Breeding For Tb resistance in deer

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Abstract

Bovine tuberculosis (Tb), caused by *Mycobacterium bovis*, remains an intransigent problem in domestic livestock in New Zealand There are around 2 million deer farmed on over 5000 properties in NZ, and in 1999around 2% of deer herds were recorded as infected with Tb Although most Tb breakdowns in deer herds involve only a few animals, sporadic outbreaks of Tb affecting up to 50% of animals in a herd continue to occur In these severe outbreaks a range of lesions are seen, although usually less than 10% of affected animals have severe disease Similarly, when red deer are experimentally challenged with 200 - 500 colony forming units (cfu) of *M bovis* by the intra-tonsillar route we see a spectrum of disease outcomes when the animals are killed 5 – 6 months after challenge Over a series of trials we have found that around 5-10% of animals have no visible lesions, while 5 –10% have severe progressive Tb, and there is a range of severity in between Thus it appears that there is considerable variation in the degree of resistance/susceptibility to natural and experimental infection with *M bovis* in the general red deer population There is also similar evidence for genetic resistance to Tb in other species including humans, cattle, rabbits and mice We report here the results of a 3-year challenge study in red deer, which showed that there was a strong genetic basis to Tb resistance and that the heritability was 0 48 (95% CI 0.22 - 0 75, P<0 01).

We propose that "Breeding For Tb Resistance" be implemented as an additional Tb control strategy. Such a strategy would be complementary to the existing Animal Health Board strategies of test-and-slaughter, movement control and vector control It is hypothesised that marker-assisted selection would allow the breeding of resistant animals and the culling of susceptible animals This would result in a reduction in the number and severity of herd breakdowns and accelerate the rate at which infected herds come off movement control

We anticipate that the "Breeding For Tb Resistance" strategy will rely on two types of test, which we estimate could be available in 4 - 5 years if sufficient funding for research is provided We envisage a cheaper test (~$20-30) intended for screening breeding animals, so that 10 to 20% of the most susceptible can be culled at the start of programme, resulting in an immediate reduction in the risk of herd breakdown We envisage a more expensive test (~$50-100) that would be used on stud farms to select the 2-5% most resistant sires The benefits of the use of resistant sires will filter down through the industry more slowly The use of the strategy is only likely to be cost-effective in "Vector Risk Areas" and perhaps in "Fringe" areas Targeting properties that have a history of Tb breakdowns or are at increased risk could increase its efficiency The application of this technology to cattle would potentially have much larger benefits

A cost-benefit-analysis by AgResearch Invermay (Shackell, Amer and Mackintosh, unpub) for this "Breeding For Tb Resistance" strategy shows a positive benefit for the country, especially if the strategy is also applied to cattle.

Introduction

Bovine tuberculosis (Tb), caused by *Mycobacterium bovis*, remains an intransigent problem in domestic livestock in New Zealand Although the number of infected herds has been dramatically reduced over the last five years, from over 1400 cattle and 230 deer herds in June1994 to 690 cattle and 96 deer herds in June 1999, the rate of progress is expected to decline in the next few years if funding remains at the current level (Anon, 1999, Anon, 2000) While cattle-to-cattle and deer-to-deer transmission still occurs, it is believed that the majority of herd breakdowns are due to the transmission of Tb to livestock from wildlife vectors, especially possums and ferrets (Morris and Pfeiffer, 1995) There are around 2 million deer farmed on over 5000 properties in NZ and of the 96 infected deer herds recorded in June

1999, 78% are in Vector Risk Areas (VRAs) and the majority of breakdowns are thought to be due to infection from wildlife vectors (Anon, 1999).

Although most Tb breakdowns in deer herds involve a few animals, sporadic outbreaks of Tb, affecting up to 50% of animals in a herd, continue to occur (Griffin et al., 1998). In these severe outbreaks the severity of Tb lesions varies widely, although usually less than 10% of affected animals have severe disease Even in the herds that experience severe outbreaks, there is always a proportion of animals uninfected, suggesting that some animals are resistant to Tb, even when there is widespread environmental contamination with *M. bovis.* Similarly, when red deer are experimentally challenged with 200 - 500 cfu of *M. bovis* by the intra-tonsil route we see a spectrum of disease outcomes when the animals are killed 5 – 6 months after challenge, and some animals appear to be uninfected (Mackintosh et al , 1995a). Over a series of trials we have found that 5 –10% have severe progressive Tb, some have a moderate to mild degree of pathology and around 5-10% of animals have no visible lesions, including some that are Tb-culture negative. Thus it appears that there is considerable variation in the degree of resistance/susceptibility to natural and experimental infection with *M bovis* in the general red deer population

There is also similar evidence for genetic resistance to Tb in other species including humans, cattle, rabbits and mice More than 60 years ago genetic resistance to Tb was demonstrated in guinea pigs (Wright and Lewis, 1921) and in rabbits (Lurie, 1941) Subsequently a number of laboratories have developed inbred strains of mice with varying susceptibility to virulent human and bovine Tb and *M bovis* BCG. Different breeds of cattle have been shown to exhibit varying degrees of Tb resistance (Francis, 1958) Genetic resistance to Tb has also been demonstrated in humans with twin studies (Kallman and Reisner, 1943), familial clustering and racial differences (Houk *et al.,* 1968; Stead *et al.,* 1990, Abel and Dessein, 1997). A number of candidate genes have been identified including Nramp (Vidal et al , 1993), iNOS, INF-γ, TNF-α (Bloom et al., 1999), IFN-gamma receptor1 (Newport et al., 1996; Jouanguy et al., 1999), and a number of other cytokines and cell receptors (Orme, 1993; Bloom et al , 1999, Nau et al , 1999, Means et al , 1999)

We report here the results of a 3-year study in red deer (*Cervus elaphus*), which showed that there was a strong genetic basis to Tb resistance and that the heritability was 0.48 (± 0.23).

Tb Genetic Resistance Study in Red Deer

In Phase I of a 3-year heritability study, 39 red deer stags were obtained from a wide range of genetic backgrounds and brought to AgResearch Invermay Semen was collected from the sedated stags by electro-ejaculation and it was stored frozen in liquid nitrogen The stags were moved down to the Infected Deer Farm (IDF) at Milton and they received an experimental Tb challenge comprising approximately 500 cfu of *M bovis* instilled into the left tonsillar sac (Mackintosh et al , 1995a) When the stags were killed and necropsied 6 months later, they displayed a range of disease outcomes, with lesion severity ranging from nil and culture negative (Lesion Severity Score – LSS 0) to severe progressive Tb (LSS 6) (Mackintosh, 1995, Mackintosh et al , 1995b)

In Phase II, six representative stags were chosen Two were uninfected (LSS 0), two were moderately affected (LSS 2 and 4) and two had severe Tb (LSS 6) and their stored semen was used in an AI programme to breed offspring from randomly allocated red hinds on a commercial deer farm. The following autumn 70 offspring of these six stags were identified and their parentage confirmed by Genomnz blood-typing

In Phase III, these 70 offspring were challenged with Tb on the IDF in exactly the same way as described in Phase I The offspring showed similar patterns of disease to their sires (see Table 1), providing evidence for a strong genetic resistance to Tb, with an estimated heritability of 0.48 (SE 0 096, 95% CI 0 22 - 0 75; P<0.01) This is the first time that the heritability of Tb resistance has been measured in domestic livestock (Mackintosh et al., 2000)

Table 1: Identity of six selected stags, their lesion severity scores (LSS), the distribution of LSS of their offspring and the mean LSS of their offspring.

Sire Stag	406	434	415	417	416	433
Sire Lesion Severity (LSS)	**0**	**0**	**2**	**4**	**6**	**6**
Offspring LSS 0	2	0	0	0	0	0
1	8	5	8	7	0	0
2	2	0	2	1	1	0
3	5	3	0	7	6	2
4	0	1	1	3	0	0
5	1	0	2	0	0	0
6	0	0	0	1	2	0
Mean LSS score of offspring	**1.78**	**2.00**	**2.00**	**2.53**	**3.56**	**3.00**

New addition to Tb control strategy

We propose that "Breeding For Tb Resistance" be implemented as an adjunct to the existing National Pest Management Strategy for Bovine Tb in New Zealand and for Tb control overseas If a cost-effective test for a genetic or immunological marker for Tb resistance was developed it could be used in deer herds in "Vector Risk Areas" (VRAs) to reduce the risk of having a Tb breakdown by increasing the resistance of deer in their herds. If a breakdown did occur, then selection for animals with increased resistance would reduce the severity of the breakdown and minimise the time on movement control. A marker test would enable high susceptibility animals to be culled and the most resistant stags could be selected for breeding. Such a strategy would be complementary to the existing Animal Health Board strategies of test-and-slaughter, movement control and vector control.

It is anticipated that the "Breeding For Tb Resistance" strategy would rely on two types of test, which we estimate could be available in 4 – 5 years, if sufficient funding for research is provided. We envisage a cheaper test (~$20-30) for screening hinds, so that 10-20% of the most susceptible could be culled at the start of programme, resulting in an immediate reduction in the risk of herd breakdown We envisage a more expensive test (~$50-100) that could be used on deer stud farms to select the 2-5% most resistant sires These premium sires would be used to breed second tier commercial sires, thus bringing the benefits of increased resistance at an affordable price to the wider deer industry. The use of the strategy in deer is likely to be most cost-effective in VRAs and perhaps in "Fringe" areas Targeting properties that have a history of Tb breakdowns or are at increased risk could increase its efficiency The application of this technology to cattle would potentially have much larger benefits.

We believe that this strategy would have the following benefits:

a) reduced vector-to-deer/cattle transmission

b) reduced deer-to-deer, cattle-to-cattle and deer/cattle-to-vector transmission

c) fewer herd breakdowns

d) fewer animals involved in a breakdown

e) faster clearance rates

The sum total of these effects of this complementary strategy would be to reduce the number of infected herds and to help the Animal Health Board achieve its goal of 0 2% infected herds sooner and cheaper than it is likely to by relying solely on the existing strategy

Potential drawbacks

Power of selection The selection of sires for Tb resistance may slow down progress in genetic improvement for other traits However, farmers purchase or select stags for a variety of reasons, but usually to improve the antler or venison genetics of their herd or to produce fast growing offspring by using a terminal sire such as a wapiti hybrid Currently there is little objective information on productive traits available in the deer industry and a lot of the selection is based on "fashion" and

phenotype If markers can be found for Tb resistance, then it should be possible to screen a range of stags that have been selected for productive traits and rank them for Tb resistance Then the most resistant could be used in high risk areas and the most susceptible could be culled or used in low risk areas of New Zealand In VRAs the risks of herd breakdown may be sufficient to encourage farmers to make selection for Tb resistance a higher priority than growth rate or velvet production for a few years It may only be necessary to use resistant stags for 5 years or so to ensure that the majority of the herd replacements have an increased level of Tb resistance and the highly susceptible animals have been culled There are a number of examples from the sheep industry in New Zealand and overseas where farmers have selected for disease resistance against parasites, footrot, facial eczema or scrapie in preference to productive traits Nevertheless, a Cost-Benefit-Analysis (CBA) carried out by Invermay (see below) included a factor that allowed for reduced rate of genetic gain for productive traits when there was selection for Tb resistance Despite this, the CBA was positive

Negative correlation between Tb resistance and productive traits Analysis of trials to date have failed to show any negative correlation between Tb resistance and production traits, such as live weight gain, suggesting that selection for one should be unlikely to directly influence the other

Multiple versus single gene effects· It is highly likely that a number of major genes control resistance to Tb. Generally speaking, the more genes involved the slower will be the progress of selection However, a heritability of 0 48 (CI 0 22 - 0 75) suggests that the genes are not recessive and that a high rate of progress can be made if sufficient selection pressure can be applied. Multiple genes may make the identification and development of genetic markers more difficult and it may be necessary to find two or three genetic markers for optimal selection Immunological markers should allow selection for a combination of resistance factors We may find that a combination of immunological and genetic tests is the most efficient

Selection without markers If markers cannot be found in the short term it will make selection of resistant animals considerably more difficult, but not impossible. Stags could be selected by progeny testing ie challenging a sample of their offspring with Tb This is currently being undertaken by us this year in order to develop selection lines for research purposes, but any resistant animals that are identified could be used immediately on a limited basis We also have a limited amount of semen available from resistant sires that have been identified in previous challenge studies.

Cost-benefit analysis

A Cost-Benefit-Analysis by AgResearch Invermay (Amer, Mackintosh and Shackell, unpub) for this "Breeding For Tb Resistance" strategy shows a positive benefit for the nation, especially if the strategy is also applied to cattle While the analysis was sensitive to a number of assumptions, simulations of risk sensitivity around the base assumptions indicated that the chances of a more favourable than expected outcome balances the risk of a less favourable than expected outcome There were also a number of positive benefits, such as farmer confidence and peace-of-mind, marketing opportunities for Tb resistance testing overseas, and spin-offs for similar diseases such as Johne's disease and human Tb, that could not be quantified

Tb resistance marker research

Currently genetic and immunological research is being undertaken in deer, albeit at a reduced level because of funding constraints However, we believe that the benefits of breeding for resistance are not confined to deer It is highly likely that the genes (and cell-mediated killing mechanisms) for Tb resistance are "conserved" and are carried by all ruminants and higher mammals. They are also highly likely to be related to resistance to other intra-cellular pathogens such as other *Mycobacterium* sp (Johne's disease, avian Tb, human Tb, leprosy etc), *Brucella* sp., *Leishmania* sp , etc.

Our aim is to develop two selection lines of deer, with extreme resistance or susceptibility to Tb, to enable the study of protective mechanisms against intracellular pathogens Deer provide the ideal study model because they are naturally susceptible to Tb, the intra-tonsillar inoculation model efficiently and repeatably reproduces "natural" Tb, deer display a wide range of resistance/susceptibility to Tb and this experimental Tb challenge model relates directly to the Tb

problem in a farm animal (Griffin et al , 1995). None of the other existing Tb models (mice, rabbits, guinea-pigs or even cattle) fulfil all these criteria.

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