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Studies on the Efficiency of Absorbed Bovine PPD in Tuberculin and Serological Tests

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With 4 tables

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Summary

The passive haemagglutination, enzyme-linked immunosorbent assay and indirect fluorescent antibody tests were applied to study the non-specific reactions in experimentally infected guinea pigs and tuberculin positive bovines. These cross-reactions were greatly decreased after absorption of either sera with avian PPD or bovine PPD antigen with anti-avian PPD serum. The use of both absorbed sera and antigen raised the specificity of PHA and ELISA to 100 %. The use of absorbed sera rendered the IFA specific in 95 %. The absorption has reduced the sensitivity of ELISA, IFA and PHA by 14, 27 and 29 %, respectively.

Key words: Tuberculosis, serodiagnosis, PPD, cross-absorption

Introduction

Delayed hypersensitivity to tuberculin has been utilized as an index of tuberculosis in animals. However, no tuberculin has so far been produced that detects all tuberculous animals without inducing non-specific reaction in a varying proportion of animals. This defect is also observed in serological tests used for diagnosis of tuberculosis as the PPD tuberculin is commonly used as antigen. It is known that bovine PPD contains antigens common to other *Mycobacteria* species.

The present work was aimed to get rid of such non-specific reactions by cross-absorption of common antigens in the bovine PPD and for cross-absorption of cross-reactive antibodies from sera before testing.

Material, Methods and Results

1. *Animals:* 25 tuberculin negative guinea pigs.
2. *Sera and lymph nodes:* Sera were collected from 43 tuberculin positive bovines and after slaughtering the lymph nodes were collected for isolation of the organism.

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3. *Antigens*: Bovine PPD batch no. 291 and avian PPD batch no. 290 (Weybridge Laboratories).
4. Immunoglobulins against avian PPD and cross-absorption of common antigens from bovine PPD were prepared according to TURCOTTE (1975).
5. Cross-absorption of cross-reactive antibodies from sera using avian PPD was done according to MINDEN et al. (1971).
6. The following serological tests were carried out:
 - a) Passive haemagglutination.
 - b) Enzyme-linked immunosorbent assay, ELISA (NARAYANAN et al., 1983).
 - c) Indirect fluorescent antibody technique (LEPPER and PEARSON, 1975).
7. Isolation and identification of Mycobacteria were done according to MARKS (1972) and KUBICA (1973).

Experiment No. 1 (Guinea pigs)

Guinea pigs were divided into 5 groups, 5 animals each. The first group was infected with *Mycobacterium bovis* (standard strain AN5), the second with *M. bovis* (local Egyptian strain), the third with *M. avium* (standard strain D₄), the fourth with a local atypical Mycobacterium and the fifth was left as control.

Sera were collected one month after infection and the serological tests were carried out on sera before and after cross-absorption using untreated and cross-absorbed bovine PPD as antigens.

The following results were obtained:

Passive haemagglutination and ELISA

From Tables 1 and 2 it is clear that absorption of sera of *M. bovis* infected guinea pigs with avian PPD (groups 1 and 2) showed still high titres when tested by untreated or cross-absorbed bovine PPD antigen. On the other hand the guinea pigs infected with *M. avium* or atypical Mycobacterium were completely negative in PHA test and showed negligible titres in ELISA when their sera were absorbed with avian PPD and tested with cross-absorbed bovine PPD.

Control animals were all negative.

Table 1. Geometric means of PHA titres in guinea pigs experimentally infected with Mycobacterium

Groups	Untreated bovine PPD antigen		Cross-absorbed bovine PPD antigen	
	unabsorbed sera	absorbed sera	unabsorbed sera	absorbed sera
1	1:640	1:368	1:368	1:160
2	1:320	1:211	1:211	1:121
3	1:61	1:13	1:26	—
4	1:46	1:15	1:20	—

Table 2. Geometric means of ELISA titres in guinea pigs experimentally infected with Mycobacterium

Groups	Untreated bovine PPD antigen		Cross-absorbed bovine PPD antigen	
	unabsorbed sera	absorbed sera	unabsorbed sera	absorbed sera
1	1:1280	1:970	1:970	1:557
2	1:1114	1:844	1:640	1:485
3	1:320	1:80	1:160	1:30
4	1:243	1:105	1:139	1:40*

* Only one out of the 5 animals was positive.

Indirect fluorescent antibody test (IFA)

In Table 3 the results of the untreated sera of experimentally infected guinea pigs with the homologous and heterologous antigens are shown. The absorbed sera on the other hand reacted mainly with the bovine antigen. Only one out of the 5 absorbed sera from guinea pigs infected with *M. bovis* reacted positively with *M. avium* antigen. The sera of control animals were all negative.

Table 3. Indirect fluorescent antibody test of serum from guinea pigs experimentally infected with Mycobacterium (Number of positive animals)

Groups 5 animals each	untreated sera			treated sera		
	bovine	avian antigens	atyp. Myco.	bovine	avian antigens	atyp. Myco.
1	5	4	2	5	1	—
2	5	3	2	4	1	1
3	3	5	5	—	1	—
4	3	5	5	—	—	2

Tuberculin-testing of guinea pigs with untreated and cross-absorbed PPD

The treatment of bovine PPD with anti-avian PPD serum resulted in loss of tuberculin activity by 30 % when tested in guinea pigs infected experimentally with *M. bovis*. In animals infected with *M. avium* or atypical Mycobacterium the loss of activity was 56 and 44 %, respectively.

Experiment No. 2 (Tuberculin positive bovines)

Sera from 43 tuberculin positive bovines were examined before and after absorption with avian PPD by PHA, ELISA and IFA using untreated and bovine PPD absorbed by anti-avian PPD serum. The following results were obtained:

PHA and ELISA

Table 4 clearly demonstrates the high specificity (100 %) of both PHA and ELISA when sera from tuberculin positive bovines were absorbed by avian PPD and tested by an antigen (bovine PPD) treated with anti-avian PPD serum. The sensitivity of both tests was reduced by 29 % in case of PHA and by 14 % in case of ELISA.

Table 4. Sensitivity and specificity of PHA and ELISA in testing of sera from tuberculin positive bovines

	Sensitivity		Specificity	
	PHA	ELISA	PHA	ELISA
untreated sera × untreated PPD	95	100	64	82
Absorbed sera × untreated PPD	81	95	86	91
Untreated sera × absorbed PPD	95	95	95	91
Absorbed sera × absorbed PPD	71	86	100	100

$$\text{Sensitivity} = \frac{\text{No. of positives}}{\text{No. of positives} + \text{false negatives}} \times 100$$

$$\text{Specificity} = \frac{\text{No. of negatives}}{\text{No. of negatives} + \text{false positives}} \times 100$$

IFA

The IFA was highly sensitive (100 %) when untreated sera were used but the specificity was 71 %. Absorption of the sera raised the specificity to 95 % but the sensitivity was reduced to 73 %.

Isolation and identification of *Mycobacterium*

M. bovis could be isolated from the lymph nodes of 21 tuberculous bovines. From 15 animals with non-visible lesions (NVL) atypical *Mycobacteria* were isolated which were identified as *M. phlei* (5), *M. aurum* (4), *M. fortuitum* (2), *M. runyonii* (2) and *M. parafortuitum* (2).

The remaining 7 animals were culturally negative.

Discussion

The experimentally infected guinea pigs showed higher PHA and ELISA titres with the homologous than with the heterologous antigens. This is in agreement with the results obtained by SCHAEFER (1967). However, the absorption of sera with avian PPD or bovine PPD antigen with anti-avian PPD serum resulted in significant reduction of non-specific reactions, though it has been reported by SCHAEFER (1967) as well as CHAPARAS and MALONEY (1978) that *M. avium* strains have a low absorbing capacity. Optimum results could, however, be obtained when both sera and antigens were absorbed. The low titres observed in case of ELISA, however, should be considered as negative. In case of indirect immunofluorescence test the absorption of sera has raised the specificity to a satisfactory extent.

The sensitivity and specificity of PHA and ELISA have been a matter of controversy and disagreement among various authors (REGGIARDO and MIDDLEBROOK, 1975 and JAGANNETH et al., 1983). In the present work ELISA test was superior to PHA both in sensitivity and specificity. The specificity of both tests increased significantly when either sera or antigen were absorbed. The use of both absorbed sera and antigen raised the specificity of both tests to 100 %. This was, however, on the expenses of the sensitivity which was reduced by 29 % and 14 % in PHA and ELISA, respectively. Nevertheless, this technique seems to be a step forward in the improvement of tuberculosis diagnosis.

Zusammenfassung

Untersuchungen zur Wirksamkeit von absorbiertem bovinem PPD bei der Tuberkulinreaktion und serologischen Verfahren

Passive Haemagglutination (PHA), Enzyme-linked immunosorbent assay (ELISA) und indirekte Fluoreszenzantikörpertechnik (IFA) wurden zur Untersuchung unspezifischer Reaktionen bei experimentell mit Mykobakterien infizierten Meerschweinchen und Tuberkulin-positiv reagierenden Rindern eingesetzt. Zum großen Teil wurden Kreuzreaktionen entweder durch Absorption der Seren mit aviärem PPD oder durch Absorption von bovinem PPD mit antiaviärem PPD-Serum eliminiert. Der gleichzeitige Einsatz von absorbiertem Serum als auch absorbiertem Antigen erhöhte die Spezifität von PHA und ELISA auf 100 %. Durch die Anwendung absorbierter Seren wurde die Spezifität der IFA auf 95 % gesteigert. Die Sensitivität von ELISA, IFA und PHA wurde durch die Absorption um jeweils 14, 27 und 29 % reduziert.

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