TUBERCULOSIS IN DEER: NEW CONCEPTS IN TEST USAGE

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Introduction

Intradermal tuberculin skin tests has been used for over a century to diagnose tuberculosis in humans, cattle, farmed deer, exotic and experimental animals, infected with *M.tuberculosis* or *M.bovis* Whereas this *in vivo* test has been extremely effective in diagnosing tuberculosis in groups of animals with a high prevalence of disease, it has been less effective in identifying individuals in a population when the incidence of TB is low (Francis *et.al.* 1978) The test has limitations in sensitivity wherein it fails to identify 'anergic' animals. This may be compounded by factors such as pregnancy, stress or intercurrent disease, which further reduce the sensitivity of the test (Lepper *et.al.* 1977) The high doses of tuberculin (5,000 i u) used for tuberculin testing of domestic livestock, compared with levels (2 5iu) used in humans, reduce the specificity of the test and cause false(+) reactions, especially in deer which have a propensity for sensitisation with non-specific mycobacteria when the single tuberculin test is used To circumvent this, the cervical skin test (CCT), which uses two injection sites at which purified protein derivative,*M.bovis* (PPD) and *M.avium* (PPD) are applied, was developed to improve the specificity of intradermal skin testing (Corrin *et.al.* 1993) While the CCT has improved specificity, it has reduced ability to identify 'anergic' animals As with all tests that use a single parameter for immunodiagnosis, the cut-points for skin tests may be adjusted to maximise sensitivity or specificity, but never both While it is accepted that intradermal skin test is an adequate screening test to identify TB infected deer herds, its limited sensitivity and specificity reduce its value in populations with a low prevalence of disease, especially when non-specific mycobacterial sensitisation is prevalent (Stuart *et.al.* 1990) Studies carried out over many years have attempted to develop *in vitro* immunoassays to improve TB diagnosis in humans and domestic livestock

Laboratory tests for TB

The central immunological pathway involved in the host response to TB and other intracellular parasites, typified by the intradermal delayed type hypersenstivity (DTH) skin test reaction, involves activation of the T-cell component of the immune system *In vitro* tests have concentrated on cell-based assays of immunity for TB diagnosis (Muscoplat *et.al.* 1975) The intradermal skin test reaction, which measures T-cell activation *in vivo*, is extremely complex, and may either reflect a protective immune response (*immunity*) or be indicative of disease related reactivity (*hypersensitivity*) By contrast, laboratory based tests can be refined to more critically define qualitative aspects of cellular immunity to infection In addition, laboratory tests can be standardised and replicated to evaluate a variety of reactions using different antigens

Apart from cellular assays, tests which measure antibody responses to TB infection have also been evaluated over many years (Krambovitis, 1987) Whereas these assays have been shown to be helpful in identifying seriously affected individuals, they have generally been considered insufficiently sensitive for TB diagnosis as stand alone tests

In 1985 our laboratory was commissioned to develop laboratory tests for TB diagnosis in deer, to supplement the single mid cervical skin test (MCT), as a result of the poor specificity of MCT for TB diagnosis in cervidae (Corrin *et.al.* 1993) In New Zealand farmed deer, less than 10% of MCT(+) animals are found to be tuberculous

The criteria we adopted to develop ancillary tests was that they must

(a) Have sensitivity at least as high as MCT (>80%)
(b) Have specificity superior to MCT (>98%)
(c) Be capable of being applied soon after MCT, either in parallel or in series
(d) Be sufficiently robust to be applied under diverse field conditions.

Based on published data on cellular and antibody tests, it was considered that no single laboratory test or antigen would provide acceptable sensitivity and specificity for TB diagnosis in deer (Griffin and Cross, 1989) Consequently, it was decided to evaluate composite tests which would monitor both cellular and humoral immune factors and inflammatory markers, in naturally infected deer and in deer with saprophytic sensitisation Tests were evaluated in three types of herds, (a) those with a high prevalence of TB, (b) ones with a low prevalence of TB, but with a significant level of non-specific sensitisation, and (c) herds with significant non-specific sensitisation without any tuberculosis *Sensitivity* values of the tests were developed only in herds where TB was present, where culture of *M.bovis* was taken as the '*gold-standard*' for classification of individual animals as tuberculous *Specificity* values were developed using animals in herds where no *M.bovis* was present

The blood test for tuberculosis (BTB)

A multifaceted test has been developed which measures, in parallel, three independent but complementary aspects of immunity and inflammation in deer blood (Griffin and Cross 1989 and Griffin *et.al.* 1994) A lymphocyte transformation (LT) reaction is used to measure the relative cellular reactivity to purified protein derivative (PPD), *M.bovis* (PPD-b) and *M.avium* (PPD-a), as a laboratory analogue of the comparative skin test (CCT) reaction Antibody levels of PPD-b and PPD-a and a *M.bovis* specific protein (MPB-70) were monitored using an ELISA assay (Griffin *et.al.* 1991) Plasma fibrinogen and haptoglobin levels were also monitored as indicators of acute inflammatory reactivity (Cross *et.al.* 1991)

While it would be ideal to use a single assay, incorporating an unique specific *M.bovis* antigen, successive studies have shown that it is necessary to carry out composite complementary assays, interpreted in parallel, to retain both specificity and sensitivity in the test (Griffin *et.al.* 1994)

Critical end points to define positive or negative test values, have been developed for each assay by retrospective discriminant analysis, following *post mortem* diagnosis or exclusion of tuberculosis, due to *M.bovis* in each animal used in the diagnostic database Background values for normal non-infected populations must be established so that they can be subtracted from test values to standardise overall test values and provide uniform test parameters

Test performance values developed from a number of datasets over the previous six years, show sensitivity values for BTB of >95% and specificity values >98.5%. Summary findings on the sensitivity of MCT and laboratory tests for 345 animals, taken from 54 *M.bovis* infected herds throughout New Zealand show individual sensitivity for tests as follows, ST(82%), LT(94%), ELISA(87%) and BTB(>95%) Tuberculosis was diagnosed histologically and animals were segregated into groups with moderate or severe disease MCT performed considerably worse in groups with severe disease compared with disease overall (67% vs 82%), while ELISA was best in seriously diseased animals (87% vs 95%) The initial diagnostic data was obtained using histopathological confirmation of TB, so it was necessary to re-evaluate the performance of BTB has been re-evaluated using data only from animals which had TB confirmed by *M.bovis*(+) culture following slaughter

Data sets have been obtained from two diseased populations, 48 infected deer [*M.bovis*(+)] from 15 herds throughout New Zealand, and 107 infected animals [*M.bovis*(+)] from one herd subjected to depopulation The prevalence of disease in the first group of herds was <2 0%, whereas the prevalence of disease in the depopulation herd was >30% Sensitivity values obtained for LT show a highly consistent performance with the LT, which had sensitivity values of 88 1% vs 90 7% for the two groups of animals The ELISA sensitivity values were lower (74 5%) for the mixed herds than for the depopulation herd (85 0%) This may be due in part to the fact that not all animals in the first group of herds had been skin tested prior to BTB testing Prior skin testing has a significant impact on the incidence and level of the ELISA reactions in TB infected deer However, when the ELISA was used in parallel to produce a BTB assay, the BTB had overall sensitivity values of 95 7% and 95 1% for the two group of animals, indicating that ELISA identified a similar proportion of MCT(-) animals in both test populations Considering the different prevalence of disease and varying degrees of background exposure of these animals to saprophytic mycobacteria, the sensitivity values obtained with BTB are very consistent

Specificity values were also obtained for LT, ELISA and BTB using 218 animals selected from nine disease free deer herds Specificity levels were 98% for LT, 100% for ELISA, 98 0% for BTB Of these animals, 118/218 were MCT(+), indicating significant levels of cellular sensitisation to saprophytic mycobacteria, the specificity of 98 0% for LT is very high

Not only did the BTB distinguish *M.bovis* reactivity from non-specific sensitisation, but it could also stratify disease risk in *M.bovis* infected animals, using the quantitative test values obtained with LT and ELISA Positive predictive values associated with BTB test parameters allow for disease patterns to be charted within a group of *M.bovis* infected deer The positive predictive values for the individual tests and the composite BTB were, LT(+)/ELISA(-)[<50%], LT(-)/ELISA(+)[>80%], LT(+)/ELISA(+)[>90%] These results show a high positive correlation

between ELISA reactivity to *M.bovis* and disease In contrast, MCT was less efficient in detecting severely disease animals (67%), than those with moderate disease (82%)

Overall there was a high degree of consistency in the sensitivity and specificity of BTB using datasets from animals where TB was diagnosed histologically or by isolation of *M.bovis* from deer in herds with a low or high prevalence of infection The reason for this concordance is due to the high positive correlation between histopathological and microbiological diagnosis

The positive predictive value for histology identifying animals that were *M.bovis*(+) on culture, was 93% In the heavily infected herd, 93% of animals with no visible lesions (NVL) did not yield *M.bovis* cultures, while 100% of animals with histological lesions atypical of TB were *M.bovis*(-) It was not unexpected to find that 7% of NVL animals from the diseased farm had *M.bovis*(+) cultures Early studies by Beatson (1984) suggested that *M.bovis* could be isolated from 5-10% of NVL animals in a seriously diseased deer herd

The ELISA test

The composite findings using the BTB test showed that the LT and ELISA tests identified different, though complementary markers of immunity, which were additive when used in parallel The ELISA test appeared to be an especially good predictor of severe TB disease Further studies have been carried out to see if the ELISA test alone, or in parallel with MCT, could be used as a simple parallel screening test to identify [False(-)] tuberculous animals which are 'anergic' to MCT The MCT had a sensitivity 82 4% and the ELISA was 85 3% sensitive When combined to produce a parallel test [MCT(+) and/or ELISA(+)] an overall sensitivity of 95% was obtained The ELISA test identified 11 infected animals which were MCT(-) Of these, nine had multiple lesions, six with generalised TB at multiple sites and three with suppurating lymphatic abscesses draining onto the skin This showed that not only could ELISA detect MCT(-) diseased 'anergic' animals but that most of these animals had severe disease and would have remained as a serious infectious reservoir, had MCT alone been used to eradicate TB from this herd. The disease status of the five double test negative [MCT(-)/ELISA(-)] animals was also examined Three of these five animals has a single lesion and two were NVL This showed that test negative animals had early or contained infection and would be unlikely to act as a reservoir of infectious spread following testing

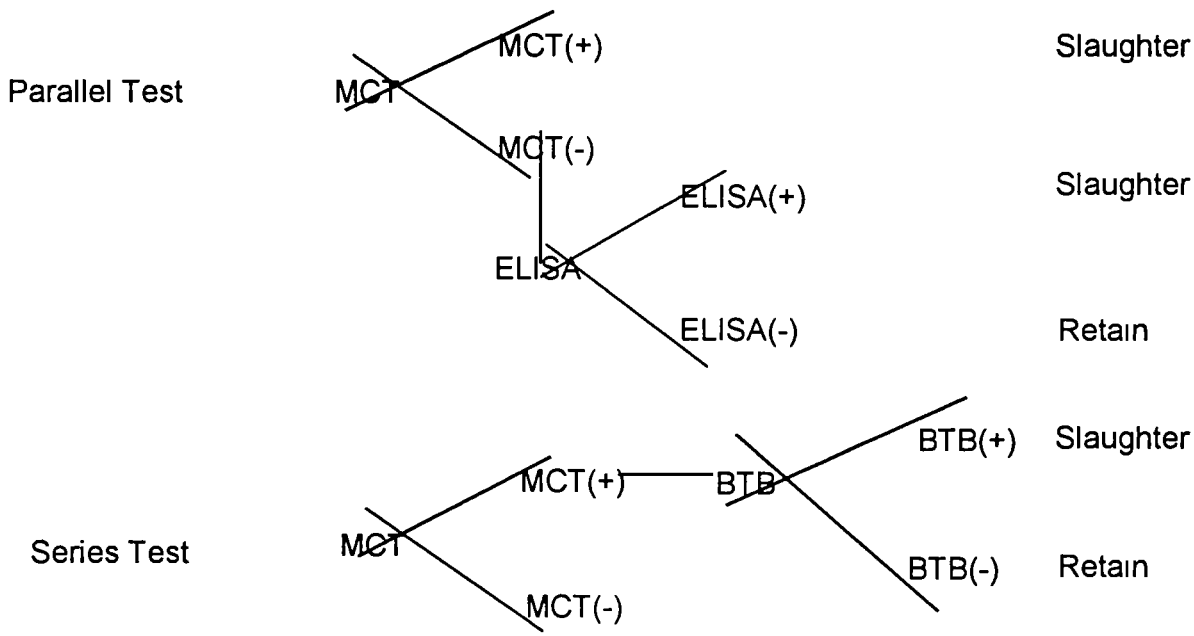
There was evidence that the ELISA titres were significantly influenced by exposure to PPD at skin testing To evaluate this, a comparison was made between ELISA results obtained just before MCT and 10 days after reading the MCT The results show that the sensitivity of ELISA was significantly ($p<0.001$) lower (45 7%) pre-MCT than 10 days post MCT (85.3%) When the ELISA results were combined with MCT in parallel, the pre-MCT ELISA and MCT had a overall sensitivity of 91 2% while the post MCT ELISA and MCT had a combined sensitivity of 95 0% The pre-MCT ELISA identified nine MCT(-) animals, seven of which had multiple lesions or generalised TB Among a group of nine animals which were double negative [pre-MCT ELISA(-)/MCT(-)], eight had single lesions, five of which had extensive calcification Whereas the predominant pattern of disease in ELISA(+)/MCT(-) animals was typical of active disease, the

ELISA(-)/MCT(-) animals had lesions more compatible with contained infection

While the ELISA test is an excellent indicator of disease even this test required the inclusion of three separate antigens to produce a robust assay with acceptable sensitivity and specificity We had hoped that a single *M.bovis* specific protein such as MPB70 would have produced an acceptable assay system, but when used alone test sensitivity was never greater than 70% (Griffin *et.al.* 1991) While the use of this single antigen produced an assay with high levels of specificity it gave significantly lower sensitivity than could be obtained with PPD-b It appears that the minimal antigenic mixture required to produce an adequate ELISA would involve a cocktail of three or more *M.bovis* specific antigens
For clarification the principle of using multiple tests for TB diagnosis is outlined in Table 1

TABLE 1

Use and Interpretation of Laboratory Tests



Parallel tests maximise sensitivity while *series tests* maximise specificity

Conclusion

Accurate immunodiagnosis of TB in deer, and the exclusion non-specific reactions, requires multiple tests to be used in parallel, or serially, to produce effective disease control The BTB test which uses measurements of cellular (LT) and humoral (ELISA) reactivity to mycobacteria, when interpreted in parallel, gives a composite test with both high levels of sensitivity (>95%) and specificity (>98 0%) This test can be used in series with MCT, to

clarify the status of skin test (MCT) positive deer, where it will accurately discriminate between False(+) and True(+) MCT reactions BTB can stratify disease severity and is able to distinguish between disease related hypersenstivity and 'putative' protection in *M.bovis* infected deer (Griffin and Buchan 1994)

An ELISA test has been developed which has a sensitivity of >85 0% for TB diagnosis in deer when used 10-40 days post skin test The special value of the ELISA is that it can identify seriously diseased animals which are 'anergic' to MCT [False(-)] ELISA titres are increased following MCT and this test can be used in with MCT, to identify MCT(-) infected deer The combined sensitivity of MCT and ELISA used in parallel is 95 0%, very similar to values obtained using LT and ELISA in parallel

Considering that independent parameters are being evaluated within the laboratory test, BTB may be used in series with MCT to identify animals with non-specific sensitisation with saprophytic mycobacteria or animals infected with *M.bovis* The fact that the LT and ELISA components of BTB are interpreted in parallel provides high levels of sensitivity and specificity within the composite assay By contrast, in known infected herds where false(-) MCT's are suspected, it is possible to use ELISA, on blood taken two weeks post MCT, to provide results where MCT and ELISA are interpreted in parallel, to maximise the sensitivity of disease detection

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