serum Samples: about 10 ml of blood were collected aseptically from life cattle then left at room temperature for 2 hours, then kept at 4°C.

Whole blood Samples: About 5ml of whole blood were collected from life cattle aseptically in to heparinized tubes for gamma interferon assay.

Single Tuberculin Test: Was done according to (OIE 2013).

Serological examination:

ELISA procedures according to : (Aagaard *et al.*, 2006) as follow: Flat bottom maxi sorb microtitre plates were coated by one of the previously mentioned antigens which diluted (1:1000 carbonate bicarbonate buffer) at PH 9.6, then incubated at 37°C over night. The plates washed 3 times with wash solution (KPL®) then the plates blocked by using bovine serum albumin (KPL®) which diluted (1:10 distilled water) and put 100 µl/well then incubated at 37°C for 1 hour. The previously collected serum diluted (1:20 ELISA diluent) (BSA 1:15) and put 100 µl/well of plates then incubated at 37°C for 1 hour. Then the plates washed 3 times by ELISA washing solution (KPL®). Add 100µl Goat anti bovine IgG horseradish peroxidase conjugate (KPL®) /well then incubate the plates for 1hours at 37°C, then washed the plates 3times with ELISA washing solution. Add 100µl from freshly prepared ABTS substrate /well and then incubate the plates in for 15 minutes. The recorded results obtained at optical density (OD) 450 nm by using spectra III reader.

Antigens:

Bovine purified protein derivative antigen (PPD - Bantigen): Obtained from bacterial diagnostic products department, Veterinary Serum and Vaccine Research Institute (VSVRI), Abbasia, Cairo, Egypt.

Commercial mixture antigen: (Prionics®, AG Schlieren, Switzerland) provided by bovine tuberculosis unit, bacteriology department, Animal Health Research Institute (AHRI),

Dokki, Giza, Egypt.

Gamma interferon assay was carried out according to (Riad *et al.*, 2014): By using gamma interferon assay kit and sensitizing antigens (Sensitizing PPD-B and Sensitizing PPD -A- Prionics - Germany). The test was applied on the whole blood samples of tuberculin positive. The test was standardized as the instruction of the kit.

Stage one – whole blood culture method:

Blood samples collection: A minimum 5 ml of blood from each cattle into a heparinized collecting tube with mix the blood and invert it for several times. The sample should be transferred to the lab within 3 hours.

Dispensing the blood: Dispense three 1.5 ml aliquots of heparinized blood from each animal into wells of a 24 well tissue culture tray.

Addition of antigens: add 100 µl of PBS as negative control, PPD-A and PPD-B to 3 wells containing the blood previously dispensed in step 1. Shake the plate ten times clockwise or anticlockwise but avoid frothing the blood.

Incubation: Incubate the mixture of blood and antigens included in tissue culture tray for 24 hours at 37°C

Harvesting the plasma: plasma obtained by centrifugation the tissue culture tray at 500 rpm for 10 min. after incubation at room temperature. Each sample should be duplicated.

Stage two - Bovine IFN-γ.

Reconstitute freeze dried components

Add 50 µl of green diluent to every well.

Add 50 µl of tested and control samples to the wells containing green diluents. Mix the plate for 1 min.

Incubate at room temperature on a plate shaker at 600 rpm for 60 min.

Wash the plates for 5- 6 times by washing buffer solution and dry it well.

Add 100 µl of freshly prepared Goat anti-bovine IgG horseradish peroxidase conjugate to each well.

Incubate at room temperature on a plate shaker at a setting of 600 rpm for 60 min.

Wash the plates for 5- 6 times by washing buffer solution and dry it well.

Add 100 µl of freshly prepared enzyme sub-

strate solution to each well then mix the plate for 1 min

Incubate at room temperature on a plate shaker at a setting of 600 rpm for 60 min.

Add 50 µl of stopping solution to each well with gentle agitation.

Read the absorbance of each well within 5 min. The absorbance values will then be used to calculate results.

The interpretation results of Gamma interferon assay according to the following equation:

Positive= OD bovine PPD - nil antigen $\geq$ 0.1; and

OD bovine PPD- avian $\geq$ 0.1

Negative = OD bovine PPD - nil antigen $\leq$ 0.1; and

OD bovine PPD- avian $\leq$ 0.1

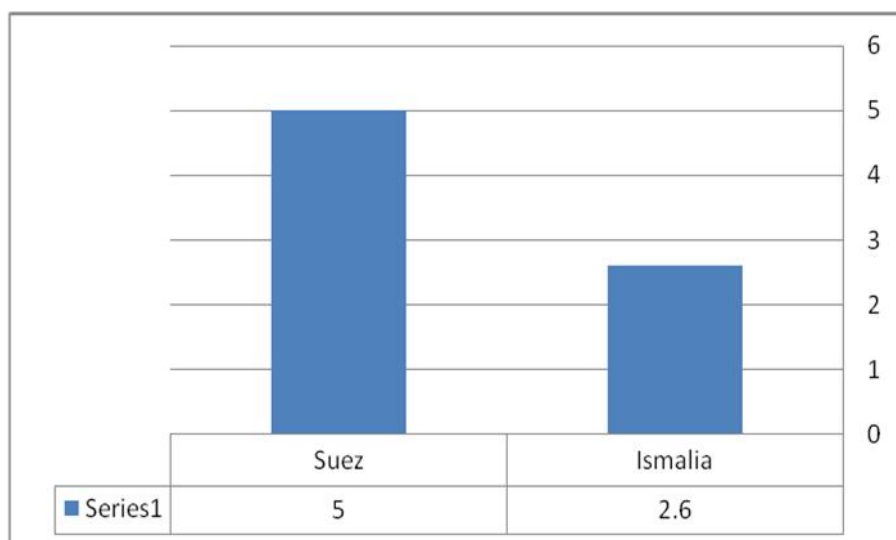
Results

Results shown in table (1) and Fig. (1) recorded the prevalence of bovine tuberculosis in Suez Canal area. The highest prevalence recorded in Suez Governorate (5%). While the lowest prevalence of tuberculin skin test in Ismailia Governorate was (2.6%).

Table (1). Results of tuberculin skin test in different dairy cattle farms at Ismailia and Suez Governorates.

Governorate	No. of tested cattle	Positive tuberculin reactors	
		No.	%
Ismalia	1340	35	2.6
Suez	593	30	5

Fig. (1). Percentage of positive tuberculin skin test at Ismailia and Suez governorates.

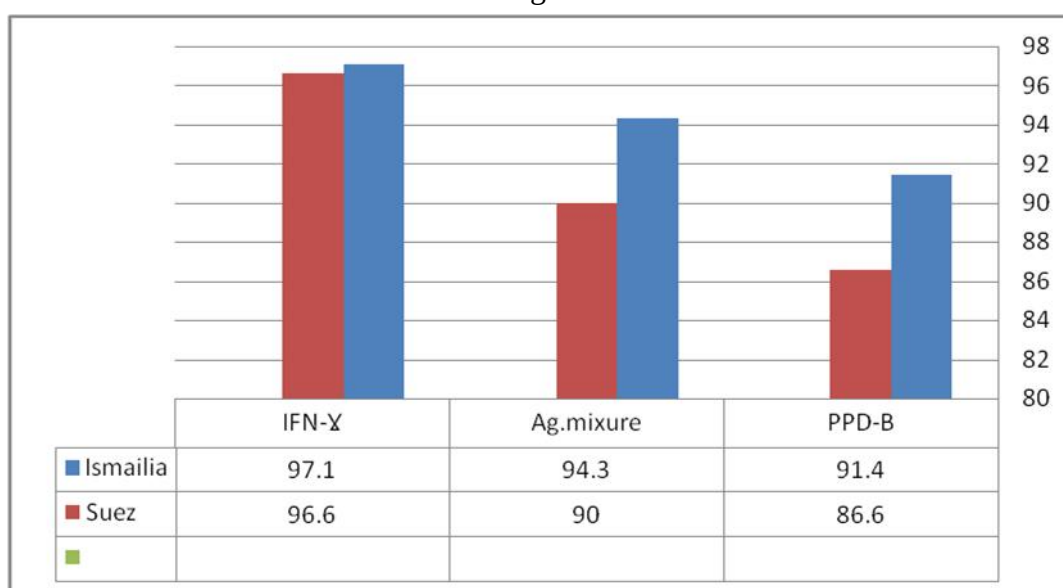


Results shown in table (2) and fig. (2) revealed that by using ELISA technique on the serum samples of tuberculin positive reactors before being slaughtered from different governorates showed that the sensitivity of commercial mixture antigen were 94.3% and 90% at Ismailia and Suez respectively. While the sensitivity of PPD - B antigen were 91.4% and 86.6% at Ismailia and Suez Governorates respectively. On

the other hand, by using gamma interferon assay on whole blood samples of tuberculin positive reactors before being slaughtered from different governorates was 97.1% and 96.6% at Ismailia and Suez respectively.

Table (2). Results of ELISA and gamma interferon results on tuberculin positive cattle at Ismailia and Suez governorates.

Governorates	No. of tuberculin positive cattle	ELISA				Gamma Interferon assay	
		Bovine PPD		Ag. Mixture			
		+ve	%	+ve	%	+ve	%
Ismalia	35	32	91.4	33	94.3	34	97.1
Suez	30	26	86.6	27	90	29	96.6

Fig. (2). Comparison between Gamma interferon assay and ELISA technique using different anti-gens.

Discussion

Bovine tuberculosis poses a threat to animals as well as humans as it cause severe economic loses and has public health importance. Sorapid identification of bovine tuberculosis in infected cattle is important to eliminate the infected animals from a herd and prevent transmission of the disease (Riad *et al.*, 2014)

The result recorded in table (1) and fig (1) showed the high prevalence of tuberculin cattle in Suez governorate (5%) than that obtained by other investigation in Egypt (Ereny, 2011 (2.2%) and Dandash, 2012 (2.3%)). It could be attributed that the highest prevalence in large herds then in medium herds while, small herds showed the lowest prevalence, density of animals (Freddy *et al.*, 2006) .Also another

reason of high prevalence is sensitizations as a result of the presence of *Corynebacterium*, *Fasciola hepatica* and some *Nocardiaspp.* (Bezoz *et al.*, 2014).

Low prevalence was found in Ismailia governorate (2.6%) than that given by El Battawy, 2008 (4.6%) while these results agree with those obtained by (Ereny, 2011 (2.3%)). This low prevalence may be due to that farms apply tuberculin test regularly. Also there are many factors which could cause false negative as in newly infected cases (Diaz-Otero *et al.*, 2003). In this study, as shown in table (2) and fig (2) revealed that the sensitivity of commercial mixture antigen were 94.3%and 90% at Ismailia and Suez Governorates respectively. While the sensitivity of PPD-B antigen were

91.4% and 86.6% at Ismailia and Suez Governorates respectively. The results were in agreement with that obtained by (Ereny, 2011) who reported that ELISA by using cocktail antigen could detect 95.3% of the tuberculin positive cattle showed lesions in the post mortem examination compared to (90.6) by PPD-B. And these results disagree with that obtained by (El-Seedy *et al.*, 2013) who stated ELISA by ESAT6-CFP10 mixture could detect 83.7% of the tuberculin positive cattle compared to 89.8% with the PPD-B antigen.

The commercial mixture antigen consists of ESAT-6 antigen which released during early stage of infection by bovine tuberculosis. ESAT-6 not presents in environmental mycobacteria spp. and absent in strains of *M. bovis* BCG. MPB-70 antigen considered another extracellular antigen which present in *M. bovis* and minimally present in *M. tuberculosis*, also it presents in MTC (Pollock, 2006).

False negative ELISA results due to the low titer of antibodies produced against mycobacterial infection, which may be due to heavy infection and those different antigens may be found in blood circulation and cause suppression of antibody production (Amadori *et al.*, 1998). While the false positive ELISA results due to greater number of antigens of PPD, so PPD-B more sensitive (Fernando *et al.*, 2003). The results shown in table (2) and fig. (2) revealed that the sensitivity of gamma interferon assay were 97.1% and 96.6% at Ismailia and Suez Governorates respectively. These results agree with that obtained by (Riad *et al.*, 2014 and Abd ellrazeq *et al.*, 2014). The advantages of the IFN- γ assay are increase the sensitivity, the possibility of more rapid repeat testing, and no need for a second visit to the farm and more objective test procedures

The results obtained in this study suggested that the tuberculin test and the IFN- γ assay working in parallel might be the best option for disease detection and control as mentioned by (Abd ellrazeq *et al.*, 2014), both the tuberculin test and IFN- γ assays have advantages and limitations, and both tests appear to be useful.

No one of them suits all conditions.

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تقييم طرق الكشف عن السل البقري على أساس إختبارات الدم في محافظتي السويس والإسماعيلية محمد السيد عناني ، **عماد مختار رياض ، *على وهدان على إبراهيم

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المخلص العربى

في هذه الدراسة تم فحص عدد ١٩٣٣ من ماشية الألبان (١٣٤٠ حالة من محافظة الإسماعيلية و٩٥٣ من محافظة السويس) باختبار التيوبركلين. وتم تجميع ٦٥ عينة من السروم وكذلك من الدم لفحصها باختبار الإليزا والجاما انترفيرون على التوالي. وعند استخدام اختبار الإليزا كانت حساسية الاختبار باستخدام PPD-B (٩١.٤% و ٨٦.٦%) في محافظات الإسماعيلية والسويس على التوالي، في حين بلغت حساسية خليط الانتجينات التجارية (٩٤.٣% و ٩٠%) في محافظات الإسماعيلية والسويس على التوالي. ومن ناحية أخرى كانت حساسية اختبار الجاما انترفيرون (٩٧.١% و ٩٦.٦%) في محافظات الإسماعيلية والسويس على التوالي وقد تضمنت الدراسة ضرورة الماسة لاستخدام الجاما انترفيرون كوسيلة تشخيصية بالإضافة الي اختبار التيوبركلين بالتوازي للكشف عن العدوى بمرض السل في المزارع.