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THE SPECIFICITY AND SENSITIVITY OF ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) AND INDIRECT FLUOR ESCENT ANTIBODY TEST (IFA) IN TUBERCULIN POSITIVE BOVINES

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SUMMARY: Twenty one out of 43 tuberculin-positive bovines were found in P.M. examination, to be tuberculous with the isolation of *M. bovis*, while 22 had no visible lesions (NVL) with the isolation of atypical mycobacteria from most cases.
Sera from all the tuberculin-positive bovines were tested for the presence of mycobacterial antibodies using enzyme linked immunosorbent asay (ELISA) and indirect fluorescent antibody test (IFA).
The cross-absorption of either sera or PPD resulted in a great decrease in cross-reactions, moreover the use of both cross-absorbed sera and PPD led to the disappearance of such cross-reactions.

INTRODUCTION

Purified protein derivative (PPD) tuberculin is the most widely used antigen for diagnosis of tuberculosis. However, it contains many antigenic constituents of broad specificity, which lead to the appearance of non-specific reactors among cattle i.e. presence of false positive and/or false negative cases.

Turcotte (1975) was able to cross-absorb such common antigens from protoplasmic extract of a Mycobacterium strain suing an

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antiserum raised against a heterologous strain of mycobacteria. He found that this cross-absorbed antigen was highly specific in tuberculin skin test. The work done here was designed to study the effect of cross absorbtion of bovine PPD on the sensitivity and specificity of enzyme linked immunosorbent assay (ELISA) and indirect fluorescent antibody test (IFA). Also the effect of cross-absorption of sera (using avian PPD) on both tests was studied.

MATERIALS AND METHODS

Fourty-three serum samples were collected from tuberculin positive bovines tested in the frame of the National Control of Tuberculosis in cattle (courtesy of Dr. A.T. Adawy). All animals were slaughtered and lymph nodes were collected for isolating the causative organism according to Marks (1976). The isolated acid fast bacilli were subjected to biochemical identification (Kubica, 1973).

The bovine and avian PPD were obtained from Weybridge laboratories. U.K.

Cross-absorption of bovine PPD: The bovine PPD was absorbed with an antiserum raised against mycobacterium avium according to the method of Turcotte (1975).

Cross-absorption of sera: Sera collected from tuberculin positive bovines were absorbed with avian PPD by the method of Minden et al. (1971).

Enzyme-liked immunosorbent assay: It was done according to Narayan et al. (1983) using protein A-peroxidase as conjugate and orthophenylene diamine (OPD) as substrate. The optical density values were read on titertek multisken MCC spectrophometer at 492-nm.

Indirect fluorescent antibody technique: The test was that described by Lepper and Pearson (1975).

RESULTS

1. Isolation:

Out of 43 tuberculin-positive bovines,generalized tuberculosis was found in 7 cattle, localized tuberculosis in 14 cattle and no visible lesions (NVL) were found in 22 bovines (6 cattle and 16 buffaloes). A total of 36 isolates were isolated and identified as: 21 M. bovis (from 21 tuberculous cattle), 5 M. phlei, 4 M. aurus, 2 M. fortuitum, 2 M parafortuitum and 2 M rungonii **(Table 1)**.

2. Serological examination:

a) Enzyme-linked immunosorbent assay (ELISA):

Table (2) shows the number of animals with positive ELISA titres in case of untreated and cross-absorbed sera. By using untreated sera, all the tuberculous animals were positive, while 4 animals only (18 %) of the NVL animals were positive. In case of cross-absorbed sera 20 tuberculous bovines (95 %) and 2 NVL animals (9 %) were positive.

Using cross-absorbed antigen and untreated sera, 95 % positivity in the tuberculous animals was recorded as 20 animals showed titres equal to or higher than 1/80. Only two NVL animals (9 %) were positive. After cross-absorption of both sera and antigen 3 tuberculous animals were negative and 18 were positive (86 %), while all the NVL animals were negative **(Table 2).**

Table (3) shows the sensitivity and specificity of ELISA in different combinations. In case of untreated sera and antigen the sensitivity and specificity were 100 % and 82 % respectively. After the cross-absorption of sera the sensitivity was 95 % while the specificity reached to 91 %. Similar results were obtained in case of untreated sera against the absorbed antigen. When absorbed sera were used against absorbed PPD the sensitivity and specificity of ELISA were 86 % and 100 % respectively.

b) Indirect fluorescent antibody test (IFA):

The results are shown in **Table 4** and it is evident that all tuberculous animals were positive (using untreated sera) resulting

Table (1): Acid-fast bacilli isolated from lymph nodes of tuberculous and "NVL" animals.

Strain isolated from		Number
Tuber culous	M. bovis	21
	M. phlei	5
	M. aurum	4
NVL	M. fortuitum	2
	M. runyonii	2
	M. parafortiutum	2
	Total	36

Table (2): Results of ELISA testing of sera (diluted 1:80) before and after cross-absorption of sera or antigen.

State of animals	No.	Unabsorbed sera +	%	absorbed sera +	%	unabsorbed PPD +	%	absorbed PPD +	%
Tuberculous	21	21	100	20	95	20	95	18	86
NVL.	22	4	18	2	9	2	9	-	-
Total	43	25		22		22		18	

Table (3): Sensitivity and specificity of ELISA after cross-absorption of sera or antigen or both.

Combination	Sensitivity	Specificity
Unabsorbed sera x unabsorbed PPD	100 %	82 %
Absorbed sera x unabsorbed PPD	95 %	91 %
Unabsorbed sera x absorbed PPD	95 %	91 %
Absorbed sera x absorbed PPD	86 %	100 %

Table (4): Results of IFA on unabsorbed and absorbed sera.

State of animals	No.	No. of +ve cases		Sensitivity		Specificity	
		Unabsorbed sera	absorbed sera	unabsorbed sera	absorbed sera	unabsorbed sera	absorbed sera
Tuberculous	21	21	15	100 %	71 %	73 %	95 %
NVL	22	6	1				

in sensitivity of 100 %. The specificity in NVL animals was 73 % as 16 out of 22 animals were negative. After cross-absorption of sera, only 15 tuberculous animals were positive (71 % sensitivity), while all but one NVL animals were negative with a specificity of 95 %.

DISCUSSION

Mycobacterium bovis was isolated from all tuberculous animals, while 22 hout of 43 tuberculin-positive bovines were found to have non-visible lesions (NVL) where atypical mycobacteria were isolated from most cases. The positive tuberculin in the latter group may be explained by the statement of Francis et al. (1978) that the single intradermal tuberculin test in the cervical site (This test is used in Egypt) detectes all tuberculous animals, but it is not highly specific. In tuberculin positive tuberculous animals the ELISA test was positive in all sera when used without absorption and diluted to 1:80. This agrees with the results of Jorgensen and Jensen (1978) and Thoen et al. (1981).

In tuberculin positive NVL animals the false positive cases were 18 % this comes in accordance with the results of Nassau et al. (1976) and Radin et al. (1983) as they recorded 16 % and 13 % false positive cases respectively. The cross-absorption of sera resulted in 5 % false negative and 9 % false-positive cases. The same results was obtained using cross-absorbed PPD against untreated sera, while using of both absorbed sera and PPD led to the disappearance of false-positive reactors, but the sensetivity of the test was reduced by 14 %. The disappearance of false-positives is probably due to the removal of common antigen (Nassau et al. 1976). These results are in accordance with that of Kalish et al. (1983). The increase in specificity by cross-absorption may be supported by Nassau (1976) who reported that the use of specific (Purified) antigen will increase the specificity of ELISA. Also the decrease in sensitivity may be explained by that the conjugate used in this work (Protein A-horseradish peroxidase) binds mainly with IgG (Goding, 1978),while cross-absorption of either sera or PPD antigen reflects only on IgM which plays the main role in cross-reactions (Lepper

and Pearson, 1975) . Concerning IFA test all tuberculous animals were positive and this agrees with the results of Affronti et al. (1973). The false positive cases in our results may be referred to the atypical mycobacteria isolated from most of the NVL animals as their sera contained cross reactive antibodies, mainly IgM. After cross-absorption of sera 29 % false negative cases were recorded at the cross absorption of sera resulted in converting some positive animals to negative (Lepper and Pearson, 1975). The specificity of IFA in our work was 73 % but after cross absorption of sera it increased to 95 % as a result of the removal of most cross reacting antibodies. The sensitivity in case of crossabsorbed sera was 71%. This decrease may be explained by that some animals may had originally low titres which were further lowered as a result of cross-absorption, which removed not only the heterologous antibodies but also some of the specific antibodies.

REFERENCES

1. Affronti, L.F.; File, E.H. and Grow, L. (1973): Serodiagnostic test for tuberculosis. Amer. Rev. Resp. Dis. **107,** 822-825.

2. Francis, J.; Seiler, R.J.; Wilkie, I.W., O'Beyle, D.; Lusden, M.J. and Frost, A.J. (1978): The sensitivity and specificity of various tuberculin tests using bovine PPD and other tubercalius. Vet. Rec. **103,** 420-435.

3. Goding, J.W. (1978): Use of staphylococcal protein A as an immunological reagent. J. Immunol. Meth. **20,** 241-253.

4. Jorgensen, J.B. and Jensen, P.T. (1978): Enzyme-linked immunosorbent assay (ELISA) for detection of antibodies to M. paratuberculosis in cattle Acta. Vet. Scand. **19,** 310-312.

5. Kalish, S.B., Radin, R.C., Phair, J.P., Levitz, D., Zeiss, C. P. and Metzgor, E. (1983): Use of enzyme-linked immunosorbent assay technique in the differential diagnosis of active pulmonary tuberculosis in humans. J. Inf. Dis. **147,** 523-530.

6. Kubica, G.P. (1973): Differential identification of mycobacteria. VII. Key features for identification of clinically significant mycobacteria. Amer. Rev. Resp. Dis. **107**, 9-21.

7. Lepper, A.W.D., and Pearson, C.W. (1975): The indirect fluorescent antibody test for the detection of circulating antibodies in bovine tuberculosis Aust. Vet. J. **51**, 256-261.

8. Marks, J. (1976): A system for the examination of tubercle bacilli and other mycobacteria. Tubercle. **57**, 207-225.

9. Minden, P.; McClatchy, J.K.; Bardana, E.J. and Farr, R.S. (1971): Antigenic differences between M. bovis strain BCG and an isoniazid-resistant mutant. Infec. Immun. **3**, 524-529.

10. Narayanan, P.R.; Acharyulum, G.S.; Krishnomurthy, P. V.; Abdel-Rauoof and Tripathy, S.P. (1983): Evaluation of Elisa as a diagnostic test in pulmonary tuberculosis. Ind. J. Tuber, **30**, 29-32.

11. Nassau, E. and Parsons, E.R. (1976): Detection of antibodies to *M. tuberculosis* by solid phase radioimmuno assay. J. Immunol. Meth. **6**, 261-271.

12. Radin, R.C.; Zeiss, C.R. and Phair, J.P. (1983): Antibodies to purified protein derivative in different immunoglobulin classes in the diagnosis of tuberculosis in man. Int. Arch. Allerg. Appl. Immunol. **70**, 25-29.

13. Thoen, C.O.; Malstrom, C. Himes, E.M. and Millsik. (1981): Use of enzyme-linked immunosorbent assay for detecting mycobacterial antigens in tissues of M.bovis infected cattle. Amer. J. Vet. Res. **42**, 1814-1815.

14. Turcotte, R. (1975): Absorption of tuberculin-active components with mycobacterial antibodies. Can. J. Microbiol. **21**, 764-773.