Advances in Tb diagnosis and prevention: lessons gained from the New Zealand deer industry.

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Abstract

In 1985 the Deer Research Laboratory (DRL) first began to develop ancillary blood tests as an aid for Tb diagnosis in deer The resulting blood test for Tb (BTB) for deer has been used widely for Tb diagnosis throughout the national herd This test has also been used overseas for Tb testing in free range wildlife species BTB has used the concept that cellular immune reactions can be used in conjunction with ELISA based antibody assays to produce a composite (parallel) test, which has superior sensitivity and specificity, to that obtained with the conventional mid-cervical skin test (MCT). BTB has played a major part in clarifying the reactor status of MCT(+) deer, resulting in confirmation of tuberculosis or salvage of non-specific reactors. However, the high unit cost of BTB precludes its widespread use in non-reactor animals Instead an ELISA test has been developed which is used in parallel for MCST(-) animals to identify skin test anergic animals in Tb infected deer herds. A modified version of the ELISA which measures different antibody classes has recently been developed, and is being used to add increased precision to Tb diagnosis.

In addition to diagnosis we also have an active programme studying vaccination as a means for Tb prevention, using BCG as the prototype vaccine Thes studies have involved a deer model for vaccination, where protection in BCG vaccinated animals is measured by exposing them to experimental infection This model has established which parameters are important in maximising protection following vaccination The influence of vaccine, dose, route, formulation, boosting and viability have been studied, and the findings will be discussed Protective memory has been shown to be sustained for at least one year post vaccination While the BCG vaccination results from our deer studies show highly significant protection against TB infection and disease, it remains to be established whether vaccination could best be used for domestic livestock or wildlife vectors

Background

The New Zealand deer industry adopted a proactive position with respect to Tb eradication from1985 onwards Faced with the prospect that tuberculosis could threaten the survival of this fledgling industry the farmers actively supported new research initiatives to allay this fear They adopted the policy that the existing Tb control measures used by cattle farmers were insufficient to achieve the goals required by the deer sector They actively advocated for ancillary diagnostic tests which would improve upon the accuracy of the existing intradermal skin test. They were faced with the dilemma that intradermal skin testing produced a high proportion of False (+) reactions while failing to identify every Tb infected animal They took the view that ancillary tests were essential to avoid unnecessary wastage of valuable livestock and to improve the sensitivity of test protocols to identify all infected stock within infected herds

Approved ancillary tests, namely the Comparative Cervical Test (CCT) (Corrin *et al* 1993) and the Blood Test for Tb (BTB – a combined Lymphocyte Transformation Test and the antibody ELISA test) (Griffin *et* al 1994) were introduced in 1986 and 1987, respectively These tests led to a reduction in wastage due to non-specific reactivity to the tuberculin tests and were instrumental in gaining farmer support for a control scheme (Carter *et al.* 1995) This support, together with a reduction in the value of deer and a more stable deer industry, lead to the introduction of the Compulsory Tb Control Scheme for deer in 1990 Since that time all deer herds have come under test and the prevalence of Tb in deer has steadily declined Another major change over the last 15 years has been the formation of the Animal Health Board, which has taken over the role of managing the Tb control programme from the Ministry of Agriculture and Fisheries (MAF) After a series of changes in the late 80s and early 90s the National Bovine Tuberculosis Pest Management Strategy came into

effect in 1998 and the Animal Health Board (Inc) has the responsibility for its implementation. The strategy is implemented through the National Tb Operational Plan. The objectives are: to reduce the number of infected herds to 0 2% of total herds in Tb Vector-Free Areas (TbVFA) of NZ, to prevent any TBVFA becoming a Tb Vector Risk (infected wildlife) Areas (TbVRA) and to keep the number of infected herds <11% in TbVRA (Animal Health Board Annual Report, 1998). Using these programmes, the number of infected deer farms has declined from 445 (13 9%) in 1985, to 274 (4.7%) in 1990 to 95 (1 72%) in the year ending December 31, 1999 By comparison the number of infected cattle herds in New Zealand in December 1999 was 635 (0 98%) The prevalence of infected deer herds in TbVFA was 0.36% and in TbVRAs was 5.97% in this last year. Around 24% of deer herds in NZ are in TbVRAs and are at increased risk of breaking down (ie having at least one animal in a clear herd becoming infected) due to the introduction of Tb into the herd from a feral/wild vector source

Blood tests for Tb diagnosis

Laboratory based Tb tests have been developed to compliment whole herd skin testing programmes for farmed deer A composite blood test for Tb (BTB), which uses a cell based lymphocyte transformation (LT) test and an antibody based ELISA test has been developed (Griffin *et al* 1994, Griffin, 1989) LT and ELISA measure relative responses to bovine purified protein derivative (PPDb) *vs* PPDa (avian) with mononuclear cells or serum from deer, to distinguish between non-specific background reactivity and specific responses to *M bovis* Test parameters and specifications for these assays have been developed using blood samples obtained from the field Tb infected animals were used to determine test sensitivity values. Detailed necropsy, histological and microbiological studies have been carried out on more than 200 animals with tuberculosis, confirmed by the isolation of *M. bovis* from necropsy specimens, obtained from Tb infected animals A similar number of animals from Tb-free herds have been studied to determine test specificity values. The BTB test is used as a serial ancillary test to clarify the status of mid-cervical test (MCT) (+) animals while the ELISA test alone is used in parallel with MCT to identify MCT(-) tuberculous animals, which fail to react to the skin test There is considerable advantage in combining cell based tests (LT or MCT) with antibody (ELISA) tests as they produce composite results which are additive and can improve the overall sensitivity to diagnose infection, without causing a major reduction in overall test specificity. In one group of 102 tuberculous deer, the sensitivity of LT alone was 90% and that for ELISA was 85%, but when combined, the composite BTB test had a sensitivity of >95% Independent studies (Hutchings and Wilson, 1995) showed that LT tests measuring comparative responsiveness to *M bovis* and *M paratubeculosis* antigens had a sensitivity of 76% for Tb diagnosis in Canadian elk (*Cervus elaphus)*

Early studies (Sutton *et al* 1985) evaluated the performance of ELISA for Tb diagnosis in naturally and experimentally infected deer, showed that the assay had a sensitivity of 72% The assay was 100% sensitive in identifying animals with generalised tuberculosis. A more recent evaluation of a competitive ELISA for Tb diagnosis in deer showed sensitivity values around 80% (Sugden *et al* 1997). An increase in sensitivity is evident, when ELISA and MCST results are combined (Griffin, 1989) While MCT was 82% sensitive for diagnosis of Tb in a group of infected animals, when combined with ELISA (85% sensitive), the composite sensitivity of the tests, interpreted in parallel, was 95% Other studies involving ELISA based tests in deer (Gaborick *et al.* 1996) show that whereas an ELISA test had a sensitivity of 70 % alone, its sensitivity was enhanced when used in parallel with the skin test

Specificity values (Griffin *et al.* 1994) for LT and ELISA were 98% and 100% respectively, for a group of 200 animals from nine non-infected deer herds. In this group the composite specificity of BTB was 98% The specificity of BTB obtained using the test on more than 50,000 deer over a ten year period is >98.5%, indicating that the test can accurately identify non-infected skin test (+/-) deer. The concept of using cell based and antibody tests in combination, allow for a gain in sensitivity without any significant drop in specificity

Considering that blood tests are designed to be used in conjunction with skin tests, it was important to establish what influence the intradermal injection of tuberculin had on subsequent laboratory

parameters measured by blood tests Samples taken prior to skin test and for the succeeding weeks show that MCT causes significant suppression in LT for 3-14 days post injection of tuberculin. MCT also has a significant effect on ELISA, where antibody levels increase dramatically in Tb-infected animals, from 4-28 days post skin test Sensitivity values for ELISA carried out prior to and at 14 days post MCT, show that the sensitivity of ELISA is significantly enhanced by MCT. Whereas ELISA was 46% sensitive pre-MCT, its sensitivity increased to 85% after skin testing. The suppression caused by MCT on LT in the period immediately following skin testing and the enhanced level of ELISA reactivity following skin testing in infected animals led to the recommendation that blood tests should be carried out between 14 and 28 days post MCT injection The sensitivity values obtained when the test is carried out 2-4 weeks post skin testing were sufficiently high (85%) to allow the assay to be registered as a stand alone parallel test for use in MCT (-) animals in TB infected deer herds (Griffin *et* al. 1994). The fact that antibody tests target chronically infected animals with generalised Tb is important, as these are the animals most likely to be 'anergic' to skin tests

New generation tests for Tb in deer

While the ancillary Tb tests developed for Tb diagnosis in deer have provided assurance that alternative ancillary tests (BTB or ELISA) are available for skin test (+/-) animals, respectively, two significant impediments remain concerning the currently available tests BTB requires separation of blood mononuclear cells, making the test expensive and difficult to automate The less expensive ELISA test is sensitive (85%) in diagnosing TB, when used 14-28 days post skin test, because the ELISA is amplified by the anamnestic response produced by intradermal injection of Tuberculin Herd screening tests that could be applied, independent of skin testing, would provide increased flexibility for the testing of MCT (-) animals in infected herds. We are currently evaluating two new tests that show promise in addressing the two major concerns raised above

Cervigam Test

This test is based on the original whole blood Interferon-γ (Rothel *et al* 1990) test *[Bovigam]* developed in Australia, for diagnosis of Tb in cattle To date we have trialed this test on MCT(+) animals, in a number of infected deer herds. The preliminary sensitivity values (80%) given in Table 1 show that this assay has considerable potential to diagnose Tb infection in deer. The test also has high specificity values, suggesting that it will not result in undue wastage of False (+) MCT reactors.

Table 1. Performance of laboratory assays for Tb diagnosis in deer

Test	Sensitivity %	Specificity %
BTB	95	> 98
ELISA	86	> 99
Cervigam (Cg)	80	> 99
CG + ELISA	88	> 99

Modified ELISA Test

The original ELISA test (Griffin *et al* 1991) developed to compliment the Lymphocyte Transformation Test, has a sensitivity of around 85% when carried out 7-30 days after skin test injection However, it has poor sensitivity (46%) when used prior to MCT. The standard test uses a polyclonal antibody that recognises deer IgG. We have developed a new ELISA that measures sublclasses of IgG [IgG1 & IgG2]. While the IgG2 response provided little diagnostic potential, we have obtained some very promising results using an ELISA specific for deer IgG1 antibody. The results (Table 2) show that the assay was significantly more sensitive in detecting tuberculous animals than the standard (IgG) ELISA, prior to skin test Sensitivity values were extremely high

when the test was carried out 14-28 days following the skin test Specificity values are not yet been established

Table 2. Sensitivity of IgG ELISA *vs* IgG1 ELISA for Tb lesion diagnosis

	IgG ELISA		IgG1 ELISA	
	Pre MCT	*Post MCT*	*Pre MCT*	*Post MCT*
Herd 1 (47)	30 (65%)	44 (95%)	39 (85 %)	46 (100%)
Herd 2 (54)	4 (7%)	39 (72%)	33 (61%)	53 (98%)

The IgG1 ELISA has the added advantage that it can identify infected animals, that have been vaccinated prior to infection Results given in Table 3 show that the modified (IgG1) ELISA could detect all of the vaccinated animals that were infected By contrast, the IgG ELISA performed very poorly with vaccinated animals that became diseased [Lesion (+)/culture (+)] and failed to detect infected animals [NVL/Culture (+)]. The ability of this assay to identify vaccinated animals that become infected, would be essential for quality assurance should Tb vaccines ever be released into the environment, for use in domestic livestock or wildlife.

Table 3. Immune markers in experimentally infected deer

Controls	Disease	Culture	LT	ELISA (IgG)	ELISA (IgG1)
801	+	+	B8	0/65	38/180
819	+++	+	B8	30/101	52/181
869	+++	+	B8	0/27	12/128
815	-	+	B6	0/0	0/27
827	-	-	A1=B1	0/0	0/13
Vaccinates					
808	++	+	B4	0/16	10/132
830	++	+	B4	0/0	0/93
803	-	+	B6	0/0	33/58
836	-	-	B5	0/0	26/13
811	-	-	B2	63/1	146/67

There is sound theoretical rationale for using the IgG1 ELISA test. This subclass of antibody is produced following activation of TH2 cells (Romagnani, 1996), the T cell subpopulation associated with antibody production, rather than cell mediated immunity. This pathway of immunity has no protective effect, so its potential to diagnose infection is logical The corollary, that IgG2 was of little value in diagnosing infection is also logical, considering that IgG2 is associated with TH1 cells, which produce cell mediated immunity, and are protective against tuberculosis.

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