An Alternative IgG_1 ELISA Test for Tb Diagnosis in Deer

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Abstract

An IgG-ELISA (Griffin et al 1991) assay has been approved by the CVO as a parallel test for Tb diagnosis in problem herds (> 5% reactors) and it is an integral part of the BTB assay used as a serial test for Tb diagnosis in MCST(+) deer. The IgG ELISA has a sensitivity around 75 – 80% when used as a stand alone assay. In-house research suggested that an ELISA test which specifically measures the IgG_1 isotype of IgG may have superior sensitivity for diagnosing Tb in deer (Griffin et al 2001). No such effect was seen with the IgG_2 isotype.

To evaluate the performance of the IgG_1 assay, animals were selected to establish test sensitivity and specificity values. Animals included for the specificity dataset were from herds with a history of non-specific reactivity and were all MCST(+). Two of these herds had a history of Johne's disease. Sensitivity datasets included animals that were either culture (+) or histologically typical of Tb.

Specificity values for IgG_1 ELISA between 95 – 98% were obtained with samples from non-infected herds sampled throughout the country. The lower specificity value was seen in herds with a known history of Johne's disease. Sensitivity values, in infected herds, were 87 to 93% in animals with M. bovis (+) culture and 100% for animals classified as positive on histology alone. Overall the IgG_1 ELISA test gave a sensitivity and specificity of 95% with a 20 unit cutpoint. The assay gave a sensitivity of 89% and a specificity of 98%, respectively, when the assay cutpoint was ^{3}30 units, classified reactions as positive and 20-30 units as equivocal.

The False(+) serological response seen in most animals that were presumed non-tuberculous, but had a B-A response ^{3} 20 units, is of special interest. All of the False(+) animals (B-A > 20) had J > B responses, indicating that Johne's infection may be a significant confounder in diagnosing M. bovis infection.

We premise that an assay with a sensitivity of 95% and the ability to identify non-tuberculous animals, likely to be infected with Johne's disease, has important diagnostic implications. We recommend that further studies be carried out to evaluate IgG_1 ELISA patterns of responsiveness in deer herds co-infected with M. bovis and M. paratuberculosis; the causative agent for Johne's Disease.

Terms of Reference for test development

Deer farmers consider the BTB test (Griffin *et al* 1994) too expensive for widespread field use. Based on the propensity of deer to produce significant levels of IgG antibody following exposure to saprophytic or pathogenic mycobacteria, further refinements of the ELISA test were considered. A modified IgG_1 ELISA-based antibody assay was developed (Griffin *et al* 2001) and trialed to determine whether it could be used as an alternative to the BTB for Tb diagnosis in deer. All laboratory costs for the development of the IgG_1 ELISA test were underwritten by DRL.

1.0 Introduction

Laboratory based immunoassays for Tb infection caused by *M.bovis* have traditionally been based on cellular (T cell) or antibody (B cell) assays. While T cell assays mimic the patterns of immune reactivity seen in the conventional intradermal skin test, antibody tests have the potential to monitor different pathways of immunity relevant for Tb diagnosis. Deer produce antibody following exposure to *M.bovis* and the antibody titre is enhanced by skin testing. Our laboratory has used an IgG-ELISA test as part of the BTB test and as a stand alone test for parallel testing of MCST(-) deer in seriously infected herds. While the IgG-ELISA has a sensitivity around 75 - 80%, it is not considered sufficiently sensitive for use in MCST(+) deer. Preliminary studies carried out over the past two years have shown that IgG_1 subclass of IgG antibodies appears superior as a marker for Tb infection in deer (Griffin *et al* 2001). This has been found in independent Tb diagnosis cattle studies (Lightbody *et al* 1998). It is also

supported by the theoretical finding that IgG_1 antibody is produced under T_H2 cell regulation. T_H2 cell pathways are most likely to be involved in non-protective immune responses to chronic intracellular bacterial infections such as mycobacteria.

2.0 Methodology

In this assay serum/plasma samples diluted to 1/100 were analysed with a B-A value ≥ 20 ELISA units, B-A value ≥ 30 ELISA units and a B-A ≥ 20 and < 30 ELISA units. An ELISA unit is equivalent to the ELISA OD value multiplied by 100. Data was obtained using serum / plasma from reactor animals.

2.1 Specificity

- 308 deer samples recruited from the field (non-specific and Johne's infected herds) where the testing veterinarian expressed interest in the assay development and the farmer was keen to cooperate. Large numbers of reactors in these herds precluded the use of BTB on all the animals. These were animals from herds greater than C3 that had a high incidence of reactors due to non-specific sensitisation.
- 147 deer samples from herds with known Johne's infection, PPD J antigen was included in the IgG_1 assay.

3.0 Results

1.1 Test Specificity

Table 1. Comparison of the specificity of the IgG ELISA and IgG_1 ELISA in herds with a history of non-specific reactivity and herds with a history of Johne's disease.

	ELISA (IgG)	IgG_1		
		³ 20	³ 20 < 30	³ 30
No. Positive	0	14	7	7
No. Negative	308	294	294	301
Specificity (%)	100	95	98	98
Total no. Analysed	308	308	301	308
No. Equivocal			7	

Table 2. Comparison of the specificity of IgG ELISA and IgG_1 ELISA on two herds with known Johne's Disease.

	ELISA (IgG)	IgG_1		
		³ 20	³ 20 < 30	³ 30
No. Positive	0	7*	2	2
No. Negative	147	140	140	145
Specificity (%)	100	95	98	98
Total no. Analysed	147	147	142	147
No. Equivocal			5	

This data set is a subset of Table 1 * In all these animals, whereas B > A; J > B.

3.2 Test Sensitivity

- 104 plasma/serum samples from infected herds *M.bovis* culture (+), with a known BTB status.

- 24 plasma/serum samples stored frozen from known infected herds where the individual samples were considered Tb infected, as they had lesions that were histologically typical of Tb, and a known BTB status.

3.3 Sensitivity values

Table 3. Comparison of the sensitivity of BTB, IgG ELISA and IgG_1 ELISA on *M.bovis* culture (+) animals.

	BTB	ELISA (IgG)	IgG_1		
			³ 20	³20 <30	³ 30
No. Positive	88	67	97	90	90
No. Negative	5	27	7	7	14
Sensitivity (%)	95	71	93	93	87
Total no. Analysed	93	94	104	97	104
No. Equivocals	11	10		7	

Sensitivity values obtained only for animals culture (+) for *M. bovis*

Table 4. Comparison of the sensitivity of BTB, IgG ELISA and IgG_1 ELISA on animals with lesions histologically typical of Tb

	BTB	ELISA (IgG)	IgG_1		
			³ 20	³20 <30	³ 30
No. Positive	23	22	24		24
No. Negative	1	1	0		0
Sensitivity (%)	96	96	100		100
Total no.Analysed	24	24	24		24
No. Equivocals		1			

Sensitivity in animals histologically typical of Tb

Table 5. Comparison of the sensitivity of BTB, IgG ELISA and IgG_1 ELISA on M.bovis culture (+) animals and animals with lesions histologically typical of Tb

	BTB	ELISA (IgG)	IgG_1		
			³ 20	³20 <30	³ 30
No. Positive	111	89	121	114	114
No. Negative	6	28	7	7	14
Sensitivity (%)	95	76	95	94	89
Total no. Analysed	117	117	128	121	128
No. Equivocals	11	11		7	

4.0 Discussion

The IgG_1 assay has specificity values of 95–98% and sensitivity of 89–95% using the datasets as outlined. Specificity dataset (Table 1) produces a total of 308 animals. Specificity is 95% for a cut point ≥ 20, 98% for a cut point of ≥ 30 and 98% when an equivocal category is used. The sensitivity dataset combined (Tables 5) gives a total of 128 animals. Sensitivity is 95% for a cut point ≥ 20, 89% with a cut point ≥ 30 and 94% when an equivocal category is used.

When submitting the BTB test for approval by the CVO, our laboratory was insistent that an "equivocal" (suspect) category be used for reporting results. While it is inconvenient to have

to recheck equivocal animals, it is important to use this category to allow for biological variation inherent in both the animal's responses and laboratory assays. The ability to recheck an animal to determine its true disease status has economic and management implications for the farmer. It is important therefore, in diagnostic testing, using biological assays, that an "equivocal" category remain. The issue with an "equivocal" category in the IgG_1 ELISA will be to determine what the appropriate method for retesting should be. The "equivocal" could be retested with a BTB or sent for slaughter as a reactor.

The IgG_1 methodology offers new insights into the complex interface between Johne's disease and tuberculosis. This has important ramifications as NZ herds move to lower levels of Tb and Johne's cross-reactivity becomes a major issue in disease eradication. Johne's infection has become the main confounding disease which could compromise the future success of the Tb eradication scheme for bovines and cervids. Currently an increasing number of deer herds with recurring non-specific MCST(+) reactors have evidence of underlying Johne's disease. The inclusion of *M. paratuberculosis* (Johnin–J) in the IgG1 ELISA would increase the specificity of the test for Tb diagnosis in deer. It could also provide confirmatory evidence of infection by *M. paratuberculosis*, and provide a useful adjunct to Tb diagnosis.

5.0 Conclusions

Further studies need to be undertaken to evaluate IgG_1 ELISA patterns of responsiveness in deer herds co-infected with *M.bovis* and *M.paratuberculosis.* Investigation of blood sampling time points would also be relevant. The current dataset was collected from samples obtained at timepoints equivalent to those used for BTB testing. Communications from the field indicate a sample taken at time of MCST read date would be beneficial. It is important to clarify test performance for different timepoints relative to intradermal skin testing. Comparisons should be made between samples obtained prior to, at skin test reading, and at a period following skin testing

6.0 Acknowledgements

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7.0 Bibliography

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