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COMPARATIVE STUDY OF TUBERCULOSIS SURVEILLANCE USING GAMMA INTERFERON DETECTION IN DAIRY FARMS IN EGYPT

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ABSTRACT

Bovine tuberculosis is significant infectious disease that produces large economic losses as well as is considered one of the major public health concerns. Therefore, there is an urgent need for improved methods to combat bovine tuberculosis. Skin testing with tuberculin and slaughter of test-positive cattle has been the mainstay of national eradication programmes for many years. However, new tools, including additional diagnostic methods, are required in countries where skin testing has not led to full eradication. Diagnostic blood tests are now available, e.g. the gamma interferon (γ-IFN) assay, which uses an ELISA as the detection method for interferon. In this study, 5700 cattle representing different 15 dairy farms were examined by the single intradermal comparative tuberculin skin test (SICTT). Both positive and suspected animals were tested for serum γ-IFN. Our finding revealed very high correlation between SICTT and γ-IFN testing results where γ-IFN showed 98.3% positive out of total 121 animals were positive for SICTT. Moreover, 35 animals that were suspected by SICTT, demonstrated positive results in 33 animals (94%) when their sera tested for γ-IFN. Interestingly, these suspected animals when retested by skin test after 60 days; they showed 30 positive reactor cattle. Thereby, it was concluded that use of γ-IFN in surveillance for TB in cattle farms is reliable, time-consuming test and can minimize dissemination of infections that could be resulted when keeping many suspected animals for months until retesting.

Key words:

Bovine tuberculosis; tuberculin skin test; gamma interferon; BoviGam®; γ-IFN ELISA

INTRODUCTION

Bovine Tuberculosis (bTB) is a chronic infectious disease that affects a broad range of mammalian hosts, including humans, cattle, deer, pigs, domestic cats, wild carnivores and omnivores. The disease can be transmitted in several ways; by direct contact, contact with the excreta of an infected animal, or inhalation of aerosols, depending on the species involved **(Phillips *et al.,* 2001; Delahay *et al.,* 2002)**. World Health Organization **(WHO, 2013)** identified bTB as one of the eight worldwide-neglected zoonosis that need more attention; especially in developing countries. In Egypt, bTB infections represent a very high percentage among zoonotic diseases **(OIE, 2009)**. Disease control programs implemented in many countries are based on the testing of cattle with PPD-tuberculin and slaughter **(Monaghan *et al.,* 1994).** .Ante-mortem testing is primarily based upon measures of cell-mediated immunity such as tuberculin skin test (e.g. Caudal fold test (CFT),single intradermal comparative tuberculin test (SICTT) and gamma interferon (γ-IFN) release assays **(De la Rua-Domenech *et al.,* 2006).** In worldwide, intradermal tuberculin test is used as standard method for detection of bovine tuberculosis **(Cagiola *et al.,* 2004)**. It has been reported that, the use of two tests together could assist to the early detection of bovine tuberculosis in infected cattle **(Gonzales-Llamazares, 1999)**. Gamma IFN is approved as an official test for diagnosis of bovine tuberculosis in New Zealand and Australia. It has been approved also that it can be used together with intradermal tuberculin test in eradication programs of bovine tuberculosis in many countries **(Palmer, 2006)**. In Egypt, bTB in cattle is regarded as one of the most serious animal health problems since cattle, being a major source of meat and milk, play an important role in both economic and social life. The prevalence of bTB in cattle and buffaloes during the 1980s ranged between 6.9% and 26.2% and it was reduced to 2.6% during the 1990s **(WHO, 1994).** According to a recent official report of the **General Organization of Veterinary services (GOVS) (1999)**, the annual proportion of bTB-infected cattle has increased, with the import of live animals from countries where bTB is prevalent and is recognized as a potential source of bTB transmission into Egypt. The current programme in Egypt relies on two methods of detecting bTB. The first is skin single cervical tuberculin, testing of cattle through the brucellosis and bTB surveillance programme that mainly covers the individual cattle of smallholders. The second method is

through slaughter surveillance that is entirely based on meat inspection at the slaughterhouse **(Corner, 1994).** In our study, we tried to evaluate use γ-IFN release assay as parallel testing for bTB monitoring in the field.

MATERIAL AND METHODS

Single Intradermal Comparative Tuberculin test: (OIE 2009). A total number of 5700 cross breed cattle were examined by SICTT test as follows; two narrow zones at the middle third of the neck of the tested animals were marked by clipping the hair using curved scissors with rounded ends. The skin thickness was measured using the caliper. Using an automatic syringe 0.1 ml of Bovine tuberculin was injected intradermal at the upper zone and 0.1 ml of avian tuberculin at the lower zone. The skin thickness was measured 72 hours post injection and the results were interpreted according to GOVS as follows; an increase in the skin thickness of 4 mm or more was considered positive, less than 3 mm was considered as negative, and from 3-4 mm was considered as doubtful. Gamma IFN Assay ***(Coad et al.,* 2008),** using BoviGam® ELISA kit. The procedure was applied according kit manual and brochure; however, it could be summarized as follows. A lithium heparinized blood sample (5 ml) was collected from each animal before application of the intradermal tuberculin test and brought to the laboratory within 8 h of collection. Blood samples collected from each animal were dispensed in 3x1.5 ml into a 24 well tissue culture plate. Then, 100 µl nil antigen (Phosphate Buffered Solution) as non-stimulating control to the first well, 100 µl bovine PPD to the second well and 100 µl avian PPD to the third well of each sample were added, and the plates were incubated in humidified atmosphere at 37°C for 16-24 hours. Then, plasma samples were harvested from the cultures and tested with the Bovigam ELISA test kit (Prionics, Switzerland) according to the instructions supplied with the kit. The samples in the ELISA were run in duplicate. Positive and negative controls were used in each plate. The absorbance within 5 min of terminating the reaction was recorded using a 450 nm filter. The mean absorbance values of positive and negative controls were determined for the test validation and compared with positive and negative values provided by the kit for validation of the test (negative bovine γ-IFN control<0.130; positive bovine γ-IFN control>0.700). The mean nil antigen, avian and bovine PPD optical density (OD) for each sample were calculated and compared with the mean absorbance values of the nil antigens, avian and

bovine PPD controls. A sample was considered as positive when the difference between OD value of a sample stimulated with bovine PPD and OD value of the same sample stimulated with avian PPD and nil antigen is equal or higher than 0.100. A sample was considered as negative when this difference is less than 0.100.

RESULTS

Results skin test (SICTT) .Thirteen out of 15 farms, in this study, were positive when tested by Skin test (Table 1). No farm was 100% negative for SICTT, while there is only one farm showed only suspected results in 1% of the tested animals. Out of 5700 cattle, only 2.1% of them were positive. Meanwhile, 0.6% (35 animals) were suspected and undergo re-testing after 60 days.

Table (1): Results of SICTT in tested dairy farms

Farm	No. of animals	SICTT (skin test)							
		Negative (-)		Positive (+)		Suspect (±)		Total (+ and ±)	
		No.	%	No.	%	No.	%	No.	%
1	650	633	97.4%	13	2.0%	4	0.6%	17	2.6%
2	850	826	97.2%	19	2.2%	5	0.6%	24	2.8%
3	450	436	96.9%	11	2.4%	3	0.7%	14	3.1%
4	400	386	96.5%	12	3.0%	2	0.5%	14	3.5%
5	200	298	99.0%	0	0.0%	2	1.0%	2	1.0%
6	150	143	95.3%	7	4.7%	0	0.0%	7	4.7%
7	300	288	96.0%	10	3.3%	2	0.7%	12	4.0%
8	400	392	98.0%	6	1.5%	2	0.5%	8	2.0%
9	250	241	96.4%	8	3.2%	1	0.4%	9	3.6%
10	750	730	97.3%	15	2.0%	5	0.7%	20	2.7%
11	450	440	97.8%	7	1.6%	3	0.7%	10	2.2%
12	200	298	99.0%	0	0.0%	2	1.0%	2	1.0%
13	250	243	97.2%	5	2.0%	2	0.8%	7	2.8%
14	210	205	97.6%	4	1.9%	1	0.5%	5	2.4%
15	190	185	97.4%	4	2.1%	1	0.5%	5	2.6%
Total	5700	5544	97.3%	121	2.1%	35	0.6%	156	2.7%

Results of serum γ-IFN detection (BoviGAM)

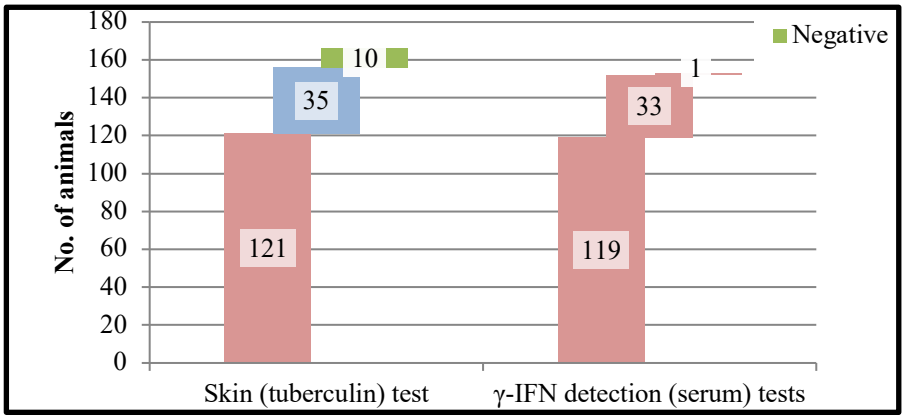
All animals that were tested by BoviGam showed positive results (121) and suspected (35) for skin test (Table 2), Fig. (2) showed 98.3% and 94% γ-IFN positive in both positive and suspected skin tested animals respectively.

Table (2): Results of γ-IFN detection in proportion to skin test examined animals.

SICTT tested Animals		γ-IFN results			
		Positive (+)		Negative (-)	
Results	No.	No.	%*	No.	%*
Positive	121	119	98.3%	2	1.7
Suspected	35	33	94%	2	6%

*** In relation to results of SICTT tested animals.**

Fig.(2): Chart showing positive results of serum γ-IFN detection according to skin test examined animals.



Results of skin test suspected animals (SICTT and serum γ-IFN detection tests). results of suspected animals that primary tested by skin test showed 86% (of total 35 cattle become positive when tested again by skin test. While 94% were positive when their sera tested for γ-IFN detection (Table 3), Fig. (3).

(Table3): Results of Skin test (SICTT) and (γ-IFN) of re-testing of suspected animals (after 60 days).

Tuberculin suspected animals	Skin (tuberculin) re-test				γ-IFN detection tests			
	Positive		Negative		Positive		Negative	
	No.	%	No.	%	No.	%	No.	%
35	30	86%	5	14%	33	94%	2	6%

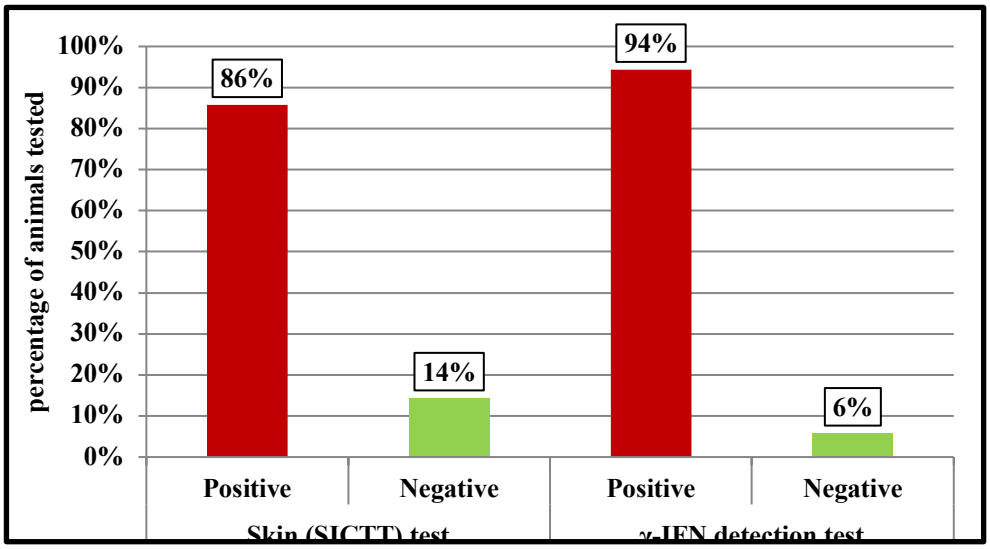


Fig. (3): Chart showing correlation between results of Skin test (SICTT) and (γ-IFN) of re-testing of suspected animals (after 60 days)

DISCUSSION

Bovine tuberculosis is a significant infectious disease that produces large economic losses as well as is considered one of the major public health concern **(Pollock *et al.*, 2005)**. Skin testing with tuberculin and slaughter of test-positive cattle has been the mainstay of national eradication programs for many years **(Adams, 2001)**. In spite of its wide use, the intradermal tuberculin test has some important limitations, related to its sensitivity and specificity **(Whipple *et al.*, 1995and Rua Domenech *et al.*, 2006)**. The sensitivity of the IFN-γ assay is significantly greater than that of the tuberculin test **(Rothel *et al* 1992 and Cousins *et al* 1998)**. The IFN- γ release assay was developed to aid in the diagnosis of bovine tuberculosis and is currently used mainly as a supplemental assay to the skin test in most TB eradication/control programs **(Wood,2001)**. IFN- γ is a good biomarker for use in tuberculosis diagnostic tests **(Mihret *et al.*, 2012)**. This study aimed to evaluate IFN-release assay compared to skin test for bovine tuberculosis in field cases ofEgyptian cattle **(OE, 1993)**. The primary skin test (SICTT) which applied on dairy farms in this study showed high percentages of TB positive animals among Egyptian cattle as reported before **(Naser *et al.*, 2008 and Hassanain *et al.* (2009)** . Whereas there were 13 out of 15 flocks showed positive results as well as no farm was 100% negative (two flocks only were suspected), a close similar finding was recorded. When the plasma of same animals were examined for

γ-IFN release, it showed very high sensitivity to detect positive SICTT tested animals (98.3%) when compared to skin test in parallel with previous results as **(Downs *et al.,* 2011, *(*** 96%) and **Cockle *et al.,* 2006, *(*** 96.6%). Moreover, these suspicious animals when retested by SICTT after two months (according to testing regulations FAO 1993), they showed 86% positive reactors (Table 3). Interestingly, γ-IFN release assay was able to detect 98% positive animals, 60 days earlier (Table 2) when they primary tested for both SICTT and γ-IFN. The suspicious animals were observed in most farms (13 out of 15 farms), as a result, long time and cost losses (housing, managing and feeding…etc.) could obtained until confirm these suspicious animals. In addition, the suspicious animals should be isolated away and retested after 60 days **(OIE1993);** if no proper isolation, there is a high possibility of disease transmission from suspected animal (in case it were infected) to the healthy one; hereby, significance of γ-IFN detection is understandable to avoid this risk. One of the important advantages of γ-IFN testing, it can detect the TB infection in the animal when occurred as early as 14 days **(Gonzales-Llamazares, 1999; Ryan *et al.,* 2000)**. This may explain the positive results of skin test suspected animals that required more time to show positive by SICTT in this study until reach enough immune response for skin reaction **(Dean *et al.,* 2005)**. The slight increase in positive results by γ-IFN more than skin test at repeated testing after 60 days could resulted from these reactor animals are still in the incubation period or slight less specificity **(Gormley *et al.*2004)**. In conclusion, this study with consistence of other studies, verified high sensitivity of γ-IFN to be used for bTB surveillance of cattle. farms. One of the practical drawbacks of skin test is coming out when interpreting the result The results are obtained within less 24 hours including blood sampling without operators' errors which common in skin test .In addition to time, monetary saving, and avoid risk of infection spread.

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