DO DEER DEVELOP IMMUNITY TO TUBERCULOSIS?

J. Frank T. Griffin, D. Ngaire Chinn and Glenn S. Buchan.

Department of Microbiology, University of Otago, Dunedin, New Zealand.

A major unresolved issue in veterinary medicine today is the failure to develop preventative strategies to control infectious diseases such as tuberculosis. Tuberculosis has presented a consistent challenge to clinicians and scientific researchers throughout the past century. Although Robert Koch first advanced the concept of the germ theory of clinical infection, by his discovery of the infectious basis of human Tb, the control of the disease has remained largely elusive. Because of this, veterinary diagnostic strategies have been devised which involve a 'test and slaughter' policy to control the disease. Not only is this approach very expensive in the short term, but it has the implicit limitation that reduced levels of endemic infection are tolerated, resulting in long term costs associated with disease surveillance and the wastage of uninfected stock. By contrast effective preventative strategies can only be advanced by the marriage of the best diagnostic technology allied with new research programmes which advance our knowledge of the underlying mechanism of disease transmission and patterns of disease aetiology, pathogenesis and host reactivity, following exposure to infectious organisms such as *M. bovis*.

With the rapid advances in immunology and microbiology in recent years it may now be possible to marshall these skills for a new assault on an infectious disease which remains one of the major international health issues. The success of such an approach can be further advanced by introducing systems which select for individual animals with genetic resistance to specific disease and management regimes involving the use of vaccines and immune modulators which optimise the development of immune protection in animals exposed to infection.

The reality presented by the emergence of deer farming within NZ agriculture, is that traditional diagnostic strategies may be inadequate to control diseases such as tuberculosis. Endemic levels of Tb in cattle and feral animals may therefore pose a unique challenge to the control of this important disease in a species considered to be especially susceptible to this disease. It is timely therefore to evaluate the alternative strategies for Tb management to advance the prospect of a Tb free National Deer Herd in NZ in the immediate future.

Host reactivity to tuberculosis.

It is widely recognised that resistance to tuberculosis is influenced by both non-specific and immunological mechanisms. Whereas some species of animals do not contract tuberculosis because of their innate resistance, those species known to contract Tb naturally show varying degrees of immunity varying from highly susceptible to only slightly susceptible. It is logical therefore that the study of immune mechanisms has remained a central area of research in the diagnosis and possible prevention of Tb in man and animals. In most of this work the delayed-type hypersensitivity (DTH) skin reaction has been central in studies involving Tb diagnosis and vaccination studies attempting to establish immune protection.

A real dichotomy exists in human and veterinary medicine concerning the interpretation of what a positive skin test means in Tb infection. While both proponents agree that a positive DTH, in the absence of prior immunisation, indicates exposure to mycobacteria, a positive result in the absence of clinical pathology over a period of time is interpreted as indicating immunity in humans. In traditional disease control programmes in veterinary medicine all reactor animals in a herd are considered to be diseased and a lack of pathology upon autopsy (NVL) is attributed to subclinical infection which would constitute a threat to the health of the herd. This has resulted in the doctrine within veterinary circles whereby naturally acquired protective immunity to *M. bovis* is not considered. In essence a positive tuberculin test may indicate nothing more than hypersensitivity which results from past or present exposure to mycobacterial infection.

If we ignore the prospect that all skin test positive animals are diseased and entertain the view that many of them, especially in herds in which Tb is known to exist, are in fact immune or in the process of developing protective immunity, the possibility of saving many hither-to slaughtered animals can be entertained, with little or no danger to the disease eradication prospect.

That the development of immune protection to Tb is possible has been shown by Lefford (1970) in which immunity could be adoptively transfered by spleen cells from sensitised mice. Transfer of protective immunity to *M. tuberculosis* or *M. bovis* is usually accompanied by an increase in the hosts sensitivity to tuberculin proteins as expressed in the DTH response (Skin Test +). There is now little doubt that protective immunity and DTH are dissociable, and mediated by separate populations of T-lymphocytes (Orme and Collins, 1984). In the mouse it has been shown that Ly2+ T-cells (Tc/s) taken from the spleens of mice at the peak of the CMI response to an immunizing dose of BCG could mediate the adoptive transfer of protective immunity to aerogenic infection with Tb. DTH however could be transfered by cells depleted of Ly2+ T cells but containing Ly1+ (Th/Td) cells.

Two types of CMI response against Tb were identified by Rook (1978) and Stanford *et al* (1981). The first called 'Listeria type' reactivity was evident 10 days after exposure to *M. bovis* bacteria and gave good levels of protection against Tb. It was also produced after BCG vaccination and exposure to environmental mycobacteria such as *M. vaccae* and *M nonchromogenicum*. Both organisms had a limited capacity to induce cross reactive tuberculin DTH sensitivity. The second ('Koch type') was evident 4-6 weeks after exposure to virulent *M. bovis* and the response was not enhanced by BCG vaccination, was prominent in the development of the hypersensitivity reaction (DTH) and was enhanced by exposure to environmental mycobacteria such as *M scrofulaceum*, which induced a strong cross reactive hypersensitivity response to tuberculin. The development of this reaction which probably reflects the central mechanism involved the (DTH) skin test response, was not considered to be an indicator of immune protection. Chaparas (1982) considered the two responses to reflect quantitative or qualitative differences of the same response. Anecdotal evidence would support the concept that the development of an immune response to Tb that tends toward a strong hypersensitivity to tuberculin is suggestive of a poor protective response.

The available evidence seems to suggest that the ST measures the 'Koch type' reaction and as such has little value in distinguishing immune from diseased animals.

The BTB as an alternative system for diagnosis of disease and protective immunity to tuberculosis.

Although the skin test is useful in disease diagnosis, the development of *in vitro* tests that more accurately reflect the ability to resist infection are required. It appears unlikely that the lymphocytic tests and the skin test are measuring totally different reactions. Several factors however suggest that the blood test is in fact a superior indicator of immune responsiveness to PPD . The first is that skin contains numerous resident, tissue macrophages which in comparison to blood monocytes are more resistant to activation by lymphokines and are known to be capable of suppressing immune responses with prostaglandin E (PGE). This difference in cell composition may explain the blood tests' ability to identify diseased animals that remain skin test negative. These tissue macrophages may also be responsible for the post-skin test suppression of DTH often observed by those in the field, and their absence from the samples used in the blood test mean that it is not subject to this suppression. While those animals made anergic by overwhelming disease are not identified in the ST, they remain highly reactive to *M. bovis* PPD in the BTB.. Any other differences between the tests are undoubtably due to the definite advantages the BT has over the ST in its sensitivity and superior capacity to be standardized. Variations in the site of inoculation, dose of antigen, experience of the veterinarian in both administering

and reading the ST, introduce error into the skin test diagnosis. The development of an *in vitro* assay such as the BTB minimises many of these factors and thus has a much more reliable predictive capacity than the ST.

The BTB has the potential to detect both the 'Koch type' and the 'Listeria type' of Tb reactions and can therefore be considered as a means of distinguishing immune protection and disease.

Assays to monitor Immunity to Tuberculosis in Deer

There is evidence that whereas the intradermal tuberculin DTH reaction offers a worthwhile system to monitor disease in up to 80% of affected individuals. Its limitation is that among the 20% of diseased individuals which are undetectable by ST many suffer from serious disease. This highlights the prospect that if disease control is attempted solely using the ST, the residue of seriously diseased (False -) animals may confound the best efforts to eradicate disease. Because the ST cannot be used quantitatively to monitor immune reactivity it has little ability to monitor other facets such as immune protection.

A laboratory assay such as the BTB has superior sensitivity to the ST in Tb disease diagnosis, because it is especially effective in the early diagnosis of infection and in identifying seriously diseased animals. The BTB assay can be used as a quantitative assay because of the direct relationship between the level of reactivity to tuberculin *in vitro* and the severity of disease. Another advantage of the BTB is that low grade reactivity in this test can be used to monitor exposure of an animal to Tb and the development of immune protection rather than disease.

Recent studies in our laboratory have focussed on questions as to how the ST and BTB can be used as complimentary systems to more effectively contribute to Tb diagnosis and identify mechanisms of protective immunity. The areas in which the BTB has been used are as follows:

a) BTB testing to confirm Tb infection in ST(+) animals, suspected as tuberculous.
b) BTB repeat testing in animals which are ST(-) to define predictive markers for disease or protective immunity.

Experimental Studies

The central issue under consideration in this paper is whether the BTB can be used quantitatively to distinguish immunological reactivity, which first identifies diseased animals and secondly can distinguish between disease and protective immunity.

Because the BTB can detect very low levels of immune reactivity which are undetectable by the ST it is conceivable that the BTB could detect immunity following exposure of animals to *M. bovis*.

Using quantitative differences in the level of BTB reactivity in animals within infected herds, the results given in Table 1 show that there is a good correlation between levels of BTB reactivity specific for *M. bovis* and whether animals will show disease or immunity upon autopsy.

Table 1.

The value of a single BTB in categorising animals as diseased, immune or uninfected

	Level of BTB Reactivity		
	High	Low	Negative
Incidence of Tuberculosis	48/58(82%)	8/41(20%)	2/45(4%)

This data shows that animals with a high level of BTB reactivity (B≥4) have a poor prognosis and the vast majority harbour infection following exposure to *M. bovis*. Whereas 20% of the animals with low grade BTB reactivity to *M. bovis* develop disease. These animals can be identified by carrying out a repeat BTB, where the response will invariably increase if disease has become established. There is then the real prospect of identifying animals with immune reactivity to *M. bovis* which are unlikely to develop disease or pose a threat to disease management. The negative BTB group show a low incidence of disease and the absence of reactivity may be due to lack of exposure or resolution of earlier exposure reactivity.

Accepting that animals with low grade reactivity to BTB could be salvaged it then remains to be established as to what would happen to such animals should they be kept in contact with diseased stock. To test this we have studied the reactivity of a group of low grade BTB reactors farmed in direct contact with diseased animals. The results given in Table 2 show the changes which occur in the BTB reactivity of low BTB reactors exposed to diseased animals and sampled three times over a six month period.

Table 2

Changes in the BTB reactivity in low BTB reactors in contact with Tb infected animals over a six month period.

	BTB Status over three Samples		
	L-H-H	L-H-L	L-L-L
Number	3	9	64
Percentage	4%	12%	84%

* L- Low (B<4) H- High (B>4)

The results given in Table 2 show that the vast majority (84%) of low BTB reactors do not develop high reactivity while in contact with *M. bovis* infected animals. A significant minority (12%) show an intermittent increase which returns to a low level and these animals are uninfected. There is a high risk of disease developing in the small group (4%) of animals which develop persistently high levels of BTB. Obviously it would be necessary to introduce a similar group of naive animals with no prior exposure to *M. bovis* to determine if the low grade BTB reactors have any increased resistance to contracting Tb due to a protective immune memory response.

Whereas low grade BTB reactivity to *M. bovis* appears to be compatible with immunity rather than disease, it remains to be determined what significance high levels of BTB reactivity have in the context of disease and immunity to Tb.

A group of 79 animals were examined, which had high levels of BTB reactivity, and were sampled repeatedly prior to autopsy. The results given in Table 3 show that a minority (18/79) of these anmimals had any significant decrease in BTB reactivity on repeat sampling. Of these over 50% (10/18) were diseased on autopsy, showing a poor prospect of salvage of this type of animal. There was a strikingly high incidence of disease (74%) in animals which sustained high BTB levels on repeat sampling. It appears likely therefore that high grade BTB reactivity is strongly associated with disease and whereas some high BTB reactors do not yield lesions, they generally present as an unacceptable disease risk and should be slaughtered. Obviously this does not present any issue when we are dealing with ST(+) animals as we would invariable recommend autopsy. It is however an issue when we identify ST(-) animals with high BTB responses.

Table 3

Changing levels of BTB reactivity on repeat sampling in animals following their classification as high grade BTB reactors

	Changing BTB Status over Six Months	
	Decrease	No Change or Increase
Total	18	61
Number with Tb	10/18(56%)	45/61(74%)

In an attempt to determine what measure of correlation exists between ST reactor status and the level of BTB, we have examined a group of animals prior to autopsy and compared their ST and BTB status. These animals all had a prior history of exposure to *M. bovis* and known BTB reactivity. The results given in Table 4 show the findings obtained from BTB samples obtained just prior to skin testing and the resulting ST response.

Table 4

BTB reactivity and ST status in a group of animals with high grade BTB responses

	ST Status	
	+	-
Number	34	29
Mean BTB Reactivity	5.4	7.3
Number with Tb	23(69%)	12(41%)

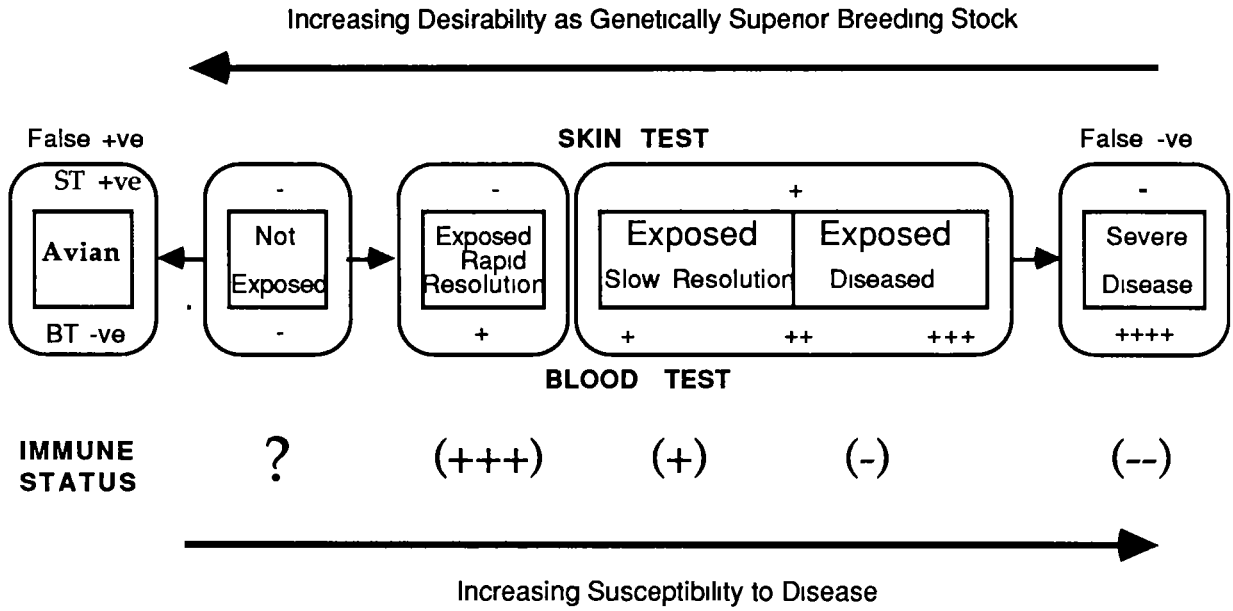
The surprising finding from this experiment was that the BTB reactivity was even higher in the ST(-) animals (7.3) than in the ST(+) reactors (5.4). This shows a total lack of correlation between skin test negative status and BTB reactivity. There is also the interesting prospect that if ST had been used for disease diagnosis in this group of animals it would have yielded a high incidence of False(-) ST reactors (41%). The higher level of BTB in ST(-); 7.3, is associated with more serious disease in this group of animals, although at a lower incidence. From this data it appears that not only can BTB reactivity make an important contribution to disease diagnosis but it can be present at high levels in ST(-) animals, many of which harbour disease. It may be important therefore to look critically at the relationship between these two tests and what they measure. It is important to subjectively analyse data depending on whether the ST or the BTB has been used to select reactor animals.

The general conclusions to emerge from the quantitative study or BTB reactivity are as follows.

1. High grade BTB reactivity is a good predictor of disease irrespective of ST status.
2. Animals with high grade BTB usually retain this reactivity over long periods.
3. High BTB is likely to result from endogenous *M. bovis* disease rather than intercurrent exposure to other infected animals.
4. In seriously infected animals there is a poor correlation between the absolute level of BTB reactivity and ST+ or - status.
5. Repeat ST are highly inconsistent and an extremely poor indicator of disease. Repeat high grade BTB is an excellent marker of disease.
6. There are good prospects of salvaging low grade BTB reactors as uninfected stock irrespective of ST status. Low grade BTB appears to be stable and may be a good marker of immunity following exposure to *M. bovis*.
7. Low BTB reactors do not pose a significant risk of harbouring *M. bovis* infection and do not appear to develop tuberculosis when kept in contact with tuberculous animals.
8. Results from the BTB at appropriate levels may be an important indicator of disease or immunity and its reactivity is largely dissociated from ST reactivity.

Selection of genetically resistant animals

There are two main points to be considered when suggesting the retention of immune animals within a herd. One is that there is some evidence that animals can be selected for genetic resistance to Tb. The elimination of non-diseased immune animals from herds may eliminate genetically elite animals with increased potential to resist Tb infection.



The second consideration is that recrudescence of Tb resulting from long dormant infections have been reported (Charapas, 1983) and therefore no herd containing previously exposed animals can be considered permanently clean. In New Zealand with its endemic opposum and bovine problem this concept already applies to most herds anyway. The low incidence of recrudescence (0.03% - 0.6% in humans with the risk mainly associated with old age) makes it reasonable to consider retention of immune animals within a herd or as a seperately managed herd.

The future undoubtedly lies in a better understanding of the mechanisms that determine the quality of the immune response. Tests that will differentiate cell populations (e.g. monoclonal antibodies) and cell function (e.g. assays to detect lymphokines) will allow us to more accurately differentiate immune from diseased animals and may eventually allow us to modulate the hosts immune response, are an immediate priority for research.

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