EVALUATION OF THE TUBERCULIN TEST IN DEER

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BACKGROUND

Experiences in a few herds have shown that some deer with tuberculosis do not react to the tuberculin test and eradication of the disease has been hindered. This has created some loss of confidence in the test and the Deer Farmer's Association has requested that these problems be investigated.

A review of testing results, however, gave a sensitivity estimate of 85%. Consideration was therefore given to the possibility that the disease was highly infectious in deer and transmission was occurring between tests. There would, therefore, be some advantage in decreasing the testing intervals to 3-6 weeks. As deer can only be tested at certain times of the year, the ability to test intensively over a short time interval would also be an advantage from this point of view.

A trial was designed to determine whether a programme of high frequency testing was practical and could identify infected deer before they became infective. The opportunity was also taken to compare two strengths of tuberculin, the application of the test, some aspects of the pathogenesis of tuberculosis and the humoral response of deer.

MATERIALS AND METHODS

This experiment spanned an 18 month period (June, 1982 to October, 1983) and involved 72 red deer (Cervus elaphus). They ranged in age from 8 months to 2½ years and were obtained from properties where Mycobacterium bovis (M. bovis) had not been diagnosed. Each had been negative to a single (2 mg/ml) bovine tuberculin test prior to the start of the experiment.

TESTING PROCEDURE

The deer were yarded into a darkened shed and individual animals were restrained in a hydraulic crush (Hydro Lift, Taranaki Deer Services) which allowed site preparation, measurement, tuberculin testing and reading, blood testing, nasal swabbing and inoculation to be carried out.

Tuberculin tests were applied to the skin on the lateral side of the neck; previous work had demonstrated the cervical area to be the most sensitive to bovine tuberculin (6).

The hair over the site was removed for an area approximately 10 x 12 cm using mains operated clippers (Sunbeam Clipmaster). An area of clipped hair (6 x 6 cm) was then shaved. Care was taken to avoid using the same site twice and the centres of each site were at least 7 cm apart.

Prior to injection, the double skin thickness of the shaved site was measured to the nearest 0.1 mm using an engineer's spring-tensioned caliper (Hauptner). Tuberculin (0.1 ml) was injected intradermally

using a pre-set syringe (McLintoch) with a 26 gauge 8 mm medium bevelled needle. One mg/ml and 2 mg/ml Commonwealth Serum Laboratories bovine Purified Protein Derivative (C.S.L. bovine P.P.D.) tuberculin was used.

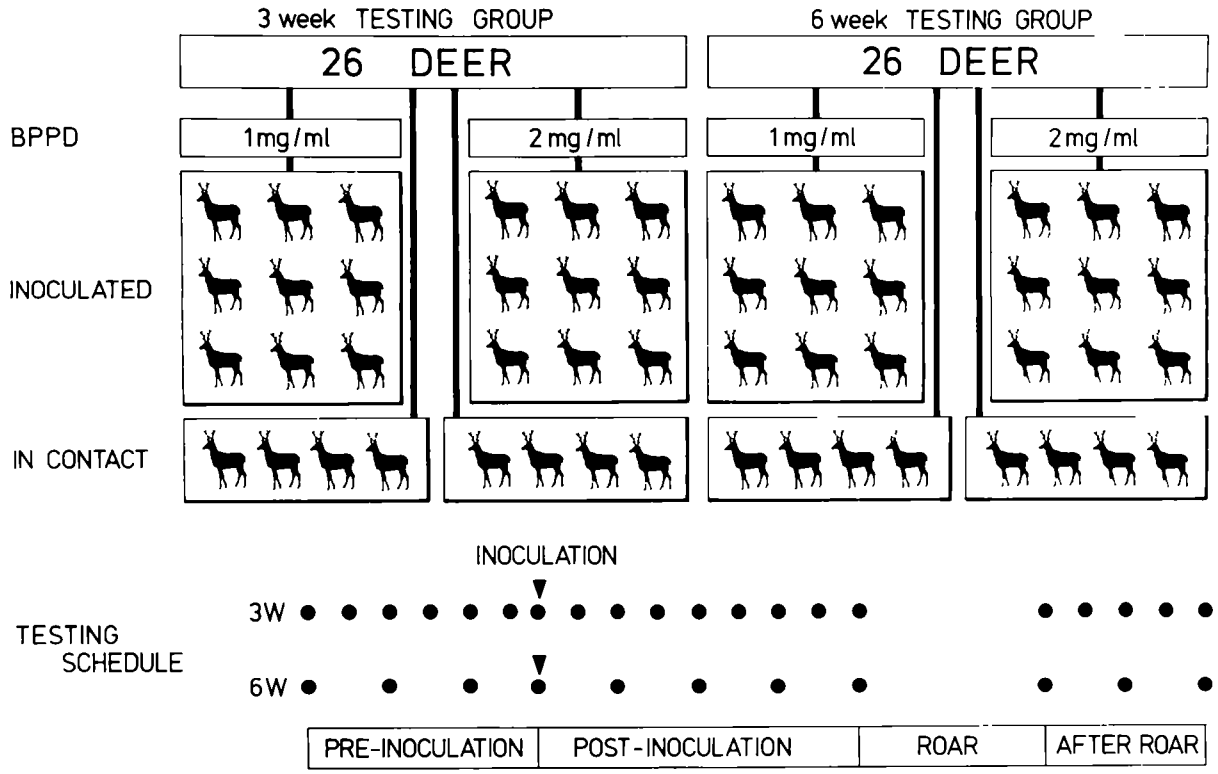
On the day of reading the injection site was observed, palpated and the double skin thickness measured. When the reaction was palpable, the length and width of the skin thickening was measured and the reaction described.

EXPERIMENTAL DESIGN

Part 1.

Fifty-two stags were randomly separated into two equal groups, one of which was skin tested at three weekly intervals and the other at six weekly intervals. Each group was grazed separately.

Figure 1: Experimental design



Within each group, half were tested with 1 mg/ml C.S.L. P.P.D. tuberculin and the other half with 2 mg/ml C.S.L. P.P.D. tuberculin. This testing schedule was maintained for 42 weeks but stopped for 14 weeks over the 'roar'. When testing was resumed for a further 12 weeks, only 2 mg/ml C.S.L. P.P.D. tuberculin was used.

Skin tests were read 72 hours after injection. An additional reading at 48 hours was made at each six week test after the 'roar'. At the

first test after the roar additional readings were made at 24, 48 and 96 hours. Thereafter at the six week tests readings were made at 48 and 72 hours.

After 18 weeks of testing, 18 deer from each group were randomly selected for intratracheal inoculation with M. bovis (2). The remaining deer were kept as in-contact controls.

Nasal swabs were regularly taken prior to, and after, inoculation. These were cultured for mycobacterial species.

Serum was collected from the jugular vein of all deer at six weekly intervals from the date of inoculation. An Enzyme Linked Immunsorbent Assay (ELISA) and Gel Diffusion (GD) test were used to examine the deers' serological response to M. bovis (3).

Two inoculated deer from each of the 3 and 6 week test-interval groups were slaughtered and autopsied at 6, 12 and 18 weeks post-inoculation. Fresh and fixed (10% formalin) samples of all suspect tuberculous lesions were taken, as well as representative lymph node samples from each of the head, thorax, abdomen and body areas.

Forty eight weeks after inoculation all in-contacts and the remaining inoculated deer were post mortemed.

Part 2

On an adjacent farm, 17 hinds and stags were grazed as a control group. They were also divided into two groups and each was subjected to a testing frequency of either three or six weeks. One and 2 mg/ml C.S.L. P.P.D. tuberculin were used.

Their testing schedule paralleled that of the experimental group for 36 weeks. None of these deer were autopsied.

RESULTS

1. Some non-inoculated non-exposed (ie non-infected) controls had skin test reactions which could be visualised and palpated (107/454 tests). However, the skin thickness difference (STD) was always small (103/107 were equal to or less than 2 mm). See figure 2.

2. These reactions in non-infected deer were hard, circumscribed and nodular, and ranged from 1 to 5 mm in diameter. This was in contrast to the reactions in infected animals, which were invariably greater than 10 mm in diameter. Reactions in infected animals were either circumscribed or diffuse, and "oedematous" or hard.

3. Fifty percent (9/18) of the inoculated deer in the 3 week group reacted at 3 weeks post-inoculation and all except one (35/36) inoculated deer reacted at 6 weeks P.I. (Figure 2). Reactions were large (mean STD 6.3 mm) and easily palpable. At subsequent tests reaction size decreased until at the last test before the

roar, 24 weeks PI, 16/21 of the surviving inoculated deer were positive to the test and the mean STD was 2.6 mm.

4. Some recovery in skin sensitivity occurred after the roar (an interval between tests of 14 weeks). The proportion of deer reacting increased to 18/21 and the mean STD increased to 4.0 mm. However, reactions declined at subsequent tests (Figures 2 and 3).

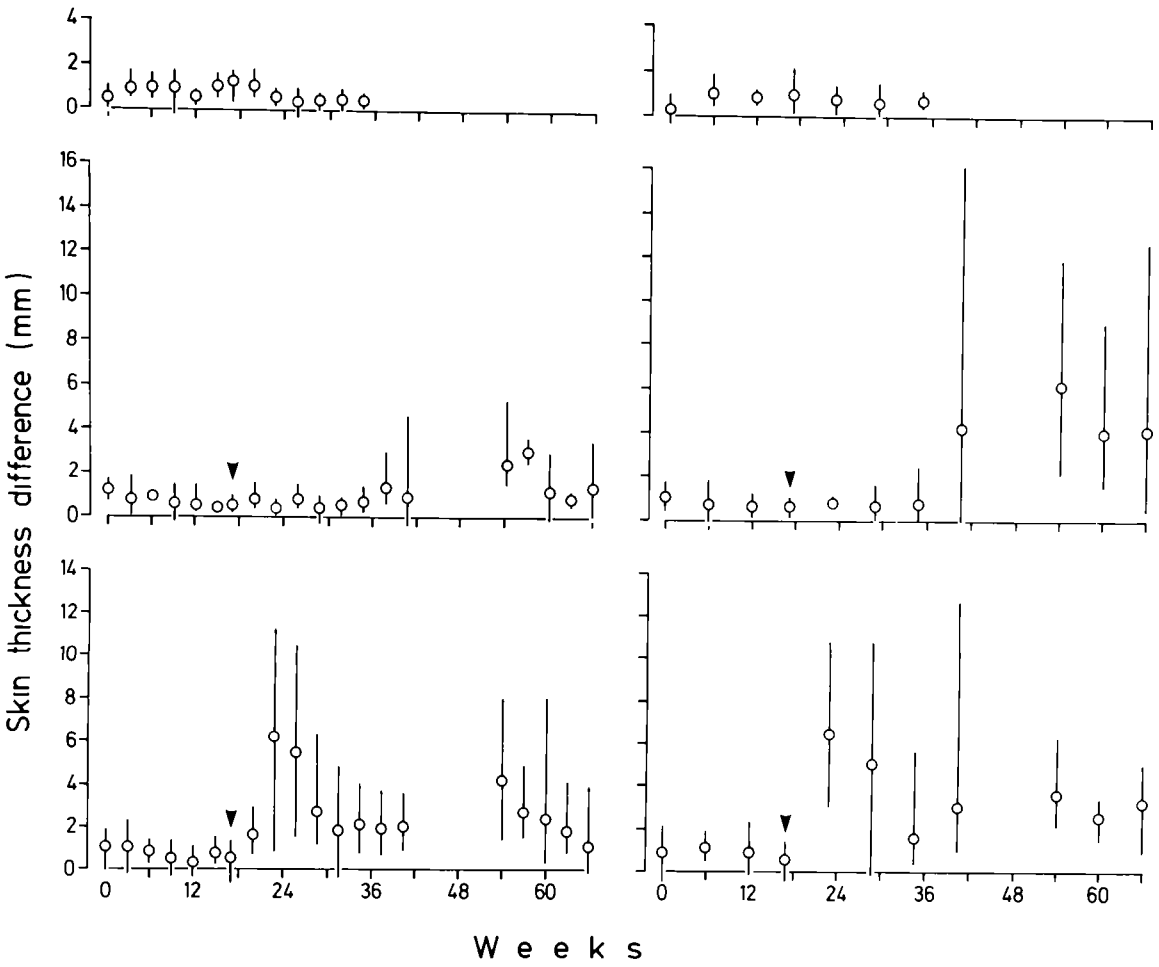


Figure 2. Skin test results; mean skin-thickness difference plus range.

Top, non-infected deer; left 3 week group, right 6 week group.
Middle, incontact deer, left 3 week group, right 6 week group.
Bottom, inoculated deer, left 3 week group, right 6 week group.

5. The earliest reactions consistent with tuberculosis (> 2 mm) in the in-contact control deer occurred between 18-21 weeks PI. At autopsy 11/15 were shown to have Tb. Their skin test reactions are shown in figure 2.

6. There was no statistical difference in skin response to the 1 and 2 mg/ml bovine PPD tuberculin.

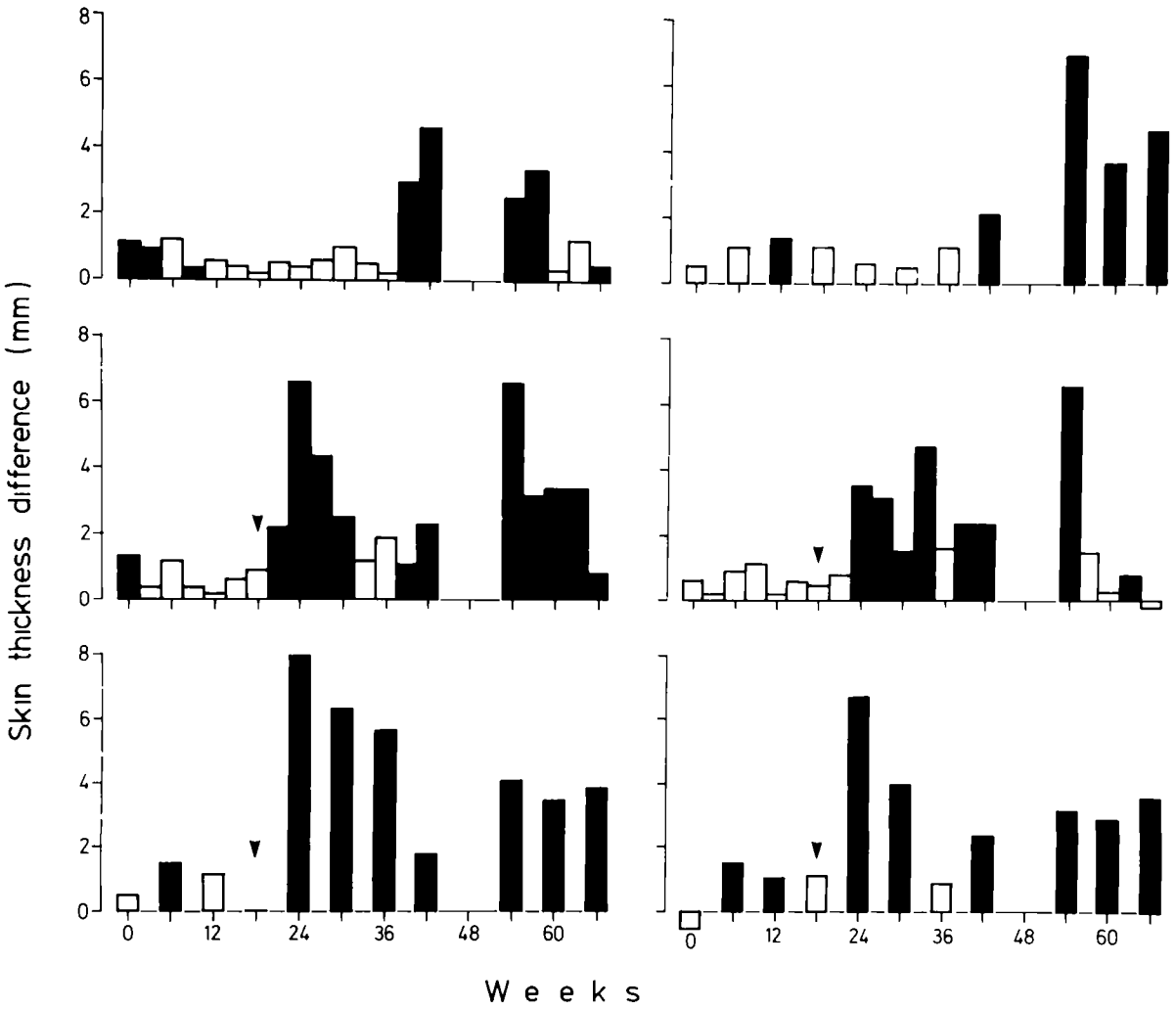


Figure 3. Skin test results of individual animals. Black bar indicates presence of a reaction; open, no reaction. Arrow indicates the day of inoculation. Top, incontact animals; left, 3 week group; right, 6 week group. Middle, inoculated deer, 3 week group. Bottom, inoculated deer, 6 week group.

7. Forty eight hour and 72 hour readings were clearly better than those done at 24 and 96 hours. There was no real difference between the 48 hour and 72 hour readings.

8. Three inoculated deer developed terminal signs of tuberculosis at 18-22 weeks P.I. Weight loss and clinical deterioration only became apparent within 1-2 weeks of death. The autopsy results will be presented by H. Brooks (1).

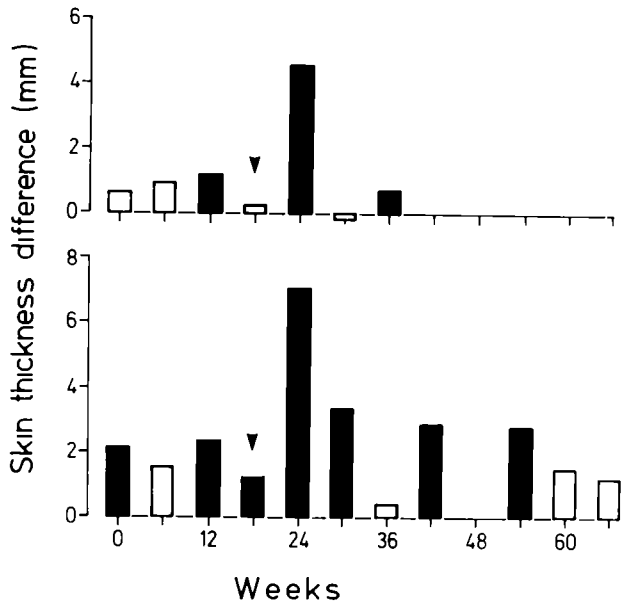


Figure 4. Skin test results of two inoculated deer which had no evidence of M. bovis infection when slaughtered. Black bar indicates presence of a reaction, open, no reaction. Arrow marks the day of inoculation.

DISCUSSION

Two of the most important factors affecting intradermal skin testing in deer are restraint and skin site preparation. Intradermal injection of tuberculin is difficult if the skin test site is not adequately prepared. If it is not closely clipped some positive skin test reactions will be missed. Positive reactions may be grossly diffuse or circumscribed, and firm or "oedematous". Those reactions with a diffuse margin need to be palpated, as well as visually inspected. Shaving greatly enhances the ability to detect these reactions. A good light source is essential to ensure that site preparation, tuberculin injection and reading are carried out correctly.

A large number of small reactions were seen in non-infected deer. Possible causes for these reactions include sensitization to bacteria which share antigens with M. bovis or "needle trauma" (5). There is now good field evidence, supported by laboratory findings that at least some deer infected with M. avium react to bovine PPD (de Lisle pers com). A second series of experiments is being conducted to see if "needle trauma" can produce small reactions. In this experiment the specifity of the skin test was reduced when the presence of a reaction was used as the sole criterion for a positive test. The

problem could be overcome by defining the criterion for a positive reaction as an increase in skin thickness of 2 mm or more. Only 4 of 107 tests on uninfected deer exceeded this range.

Two of the inoculated deer which reacted to the tuberculin test (STD > 4 mm) showed no lesions of tuberculosis at autospy and were negative for M. bovis on culture. Although these skin reactions are indicative of infection by M. bovis the deer may no longer have been infected at slaughter (Figure 4).

Assuming that the in-contact deer took about the same time as the inoculated deer to develop sensitivity, then they acquired an infection 12 - 15 weeks after inoculation of their cohorts.

Short interval repetitive testing in M. bovis infected deer appears to cause suppression of subsequent skin test reactions. This has also been recorded in other species (4). Evidence for suppression includes the decline in reaction sizes following inoculation, which was greater in the 3 week group, and the increase in reaction sizes following the 14 week break in testing at the 'roar'. Our experimental protocol did not allow us to eliminate the possibility of a natural decline in reactivity associated with age of infection.

Short interval repetitive testing did not inhibit the ability of the inoculated deer to develop sensitivity to tuberculin. Development of sensitivity in the in-contact animals may have been affected by short interval testing. The post 'roar' reactions of the 3 week in-contact group were less than those of the 6 week animals. This may in part be due to differences in the degree of exposure to M. bovis.

Testing at 3 weekly intervals is contra-indicated but the use of a 6 weekly interval may be advantageous for disease control in infected herds.

Acknowledgements

The following livestock officers gave their willing support in ensuring the success of the project: R.M. Brockbank, R.L. Broughton, K.M. Comer, G.G. Cracroft-Wilson, R. E. Henman, R.P. Kovaleski, P.C. Oden, A.F.C. Paull, O.M. Walter. The farm manager at Flock House, P. Burke, looked after the deer and helped solve many practical problems. R. Kissling, our biometrician analysed the data.

We acknowledge the assistance given by these people. Without their help this project would not have been possible.

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