Recent advances in deer Tb research: Diagnosis, vaccination and heritability of resistance

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Abstract

Over the past year, continuing research programmes at Otago University have examined new methods for Tb diagnosis, evaluated immune memory post vaccination and extended the studies on heritable resistance to Tb in deer

Diagnostics. A major study has been undertaken to determine if a cheaper blood test could be developed for Tb diagnosis in farmed deer Two types of test have been evaluated, 1) A cell-based test measuring Interferon-γ (IFN-γ), and 2) A modified antibody based test (ELISA) which measured IgG1 antibody to *Mycobacterium bovis* antigens. The specificity of these tests were evaluated using blood samples obtained from 50 MCT(+) non-infected deer, obtained from a number of C3 herds that were from 'vector free' regions. An equivalent number of animals from infected herds were used to determine test sensitivity values Estimated specificity and sensitivity values were determined for each test, compared with the data obtained with the 'gold standard' BTB test

Vaccination Long-term immune memory data was obtained for animals challenged 26 and 52 weeks post 'prime-boost' vaccination The efficacy of long-term immune memory was evaluated by comparison with positive control vaccine groups of animals challenged 6 weeks after 'prime-boost' vaccination with BCG, and with unvaccinated control animals

Tb resistance Heritability of resistance was confirmed in weaner deer selected from herds considered to have a significant bias towards a **R** or **S** phenotype These were compared with animals from Non-selected **(N)** herds and from a herd which had a serious outbreak of Tb infection in the past three years **(PI).**

Diagnostic test data

Non-infected animals were selected from herds with at least C3 Tb-free status from regions within New Zealand that were free of infected vectors These animals were used to estimate the specificity of alternative blood tests for Tb diagnosis. Sensitivity values were determined using MCT (mid-cervical skin test) (+) animals from infected herds Test results were compared with the standard BTB test (Griffin *et al* 1994), to establish the validity of the assays within the data sets and to provide some comparison with the overall database available for the BTB test The BTB test is comprised of a composite of the lymphocyte transformation test (LT) and standard IgG ELISA test The BTB measures the differential responses to *M bovis* and *M avium* tuberculin, respectively (PPD-B *minus* PPD-A) While this test is very precise in diagnosing Tb and in excluding non-specific reactors, it is expensive and technically demanding to perform

A modified version of the Bovine Interferon-γ (IFN-γ) [Bovigam™] developed by CSL, Australia (Wood *et al* 1990), was used to determine the precision of whole blood lymphocyte (T-cell) tests for Tb diagnosis in deer This will be referred to subsequently as the ""Cervigam"" test A modified version of the standard IgG ELISA test (Griffin *et al* 1994) which measured IgG1 levels was used to determine if the precision of the ELISA test for Tb diagnosis in deer could be improved upon. The rationale for choosing an IgG1 based ELISA was that this antibody isotype (IgG1) is associated with TH2 cell regulation of B-cell activity TH2 cells are associated with the non-protective pathway of immune reactivity to intracellular pathogens such as *Mycobacterium bovis* (Griffin *et al* 1998) By contrast, the IgG2 isotype is associated with TH1 cell regulation, the pathway considered to generate protective immunity following infection with *M bovis* The data presented in Table 1 shows the comparative data set for the sensitivity of BTB, the standard IgG-ELISA and the modified IgG1-ELISA.

Table 1 Comparison of Sensitivity of BTB, ELISA and IgG1 ELISA for M. bovis culture positive animals

	BTB	ELISA	IgG1 ELISA		
			>20	*>30**	*>30*
No positive	48	38	47	43*	43
No negative	5	15	6	6	10
Sensitivity (%)	91	71	88	88	81

The results given in Table 1 show that the sensitivity of BTB (91%) was similar to that obtained with this assay in successive studies out over the past 15 years (90-95%). The sensitivity of the standard IgG-ELISA (71%) was lower than found in earlier studies where sensitivity was normally around 80%. In this study the sensitivity of the modified IgG1-ELISA was 88% when a cut point of 20 units was used for PPD-B minus PPD-A It was the same when a cut point of 30 was used, and animals with values of 20-30 units were classified as equivocal* Sensitivity for the IgG1-ELISA was 81% when a cut point of >30 was used

The specificity values for these assays was determined using blood samples from MCT (+) animals obtained from a series of Tb status All of these herds had a status of at least C3, and they were farmed in areas with Tb-free vectors The specificity values are given in Table 2 below.

The results given in Table 2 BTB and IgG-ELISA had 100% specificity. The IgG1-ELISA had a specificity of 97% using a 30 cut point or a 30 cut point with animals showing reactions between 20 and 30 being classified as Equivocal*** The assay showed a specificity of 94% when a cut point of 20 was used

Table 2: Specificity of BTB, IgG-ELISA and IgG- ELISA Assays for MCST Reactor animals from C3 or greater herds in vector free areas

	BTB	IgG-ELISA	IgG1 ELISA		
			> 20	*>30****	*>30*
No negative	60	63	61	61	63
Equivocal/No Data*	5	2	0	2	0
No Positive	0	0	4**	2	2
Specificity (%)	100	100	94	97	97

* All animals showing Equivocal or No data status returned negative results on retesting

**These four animals were tested with an extended range of antigens including PPD-A, PPD-B and PPD-J, Johnin obtained from M paratuberculosis

**An attempt was made to elucidate further the basis of the " False (+)" reactions in the four animals with B-A values of > 20 The results obtained, when PPD-J (Johnin) was included in the assay show that whereas these animals had B-A reactions that was indicative of a positive reaction to M bovis, each had J reactivity that was greater than the B reaction The J >B >A pattern of humoral reactivity has been seen consistently in cattle and deer vaccinated with Johne's vaccine ("Neoparasec") or animals naturally infected with M paratuberculosis This provides evidence, that whereas the IgG1-ELISA appeared to produce a 'False (+)' B-A reaction, the J >B response suggested that these animals may have reacted due to an underlying M paratuberculosis infection

Caveat: The results presented here have not yet been subjected to an independent audit The final specifications for the IgG1 ELISA will be published after completion of the audit "Cervigam" data remains to be audited and will be published in due course.

Vaccination studies

Vaccine studies carried out over the past ten years have shown that subcutaneous vaccination with live BCG produces significant protection against infection and disease, providing a two dose prime-boost protocol is used (Griffin *et al* 1999) A single dose of vaccine produced protection against disease but did not protect against infection The efficacy of protection has been determined by challenging vaccinated animals with virulent *M bovis* by the intratonsilar route, 6-8 weeks post vaccination The current study examined the persistence of protective immunity in animals challenged 6 weeks, 6 or 12 months post-vaccination The results are given in Table 3

Table 3 Protective Immunity at 26 and 52 weeks following prime-boost vaccination with BCG

Treatment	Control	Vaccinated (6 weeks)	Vaccinated (26 weeks)	Vaccinated (52 weeks)
Non-Infected	1/14	11/15	11/15	9/14
Infected	13/14	4/15	4/15	5/14
Log No M bovis / Gm of LMRP*	2 9	2 5	1 9	1 83
Diseased	11/14	1/15	1/15	0/14

*LMRP- Left Medial Retropharyngeal Lymph Node; The infection level is represented by the numbers of virulent M. bovis recovered per gram of infected tissue LMRP tissues best reflect the level of infection found following experimental challenge of the left tonsil

The results given in Table 3 show that the levels of protection seen at 6 and 12 months post vaccination were equivalent to the levels seen at 6 weeks post vaccination. All showed significant protection against infection and disease compared with the non-vaccinated control group This data suggests that protective immunity persists for at least 12 months after 'prime-boost' vaccination. The absence of any waning of immunity between 6 weeks and that found at 6 or 12 months, infers that protective immunity may be sustained for considerably longer than 12 months.

Heritable resistance to Tb

Studies have been carried out over the past five years (Mackintosh et al 2000) using artificial insemination of out-bred females with semen from stags shown to be resistant (R) or susceptible (S) to experimental M bovis infection The estimated heritability of resistance to Tb in the progeny was 0 48 (+/- 0.26), indicating that 48% of the resistance trait was likely to be associated with the genotype of the host. This has allowed us to identify breed lines of deer considered to be either resistant or susceptible to Tb Our most recent study was designed to confirm that the putative R and S bloodlines breed true to their genotype

Weaners were selected from herds considered to have a R or S genotype. In addition animals were chosen from herds where no selection had been carried out. A further group of animals were selected from a herd which had a major outbreak of Tb three years ago In this herd, upwards of 20% of infected adult animals had been culled after the initial Tb outbreak. The level of infection found at the initial Tb outbreak provided significant selective pressure against susceptible animals. Progeny from this herd were studied on the premise that uninfected, surviving adults (hinds or stags) might show

evidence of increased resistance, compared with unselected stock. Results from this study are given in Table 4

Table 4. Mean group Lesion Severity Score (LSS)* in animals challenged with virulent M bovis via the tonsil

Herd Status	'Resistant'	'Post-Infection'	'Unselected'	'Susceptible'
LSS	1 60	2 05	3 20	4.80

The response to intra-tonsilar infection with virulent *M bovis* was evaluated at necropsy, 4 months after challenge The classification is on a scale of 1-6, where 1 represents the absence of lesions and 6 represents generalised Tb (GTB) The results show that the status of the "Resistant' and 'Susceptible' animals was confirmed Unselected animals showed no bias towards resistance or susceptibility. By contrast, animals from the "Infected" herds showed a significant trend towards a resistant phenotype

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