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Evaluation of ELISA and Gamma Interferon Techniques for Diagnosis of Tuberculosis in Cattle

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Abstract

In the present study, two groups (I and II) of animals were tested. (Group I) composed of 170 tuberculin reactor cattle and out of them 88 animals (51.8%) were positive to bTB by microscopic examination of direct smears while by culture on L-J medium (4% pyruvate) *M.bovis* was isolated from 114 animals (67.1%). The bacteriological examination of tissue samples collected from different organs showing tuberculous lesions from (group I) (170 lymph node, 40 lung, 34 liver and 20 spleen samples) revealed that by microscopic examination spleen showed the highest percentage of positive results, 55% (11/20), followed by lung 52.5% (21/40) then lymph nodes 51.8% (88/170) and finally liver at a percentage of 50% (17/34), also by culture method Spleen had the highest percent of positive results, 70% (14/20), followed by lung, lymph node and liver in a percentage of 67.5% (27/40), 67.1% (114/170) and 61.8% (21/34) respectively. On the other hand, (group II) composed of 200 animals which were subjected to SICTT on two phases, out of them 26 animals were positive reactors, 14 from the first phase and 12 from the second one, of them 19 (73.1%) animals were positive to bTB by culture on L-J

(4% pyruvate). Serum samples were collected from (group II), before being tested by SICTT, to be tested by ELISA and results revealed that out of the total 200 animals, 20 (10%) animals were positive to bTB when using PPD-B as coating antigen compared to 23 (11.5%) positive animals by using a commercial mixture antigen. The use of IFN-γ assay, on heparinized whole blood samples collected from (group II) before SICTT, detected 26 positive animals out of the total 200 animals at a percentage of (13%). By comparing results of the IFN-γ assay to results of the first phase SICTT, it appears that the IFN-γ assay could detect 12 positive cases that tested negative by the first phase SICTT, of them 9 animals were confirmed to be positive by the culture method. It can be concluded that the use of mixture antigen in ELISA helps giving more efficient diagnosis than using PPD-B antigen. Also it can be added that the IFN-γ assay detected more positive cases than other tests which indicates that it appears to be more sensitive than both SICTT and ELISA and should be used in parallel to SICTT to allow detection of more positive animals before they become source of infection to other animals and human.

Keywords: *M.bovis*, bovine tuberculosis, ELISA, Gamma interferon assay, SICTT

Dedicated with gratitude to

My mother

My father

My wife

My daughter Laila

My brother and sister

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INTRODUCTION

Bovine tuberculosis is a chronic debilitating highly contagious disease of cattle, buffaloes and many wild species **(Hardstaff *et al.*, 2013)**. It is characterized by the formation of tuberculous lesions which appear most frequently in lymph nodes, lung, liver, spleen, intestines, pleura and peritoneum **(OIE, 2009)**.

The disease causes severe economic losses, where it causes reduction of 10 - 20 % in milk production and weight in cattle, in addition to infertility and condemnation of meat. Without considering the death rate, there is 10 - 25 % loss of the productive efficiency of cattle **(Lilenbaum *et al.*, 1999(b))**. The economic impact is not only as a result of the disease directly on livestock, but also due to high cost of eradication programs and serious consequences for movements of animals and their products **(Rodriguez-Campos *et al.*, 2014).**

Bovine tuberculosis, as well as its economic impact, is a significant zoonoses where it can be transmitted to human either by inhaling infective droplets or direct exposure to infected animals (**Perez-lago *et al.*, 2013),** or by consuming raw milk, meat and their products from infected animals **(Malama *et al.*, 2013)**.

The diagnosis of bovine tuberculosis still have challenges where there is nowadays no single test that will meet all the criteria necessary to identify all infected animals **(Sam *et al.*, 2011)**. The methods for the diagnosis of tuberculosis in cattle could be expressed in two broad categories namely direct and indirect tests.

The direct tests depend on the detection and identification of the organism in host animal, which primarily relates to the post mortem examination of animals **(Sam *et al*., 2011),** and associated tests to confirm the infection by demonstration of acid fast bacilli by microscopic examination of smears stained with Ziehl–Neelsen stain, the isolation of mycobacteria on selective culture medium and their subsequent identification by cultural and biochemical tests **(OIE, 2009)**.

Although, the diagnosis based on isolation and identification of Mycobacterium is tedious and time consuming **(Cedeno *et al*., 2005)**, it remains the gold standard for tuberculosis diagnosis where its specificity assumed to be very high but the sensitivity is relatively low due to insufficient materials submitted for culture and failure of culture to isolate the organism when it presents at low levels **(Sam *et al*., 2011)**.

The Indirect tests depend on identifying and measuring the immune responses in animal to the *M.bovis* organism. Either those based on the cellular immune response (Tuberculin skin test and the interferon gamma assay) or those base on the antibody responses (**Sam *et al*., 2011)**.

The tuberculin test based on a delayed type hypersensitivity to mycobacterial tuberculoprotein **(Carter, 1984).** It is convenient, cost effective and it is "gold standard" for diagnostic screening for detection of new or asymptomatic *Mycobacterium tuberculosis complex* infection

(Katial, *et al*., 2001). But it has poor specificity due to cross reactions with other non-pathogenic mycobacteria **(Praud *et al*., 2014).**

Enzyme Linked Immunosorbent assay appears to be the most suitable of the antibody-detection tests for diagnosis of bovine tuberculosis can be used as a complement, rather than an alternative, to test based on cellular immune response **(OIE, 2009)**. ELISA is a valuable complementary tool in order to identify possible anergic cows that may act as reservoirs of infection (**Lilenbaum and Fonseca 2005)**. An advantage of the ELISA is its simplicity, but sensitivity is limited mostly because of the late and irregular development of humoral immune response in cattle during the course of the disease. Specificity is also poor in cattle when complex antigens such as tuberculin or culture filtrates are used. *M.bovis* has been shown to be useful in increasing the specificity in the ELISA. Improvement may be possible by using combination of different antigens including proteins which are specific but lack sensitivity (**Lilenbaum *et al*., 2011).**

As a result of the role of cytokines in tuberculosis immunology, the bovine interferon gamma (IFN-γ) production assay was developed which enables the detection of the release of IFN-γ in response to bovine PPD in a whole blood sample **(Rothel, *et al*., 1990; Wood and Jones, 2001)**. The advantages of the IFN- γ assay are the increased sensitivity, the ability of more rapid retesting and no need for a second visit to the farm. While reduced specificity, high logistical demands (culture start is required within 24 hours after blood sampling), increased likelihood of non specific response in young animals (due to

natural Killer cell activity) and its high cost may comprise some limitations of IFN-γ (**Wood and Jones, 2001; Cyrithia, 2003).** The IFN-γ test is used for serial testing (to enhance specificity) and parallel testing (to enhance sensitivity) **(OIE, 2009)**.

Since diagnosis of bovine tuberculosis is challenging, the aim of this study was directed to evaluation of ELISA and gamma interferon assay for diagnosis of tuberculosis in cattle which was carried out through:

- Follow up the positive cases of tuberculin test in the abattoir in some Egyptian Governorates.
- Isolation and identification of *Mycobacterium bovis*.
- Comparative study on ELISA, using different antigens and Gamma interferon assay.