COMPARATIVE CERVICAL TEST IN DEER

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INTRODUCTION

Under New Zealand conditions the sensitivity and specificity of the cervical test (CT) in deer, using 0.1 ml of 2 mg/ml bovine purified protein derivative (PPD) have been estimated as 85% and 99.5% respectively. However in a limited number of herds where there is no evidence of Mycobacterium bovis the specificity of the test is considerably less than 99.5%.

In 1984 0.86% of 93233 deer tested reacted to the CT and it is estimated that the predictive value or validity of the test is approximately 30%. This will be further reduced as the incidence of cervine tuberculosis decreases. It would therefore be desirable to improve the specificity of tuberculin testing. This may be acheived by two methods.

1. A modified interpretation of the CT.

   A modified interpretation classifies animals as having reacted to the test if there is a skin thickness difference greater than a specified amount. This eliminates many of the small non-specific reactions such as those described in the first Flock House experiment (1).

2. The use of the comparative cervical test (CCT).

   Tuberculins contain a range of different antigens, a portion of which are common to many different species of mycobacteria. Skin test reactions may therefore be induced by sensitisation to M.bovis or some other mycobacteria. While the cause of non-specific sensitisation is often unknown, avian tuberculin has been found to be the most suitable reagent to use concurrently with bovine tuberculin in comparative tests. If the response to bovine tuberculin is greater than that to avian tuberculin in the CCT then this is indicative of a M.bovis infection. Non-specific reactions are those where the larger response is to avian tuberculin. They may be induced by sensitisation to Mycobacterium avium complex or a variety of other mycobacteria.

While the modified interpretation and the CCT increase the specificity of skin testing they will reduce the sensitivity of the tests. The results presented in the paper are some initial findings in a study for improving the specificity of tuberculin testing in deer.

MATERIALS AND METHODS

1. Comparative testing in M.bovis inoculated deer

Sixty castrated stags were subjected to various treatments (tuberculin tests) within four stages of the trial. Each stage was 8-12 weeks apart.

At each stage of the trial deer were allocated to specific treatment groups according to a predetermined experimental design. This design enabled not only the comparison of testing procedures within each stage but also allowed the estimation of any possible "carry-over" effects from one stage to another.

The four stages with their specific treatments were:

Stage A Two weeks before intra-tracheal inoculation of M.bovis .

group 1	1.0 mg/ml	bovine PPD
	0.5 mg/ml	avian PPD
group 2	1.0 mg/ml	bovine PPD
	1.0 mg/ml	avian PPD

Stage B Six weeks post-inoculation

group 1	1.0 mg/ml	bovine PPD
	0.5 mg/ml	avian PPD
group 2	1.0 mg/m	bovine PPD
	1.0 mg/ml	avian PPD

Stage C

group 1	CCT
group 2	CT followed 3 days later by a CCT
group 3	CT followed 7 days later by a CCT

Stage D

group 1	CCT
group 2	CT followed 28 days later by a CCT

2. Comparative testing of CT reactors in deer with no evidence of M.bovis

CCTs were performed on CT reactors from four commercial deer farms. These tests were conducted at a minimum interval of 21 days following the initial CT and 1.0 mg/ml bovine PPD and 0.5 mg/ml avian PPD was used. Following this CCT the deer were necropsied and examined for evidence of M.bovis.

RESULTS

1. Comparative testing in M.bovis inoculated deer

1.1 Stage A of experiment
There was a large difference between the two groups for both the avian and bovine reactions (Table 1). This was due to a problem encountered in preparing the 1.0 mg/ml avian tuberculin.

1.2 Stage B of the experiment
At the first test after inoculation no difference was found between the CCT using the high concentration of avian PPD (group 2) and the lower level (group 1). See Table 2. The lower level of avian PPD (0.5 mg/ml) is commercially available and was consequently used in the remaining treatments.

Results of the CCT carried out prior to and following inoculation are shown in the scattergram (Fig 1).

Table 1

Mean skin thickness difference to the CCT, using 1.0 mg/ml bovine PPD, and two strengths of avian PPD before inoculation (Stage A).

Site	Avian PPD 0.5 mg/ml n=30	Avian PPD 1.0 mg/ml n=30
Avian site	0.56 mm	1.61 mm
Bovine site	0.44 mm	0.73 mm
Bovine-Avian	-0.12 mm	-0.88 mm

Table 2

Mean skin thickness difference to the CCT, using 1.0 mg/ml bovine PPD and two strengths of avian PPD after inoculation (Stage B).

Site	Avian PPD 0.5 mg/ml n=29	Avian PPD 1.0 mg/ml n=30
Avian site	3.28 mm	3.74 mm
Bovine site	6.76 mm	6.58 mm
Bovine-Avian	3.48 mm	2.84 mm

Fig. 1: Scattergram of the comparative skin test reactions of 59 deer which had been inoculated six weeks previously with M.bovis. The shaded box delineates the area of the comparative test reactions conducted on the same animals two weeks prior to being inoculated.

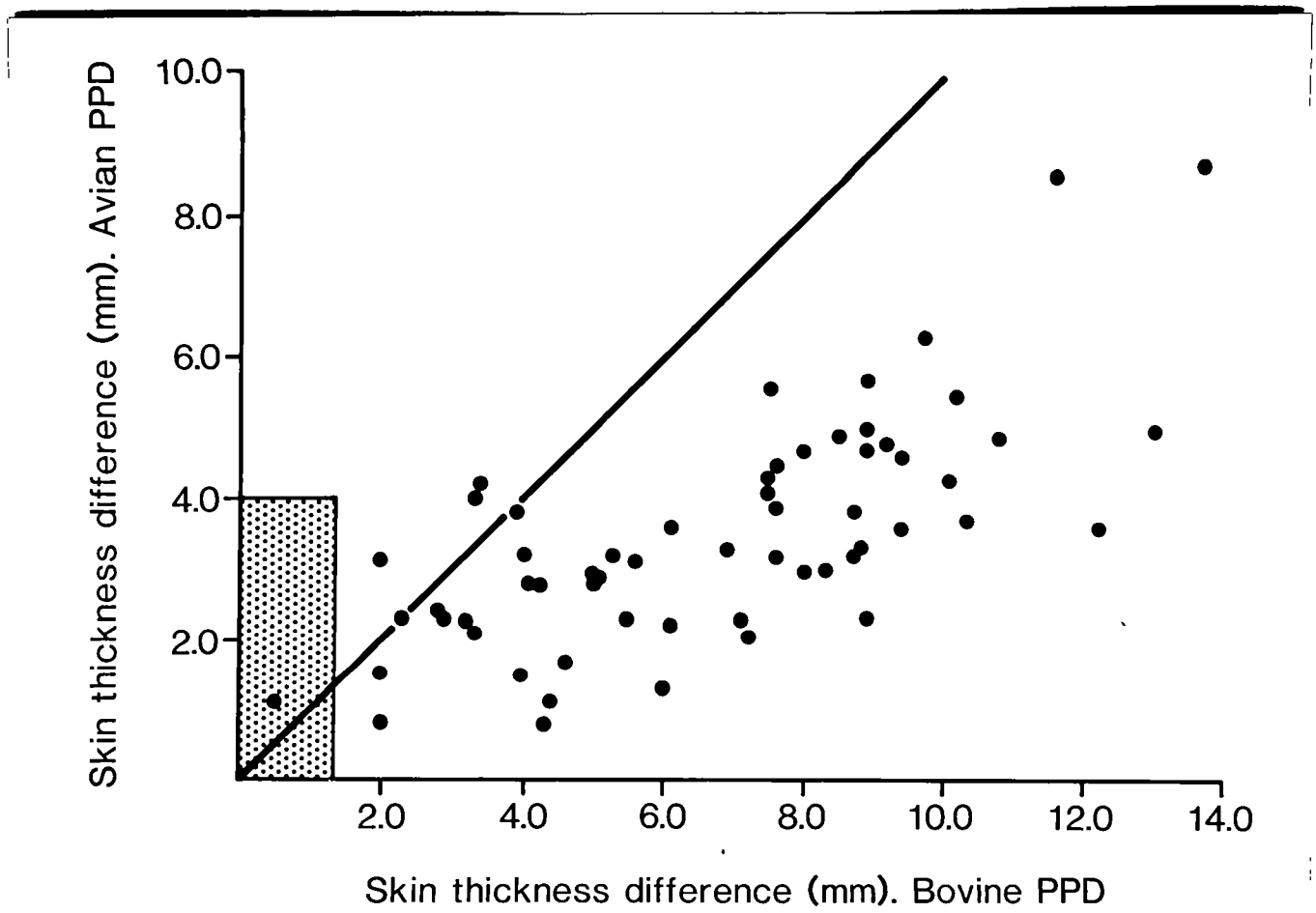
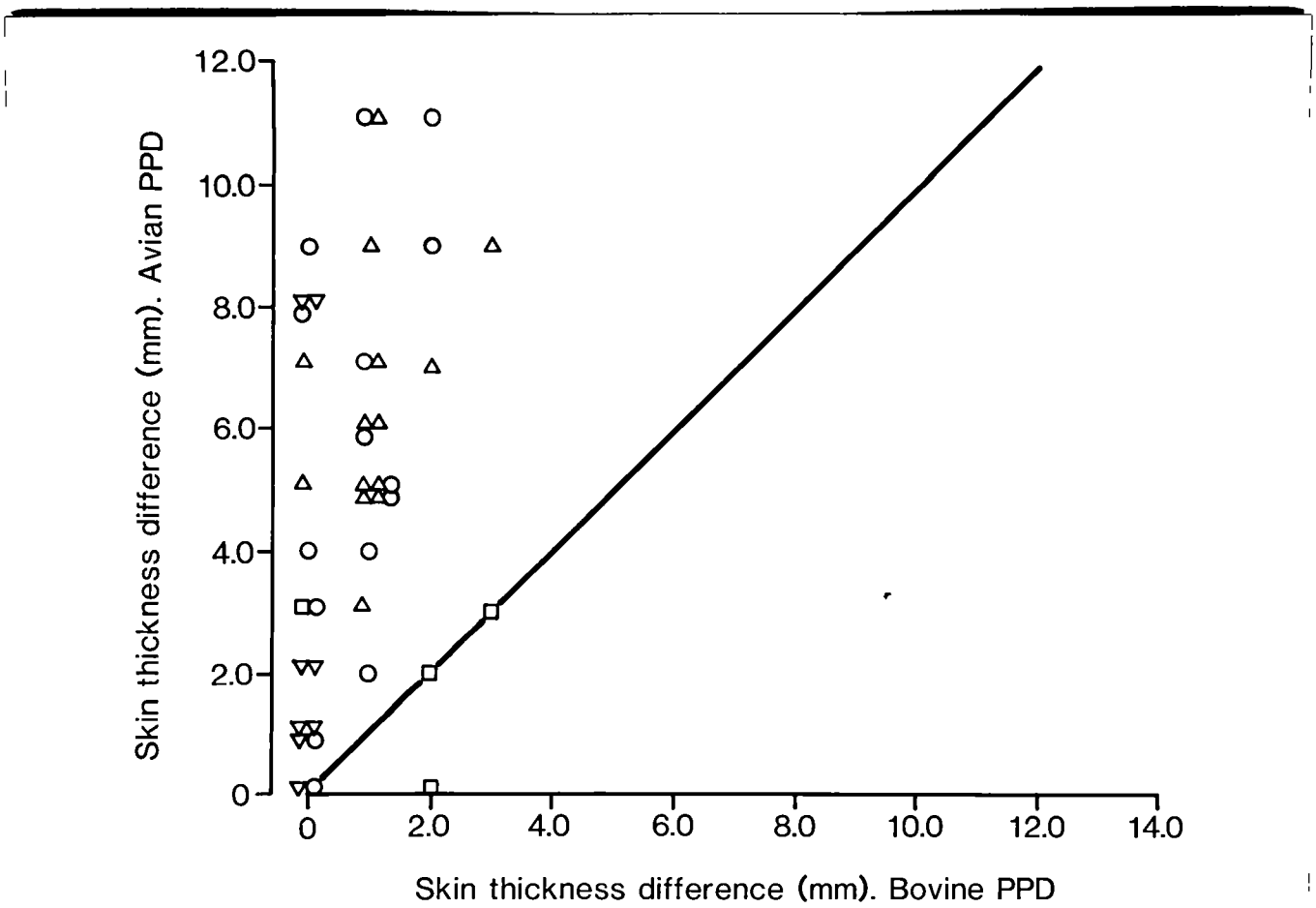


Fig. 2: Scattergram of the comparative test reactions of deer which were previous CT reactors but were not infected with M.bovis. These animals came from four properties which are represented by different symbols.



1.3 Stage C of the experiment
The initial CT was found to suppress the subsequent CCT at both 3 and 7 days. The suppression was greater at 3 days (Table 3).

1.4 Stage D of the experiment
The suppressive effect was still apparent 28 days after an initial CT (Table 3).

2. Comparative testing of CT reactors in deer with no evidence of M.bovis

The results of the CCT are shown in the scattergram (Fig 2). In farm A there was no gross evidence of M.bovis infection in the 15 animals examined. No bacteriological examination was performed on this group. In the remaining groups there was no evidence of M.bovis infection based both on critical post-mortem and bacteriological culture of pooled lymph nodes.

M.avium complex was isolated from 22 of 29 animals from farm B. No significant isolates were found in animals from the remaining two farms.

DISCUSSION

Although the results presented in this paper are preliminary in nature a number of important points can be made on the use of the CCT in deer. The factors affecting the CT (1) are also important when using the CCT. The CCT presents an added complication with the requirement to accurately measure skin reactions. Accurate measurement of reactions is only possible if there is good site preparation, adequate lighting and sufficient restraint.

The specificity of the CT can be increased by using a modified interpretation. If a reactor is defined as having an STD greater than 2 mm then 96.3% (103/107) of the reactions observed before inoculation in the first Flock House experiment would be classified as non-specific (1). In this trial 24% of the reactions in the inoculated infected animals were less than 2 mm, but these responses were affected by short interval testing, thus considerable caution has to be exercised when using a modified interpretation to ensure that there is not an unacceptable decrease in the sensitivity of the test.

The most important finding of the two Flock House experiments is the suppressive effect of prior skin tests. Reaction sizes to bovine PPD in M.bovis infected deer were significantly reduced in animals which had been tested up to 28 days previously. These results indicate that in deer, unlike cattle (2), the CCT cannot be used as an immediate follow up test on finding a reaction to the CT.

Only a limited number of CCTs have been performed on CT reactors which are not infected with M.bovis. These reactions are shown in the scattergram in Fig 2. These results need to be interpreted with considerable caution. While the CCT correctly identified the deer as non-specific reactors there was evidence suggesting suppression by the previous CT. In 17/41 (41%) of the deer there was no reaction to

Table 3

Showing mean skin thickness difference to treatments at (stages C and D).

STAGE	STAGE C			STAGE D	
TREATMENT	Control CCT	CCT 3 days after CT	CCT 7 days after CT	Control CCT	28 days after
mean STD* (mm) AVIAN tuberculin	1.63	0.94	1.32	2.50	1.09
mean STD* (mm) BOVINE tuberculin	4.77	1.50	2.34	7.72	2.66
BOVINE-AVIAN	3.14	0.56	1.02	5.22	1.57

* STD = skin thickness difference

bovine PPD in the CCT despite having responded to it 21 to 42 days previously. This field evidence corroborates the results of those found in inoculated deer.

M.avium complex was isolated from grossly normal lymph nodes from deer on one of the properties. The cause of the non-specific sensitisation on the remaining farms was not determined.

Before the CCT can be used in deer to identify non-specific reactions to the CT further data needs to be collected. Investigations are presently being undertaken in those herds where non-specificity is suspected and there is no evidence of M.bovis.

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