J. Egyptian Vet. Med. Ass. 48, No. 4, 565 - 574 (1988)

SERODIAGNOSTIC TESTS FOR TUBORCULOSIS IN EXPERIMENTALLY INFECTED GUINEA PIGS USING CROSS-ABSORBED SERA OR ANTIGENS OR BOTH

M.REFAI*, F.R. EL-SEEDY*; IKRAM A. KARIM** ; W.M. AWAD AND H.M. HAMMAM***

* Department of Microbiology Fac. Vet. Med. Cairo Univ.
** Animal Health Research Institute, Dokki Giza,
*** Sera and Vaccines Research Institute, Abbasia Cairo.

SUMMARY: Four groups of guinea pigs were experimentally infected with *M.bovis* standard strain (gr 1), *M.bovis* local strain (gr 2), *M.avium* standard strain (gr 3) and local atypical mycobacteria (gr 4). Passive haemagglutination test (PHA), enzyme linked immuosorbent assay (ELISA), indirect fluorescent antibody test (IFA) and tuberculin test were applied on all groups using untreated bovine PPD, Cross absorbed bovine PPD and untreated and cross absorbed sera. In case of untreated sera against untreated PPD, guinea pigs of all groups showed positive results with higher titres in the first 2 groups than the third and fourth groups. Using cross-absorbed sera against untreated pPD or untreated sera against cross-absorbed PPD, the cross-reactions were greatly decreased. After cross absorption of sera and PPD, guinea pigs of groups 1 and 2 were still positive, while cross - reaction in group 3 and 4 disappeared almost completely. Concerning the tuberculin test , the cross-absorbed bovine PPD showed increased specificity as the heterologous reactions were greatly reduced.

Received: 17.9.1988.

INTRODUCTION

The major problem in diagnosis of tuberculosis with currently available techniques is that weeks to months may be required until a positive culture is isolated and/or identification of the species is accomplished (Kalish et al., 1983).

Various serological methods have been applied to demonstrate the presence of antibodies in animals and humans infected with tubercle bacilli, but due to lack of specific antigens, most of the serological tests used were not completerly successful and were found to have some limitations.

In this work it was tried to get rid of msot of the common antigens present in the bovine PPD tuberculin, by cross-aborption with immunoglobulins against avian PPD tuberculin.

The efficiency of this cross-absorbed PPD in comparison with the untreated PPD was studied in guinea-pigs experimentally infected with different mycobacterium species. Also sera were cross-absorbed with avian PPD. The serological tests used in this study were the passive haemagglutination (PHA) test , the enzyme linked immunosorbent assay (ELISA) and the indirect fluorescent antibody (IFA) test.

MATERIAL AND METHODS

1- Strains :

M. bovis **strain AN5**	**(standard)**
M. bovis	**(local)**
M. avium **strain D 4**	**(standard)**
A typical mycobacteria	**(local)**

2- Antigens:

Bovine PPD tuberculin, batch 291 from *M. bovis*
Avian PPD tuberculin, batch 290 from *M. avium*
Both antigens were obtained from Central Veterinary laboratories, Weybridge, England.

3- Lab. animals:

Twenty five albino guinea pigs weighing from 200-300 gm. Six boskat rabbits weighing from 1.5 - 2.5 kg were used for the production of anti avian PPD immunoglobulins , antibovine and anti-guinea pig immunoglobulins for IFA test.

Four groups of guinea pigs were experimentally infected with the following mycobacterium species:

Group (1) : *M. bovis* standard strain.
Group (2) : *M. bovis* local strain.
Group (3) : *M. avium* standard strain.
Group (4) : Atypical mycobacteria local strain.

After one month, blood samples were collected by heart puncture from all animals and sera were obtained. The serum samples were tested by PHA (Thorns et al., 1982), IFA (Lepper and Pearson , 1975) and ELISA (Narayanan et al., 1983) using bovine PPD as an antigen for PHA and ELISA and dead mycobacterium cells for IFA. The same tests were applied on sera after cross absorption with avian PPD (Minden et al., 1971) using cross absorbed bovine PPD (Turcotte, 1975 a) as an antigen , PHA and ELISA were applied on sera before and after cross absorption. Also tuberculin test was applied on the experimentally infected guinea pigs using bovine PPD and cross absorbed bovine PPD.

RESULTS

Passive haemagglutination test (PHA):

Using untreated sera and antigen as shown in **Fig.** (1 - a) guinea pigs of the first group showed the highest titres that varied from $^1/320$ to $^1/1280$ with a geometric mean of $^1/640$. The second group showed a mean of $^1/320$. Third and fourth groups had low titres with mean of $^1/61$ and $^1/46$ respectively . After the absorption of sera of the infected animals with avian PPD, the titres in group 1 and 2 showed slight reduction in their mean **(Fig. 1 - b)** . On the other hand , sera of animals in the third and fourth groups presented low titres or were

negative (< $^{1}/20$). The cross-absorption of the *M.bovis* antigens with antisera against *M.avium* reduced the titres in all groups particularly that of the third and fourth ones as shown in **Fig. (1-c).** While the use of cross-absorbed sera and antigen rendered the test highly specific as guinea pigs infected with *M.avium* and atypical mycobacteria showed completely negative results (< $^{1}/20$) **Fig. (1 - d).**

Enzyme linked immunosorbent assay (ELISA):

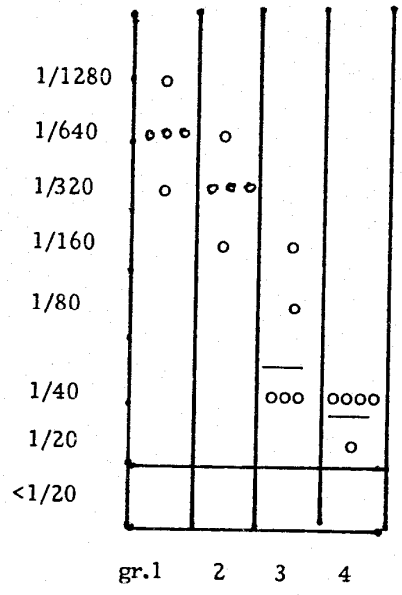
It is clear from **Fig. (2 - a)** that the titres were higher than that of PHA as the means of the four groups reached $^{1}/1280$, $^{1}/1114$, $^{1}/320$ and $^{1}/243$ respectively. After cross - absorption of sera, the titres in group 1 and 2 were still high with means of $^{1}/970$ and $^{1}/844$ respectively while that of the third and fourth groups were reduced to $^{1}/80$ and $^{1}/106$ **(Fig. 2 - b).** On the other hand , the use of untreated sera and cross - absorbed antigen **(Fig. 2 - c)** resulted in mean titres of $^{1}/970$, $^{1}/640$ $^{1}/160$ and $^{1}/139$ respectively. As shown in **Fig. 2 - d** still high titres were observed in guinea pigs of the first ($^{1}/557$) and second ($^{1}/485$) groups by using cross - absorbed sera and antigen , while the third and fourth groups showed very low titres.

Indirect fluorescent antibody test (IFA):

Using untreated sera, all guinea pigs of group 1 and 2 were positive to bovine antigen , 2 in each group were positive to atypical mycobacteria antigen while 4 from group 1 and 3 from group 2 were positive to aviun antigen. Simialr results were recorded in the third and fourth groups **(Table 1).**

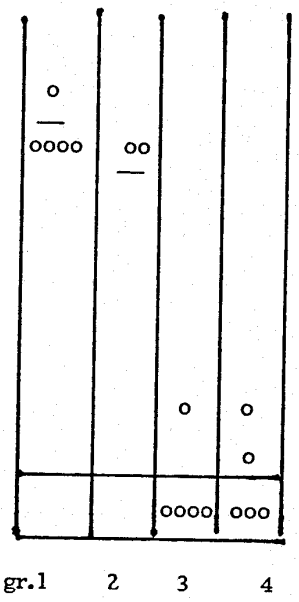
After cross-absorption of sera all guinea-pigs of the first group were positive to the bovine antigen and negative to the atypical mycobacteria antigen. In the second group 4 guinea - pigs were positive to bovine antigen , one to the avian antigen and one to the atypical antigen . Concerning the third and fourth groups , one guinea pig was positive to avian antigen while 2 of the fourth group were positive to atypical mycobacterial antigens.

Fig. (1): PHA titres in guinea pigs experimentally infected with different mycobacterium species

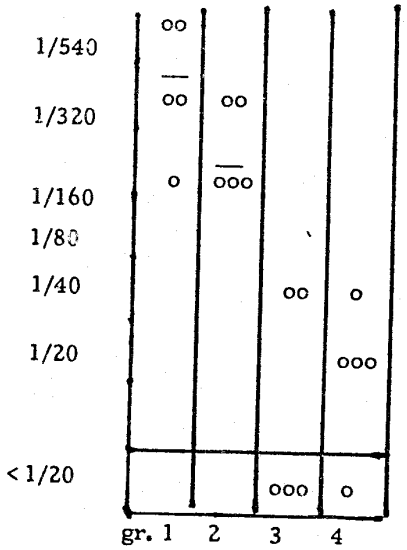


(a) Untreated sera and antigen

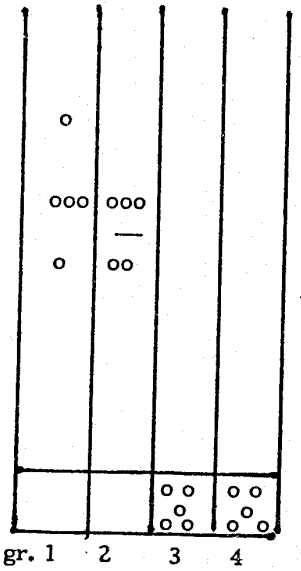
o = no of animals



(b) Cross-absorbed sera and untreated antigen

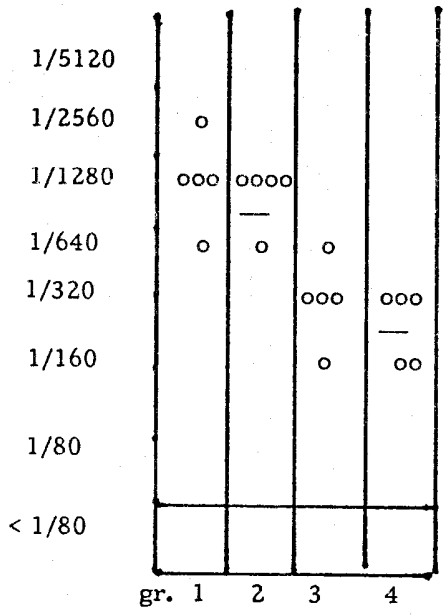


(c) untreated sera and cross-absorbed

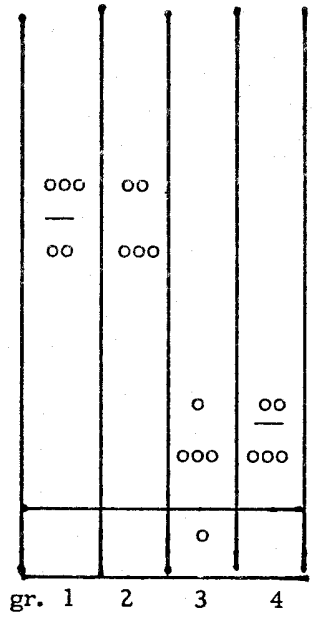


(d) Cross-absorbed sera and antigen

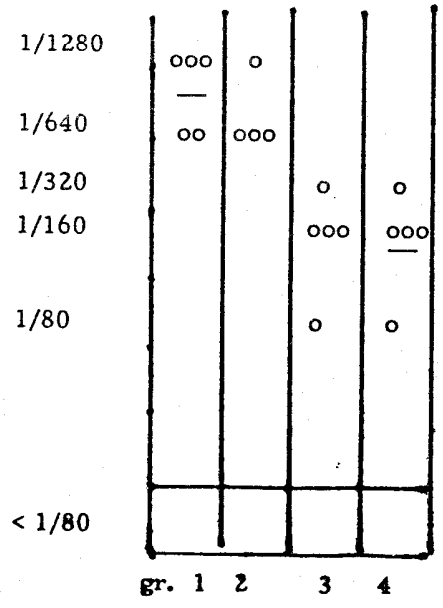
Fig. (2): EUSA titres in guinea pigs experimentally infected with different mycobacterium species



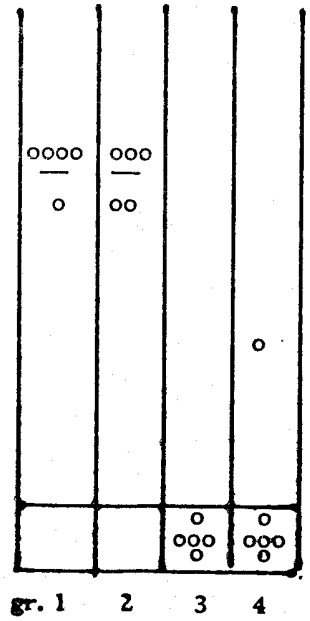
(a) untreated sera and antiges



(b) Cross-absorbed sera and untreated antigen



(c) untreated sera and cross absorbed antigen



(d) Cross absorbed sera and antigen

Table (1): Results of IFA in guinea pigs experimentally infected with different mycobacterium species using untreated and Cross-absorbed sera.

Group	Guinea pigs infected with	No.of g-pigs	Number of guinea pigs positive to					
			Bovine antigen		Avian antigen		Atyp. mycobac. antigen	
			untreated sera	absorbed sera	untreated sera	absorbed sera	untreated sera	absorbed sera
1	*M.bovis* (standard)	5	5	5	4	1	2	-
2	*M.bovis* (local)	5	5	4	3	1	2	1
3	*M.avium* (standard)	5	3	-	5	1	5	-
4	Atyp. mycobac (local)	5	3	-	5	-	5	2

Table (2): Results of tuberculin test in guinea pigs using both bovine PPD tuberculin and absorbed bovine PPD tuberculin.

Group	Guinea pigs inoculated with	Average diameter of skin reaction in mm.		loss of activity %
		Bovine PPD	Absorbed bovine PPD	
1	*M.bovis* (Standard)	20	13.8	31
2	*M.bovis* (local)	18.4	12.4	30
3	*M.avium* (Standard	13.6	6.0	56
4	Atyp. mycobac(local)	15	8.4	44

Tuberculin test :

As shown in **Table 2** the injection of untreated antigen evoked an average diameter of skin reaction to bovine PPD of 20 mm. in the first froup while it was 18.4 , 13.6 and 15 mm in the other three groups, respectively.

On the other hand , the average diameter of skin reaction (using Cross-absorbed antigen) was 13.8 mm in the first group with a loss of activity of 31%. The overage diameter in the second group was 12.8 mm with 30% loss of activity. The reaction in the third and fourth gorups was much reduced.

DISCUSION

The positive titres to bovine PPD antigen in animals infected with *M.avium* or atypical mycobacteria were expected as many researchers had reported the presence of false - positive cases (Schaefer, 1967 , Reggiardo and Middlebrook , 1975 and Rouslathi, 1976). When either sera or antigen were cross-absorbed, the non-specific reactions were greatly reduced. Both treatments removed almost all cross-reactions. In animals infected with *M. avium*, the absorption with either *M. avium* PPD or antiserum did not remove all homologous antibodies or antigens. This may be explained by the statement of Schaefer (1967) that the *M.avium* strains have a low absorbing capcity. Also Chaparas and Maloney (1978) reported that *M.avium* has somewhat small numbers of cross reactive antigens. On the other hand , the use of cross-absorbed sera and antigen showed high specificity of both tests as no cross-reactions were observed in PHA while in ELISA only one guinea pig in the fourth group had a positive titre ($^1/80$). This may be due to the failure of complete absorption and the higher sensitivity of the ELISA in comparison with the PHA

It is clear from our results that IFA test is sensitive as guinea pigs of each group showed positive reaction to the homologous antigen. On the other hand , many cross-reactions were observed in heterologous antigens. The presence of such cross-reactions was reported by Bennedsen (1966). Lepper and Pearson (1975) also stated that at least two surface antigens give rise to antibodies detectable by IFA , one of which is specific while

the other shares antigenic relationships with a number of mycobacterial species. Cross-absorption of sera resulted in removal of most of the cross-reactions observed and thus the specificity of IFA was increased. This comes in agreement with the findings of Bennedsen (1966) who could remove cross-reactions by cross-absorption of sera.

Concerning the tuberculin skin reaction in experimentally infected guinea pigs using both bovine PPD and cross-absorbed PPD it is clear that a loss of tuberculin activity ranging from 30-56% in the cross - absorbed PPD was observed. This agrees with the findings of Turcotte (1975 b) who recorded about 50% loss of tuberculin activity in cross - absorbed protoplasmic extracts. Also Chaparas and Maloney (1978) studied cross reactions among mycobacteria in tuberculin test and found that *M.avium* had somewhat small numbers of cross - reactive antigens. This may be another explanation of the observed cross - reactivity in case of the cross - absorbed PPD as anti *M.avium* antibodies for cross absorption were used. Trials of absorption should be continued with other cross - reacting micro - organisms with the hope to obtain better reactions both in the serological and allergic tests .

REFFERENCES

1 . Bennedsen ; J. (1966): Circulating antibodies in experimental mycobacterial infections demonstrated by immunoflourescence. Acta. Path. Micro. Scand. **68** , 262 - 272.

2 . **Chaparas, S.D. and Maloney, C.J. (1978): An analysis of cross - reactions among mycobacteria by in vivo and in vitro assays of cellular hypersensitivity. Amer. Rev. Resp. Dis. 117 , 892 - 902 .**

3 . **Kalish , S.B. , Radin , R.C. ; Phair , J. P. ; Levitz , D. ; Zeiss, C.P. and Metzger , 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 .**

4 . 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 .

5 . 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. Infect. Immunol . **3** , 524 - 529 .

6 . Narayanan , P.R. ; Acharyulu , G.S. ; Krishmamurthy , P.V.; Abdul Ravoof and Tripathy , S.P. (1983): Evaluation of ELISA as a diagnostic test in pulmonary tuberculosis. Ind. J. Tuberc. **30** , 29 - 32 .

7 . Reggiardo Z. and Middlebrook , G. (1975): Serologically active glycolipid families from *M.bovins*. II serologic studies of human sera. Amer . J. Epidem . **100** ; 477-486.

8 . Rouslathi , E. (1976): Antigen - antibody interaction ; antibody affinty and dissociation of immune complexes. Scand. J. Immunol . **6** , 3 - 7 .

9 . Schaefer , W.B. (1967): Type - specificity of atypical mycobacteria in agglutination and antibody absorption tests . Amer. Rev. Resp. Dis., **96** , 1165 - 1168 .

10. Thorns , C.J. ; Morris , J.A. and Little , T.W.A. (1982): A spectrum of immune responses and pathological conditions between certain animal species to experimental *M.bovis* infection. Br. J. Exp. Path. **63** , 562 - 572 .

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

12. Turcotte , R. (1975 b): The participation of common and species specific antigens of mycobacteria in the tuberculin skin reaction. Can. J. Microbiol. **21** , 774 - 783 .