INVESTIGATIONS INTO THE ROLE OF GENETIC RESISTANCE IN THE EPIDEMIOLOGY OF TUBERCULOSIS IN DEER

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Introduction

A previous Deer Branch Conference paper (Mackintosh and Griffin, 1994) reviewed the major factors found to influence or be associated with the transmission of *Mycobacterium* bovis (Tb) to deer These included -

1 The head lymph nodes, especially the medial retropharyngeal, are most commonly involved

2 The tonsil appears to be a common natural route of entry for Tb in deer suggesting that infected material commonly enters via the oral cavity when the animal is eating, drinking, licking, muzzling or grooming rather than via the respiratory tract as an aerosol, which is suggested as the most common mode of transmission in humans and cattle

3 An experimental model for Tb has been developed which relies on intratonsil inoculation Using this route as few as eight colony forming units (c f u) of virulent *M bovis* (89 MES) has resulted in Tb in 50% of inoculated animals

4 Increasing the inoculation dose to 200 to 500 c f u resulted in Tb in 70 to 100% of animals with increasing severity of pathology

5 Chronic stress, simulated by slow release synthetic corticosteroid (dexamethasone) increased the prevalence and severity of experimental infections

6 Natural transmission from experimentally infected deer to non-inoculated deer, run in contact with them, occurred under field conditions, and in the absence of possums However, transmission was at a low rate (<10%), indicating that deer in the first six months after infection by the intra-tonsil route, were not highly infectious because the majority of lesions were confined to the retropharyngeal lymph nodes Deer with serious lung lesions or open draining sinuses appeared to be more infectious

1994/1995 Trials : Investigation of natural resistance to *M. bovis* infections in deer

There is good evidence that within various animal species there are some strains or breeds that are more resistant to Tb than others Francis (1958) summarised a number of trials which showed that different strains of rabbits and breeds of cattle showed varying degrees of resistance to experimental challenge with virulent *M bovis* Natural resistance to infection with intracellular organisms such as

M bovis is controlled by a dominant gene in mice, designated initially as *Bcg* and latterly as the Nramp gene (Vidal *et al*, 1993) This appears to relate directly to the innate ability of the macrophage to kill the organisms which reside in phagolysosomes Workers at Texas A & M University believe that this gene occurs in cattle and codes for mechanisms responsible for the killing of intracellular organisms such as *Brucella* and *Mycobacteria* by macrophages There is a high degree of coincidence between resistance to both organisms as seen by 95% of animals with innate resistance to *M bovis* also showing resistance to *B. abortus*. Currently work is underway at the AgResearch Molecular Biology Unit at the University of Otago to study the Nramp gene in deer and to investigate its role in innate resistance to Tb The Nramp equivalent gene has been cloned from deer mononuclear cells (A Crawford, pers comm) Investigations of major histo-compatibility complex (MHC) Class II DRB gene polymorphisms are being undertaken to determine whether the sequence composition is associated with acquired resistance to *M bovis* challenge These studies are being carried out in conjunction with a series of selection trials carried out by Invermay The initial stag selection trial has just been completed

Phase 1 : Stag selection

Thirty nine 3-year-old stags from a variety of sources throughout the South Island were gathered at AgResearch Invermay in February/March 1994 Semen was collected from them (by electro-ejaculation under Fentazin' sedation) in April, May and June, and frozen The stags were then transported to the Tuberculosis Research Farm at Milton where they were all challenged by instilling 5 x 10^2 c f u *M. bovis* organisms into the left tonsil crypt, while the animals were sedated with Fentazin For the following six months they were monitored by regular Blood tests for Tb (BTBs), cytokine and macrophage assays and a final skin test just prior to slaughter Necropsies were performed, lesions examined histopathologically and cultures made of lesions and pools of head, thoracic and abdominal lymph nodes The results are summarised in Table 1

Table 1 Number, severity and distribution of lesions, overall results of culture of lesions and pools of NVL lymph nodes and the number of stags in each category when killed six months after challenge with virulent M. bovis by the intra-tonsil route.

Gross Lesions	Scale of Lesion Severity	Lesion and NVL Pool Culture Results	Number
No visible lesions (NVL)	0	Negative	5
NVL	0	*M bovis*	4
Single small lesion in left medial retropharyngeal lymph nodes	1	*M bovis*	6
Single moderate lesion in left medial retropharyngeal lymph node	2	*M bovis*	3
Very large single or multiple small lesions in left retropharyngeal lymph node and tonsil	3	*M bovis*	7
Multiple lesions in head lymph nodes and tonsil	4	*M bovis*	6
Large multiple lesions in head and abdomen	5	*M bovis*	5
Large, multiple lesions in head, thorax and abdomen	6	*M bovis*	3
		TOTAL	**39**

Of the 30 lesioned animals, 22 had head LN lesions, 5 had head and abdominal LN lesions and 3 had head, abdominal and thoracic lesions Sixteen of the 39 inoculated animals had either gross lesions and/or positive cultures *M bovis* detected in the left tonsil at slaughter (six months after inoculation) Thirty three of the animals were lesion positive and/or culture positive in the left medial retropharyngeal LN Four stags were kept in direct contact with the inoculated stags throughout the trial and at slaughter none had gross lesions, although one yielded *M avium* from a culture of the retropharyngeal LNs This result confirms previous findings that the transmission of Tb between stags kept under normal field conditions is poor in the first six months of infection, despite a number of animals having lesions in tonsils, thorax and abdomen

There was good correlation between BTB, skin test and necropsy results Macrophage killing assays on a limited number of animals showed good correlation between the ability of an individual's macrophage to kill *M bovis* BCG organisms and its resistance to Tb challenge

Phase 2 : Breeding offspring of resistant and susceptible sires

The semen from the three most resistant (i e NVL and culture negative) and three most seriously affected animals was used in an AI programme (synchronised oestrus using CIDR + PMSG at withdrawal and timed laparoscopic insemination 50-55 hours after CIDR withdrawal) to inseminate 220 randomly selected commercial hinds on a farm in Canterbury Ultrasound scanning at 40 days post-AI showed that 109 hinds (50%) were pregnant and they are due to calve in early December 1995

Phase 3 : Testing the hypothesis that resistance to Tb is inherited

Next autumn it is planned to take the female offspring (approx 50) and challenge them with Tb to look for inheritance of the observed 'resistance' or 'susceptibility' to Tb exhibited by their sires Concurrently the Nramp genotype and the innate intracellular BCG killing ability of each animals macrophages will be studied Similarly the MHC Class II DRB gene will be studied to determine if there are correlations between sequence composition and indication of acquired resistance to *M bovis* challenge

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