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Diagnosis of tuberculosis in New Zealand farmed deer: an evaluation of intradermal skin testing and laboratory techniques

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Farmed deer may act as a new reservoir of tuberculosis (TB), due to infection with *Mycobacterium bovis*. Deer appear to be particularly susceptible to TB infection by *M. bovis*, *M. paratuberculosis* and *M. avium*. New schemes have been devised in New Zealand for the diagnosis and control of tuberculosis in farmed deer. Use of the single cervical skin test (ST) has produced a monitoring system with a sensitivity of 85 *per cent* for diagnosis of cervine TB. Cross reactivity due to exposure of deer to other mycobacterial species has resulted in unacceptably low levels of specificity for ST, such that a comparative cervical test (CCT) has been introduced to diagnose 'atypical' reactors. Reduced sensitivity (70 *per cent*) of the CCT, and the inability of skin tests in general to diagnose the most seriously affected tuberculous animals have led to the development of a new laboratory technique for the diagnosis of TB in deer. A new blood test (BTB) for the diagnosis of TB uses a combination of immunological and inflammatory parameters. The BTB offers considerable advantages over skin testing and gives a sensitivity of 95 *per cent* and a specificity of 92 *per cent* in deer herds harbouring mixed infection due to *M. bovis* and *M. avium*. This test can be used to diagnose TB in seriously diseased animals that give false-negative reactions on skin testing. The BTB has superior sensitivity, specificity and validity to the ST or CCT, and it can detect TB reactivity much earlier than skin testing. This system can be used to effectively diagnose and manage tuberculosis in infected deer herds by minimising wastage through slaughter of uninfected stock. It is hoped that this technique may ultimately be used to distinguish between infectious disease and immunity in deer exposed to *M. bovis*.

Key Words: Deer, Tuberculosis, Tuberculin Skin Tests, Laboratory Diagnosis, Sensitivity, Specificity.

+ The BTB is registered under NZ Patent No. 214400, with patent applications pending in Europe, EEC, US, Canada and Australia.

Introduction

Immunodiagnostic techniques have been used widely in veterinary medicine for disease control programmes. Immunological reactivity is taken as presumptive evidence of infection, so that animals showing such reactions are culled. The *validity* of any given procedure is influenced by the *sensitivity* of the technique to identify infected animals, and the *specificity* of the system, which excludes uninfected animals. Generally, there is an inverse relationship between sensitivity and specificity, so it has been unrealistic to aim for 100 *per cent* validity in immunodiagnosis.

Cellular immune mechanisms are considered central in the host's response to intracellular infection by mycobacteria. The role of this response in tuberculosis (TB) first led to the discovery of cell-mediated immunity (CMI) as the protective immune response in TB-infected guinea pigs, over a century ago. The *in vivo* measure of CMI has been the indurated swelling produced intradermally in individuals inoculated with microorganisms or the soluble extracts ('tuberculin') of the prototype microorganism (Koch, 1884). The *in vivo* skin test (ST) produces a delayed-type (48 to 96 hours) hypersensitivity (DTH) response, and this response has been used extensively as the primary method for the diagnosis of TB in cattle and humans, throughout the world during the past fifty years.

Subjective considerations that influence the efficacy of methods for the control of TB in domestic animals are:

1. Endemic levels of *M. bovis*
   a) In the host species.
   b) In feral animals.
2. Genetic resistance of the host species, and individual groups of animals within a species.
3. Spurious immune reactions that result from prior exposure of animals to other mycobacterial species that cross react with *M. bovis*.
4. Technical difficulties in applying a given test.
5. Financial investment in the application and frequency of testing, and in providing incentives through compensation to farmers for slaughter of reactor stock.

Rigorous application of the ST in cattle has failed to control TB in a number of countries, including Australia, New Zealand and Ireland, where one or more of the above factors may have militated against the successful conclusion of the TB eradication scheme. Application of skin testing for TB control in farmed deer in New Zealand has had to take due consideration of all the above factors, each of which has had a direct influence on the TB control programme for deer. TB is endemic in cattle and feral animals in New Zealand, and farmed deer appear to be highly susceptible to infection, producing lesions which may facilitate spread within and between individual herds. Deer subjected to management stress (e.g., capture, breeding and weaning) may become compromised, so that some animals may become increasingly susceptible to infection (Griffin, 1987,1989). Such individuals may be predisposed to invasive infection and, when diseased, would be less

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Abbreviations:

AFB:	Acid-fast bacilli
BOV*:	Reactivity with bovine PPD.
BTB:	Blood test for tuberculosis
CCT:	Comparative cervical skin test
CMI:	Cell-mediated immunity
DTH:	Delayed-type hypersensitivity
GTB:	Generalised tuberculosis
NVL:	No visible lesion
PPD:	Purified protein derivative of tuberculin
PPD-B:	Bovine PPD
ST:	Single cervical skin test
TB:	Tuberculosis

likely to produce a positive skin test. Farmed deer also appear to be uniquely susceptible to infections caused by other mycobacteria (*M. paratuberculosis, M. avium*) that can produce pathological lesions (Griffin, 1988). Saprophytic mycobacterial species may also cause sensitisation of deer and produce false-positive reactions on testing with tuberculin.

Intradermal skin testing in the diagnosis of cervine tuberculosis

The pioneering studies (Beatson, Hutton and de Lisle, 1984) on the use of tuberculin skin testing for the control of tuberculosis in an infected herd of farmed deer in New Zealand were carried out between 1978 and 1983. During this period 3620 individual animal tests were carried out during 29 different testing episodes, at which 107 reactors were identified and slaughtered. Gross lesions, consistent with TB infection, were found in 82 (77 *per cent*) of the skin test reactors. Of 68 animals that died of natural causes throughout the study, 25 (37 *per cent*) had TB lesions, and of these 15 (60 *per cent*) had generalised TB (GTB). Up to the time that the herd was depopulated in 1983, 43/326 (13 *per cent*) of the animals that were negative to skin testing had tuberculosis diagnosed at necropsy. In the early test series 0.1ml of 1mg/ml bovine PPD was used but in the later part of the programme 2mg/ml bovine PPD was used for the intradermal cervical skin test (ST). Application of the comparative cervical test (CCT) using 0.5mg/ml avian PPD gave consistently poor results, with only 26 *per cent* of ST reactors giving a positive CCT. A subsequent study, which involved active experimental infection of deer with *M. bovis* (de Lisle, Corrin and Carter, 1984), demonstrated that the single intradermal skin test had a sensitivity of 86 *per cent* (36/44) in detecting infected deer, when positive skin test reactivity was taken as the presence of a visible or palpable skin test reaction. When an increase of ≥2.5mm in skin thickness was taken as evidence of reactor activity, the test showed only 45 *per cent* (20/44) sensitivity in detecting tuberculous animals. These data h light the desirability of classifying as reactors all animals shov any evidence of skin test reactivity, if acceptable levels of se tivity are to be achieved. A parallel study carried out on exp mentally infected animals (Carter, Corrin and de Lisle, 19 indicated that repeated short interval (three weeks) testing sig cantly reduced the sensitivity of the ST. They also showed effective skin testing was influenced markedly by the qualit animal restraint, lighting and the preparation of the skin site p to testing. An added complication is that deer have thin skir 3mm), so care must be taken to ensure that the antigen usec skin testing is applied intradermally rather than subcutaneou Unless adequate shaving of the hair is carried out diffus oedematous positive reactions may easily be overlooked. This meant that application of skin testing in deer is more technic demanding than similar tests in cattle.

Because a large number of small reactions were also foun non-infected deer (Carter *et al.*, 1984), care should alsc exercised if false-positive reactions are to be eliminated. W spread exposure of deer to *M. avium* and *M. paratuberculosis* Lisle and Havill, 1985: Griffin, 1988) would infer that sensi tion of farmed deer to cross-reactive mycobacterial antig demands caution in the development of techniques to ensure acceptable levels of specificity can be achieved using tuberc skin testing in deer.

Since the onset of herd testing for tuberculosis in New Zeal deer herds it became obvious that the use of the single intrader skin test (ST) produced an unacceptably large percentage (6(*per cent*) of false-positive reactors, due to high levels of sen sation of deer with atypical mycobacteria. Official statistics on testing of New Zealand deer in 1985 (Carter, Corrin, de Lisle Kissling, 1986) identified 2594 (1.45 *per cent*) reactors am 178,788 animals tested. Based on a validity of 30 *per cent*, it suggested that the likely prevalence of tuberculosis in farmed would be 0.5 *per cent*. It was also felt (Carter *et al.*, 1986) that occurrence of false- positive reactions in herds with no histor tuberculosis had caused many farmers to lose confidence in standard intradermal skin test (ST). This problem was fur exacerbated by the prevailing test schedule since 1985, in wl no compensation was paid for slaughter of reactor animals, because involvement of farmers in the scheme was volunt With this in view, a CCT was introduced by Carter, Corrin Lisle and Kissling (1985, 1986) to improve the specificity of testing. They used 2mg/ml bovine PPD and 0.5mg/ml avian P on animals infected experimentally with *M. bovis* and when used a 2mm increase in skin thickness and a bovine reactic avian reaction as indicative of a positive reaction, they dem strated that the CCT had a sensitivity of 92 *per cent*. They nc significant post-test suppression (40 *per cent*) of skin test reac ity, if CCT was applied within 28 days of ST and 11 *per* suppression at a 60-day test interval. Mean skin thickness bovine PPD was reduced from 7.49mm at Day 0 to 2.44mm

TABLE 1
Specificity of BTB assay in herds with ST reactors

BTB Reactivity	M. bovis	M. bovis + M. avium	M. bovis + M. paratuberculosis	M. avium
Number of herds tested	6	15	2	23
Herds with TB lesions	6	14*	2	0

BTB: Blood test for tuberculosis.

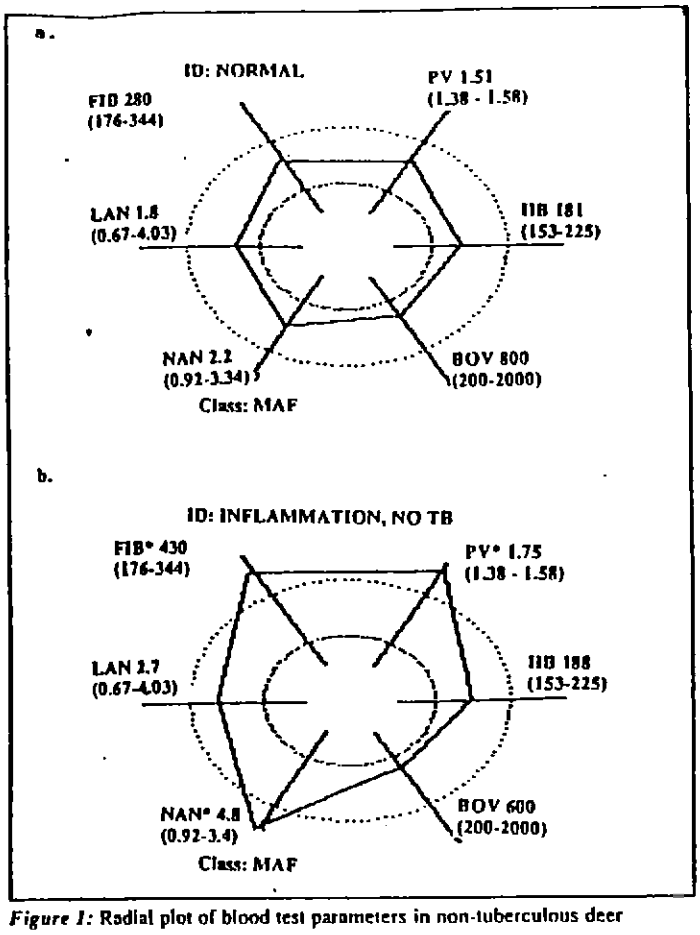

Figure 1: Radial plot of blood test parameters in non-tuberculous deer

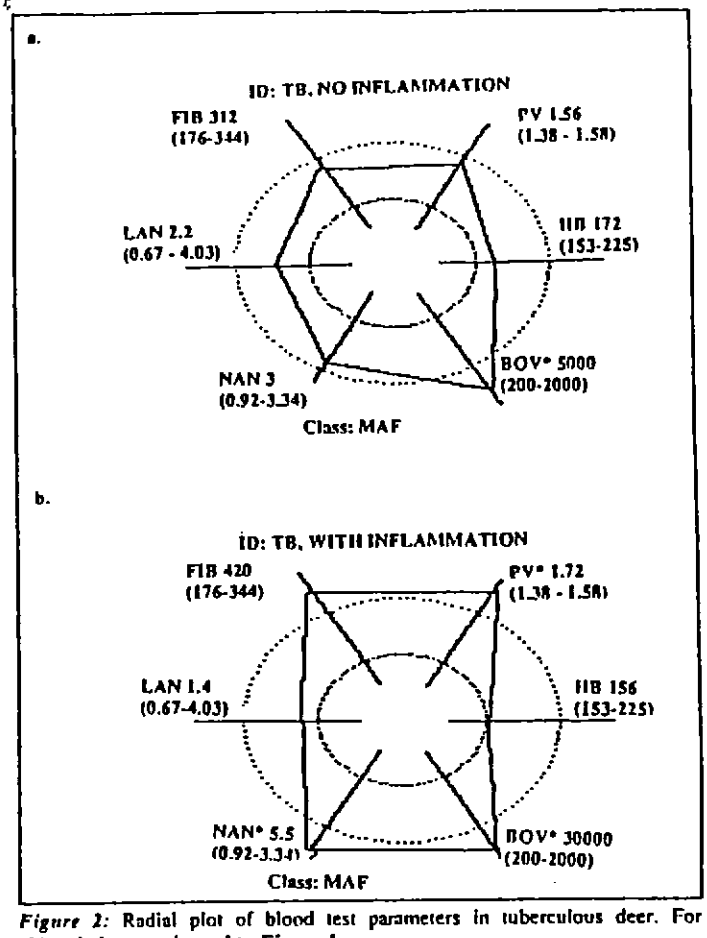

Figure 2: Radial plot of blood test parameters in tuberculous deer. For abbreviations see legend to *Figure 1*.

Abbreviations: BOV : Specific lymphocyte transformation, expressed as counts per minute
FIB : Concentration of fibrinogen
HB : Concentration of haemoglobin
LAN : Number of lymphocytes
NAN : Number of neutrophils
PV : Plasma viscosity

Day 60. A 120-day interval showed no post-test suppression; it was suggested that a 90-day test interval could provide a reasonable compromise. The additional complication inherent in the CCT is that measured increases in skin test thickness are necessary to produce a specific test, whereas it has been demonstrated by de Lisle *et al.* (1984) that high levels of sensitivity are found only when all visible or palpable skin test tuberculin reactions are deemed positive for deer. They state that, based on a sensitivity of 85 *per cent* for ST under field conditions, the likely sensitivity of composite ST and CCT should be in the region of 68 *per cent*. The specificity of CCT under experimental conditions was found to be 99 *per cent*. Of 1,157 deer tested on properties considered to be free from tuberculosis, 15 (1.3 *per cent*) showed positive CCT bovine reactions.

Some care should be exercised in extrapolating from the findings obtained from skin test parameters in deer which have assessed sensitivity levels only in animals infected experimentally with *M. bovis*. Similarly, specificity data obtained from herds known to be free of TB may not accurately reflect the complexity of reactivity which may prevail in herds with mixed infection. It is likely that, at best, the performance of tuberculin skin testing in deer will not exceed the best levels found currently in cattle. Francis, Seiler, Wilkiw, O'Boyle, Lumsden and Frost (1978), who reviewed a number of skin test trials in cattle, quote the summary findings from a number of different studies, which give an average sensitivity of 81.8 *per cent*, and a specificity of 96.3 *per cent* for ST. They suggest that available evidence infers more limited sensitivity for CCT - a contraindication for its use as a primary diagnostic assay. Local studies (de Jong and Ekdahl, 1969), using CCT in cattle herds, yielded a disappointingly low sensitivity (68.6 *per cent*) for this assay under New Zealand conditions. This figure is significantly lower than the sensitivity figures (88.8 *per cent*) for CCT in the UK, where it is used as the main diagnostic test (Francis *et al.*, 1978).

By 1986, 1147 (33 *per cent*) deer herds in New Zealand had joined the voluntary TB accrediation scheme (Corrin, 1987). Of 281,003 deer tested, 0.84 *per cent* were reactors; reactors were found in 0.52 *per cent* of herds in the accreditation scheme and in 1.1 *per cent* of herds not in the scheme. Amongst 421 reactors examined at necropsy 68 (16.1 *per cent*) had *M. bovis* lesions. During this period, 165 deer farms had confirmed infection with *M. bovis* (Walker, 1987) and 95 herds had TB-free accredited status. By November 1987, 41.5 *per cent* of all deer herds in New Zealand had joined the TB accreditation scheme and 332 herds had gained accredited status (Carter, 1988). For the first 10 months of 1987, 373,698 ST were carried out on farmed deer with a further 9356 animals being subjected to CCT, with a reactor rate of 0.56 *per cent* among all the animals tested. Currently there are plans to introduce a compulsory scheme for TB testing of all deer herds in New Zealand.

Laboratory tests for diagnosis of tuberculosis in deer

Since 1963 (Schreck, 1963; Permain, Lycett and Fitzgerald, 1963), various research workers have evaluated the prospect of using lymphocyte stimulation assays to monitor tuberculosis in humans (Verma, Gupta and Ghai, 1974), primates (Charapas, Hedrick, Clark and Garman, 1970) and ruminants (Muscoplat, Thoen, Chen and Johnsone, 1974). Whereas these workers have consistently found that lymphocyte stimulation by tuberculin *in vitro* provides superior discrimination for disease diagnosis, no system has yet been produced which has been tested in a naturally infected host under field conditions. The most likely explanations for this are that laboratory tests, using sterile culture systems, are difficult to automate for widespread application, and longitudinal studies on disease diagnosis under field conditions are very expensive.

Our laboratory has developed a blood test for TB (BTB) for use in deer (Griffin and Cross, 1986). The context within which this test was established has concentrated primarily on the development of a diagnostic system which would have improved levels of sensitivity, specificity and validity, and yet be sufficiently robust so that it could have value within the known constraints that apply in cervine tuberculosis. Rather than develop an assay using animals which were experimentally infected with *M. bovis* or *M. avium*, it was decided to focus the studies on farmed deer herds which had known levels of naturally occurring TB infection due to *M. bovis*, mixed infection with *M. bovis* and *M. avium*, or atypical reactions due to *M. avium*. Rather than use small groups of experimental animals, we have used uninfected animals to establish a complete reference database of laboratory parameters against which the performance of diseased animals could be evaluated. To date, blood samples from more than 5,000 deer have been included in this database, using groupings which include sex and age differences. Following testing, large numbers of animals have been necropsied to establish their disease status. Although this approach is very expensive, it is the only valid means to develop a diagnostic system for natural infection.

The BTB assay system

Because laboratory techniques allow for the more critical quantitation of immune reactivity in animals exposed to mycobacteria they offer increased precision in the assessment of the individual animal's response to infection. Such assays allow for the more specific definition of the immunological reactions in the host, and can be used to distinguish reactions uniquely specific for *M. bovis*, *M. avium*, *M. paratuberculosis* or saprophytic mycobacteria. They also may allow for a distinction to be made between reactivity due to disease caused by *M. bovis*, and reactivity that results from infection of an animal with tuberculosis mycobacteria, without the establishment of TB disease. Measurement of inflammatory reactions in the test animals adds further discrimination in identifying animals with lesions due to *M. bovis*, as opposed to immune clearance of bacteria from infected stock.

The basic assay used to define specific immunological f involves a modified lymphocyte transformation assay i mononuclear leukocytes are co-cultured with PPD, in tri for 5 days prior to pulsing with ^{3}H-thymidine. Cells are ha 18 hours post-labelling with thymidine using an automa harvester (PHD Cell Harvester), and the radiolabel up estimated as the counts per minutes (cpms) using a β-scint counter (LKB 1214 Rackbeta). Negative unstimulated c and mitogen (Concanavalin-A)-stimulated positive cont included in every assay. A range of PPD is used to identify s reactions due to *M. bovis*, *M. paratuberculosis*, *M. av* saprophytic mycobacteria. Repeat triplicate controls using tuberculin are incorporated into separate microculture pl validate the level of bovine reactivity in every animal under

Differential white blood cell counts (LAN: number of ly cytes; NAN: number of neutrophils) and haematological (HB: haemoglobin) are established using a Technicon H6 standardised for blood of *Cervus elaphus*, calibrated a standard techniques. Plasma viscosity (PV) is estimated modified Harkness viscometer, using a 3.60 *per cent* NaC dard, and a lyophilised human serum albumin control Fibrinogen (Fib) is calculated by thrombin clotting using a automated technique, calibrated against a heat precipitation n (Cross, 1987).

Composite immunological and inflammatory radial plots

The plots obtained for non-tuberculous (Figure 1) and tuberc (Figure 2) weaner deer are given . Figure 1 shows tha immunological response to bovine PPD is taken as the pri indicator of tuberculosis. Figure 1a shows a normal respons control animal, whereas Figure 1b shows an aberrant inflam tory response in an animal that was non-tuberculous and la specific reactivity with bovine PPD (Bov*), compatible wi berculosis.

Figure 2 shows specific transformation (Bov*) in two t culous animals. Figure 2a shows the response of an animal failed to yield acid-fast bacilli (AFB) on necropsy, but had a s

TABLE 2
Incidence of reactors in an affected herd

	ST reactors	BTB reactors	Lesions in reactor at necropsy ST+	BTB+
Positive reactions (Day 0)	6/127	47/127	3/6	6/6
Positive reactions (Day 90)	28/115	54/115	10/28	23/28

ST: Single Cervical Skin Test.
BTB: Blood test for tuberculosis.

TABLE 3
Ranking severity of lesions at necropsy

Rank at necropsy	Lesion status
0	No visible lesion
1	Lymph node enlargement, no pathology but acid fast bacilli on culture
2	A single caseous calcified lesion
3	A single liquefactive lesion
4	Liquefactive + caseous lesions
5	Multiple liquefactive lesions – Generalised TB

calcified lesion 'typical' of tuberculosis. Figure 2b shows the response representative of animals harbouring multiple caseo-liquefactive lesions and large numbers of AFB. Although there was a significant level of transformation (Bov* 30,000 cpms) with PPD-B, this animal gave repeated false negative ST reactions to successive skin tests. This indicated that the blood test can detect active immunological and inflammatory responses in an 'anergic' animal that is harbouring significant infection but is unreactive to skin test.

using data obtained from a single blood test performed within 14 days before necropsy. The results obtained in this analysis are given in Figure 3. The data obtained from a single blood test showed a high degree of correlation between the Risk Value obtained from BTB test data and the subsequent necropsy findings. The severity of the lesions was ranked according to the scale outlined in Table 3.

The results in Figure 3 show that there was a good correlation between predicted risk, as evaluated from the blood test, and the subsequent disease status of the test animals. It was particularly satisfying to see that animals with the most severe disease generally ranked high in the assay system. At the outset it was thought likely that some of the heavily infected animals might be immunologically unresponsive ('anergic') and be difficult to identify, but this was not so.

TABLE 4

Sensitivity and specificity of the composite laboratory assay (BTB) and skin test (ST) on animals prior to necropsy

	Sensitivity	Specificity
BTB	158/166 (94.8%)	420/446 (91.9%)
ST	139/166 (84.1%)	365/446 (82.3%)

Definition of herd status using a single blood sample

The ability to define the TB status of a herd using a single blood sample obtained from skin test (ST) reactors and matched control animals is shown in Table 1. The disease status of reactor animals was subsequently confirmed by necropsy findings in each of the herds listed.

The necropsy findings confirmed that a single blood test provided an accurate TB diagnosis in all herds except one, in which there was bovine reactivity but lesions were not found at necropsy. This herd had an earlier confirmed case of tuberculosis with typical lesions in an animal at necropsy, so the BTB reactivity found in the ST reactor animals was likely due to low level exposure of these animals to *M. bovis* which reflected immunity rather than spread of disease. The very high incidence of mixed infection with *M. bovis* and *M. avium* highlights the problem inherent in applying the ST in such herds, where a high loss through false-positive *M. avium* reactivity is likely to occur. The contraindication to using the CCT in such herds would necessitate continuing wastage of stock through false-positive reactions, if false-negative reactors are to be avoided in a disease control programme.

Chronology of reactivity to ST and BTB in TB infected herds

The data from two herds in which TB was known to be present at a significant level (> 10 *per cent*) is shown in Table 2. Results are given from tests carried out in an initial day test (Day 0) and from repeat tests three months later (Day 90).

The points of relevance to emerge from the comparison between ST and BTB were: firstly, that at the initial test a significantly higher incidence of reactivity was found in the BTB (37 *per cent* - 47/127) than in the ST (5 *per cent* - 6/127); secondly, the predicted incidence of lesions (*validity*) was significantly higher than BTB (85 *per cent* - 29/34) than with ST (38 *per cent* - 13/34). These data infer that the BTB provided evidence for exposure to *M. bovis* much earlier than was found with ST, and it succeeded in identifying infection with much lower wastage through slaughter than was evident with ST. The high wastage with ST was obviously due to false-positive reactors due to *M. avium*. Wastage of BTB-positive animals was due to the slaughter of animals with high levels of immune *M. bovis* reactivity, which were considered to pose an unduly high risk of infection and were culled. Significant suppression of BTB reactivity was not seen in animals tested 10 days after the application of ST.

Value of BTB in the prediction of lesion status of animals in an infected herd

In this experiment the data obtained from BTB and the inflammatory profiles were computed for each animal prior to necropsy and a Predictive Risk Value was calculated. Analysis was carried out

Sensitivity and specificity of BTB in herds harbouring *M. bovis*

The results of the BTB assay were critically evaluated in six herds in which tuberculosis was known to be present and extensive necropsies were carried out on all the animals tested, irrespective of whether they were likely to be tuberculous or uninfected. ST were carried out prior to necropsy and the results from these tests are included for comparison. Necropsies were carried out under controlled conditions on the farms or at deer slaughter premises. Animals with lesions had representative samples submitted to the laboratory for histopathological and microbiological studies. Where no AFB organisms were demonstrated on histopathology, microbial cultures were carried out. Cultures were carried out on all specimens showing any evidence of pathology compatible with tuberculosis, though not from animals where no visible lesions were detectable.

Of 612 animals examined critically at *post-mortem*, 166 were classified as having tuberculous lesions and 446 were classified as non-tuberculous. The results of the laboratory assay carried out on a single blood sample (taken within one month of necropsy) and the ST data obtained at the same time are given in Table 4.

These comparisons show the superior discrimination obtained by the BTB assay over that obtained in the ST in both its sensitivity and specificity. In the case of assay sensitivity, apart from the incidence of false reactions, another significant difference also emerges. None of the 8 *per cent* tuberculous animals that would have passed the BTB as false-negatives had liquefactive lesions.

By contrast, among the 27 (16 *per cent*) of animals that passed the ST at least half had multiple liquefactive lesions, with one quarter of these animals showing generalised TB (GTB). This infers that the BTB is effective in the management of TB, where disease can be diagnosed early and where severely infected animals, likely to cause infectious spread within the herd, are not left behind following BTB testing.

Blood sample collection and transport to the laboratory

During the development phase of this project, multiple blood sample was obtained from each animal to establish optimal conditions for sampling. Subsequent refinements in processing of these specimens now allow us to establish an immunological and inflammatory profile on each animal using one 15ml heparinised

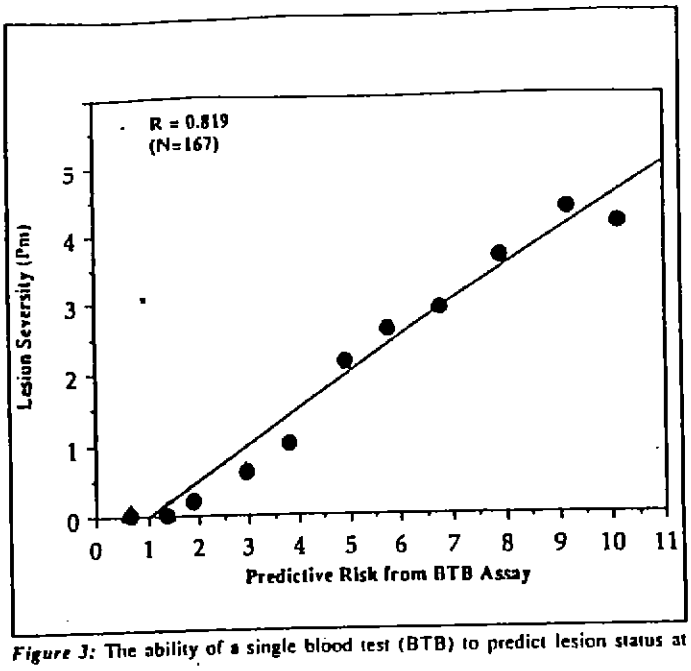

Figure 3: The ability of a single blood test (BTB) to predict lesion status at necropsy.

blood sample and one EDTA blood sample. We have placed the highest priority on minimising the blood sample volume to obviate artefacts due to sampling, and errors associated with laboratory filing and processing. There appears to be an inverse relationship between sample volume and specimen quality, so it is considered vital to request the collection of the minimal sample necessary for a proper diagnosis. To avoid artefactual changes in samples post-collection it is imperative that the blood sample is taken in one single unimpeded draw. Samples should be collected into a 15ml heparinised *Vacutainer™* using an 18G needle. An interrupted blood collection or incompletely filled tubes should not be submitted to the laboratory. All tubes should be inverted at least six times to ensure adequate admixture with the anticoagulant, prior to clear and accurate labelling of the tubes. Farmers should ensure that all test animals have distinguishable tag numbers so that accurate cross-referencing can be carried out subsequently.

Conditions that prevail during collection of blood should be stated by the submitting veterinarians. Complications in mustering or restraint of individual animals should be noted, as severely stressed animals may yield abnormal haematological profiles and confuse the *in vitro* evaluation of the animal's status. We have also established (Cross *et al*, 1988) that the use of *'Rompun'™* for sedation of animals can significantly alter the haematological values for individuals, as opposed to those sampled under physical restraint. Reference values have been obtained from animals under both conditions so no complications need ensue, provided the conditions which prevail during sampling are noted by the veterinarians.

Blood samples should be packed carefully and transported at room temperature, to arrive at the laboratory no later than 24 hours post-collection. Should further delay in transport be anticipated samples should be stored in a chilled insulated container on ice. Precipitous temperature shock through exposure to excessive heat (sunlight) or cooling should be avoided, as such conditions impair the quality of specimens. Some caution is necessary in the interpretation of inflammatory status after skin testing, as this is associated with an acute phase reaction in a proportion of deer (Cross 1987; Cross *et al* , 1988).

Conclusions

The value of alternative techniques for the diagnosis and control of TB in animals or humans should be considered in the context of the following constraints which limit the value of skin testing.

Test interval

The requirement for a 90-day interval, because of post-ST su pression, may compromise the ability of ST to eliminate TB i fection, if present at a significant level within a deer herd, becau of the ability of TB to spread rapidly within some deer herds. A obvious advantage of the BTB is that it can be used within 10 da of skin testing without undue interference through post-ST su pression. Repeat BTB can be carried out without any interferen with subsequent testing.

Sensitivity

It is widely accepted that anergy to ST may occur in seriou infected tuberculous animals, and that these false-negative re tors may compromise its ability to control TB spread. The sen tivity of the BTB is such that all seriously affected tuberculc deer can be detected, so that the anergy found in ST is r duplicated in the BTB. Independent studies in human tuberculo have also shown that *in vitro* tests can detect individuals w serious TB infection who are anergic to the ST (Verma *et al*, 19 Steiner and Rao, 1980). The strength of the BTB is that it particularly effective in identifying the seriously diseased anim that often appear as false-negative anergic animals when sl tested.

Specificity

It is recognised that the CCT offers significantly increased spe ficity over the ST in the detection of 'atypical' reactors due to *avium*. Recent recommendations (Wilson, 1986) are that the C should not be applied in herds where *M. bovis* is known to occ because of the reduced sensitivity of the CCT (de Jong a Ekdahl, 1969). In such herds significant stock losses would oc due to atypical reactors to the ST. The high degree of specific of the BTB means that it can be applied effectively in herds w mixed infections due to *M. avium* and *M. bovis*.

A further complication is that even when the CCT is appli false-positive reactions may occur, due to exposure to sun saprophytic mycobacteria. Results in Table 1 show how s problems can be eliminated by the use of BTB. Independ experimental evidence has shown that some slow growing sap phytic mycobacteria may elicit a strong cross reactive ST sponse with *M. tuberculosis* (Hattikudar & Kamat, 1985), and incidence of 1-2 *per cent* reactors to CCT in herds known to be i of *M. bovis* suggests that there are some limitations in specificity of CCT in deer using bovine or avian PPD (Carter e 1986).

Validity

Although the cellular response involved in the skin test D reaction correlates with infection, it is independent from cellular response that confers immunity following exposure to (Orme and Collins, 1984; Shapiro, Harding and Smith, 19 This means that the ST response cannot be used to accura identify immune animals. A validity of 30 *per cent* (Carter e 1985) would mean that valuable uninfected stock must be sa ficed in a ST programme. In experimental animals ther evidence that genetic resistance is an important factor in defi the establishment of disease following exposure to *M. b* (Stach, Gros, Forget and Skamene, 1984). Much work is b carried out at present to identify genetic lines of animals superior resistance to a number of important infectious disea Use of the BTB may allow for retention of genetically animals with increased resistance to TB, by identifying charac istics that may be associated with immune protection.

Feasibility

Considerable effort has been expended to simplify and stand ise the procedures involved in the BTB assay. Much work been carried out to obviate problems due to sampling, recor

and reporting. The laboratory system currently employed allows for the efficient processing of large numbers of blood samples. The main limitation of the laboratory system is that it is expensive to use because it is technically demanding and labour intensive.

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