Tb Resistance in Deer - Preliminary Results

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Abstract

Preliminary results of this study show that there is a strongly heritable basis to the natural resistance/susceptibility of red deer to tuberculosis (Tb) caused by *Mycobacterium bovis*.

Six red stags were selected from 39 on the basis of their apparent susceptibility to Tb challenge, with two having no lesions, two with moderate lesions and two having severe lesions. Offspring were bred from these 6 stags by artificial insemination of randomly selected commercial red hinds. These offspring were challenged with Tb. In general terms the offspring of the resistant sires had mild to moderate lesions, those of the susceptible sires had moderate to serious lesions and those of the intermediate sires ranged from mild to serious. The result was highly significant ($P < 0.01$) and the heritability (based on preliminary results) was calculated as 0.76 ± 0.25.

Future research efforts will be directed at identifying genetic and immunological markers for resistance and susceptibility which could be used to cull out highly susceptible hinds and select for highly resistant stags in order to reduce Tb outbreaks in high risk areas.

Introduction

A previous Deer Branch Conference paper (Mackintosh *et al.*, 1995a) described the first two phases of a 3 year investigation of natural resistance/susceptibility (R/S) to *M. bovis* infection in red deer. Phase 1. involved the collection of semen from 39 red stags of wide genetic origin and then their subsequent challenge with Tb. They were slaughtered 6 months later and ranked according to the severity of disease, ranging from animals which had no visible lesions (NVL) and culture negative, through to severe spreading Tb involving head, thorax and abdomen. In Phase 2. the two worst affected stags (416 and 433), one moderately severely affected stag (417), the two least affected animals (406 and 434) plus one animal with very mild disease (415) were selected for a laparoscopic AI programme and their frozen semen was used to inseminate hinds on a commercial property. In March the calves were weaned and parentage testing by ***Genomnz***[a] identified 67 calves known to be sired by the 6 trial stags. These calves were all moved to the Tb Research Farm near Milton and were blood sampled for testing by the Deer Research Laboratory at Otago

[a] *Genomnz* Laboratory, AgResearch Invermay, Private Bag 50034, Mosgiel

University for *in vitro* immunological indicators of resistance to Tb and by the Molecular Biology Unit at Otago University for genetic markers for resistance to Tb. In November 1996, these animals were challenged with Tb in exactly the same way as their sires, using the left intra-tonsillar challenge system reported previously (Mackintosh *et al.*, 1993; Mackintosh *et al.*, 1995b). Six months later they were all slaughtered and the carcases and viseria were subjected to careful examination. All lymph nodes and tonsils were excised and sliced finely. All gross lesions were sampled for histopathological examination and culture. All lymph nodes from animals with no visible lesions (NVL) were pooled and cultured (head, thorax, abdominal and carcase pools) together with individual cultures of the left tonsil and left medial retropharyngeal lymph node. Based on the gross, histopathological and culture results the animals were scored on lesion severity (see Table 1).

Results

The data presented here are preliminary because the culture results have not been completed at the time of writing. Lesion severity scores of the 67 offspring of the 6 sires based on gross and histopathological examinations, are presented in Table 1. The offspring of NVL stags (lesion score 0) had an average lesion score of 1, while offspring of severely affected stags (lesion score 6) had an average lesion score of 3.4. The offspring of the other two stags had intermediate scores of 1.4 and 2.2. To date we have been unable to identify *in vitro* immunological markers or genetic markers of R/S, but work is still in progress.

Analysis of these preliminary data gave an estimated realised heritability of 0.38 and a true heritability of 0.76 +/- 0.25.

Table 1 Showing the lesion severity of the offspring of the six stags

Lesion severity	406 (0)	434 (0)	415 (2)	417 (4)	416 (6)	433 (6)
0	11	5	5	6	1	
1						
2	1	1	4	1	1	
3	5	2	1	6	5	1
4			1	3	3	1
5						
6				1	2	
n=	17	8	11	17	12	2
Mean lesion severity	1	1	1 36	2 24	3 42	3 5

Lesion Severity Score
0 - NVL culture -ve
1 - NVL culture +ve
2 - small abscess in left tonsil or left medial retropharyngeal LN
3 - moderate single or multiple small lesions in head node
4 - large or multiple moderate lesions in head nodes
5 - moderate/large head and thorax or head and abdomen lesions
6 - multiple head, thorax and abdomen lesions

Discussion

The results of this trial confirm that there is a strongly inherited basis for the R/S of deer to infection with *M. bovis*. The estimated realised heritability of 0.38 suggests that there are major gene effects, which would account for the expression of R/S in the offspring of the 6 stags when bred with unselected commercial hinds. It is believed that immunity to intracellular parasites is dependant on the ability of the macrophage to stop multiplication of phagocytosed organisms and to kill them. Animals that cannot control multiplication or which produce an inappropriate humoral response to infection are doomed. The most susceptible animals develop spreading, fulminating Tb which can be fatal in less than 6 months. On the other hand highly resistant animals appear to handle moderate challenge well with an efficient cell-mediated response. *In vitro* laboratory studies in cattle selected for resistance to brucellosis have shown a correlation between the ability of these animals' macrophages to kill *M. bovis* BCG and *Brucella abortus* suggesting that the intracellular killing mechanisms are the same or similar for these two intracellular organisms (Qureshi et al, 1996).

Work is still underway to identify immunological or genetic markers of R/S which could be used to identify R/S animals without the need to challenge them with *M. bovis*. It is hoped that in the short to medium future we can identify such markers which could be used to screen animals. It is envisaged that in Tb endemic areas, where there is a high risk of Tb breakdowns, it may be possible to screen the hinds and cull out the highly susceptible animals which are most likely to become infected, quickly develop serious Tb and infect other deer within the 1 year period between whole herd tests. In these herds, highly resistant sires could be used, so that the herd replacements will be relatively more resistant, thus increasing the overall resistance of the herd. It is hypothesised that highly susceptible deer are also most likely to become anergic to skin tests and are likely to be poorly immunised by Tb vaccines.

The ability to identify R and S animals and identify genetic and immunological markers associated with R/S will provide us with extremely valuable tools with which to investigate the immune response of deer and other animals to Tb infection. This should greatly assist the development of better vaccines and other means of improving protection against Tb in animals and humans.

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