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Sero-diagnosis of Bovine Tuberculosis by ELISA Using Bovine PPD and ST.CF

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Bovine tuberculosis represents one of the very important infectious diseases in Egypt and the world. It has zoonotic importance and causes severe economic losses. Accurate and rapid diagnosis considered as the milestone for control of the disease. In this study ELISA technique was used for confirmation of positive reactors cows that tested with single intradermal tuberculin test, to detect false positive reactors. Bovine PPD and ST.CF antigens have been used as two different coating antigens for ELISA technique. 3747 cattle from dairy farms in five different governorates were subjected to the single intradermal cervical tuberculin test whereas 78 (2.24%) proved positive reactors to tuberculin. These positive reactors tested with ELISA. 64 (82.05%) animals were positive by ELISA coated with ST-CF, while by using bovine PPD as coating antigen 58 (74.35%) animals were positive. The previous results indicated that ELISA test showed higher sensitivity and specificity using ST-CF as coating antigen than in case of bovine PPD coating antigen.

Bovine tuberculosis is a chronic bacterial disease of animals and man which, caused by *Mycobacterium bovis*. In a large numbers of countries bovine tuberculosis is a major infectious disease among cattle, other domesticated animals and certain wildlife population. Transmission to humans constitutes a public health problem (OIE 2009). Its economic losses worldwide are estimated to account for over $ 3 billion annually (Steele 1995)

Tuberculosis occurs in every country of the world and is of major importance in dairy cattle. This disease under strict control in most developed countries but is still a major cause of loss in many less well endowed countries. Apart from actual deaths, infected animals lose 10-25% of their productive efficiency (Radostitis *et al.,* 2007).

Mycobacterium tuberculosis complex, including *M. bovis*, *M. tuberculosis*, *M. africanum*, *M. canetti* and *M. Microti.* They are responsible for tuberculosis, as a chronic disease that represents both animal health problem and a serious public health problem in the world. The global prevalence of tuberculosis infection is estimated to be 1.7 billion persons or about one third of the world's population, a number which is expected to grow steadily (Angela *et al.,* 2006).

The current increasing incidence of tuberculosis in humans, particularly in immunocompromised humans, has given a renewed interest in the zoonotic importance of *M. bovis*, especially in developing countries and the ease and frequency of the spread of tuberculosis from animals to humans in an uncontrolled environment make this disease as the most important zoonotic problem. (Radostitis *et al.,* 2007).

Due to increase in the percentage of reactors, which are not in fact tuberculous, to the point where a more discerning test than that based on coetaneous hypersensitivity is required. Most of the tests tried so far have been serological ones. Their aim is to identify anergic animals and cases sensitized by some other bacteria. Serological tests including complement fixation, fluorescent antibody, direct bacterial agglutination, precipitin tests have been developed but have little potential value for the routine diagnosis of tuberculosis. Early enzyme-linked immunosorbent assay (ELISA) to crude mycobacterial antigens had limited value but an ELISA which examines antibody to defined antigens of *M. bovis* before and after skin testing

appears useful in detecting the non-specific reactors (Lightbody *et al.,* 1998).

Enzyme linked immunosorbent assay (ELISA) is one of the main important serological tests for diagnosis of bovine tuberculosis. ELISA technique was applied as a sensitive method for measurement of antibodies in sera of tuberculous animals (Engvall and Perlmann, 1972).

So in this study purified protein derivatives (PPD) and short term culture filtrate (ST.CF) were used as coating antigens for ELISA and compare between their efficiency in detecting tuberculos animals.

Material and methods

A total of 3474 mixed cattle Holishtin Frisian and Brown Swiss were examined in farms of five different governorates (El-Sharkia, Menoufia, Ismailia, El-Gharbia and El – Behyra) by single intradermal (SID) as a cervical tuberculin skin test According to Ovdienkop *et al.,* (1987) by using mammalian PPD tuberculin which was prepared and obtained by Bacterial Diagnostic Products Research Department, Veterinary Serum and Vaccine Research Institute (VSVRI), Abbassia, Cairo, Egypt. Blood samples were collected from positive tuberculin cattle for preparation of serum samples which labelled and kept at -20˚C till use. Post mortem examination was done by naked eyes to detect presence of any suspected tuberculous nodules such as caseation, calcification and congestion which might be present in lymph nodes and / or organs. The serum samples were tested by ELISA according to (Hall and Thoen 1985) by using antibovine horse Radish Peroxidase conjugate (Sigma), Orthophenylene diamine (OPD) as a substrate (Sigma), bovine PPD tuberculin which was prepared according to the Central Veterinary Laboratories, Weybridge protocol, United Kingdom from M. bovis strain AN5. It obtained kindly from Bacteriological Diagnostic products Department, Veterinary serum and Vaccine Research Institute (VSVRI), Abbasia, Cairo, Egypt as coating antigen and Short Term – Culture Filtrate (ST-CF) as coating antigen which prepared according to (Andersen *et al.,* 1991; Gupta *et al.,* 1998), concentrated by freeze – drying according to (Placktt *et al.,* 1989; Andersen *et al.,* 1991), and its total protein estimated according to (Lowry *et al.,* 1951) then finally standardized according to (Heilman, 1967). The optical density (OD) was measured at 405 nm by using spectra III ELISA reader. A serum dilution was considered positive if it yielded a mean OD of each group equal to, or greater than cut off value according to Dimitri *et al.,* (1987). Cut off value was calculated according to Nassau *et al.,* (1976), which equal to the mean OD of negative serum plus 2 standard deviation.

Results

Examination of 3474 dairy cattle in farms of five governorates by SID cervical tuberculin test, showing that only 78 cattle were positive reactors with percentage of (2.24%). Table (1) illustrates results of tuberculin skin test by using Single intra-Dermal test (SID) on different dairy cattle farms in different Governorate.

The positive tuberculin animals were slaughtered, then postmortem examination was carried out on all positive tuberculin reactor cattle, 60 animals with percentage (76.9%) had visible lesion and other 18 animals with percentage (23.1%) didn′t demonstrate any lesion as illustrated in (Table 2).

The serum samples of 78 positive reactors were examined by using ELISA with PPD and ST-CF antigens. Results declared that 64 out of 78 (82.05 %) cattle were positive and 14 out of 78 (17.95 %) cattle were negative in ELISA, using ST-CF antigen at serum dilution of 1/80 and coating antigen in dilution 1/80. While 58 out of 78 (74.35%) cattle were positive and 20 out of 78 (25.65%) cattle were negative in ELISA, using Bovine PPD antigen at the same dilutions. The results of ELISA on sera at dilution 1/80 from tuberculin positive cattle by PPD and ST-CF as coating antigens are shown in (Table 3).

Table (1): Results of tuberculin skin test by using Single intra-Dermal test (SID) on different dairy cattle farms.

Governorate	No. of examined animals	No. of positive reactor animals	Percentage of positive reactors
El Sharkia	654	16	2.44%
El Menoufia	1124	24	2.13%
El Ismailia	836	14	1.67%
El Gharbia	592	18	3.04%
El Behyra	268	6	2.23%
Total No.	3474	78	2.24%

Table (2): Results of postmortem examination of positive tuberculin reactors.

No. of examined animals	No. of reactor animals	Percentage of reactors from total No.	Postmortem findings			
			V.L	V.L %	N.V.L	N.V.L %
3474	78	2.24%	60	76.9	18	23.1

V.L = Visible lesion N.V.L = Non visible lesion

Table (3): Results of ELISA using PPD and ST-CF as coating antigens.

Type of lesion	No. of tuberculin Reactor	+ve cases with Bovine PPD		+ve cases with ST.CF	
		No.	%	No.	%
V.L	60	48	80	54	90
N.V.L	18	10	55.56	10	55.56
TOTAL	78	58	74.35	64	82.05

Discussion

Tuberculosis remains one of the most prevalent and devastating diseases of human and animals, in spite of great efforts made towards its control and eradication. In Egypt the control of bovine tuberculosis depended on test and slaughter policy. In which animals tested by cervical SID tuberculin test and positive reactors slaughtered. Although the skin test is a good herd test, it is acknowledged that it is of limited use in identifying individual infected animals; moreover, obtaining a result from the test is also a slow process, due to the time needed for a reaction to show (Hancox, 2004). The main disadvantage of the test is its lack of specificity and increased number of no-visible-lesion reactors (NVLs) which occur (Radostitis *et al.,* 2007).

In the present study 3474 dairy cattle in farms of five governorates were tested by cervical SID tuberculin test. 78 cattle were positive reactors with percentage of (2.24%). This results is comparatively less than that recorded by Lotfy *et al.,* 1958 (6.9 %); EL – Sabban *et al.,* 1992 (24 %) and Hassan, 2008 (12.4 %)] in Egypt, while in other countries Waddington, *et al.,* 1965 (6.6 %) in Kenya and Eid, 1975 (25 %) in Northern Nigeria. Also these result is higher than the results recorded by Johns, 1969 (0.4 %) in New Zealand; Clay, 1971 (0.05 % to 0.15 %) in Australian and El – Sawah, 2008 (0.96 %) in Egypt. 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 movement, unhygienic local habits and stress factors due to other diseases and mass vaccination against various diseases (Abu–Eisha *et al.,* 1995).

The postmortem examination of slaughtered positive reactors indicated the presence of 60 animals with percentage (76.9%) had visible lesion and other 18 animals with percentage (23.1%) didn′t demonstrated any lesion. The percent of non visible lesion reactors exceeds 10%. So that other methods or tests for diagnosis should be used (Radostitis *et al.,* 2007). So in this study ELISA technique was evaluated for diagnosis of tuberculosis and to study the specificity and sensitivity of PPD and ST-CF as coating antigens.

ELISA results of tested serum of 78 reactor animals with tuberculin test showed that only 58 were positive representing 74.35% when using PPD as coating antigen. ELISA results when using ST-CF as coating antigen were 64 positive representing 82.05%. These results ensure the usefulness of ELISA in diagnosis of bovine tuberculosis as confirmed by Engvall and Perlmann, 1972, Lilenbaum *et al.,* 1999, Lilenbaum and Fonseca 2006 they referred that ELISA is one of the main important serological tests for diagnosis of bovine tuberculosis and applied as a sensitive method for measurement of antibodies in sera of diseased animals.

Only 48 samples (80%) from 60 positive reactors with visible lesion were positive by using ELISA with PPD as coating antigen while the number of positive samples when using ELISA with ST-CF as coating antigen were 54 (90%). Eighteen reactors without visible lesion were tested only 10 (55.56%) were positive when using ELISA with both coating antigens. Also these findings are supported by the results of Gupta and Ram 2000 who reported that the culture filtrate antigens are highly immunogenic for humoral response, being sensitive and specific when used for the diagnosis of bovine tuberculosis by ELISA technique. Also, Diaz-Otero *et al.,* 2003 and Riad *et al.,* 2010 pointed out that ST-CF of M. bovis was more sensitive than bovine PPD.

Conclusion

From previous results ELISA test showed higher sensitivity and specificity by using ST-CF as a coating antigen than in case of using bovine PPD as a coating antigen. It is clear from the preliminary results that using of ST-CF antigen has appreciable value in diagnosis of bovine tuberculosis as it is a useful antigen that minimizes non-specific reactions.

References

Abu – Eisha, A. M.; El – Attar, A. A. and Elsheary, M. N. (1995): Bovine and atypical mycobacterial infection of cattle and buffaloes in Port Said Province, Egypt. Assiute Vet. Med. J. (47):152-162.

Andersen, P.; Dorthf, A.; Leneljnngovist; Jorgen, B. and Iver, H. (1991): Protein released from *M. tuberculosis* during growth. Infect. Immun., 59 (6): 1905-1910.

Angela, D. P.; Ciccarese Giuseppina; Tony, F. V.; Bizena Bijo; Fatmira, S. and Giuseppina, T. (2006): Detection of M. tuberculosis complex in milk using Polymerase Chain Reaction (PCR). Food Control 17: 776-780.

Clay, A.L. (1971): Tuberculosis of cattle in Australian with particular reference to Queensland – Aust Vet J, 47: 409 – 414.

Diaz-Otero, F.; Banda-Ruiz, V.; Jaramillo-Meza, L; Arriaga-Diaz, C.; Gonzalez-Salazar, D. and Estrada-Chavez, C. (2003): Identification of Mycobacterium bovis infected cattle by immunological and molecular methods. Veterinaria-Mexico, 34 (1): 13-26.

Dimitri, R. A. (1987): Studies on application of some immunodiagnostic methods in bovine tuberculosis. Ph.D. Thesis, Infectious Diseases Fac. Vet. Med., Cairo University.

El- Sawah, R. M. I. (2008): Evaluation of different diagnostic tests used for diagnosis of bovine tuberculosis. M.S. in Veterinary Sciences (Infectious Diseases), Department of Internal Medicine, Infectious Diseases, Faculty of Veterinary Medicine ,Cairo Universty.

El- Sabban, M. S.; Lotfy, O.; Awad, W. M.; Souft, H. S.; Mikhail , D. G.; Hammam, H. M.; Dimitri, R. A. and Gergis, S. M. (1992): Bovine tuberculosis and its extent of spreed as a source of infection to man and animals in Arab Republic of Egypt. Pro.Int.Conf.Animal tuberculosis, GOVS, Cairo, Egypt, 198-211.

Eid, F. I. A. (1975): Some observations of tuberculosis among cattle in North – Wesatern State of Nigeria. J. Nig. Vet. Med. Ass.

Engvall, E. and Perlmann, P. (1972): Enzyme Linked Immunosorbent Assay (ELISA) III. Quantitative immunoglobulin antigen coating tubes. J. Immunol, 109: 129 – 135.

Gupta, V. K. and Ram, G. C. (2000): Fractionation and immunoreactivity of membrane associated proteins of M. bovis. Ind. J. Anim. Sci., 70 (10): 1015-1020.

Gupta, V. K.; Ram, G. C. and Bansal, M. P. (1998): Intracellular killing potential of macrophages activated with different *M. bovis* AN5 antigens.

Hall, M. R. and Thoen, C. O. (1985): detection of Mycobacterial antibodies in sera of *Mycobacterium bovis* sensitized cattle using ELISA: evaluation of assay parameters. Fed. Proc. 26th U.S.A: 934 - 943.

Hancox, M. (2004): Thirteen report of House of Commons, 637-EV / 638-EV 44. Available from: www. Parliament uk. Parliament_committees/environment_food_and_rural_affairs/efra.p_70_040.

Hassan, N. R. A. (2008): Emergency Mycobacterium tuberculosis complex organisms: advances in diagnosis and drug resistance. Ph. D. in Veterinary Sciences (Bacteriology – Immunology – Mycology), Cairo Universty, Faculty of Veterinary Medicine, Department of Microbiology.

Heilman, D. H. (1967): In vitro studies on polysaccharides of *Mycobacterium tuberculosis* and delayed hypersensitivity. Am Rev Respir Dis. 96(2):198-203.

Johns, A. T. (1969): New Zealand Report of the Department of Agriculture for the year ended 31 March – 1969, PP.69 Wellington: AR., Shearer, Govt. Printer {Animal Health Divn. PP. 18 -26}45c.

Lilenbaum, W.; Ribeiro, E. R.; Souza, G. N.; Moreira, E. C.; Fonseca, L. S.; Ferreira, M. A. and Schettini, J. (1999): Evaluation of an ELISA-PPD for the diagnosis of bovine tuberculosis in field trials in Brazil. Res. Vet. Sci. 66(3): 191-195.

Lilenbaum, W. and Fonseca, L. S. (2006): The use of Elisa as a complementary tool for bovine tuberculosis control in Brazil. Braz. J. Vet. Res. Anim. Sci. 43(2):256-261.

Lightbody, K. A.; Skuce, R. A.; Neill, S. D. and Pollock, J.M. (1998): Mycobacterial antigen-specific antibody responses in bovine tuberculosis: an ELISA with potential to confirm disease status. Vet Rec l42 (12): 295-300.

Lotfy, O.; Guindi, S. M. and Moustafa, I. A. (1958): Tuberculin testing of cows and buffaloes in Egypt. Agr. Rev., 36: 613 – 618.

Lowry, O. H.; Rosenbrough, N. J.; Farr, A. L. and Randall, R. J. (1951): Protein measurement with the folin phenol reagent. J Biol Chem 193: 265-275.

Nassau, E.; Parsons, E. R. and Johnson, G. D., (1976): The detection of antibodies to *Mycobacterium tuberculosis* by microplate Enzyme Linked Immuno sorbent Assay (ELISA). Tubercle, 57: 67 - 70.

OIE (2009): Version adopted by the World Assembly of Delegates of the OIE in May chapter 2.4.7.

Ovdienko, N. P.; Shchurevskii, V. E. M. S.; Naimanov, A. Kh.; Yokuskeva, O. V.; Sharov, A. N. and Plonikov, E. S. (1987): Frequency of tuberculin injection in cattle. Vet. Moscow, USSR, 8: 29 - 33.

Plackett, R.; Ripper, J.; Comer, L. A.; Small, K.; Witte, K.; Melville, L.; Hides, S.; Wood, P. R. and De-Witte, K. (1989): An ELISA for the detection of anergic tuberculous cattle. Aust. Vet. J. 66 (1) 15-19.

Radostitis, O. M.; Gay, C. C.; Blood, D. C. and Hinchcliff, K. V. V. (2007): Veterinary medicine 10th ed. Saunders Elsevier, London, 1007-1017.

Riad, E. M.; Mahmoud, S. Z. and Abd al-azeem M. W. (2010): Diagnostic tests for detection of bovine tuberculosis in dairy cattle farms compared to tuberculin test. Assiute Vet. Med. J. 56 (124): 102-112.

Steele, J. H. (1995): Regional and country status report. In: Thoen, C.O., and J.H Steel (Eds), *Mycobacterium bovis* infection in animals and humans, pp. 169-172. Iowa press, Ames.

Waddington, F. G.; Vitale, F.; Giuseppina, C.; Letizia, M.; Stefano, R. and Gesualdo, V. (1965): Observation on tuberculosis sensitivity in Kenya.