# TUBERCULOSIS IN DEER: NON-SPECIFICITY AND THE COMPARATIVE CERVICAL TEST

**P.R. Wilson**
**Department of Veterinary Clinical Sciences**
**Massey University**

## 1. INTRODUCTION:

Tremendous progress has been made toward tuberculosis control in farmed deer since the now classic description of this problem by Beatson and Hutton (1981). The major breakthrough came with the validation of the mid-cervical tuberculin test (CT) and the investigation of many aspects of its application and performance in the practical and research environment (Livingstone (1980), Beatson and Hutton (1981), Carter et al. (1984). The sensitivity of the CT was found to be approximately 85% and the specificity approximately 99%. The exacting requirements for the application of the test were established.

From a disease control viewpoint the sensitivity of the test is the most important factor. It became clear from experiences of Beatson et al. (1984) that the "false negative" tested deer was a threat to the value of the test as a tool for the eradication of the infection. Further to the false negative problem, in a severely affected herd the rate of spread of infection appears to exceed the rate at which the test can detect and eliminate infected deer. However, it must not be overlooked that in most situations a test and slaughter programme combined with appropriate herd management will be effective in eliminating the infection from a deer herd. An MAF circular (14.12.83) stated that Tb had been eradicated in 16 herds using the CT and that 6 herds had a false negative problem.

A further MAF circular (16.7.84) stated that ...."tuberculosis in deer can be controlled using current procedures. However, in a small proportion of herds total eradication will be impossible". Thus the CT has the qualities to provide the basis for a control programme, and it is likely that this test will remain as the primary test for this purpose. This fact must be kept in perspective by veterinarians in view of the introduction of the comparative cervical test (CCT) and current research on an in vitro lymphocyte transformation test (Griffin, 1986). The CT will continue to be the mainstay of the Tb control programme in the foreseeable future.

As the amount of tuberculosis testing in deer herds accelerated from 1980 it became increasingly apparent that from some herds with reactors to the CT no evidence of M.bovis could be found either by gross or histopathological lesions and/or culture. The first official recognition of this was given in a MAF Circular 13.12.84 which stated ....."There have been only two herds in New Zealand with an apparent non-specificity reactivity problem. They have no history of tuberculosis but 3% or more of the herd have given positive reactions at a tuberculin test. Five other herds have had non-specificity at a lower rate".

Deer farmers were quick to understand the meaning and ramifications of this phenomenon and there developed a loss of confidence in the CT (Carter et al 1984). Subsequently both farmers and veterinarians began to regard almost all reactors with suspicion. Many farmers ceased slaughter of reactors. Potential "false positives" were used as an excuse for not Tb testing. Testing veterinarians sometimes found themselves in an invidious position. Cessation of compensation for slaughtered reactors compounded the problem. The Tb control scheme was in jeopardy.

Thus the need for methodologies for investigating non-specificity was established.

## 2. OCCURRENCE OF NON-SPECIFICITY

The MAF circular (16.7.84) stated ....."a single test will correctly identify..... more than 99.5% of non-infected deer". Thus an estimate over the national herd is that 1 in 200 non-infected deer will be a reactor to the CT. Many have taken this to indicate that every herd of 200 deer must get 1 reactor at each test.

This is not the case. The distribution of non-specificity is localised i.e. not all farms have non-specific reactors, but where they occur the percentage of deer reacting non-specifically is likely to be much greater than 0.5%. (See case reports later in this proceedings.)

## 3. CAUSES OF NON-SPECIFICITY

Any deer previously sensitised to an antigenic component of any source closely resembling the antigenic component(s) of M.bovis potentially will produce a reaction to the bovine tuberculin used in the CT. It is logical to expect that other Mycobacteria would possess antigenic similarity.

de Lisle and Havill (1985) found 35 isolates of M.avium-intracellulare from deer between 1979-83. Twenty-two of these were from CT reactors, 17 of which had no gross lesions. Thus 5 reactors with M.avium-intracellulare isolated possessed gross lesions. Four isolates were made from clinically normal deer. Interestingly, two cultures were found in deer which had not reacted to the CT, suggesting as one would expect, that not all M.avium-intracellulare infected deer are sensitised to bovine tuberculin. (It is only an assumption however, that the presence of M.avium-intracellulare caused the reaction to the CT in the 22 cases mentioned above).

The relative importance of M. avium-intracellulare as a cause of nonspecificity is unknown. There are few reported isolates from the New Zealand bird population, thus it would appear that the prevalence of this organism is not high (de Lisle pers. comm.). It is commonly believed by farmers and some veterinarians that grain feeding predisposes deer to M. avium infection transmitted by bird faeces.

While there is often an association between non-specificity and grain feeding, this has not yet been proven. It appears that most deer farmers feed grain to deer at some stage, so the association may be purely coincidental.

Other Mycobacterial isolates found by de Lisle and Havill (1985) include:

| | No. |
|---|---|
| M.vaccae | 7 |
| M.smegmatis | 5 |
| M.chelonei sub abscessus | 3 |
| M.chelonei | 1 |
| M.gastri | 1 |
| M.diernhoferi | 1 |
| M.monochomogenicum | 1 |

Some of these isolates, but not all, were from CT reactors (de Lisle, pers. comm.) None were associated with lesions. It is not known whether these isolates were due to the actual presence of the organism in the tissues or whether they were post-mortem contaminants. All these bacteria survive in the environment, but possibly have a potential to sensitise deer to bovine tuberculin.

Johne's disease (M.paratuberculosis) has been diagnosed in farmed deer (personal observation and Gumbrell pers.comm.) but this organism is probably not of great importance as a source of cross-reactivity because of its apparent low prevalence in the national herd.

In addition to the above Mycobacteria already isolated from deer there are a large number of other environmental Mycobacteria, including M. phlei. Again the importance of these has not been established.

## 4. EFFECT OF CT INTERPRETATION ON NON-SPECIFICITY

The CT interpretation is that ...."any palpable or visible reaction at the site of injection is to be taken as a positive result". (MAF Circular 22.12.81). This interpretation excludes ....."a hard nodular reaction about wheatgrain size with a skin reaction diameter of 5mm or less and a skin thickness difference (STD) of less than 2mm." (Tuberculin testing of Deer, MAF/NZVA 1986).

It is likely that the specificity of the test would increase if a modified interpretation was used. Flock House research (Carter et al 1985) showed a higher specificity when the interpretation excluded small swellings as indicated above from being designated reactor status.

An alternative could be to exclude skin thickness differences of 2mm or less or reactions of less than a certain diameter. However, more research needs to be conducted before this can be contemplated since it is likely that such modified interpretation would decrease the sensitivity of the test. It must be remembered that the CT is designed to detect tuberculosis reactors, not non-specific reactors.

It has been suggested that the specificity could be improved if a lower strength tuberculin (1.0mg/ml) was used. However, earlier work has shown that this is not the case (Carter et al 1984).

## 5. EFFECT OF TESTING STANDARD ON NON-SPECIFICITY

If test sites are not large enough, if the area is not clipped cleanly, if the tuberculin is not placed accurately at the intradermal site, or if reading is performed with inadequate light the CT will be less likely to yield a visible or palpable skin thickening and therefore be less sensitive (but possibly more specific) than tests performed to the acceptable standard. However, this presupposes that non-specific reactors produce a smaller thickening than bovine Tb infected reactors. As indicated in (4) above further investigation of this possibility is needed. There may be a role in future for a modified interpretation in herds with a proven non-specificity.

It could be speculated that the higher reactor rate in 1985 may have been due to the more stringent application of the test, and that the percentage of non-specificity cases increased as a result. If this was the case, the sensitivity probably increased as well.

## 6. EFFECT OF PREVALENCE OF INFECTION ON SPECIFICITY, SENSITIVITY AND VALIDITY

Sensitivity and specificity of a test do not change with prevalence of infection. Validity (or predictive value) i.e. the proportion of test-positive animals that are actually infected, does. Validity decreases more rapidly as the prevalence of disease approaches zero.

This concept was clearly explained in Veterinary Cervus (Deer Branch NZVA Newsletter) Issue 7, March 1985 as follows:

If the prevalence of Tb was 50%, the sensitivity of the Tb test 90% and the specificity 99%, then to eliminate 90,000 of the 100,000 Tb deer from a population of 200,000 you would also eliminate 1,000 healthy deer. The validity would be 98.9% $\frac{(90,000)}{(91,000)}$

| | Tb test + ve | Tb test - ve | |
|---|---|---|---|
| Tuberculous | 90,000 | 10,000 | 100,000 |
| Healthy | 1,000 | 99,000 | 100,000 |
| | 91,000 | 109,000 | 200,000 |

If the prevalence is 1% then to eliminate 1,800 tuberculous deer you would also eliminate 1,980 healthy deer. The validity would be 47.6% $\frac{(1,800)}{(3,780)}$

| | Tb test + ve | Tb test - ve | |
|---|---|---|---|
| Tuberculous | 1,800 | 200 | 2,000 |
| Healthy | 1,980 | 196,020 | 198,000 |
| | 3,780 | 196,220 | 200,000 |

If the prevalence is 0.1% in the above situation the validity would be 8% i.e. to eliminate 180 tuberculous deer you would also eliminate 1,998 healthy deer." (Acknowledgment Dr C G Mackintosh).

At the present reactor rate (approx 1.5%) the validity is approximately 30% (Carter et al, 1985).

These are simplistic examples but demonstrate a general problem with this type of biological test.

Thus as the number of infected deer decreases, an ever increasing proportion of CT reactors will be non-specific. To achieve continued farmer confidence in and acceptance of a control or eradication scheme the role of ancillary tests becomes increasingly important.

## 7. ANCILLARY AIDS FOR DETECTION OF M.BOVIS AND/OR NON-SPECIFICITY

### (i) AUTOPSY

Brooks (1984) reported gross lesions in 89% of 36 deer inoculated with M.bovis intratracheally, and in 80% of 15 in-contact deer. No visible lesions were found in the other 4 in-contact deer, all of which reacted to the CT. Beatson et al (1984) reported gross lesions in 76.7% of 107 CT reactors in an M. bovis infected herd.

It is stated in the MAF/NZVA booklet "Tuberculosis testing of deer" that ..."field experience has shown that approximately 70% of infected deer will have visible lesions...". This figure is an estimate and is lower than the above figures. Presumably this is an acceptance that autopsies in the field may not be as accurate as those performed by teams of experienced veterinarians in the above two studies.

The MAF circular 16/7/84 stated that ..."15% of reactors have visible lesions...". With an estimated validity of the CT at 30% the sensitivity of autopsy would be only 50%.

Thus there are discrepancies, but some of these figures are based on estimates. The critical work of Brooks (1984) and Beatson et al (1984) demonstrates the potential for autopsy in aiding the diagnosis of tuberculosis or non-specificity. With these figures considered, it has been possible to estimate the probability of accuracy of diagnosis of Tb/non-specificity based on autopsy results. These figures were calculated by the MAF and one reproduced here from the booklet "Tb testing of deer" (See next page).

The sensitivity depends largely on how thoroughly the autopsy is done. The technique is outlined elsewhere (Beatson et al (1984), Brooks (1984) and in the booklet "Tb testing in Deer"). Descriptions of the distribution of lesions are published in earlier Deer Branch Course Proceedings (Beatson and Hutton (1981), Beatson et al (1984), Brooks, (1984)).

It is important to note that singleton lesions occurred in 50% of lesion positive naturally infected deer (Brooks, 1984). This highlights the precision that is needed for an accurate autopsy.

| Number of reactors | Number of postmortems | Herd prevalence of M. bovis 0.5% | 1.0% | 2.0% | 4.0% | 8.0% |
|---|---|---|---|---|---|---|
| 3 | 3 | 0.98 | 1.0 | 1.0 | 1.0 | 1.0 |
| 4 | 3 | 0.86 | 0.97 | 1.0 | 1.0 | 1.0 |
| 5 | 3 | 0.81 | 0.93 | 0.98 | 1.0 | 1.0 |
| 6 | 3 | 0.78 | 0.91 | 0.97 | 0.99 | 0.99 |
| 7 | 3 | 0.76 | 0.89 | 0.96 | 0.98 | 0.99 |
| 8 | 3 | 0.75 | 0.88 | 0.95 | 0.97 | 0.98 |
| 9 | 3 | 0.74 | 0.87 | 0.94 | 0.97 | 0.98 |
| 10 | 3 | 0.74 | 0.87 | 0.94 | 0.97 | 0.98 |
| 15 | 3 | 0.72 | 0.85 | 0.92 | 0.96 | 0.97 |
| 20 | 3 | 0.71 | 0.84 | 0.92 | 0.95 | 0.97 |
| 4 | 4 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 5 | 4 | 0.96 | 1.0 | 1.0 | 1.0 | 1.0 |
| 6 | 4 | 0.92 | 0.98 | 1.0 | 1.0 | 1.0 |
| 7 | 4 | 0.89 | 0.97 | 0.99 | 1.0 | 1.0 |
| 8 | 4 | 0.88 | 0.96 | 0.99 | 0.99 | 1.0 |
| 9 | 4 | 0.86 | 0.95 | 0.99 | 0.99 | 0.99 |
| 10 | 4 | 0.86 | 0.95 | 0.98 | 0.99 | 0.99 |
| 15 | 4 | 0.83 | 0.93 | 0.97 | 0.99 | 0.99 |
| 20 | 4 | 0.82 | 0.92 | 0.97 | 0.98 | 0.99 |
| 30 | 4 | 0.81 | 0.91 | 0.96 | 0.98 | 0.99 |
| 50 | 4 | 0.80 | 0.90 | 0.96 | 0.98 | 0.98 |
| 5 | 5 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 6 | 5 | 0.99 | 1.0 | 1.0 | 1.0 | 1.0 |
| 7 | 5 | 0.97 | 1.0 | 1.0 | 1.0 | 1.0 |
| 8 | 5 | 0.95 | 0.99 | 1.0 | 1.0 | 1.0 |
| 9 | 5 | 0.94 | 0.99 | 0.99 | 1.0 | 1.0 |
| 10 | 5 | 0.93 | 0.98 | 0.99 | 1.0 | 1.0 |
| 15 | 5 | 0.90 | 0.97 | 0.99 | 0.99 | 0.99 |
| 20 | 5 | 0.89 | 0.96 | 0.99 | 0.99 | 0.99 |
| 30 | 5 | 0.88 | 0.96 | 0.98 | 0.99 | 0.99 |
| 50 | 5 | 0.87 | 0.95 | 0.98 | 0.99 | 0.99 |

Table 3: Table showing probability that one deer of number postmortemed will have lesions.

Thus, when conducted properly, autopsy has a relatively good sensitivity and when used in conjunction with other tests and history is undoubtedly the most useful aid in the diagnosis of Tb/non-specificity.

Advantages of Autopsy

- demonstration of lesions (usually supported by culture) is the only definitive method of diagnosis of Tb.

- a tentative diagnosis can be made immediately if lesions are found.

- autopsy will help establish the acceptability of using the CCT in a herd.

- autopsy assessment will improve the accuracy of ancillary tests (i.e the CCT is more accurate if used where there is a low probability of M.bovis infection).

Disadvantages of autopsy

- a number of deer (up to 4-5) need autopsy for the diagnostic probability to be acceptable (see table). The significance of a single autopsy is limited.

- farmer acceptance of autopsy before other tests are applied is often difficult to achieve.

- sub-standard autopsy will lead to mis-diagnosis.

- cost; both in terms of deer lost and cost of autopsy.

The Significance of performing an autopsy

Application of the CCT requires a thorough knowledge of the history of the herd. It should be used only where there is little or no evidence of M.bovis infection. Autopsy will usually be the most accurate and definitive method for determining the presence or absence of infection. It will usually be necessary to perform autopsies before the CCT is used. Information gathered over the past few years now allows an assessment of the probability of a diagnosis (see table) based on autopsy.

The importance of this is that **the farmer can now be given a guarantee that only a limited number of autopsies will be necessary to establish a diagnosis within a certain probability.**

Thus, the major fear of farmers that a large number of non-specific reactor deer will be slaughtered is no longer warranted. This excuse not to test has now disappeared.

Histopathology

Histopathological signs of tuberculosis are not pathognomonic and can only be used as an aid to diagnosis. Further consideration of this is in the booklet "Tuberculin testing of deer".

## Deer Slaughter Premises (DSP) Role in Autopsy of Reactors

Tb reactors are now allowed to be slaughtered at a DSP provided they are clearly marked. The guidelines for the application of the CCT (MAF/NZVA booklet) impresses the need for accurate necropsy to determine the absence of lesions before the CCT can be applied. The MAF Meat Division veterinarian at the DSP's has an important support role for the practitioner in carrying out a thorough investigation of reactors in line with the details published in the guidelines, and in reporting back to the practitioner concerned. The practitioner will need to base his/her judgement on whether or not to apply a CCT on a property on findings of autopsy at the DSP. The Meat Division MAF has recognised its role and has instructed its veterinarians accordingly.

(ii) CULTURE

Confirmation of M.bovis infection by culture has been achieved in 84% of deer with gross lesions of Tb (Hunter, 1984). Brooks (1984) indicated cultural confirmation of infection in 100% of 32 innoculated deer with lesions at autopsy and in 2 of 4 innoculated deer with NVL autopsy results. All deer reacted to the CT. Further, lesions in 100% of naturally infected in-contact deer produced M.bovis cultures.

Beatson et al (1984) reported M.bovis isolation from 11 non-reactor deer (23% of those slaughtered) in one mob and from 3.5% of a second mob (n=194) from a heavily infected herd (average 9%). All were NVL. The MAF circular (16.7.84) indicated that ..."up to 10% of lesion-free (NVL) reactors could produce positive cultures of M.bovis."

The MAF ceased to perform cultures on lymph nodes of NVL cases because of the low probability of NVL's actually being infected. It is likely that culture will again become an option now that the Animal Health Laboratories will be charging a fee for services.

However, the practitioner must bear in mind the lower sensitivity of culture as a method of diagnosis and therefore results must be interpreted in light of all other findings.

iii) SEROLOGICAL AND IN VITRO TESTS

Results of serological tests (immunodiffusion and ELISA tests) are presented in detail elsewhere (de Lisle et al (1984), Sutton et al (1985). Both tests had a poor sensitivity under the experimental conditions investigated. There appeared to be a degree of cross-reactivity with other mycobacterial antigens.

The practicalities of in vitro testing must be considered and kept in perspective. It is likely that to be effective, whole herds will need to be sampled, particularly if false negatives are to be detected. The present skin tests (CT and CCT) are likely to be adequate for the detection, control and eradication of M.bovis in most instances and for the detection of non-specificity. Management factors which influence the incidence and spread of the disease should not be overlooked as an aid to the control and eradication of the disease.

iv) THE COMPARATIVE CERVICAL TEST (CCT)

The CCT is able to assist the detection of non-specificity. Its ability to detect M.bovis infection (approx 80% sensitivity) is significantly less than that of the CT (approx 90%). The important characteristics of the CCT are published elsewhere in this proceedings (Carter et al) in Carter et al, (1985) and in the MAF/NZVA booklet "Tuberculin testing of deer".

The following in bold type are excerpts from that booklet with further comment in normal type.

"DEFINITION OF HERD STATUS

The accreditation scheme rules give the veterinarian discretion to determine the fate of deer which react to the standard test. Whether deer are slaughtered or held for a CCT will be dependent on an assessment of the herd's Tb status.

It is essential for the testing veterinarian to establish the likelihood of an M.bovis infection before a CCT is applied. If the epidemiological assessment is not thorough, the results of any CCT applied will be more difficult to interpret and the accuracy of diagnosis reduced.

The assessment of the herd status is necessary to establish:

* the likelihood of a M.bovis infection,

* and if it appears unlikely that a M.bovis infection exists, whether a CCT is indicated.

The CCT should be used to assist in determination of non-specificity not M.bovis infections.

FACTORS TO BE CONSIDERED IN ESTABLISHING A HERD STATUS

Past tuberculosis testing history

How many deer on the property have been tested? How many whole herd tests have been carried out? What were the results of the previous tests? How long ago was testing conducted? Were animals introduced to the herd since the last test.

Note: Veterinarians must use the official Deer Tuberculosis Test Report form, AgL202, and ensure that MAF is sent its copy for the records of the accreditation scheme and national statistics.

It is important to assess the farmer's honesty in presenting details. It is well known that some farmers change veterinarians regularly. If there is any doubt the details should be sought from the other veterinarians used or the AHD of the MAF. All certificates should be viewed personally and if not available, suspicion should be aroused.

It is clearly desirable for close liaison between practitioners where it is realised that a farmer has changed veterinarians. Care must be taken to ensure that reactors found by one vet are not put up by the farmer for test by another veterinarian. Thus a high ethical standard is necessary on the part of all practitioners.

Autopsy results where previous reactors were slaughtered and autopsied

How critical was the autopsy? Were lesions found? Were cultures performed? How many postmortems were done? (See table on probabilities, but note that these figures rely on a critical postmortem). How long ago were autopsies performed? Have clear whole herd tests been conducted since autopsies confirmed infection? Have more animals been introduced to the herd since the last autopsy?

**Note: To accurately establish presence or absence of infection, it will usually be necessary to slaughter and autopsy a number of deer.**

The interpretation is more difficult when only one or two reactors are found. If a large number of reactors are found, there is possibly a role for the CCT to play in selecting those deer for slaughter. However, this is not without risk since deer must be held for 90 days before CCT and this potentially allows spread of Tb if the group is not isolated. It must be borne in mind that fewer deer will show M.bovis infection using the CCT than using the single test.

Origin of the deer

**A full history of the sources of deer should be obtained from the farmer. Veterinary certificates accompanying purchases should be examined for reference to reactors at sale tests. Animal Health Division Officers will be able to assist by providing:**

*** information on the disease control place status of the property**
*** information on whether the herd is accredited**
*** the date of the last whole herd test**

**To obtain an up-to-date history of a herd it may be necessary to contact the vendor's practitioner. If positive test reactions occur only in recently purchased deer these deer must be regarded with greater suspicion.**

**Note: Such tracebacks should be considered with caution for several reasons:**

*** There have been inadequate reporting procedures using the AGL 150s to date; e.g. reporting procedures did not state whether there were whole herd tests or repeat tests.**

*** Some tests have not been reported to the MAF.**

*** Not all deer being traded have been tuberculosis tested.**

**If deer with positive test results are from herds with known M.bovis infection they should be classed as reactors, slaughtered and autopsied. If the purchase history is dubious, slaughter may be desirable. In this instance the CCT may be useful to assist in determining which deer are to be slaughtered for autopsy.**

Reports on the "Deer Tuberculosis Test Report" (AgL/202) have overcome the shortcomings of the earlier AgL/150 form.

Further to the points of caution above, another shortcoming with requesting details from the MAF is that VO's apparently are not at liberty to disclose all the facts about a property e.g number of reactors, whether a CCT was performed, identification of reactor deer, whether M.bovis has been confirmed by gross pathology or culture, whether the last whole herd test was clear etc. It might be suggested that withholding such information is not in the best interests of the control programme and that too great an emphasis appears to be placed on the confidentiality of information held by the MAF. I believe this information should be freely available to practitioners and farmers.

## Clinical cases

**Have there been recent clinical cases of tuberculosis, deaths or abscesses which have been confirmed as M.bovis infections?**

## Slaughter

**Have there been deer which have been recently rejected at deer slaughter premises (DSPs) for gross tuberculosis?**

## Endemic area

**Deer positive to the test which live in or have been captured from areas designated as endemic for tuberculosis should be regarded with greater suspicion. Tb-infected feral deer have been captured from many areas but the most important are the Hauhungaroa range, the Wairarapa, the West coast and the Catlins.**

However, it should be remembered that not all deer from such areas are affected, and indeed that infection is usually localised to relatively small areas or small numbers of farms within that district. Blanket discrimination is inappropriate.

## Management factors

**Herds trading large numbers of deer are obviously at a higher risk of contracting infection, especially when the deer have passed from herd to herd in rapid succession. Effective isolation of mobs may also need to be considered.**

Some farms operate more than one unit. Some may have kept newly purchased mobs physically isolated from other mobs on the farm. Some herds with infection may operate a management system of set-stocked set-allocation herds over the whole property. Any ongoing occurrence of reactors must be analysed with these factors in mind.

## Tuberculosis in other animals

**If there is a tuberculosis problem in any other class of animal (e.g cattle, Tb-infected possums, and feral deer) on the property then this should be taken into account.**

There is a tendency for farmers to incriminate the local wildlife population e.g. possums as an ongoing source of infection if reactors keep occurring. There is only a small likelihood of Tb becoming established in feral animal populations outside those areas where infection is already endemic in wildlife.

**Neighbouring properties**

**If there is evidence of a tuberculosis problem in any species on neighbouring properties this should be considered and the degree of contact evaluated.**

## 8. USE OF THE CCT IN M.BOVIS INFECTED HERDS

The CCT detects approx. 80% of deer with bovine Tb (the CT detects almost 90%). Thus, if the CCT is used in the presence of M.bovis infection the rate of progress toward control could be severely impeded and in some events could even result in an increase in incidence of the disease (i.e. where initial infection rate is high). Safeguards are necessary (see below).

There will be instances in which use of the CCT disproves the epidemiological evidence supporting the existence of non-specificity i.e. use of the CCT was found to be inappropriate. This situation will usually not be too critical when all the initial reactors were kept isolated from the rest of the herd. The safeguard is provided in the guidelines for use of the test in that all reactors to the initial test be slaughtered.

If the CCT indicates M.bovis infection the veterinarian would be wise to consider further autopsies. The important concept is that the CCT results must be believed and acted upon, not ignored. Errors of diagnosis are more likely with the CCT when only small numbers of deer (e.g 1-3) are involved (See Section 11).

It is advisable for the veterinarian to instruct the farmer when the CCT is first used on a property as to the greater possibility of a mis-diagnosis. It will be easier for the farmer to accept the unexpected outcome if he/she has been alerted to the possibility in advance. It is also advisable to inform the farmer that all the original CT reactors will be designated for slaughter if any react to the CCT.

NOTE: - If the CCT indicates an M.bovis infection in a herd it must not be used again in that herd unless there is evidence that the infection has been eradicated.

## 9. USE OF THE CCT IN DUAL M.BOVIS AND NON-SPECIFIC INFECTIONS

There will be cases, albeit rare, in which dual infection occurs.

The CCT has been shown to detect non-specific reactors in a very high proportion of cases (probably more than 95%). Thus, if 100% of original CT reactors in a herd show a greater STD to avian tuberculin then the diagnosis is fairly clear. Likewise, if the majority (70% or more) of original CT reactors are shown by the CCT to be M.bovis infected as is likely in most infected herds, the diagnosis is fairly clear.

However, if a significant number of original CT reactors show non-specificity and a significant number show M bovis infection when the CCT was applied, there is a probability of a dual infection being present. (N.B. diagnosis of dual infection would be impossible if only a small number of reactors occur).

If a dual infection is apparent, the low sensitivity of the CCT will mean that some of the CCT negative deer may be carrying M.bovis. The major difficulty in dealing with a dual infection is that there is currently no way of assessing which animals these are. In vitro tests may have a role in eliminating this problem, but until these are avilable, the farmer and vet are faced with a serious problem. The options are:

1. Slaughter all original CT reactors.

2. Slaughter only the CCT positive deer, isolate the CCT negative deer and re-test. (This is potentially a very risky option as the chance of false negatives developing is probably high. Thus the chances of eliminating the M.bovis infection are limited.)

3. Hold all original CT reactors and wait for validation of in vitro tests. This may be a long wait and meanwhile the infection will spread.

## 10. USE OF THE CCT AS THE PRIMARY TEST IN HERDS WITH NON-SPECIFICITY

There is some discussion about using the CCT as a primary test in herds with proven non-specificity.

Advantages are:

(i) No 90 day waiting between CT and CCT.

(ii) Sale tests can be done with no threat of losing sales or having to wait 90 days for a CCT if reactors occur to the sale CT.

(iii) Cost and Management: only one set of veterinary visits would be necessary.

Disadvantages are:

(i) There is a greater chance of misdiagnosis in the event that M.bovis was latent in the herd. If adequate history is established, this of no concern.

(ii) Test difficulty. The application of the test is exacting and time consuming. More errors are possible. The time factor may cancel possible cost advantages.

General Policy:

**The general policy is that the CCT should not be used as the primary test.**

However, the testing veterinarian has the discretion to use the CCT as a first test if he or she so wishes. A thorough understanding and history of the property must be achieved before this should be contemplated.

## 11. FACTORS THAT INFLUENCE THE ACCURACY OF THE CCT

Diagnosis of Tb/non-specificity is an exercise in probabilities. some of the factors that influence the probability are:

(i) Accuracy of assessment of herd status.

(ii) Accuracy of autopsy and number performed.

(iii) Numbers of deer tested. The accuracy is less if a whole herd CT had not been performed.

(iv) Number of reactors to the CT. If only small numbers react, the probability of error is higher. Conversely, if large numbers of deer react the accuracy of interpretation is higher. For example:

| No. of CT reactors given CCT | No. neg | No. pos (i.e. M.bovis) | Accuracy |
|---|---|---|---|
| 2 | 1 | 1 | poor |
| 5 | 4 | 1 | fair |
| 50 | 1 | 49 | high+ |
| 50 | 49 | 1 | high* |

+ In this instance it would be probable that the CCT negative deer was a false negative.

* In this instance it is probable that the CCT positive deer was not infected with M bovis.

(v) Accuracy of performance of CCT. If facilities and technique are not satisfactory, the test becomes less accurate, e.g. if the bovine tuberculin is given intradermally and the avian subcutaneously, the test would be invalid. The deer will give a totally erroneous bovine reaction and be positive. This would confuse the situation almost beyond comprehension. **THUS BOTH INJECTIONS MUST BE GIVEN WITH ABSOLUTE ACCURANCY.**

## 12. TERMINOLOGY: REACTOR vs REACTION

The accreditation programme is designed to encourage the testing veterinarian to objectively assess the likelihood of a reactor being infected with M.bovis by the methods explained above, and to apply discretion as to whether to determine that the reactor is slaughtered or not. A distinction must be made between a reaction to the test that deems the deer to be tuberculous and one which is not. Tuberculous deer must be slaughtered.

Thus the terms Reactor and Reaction have been used. Reactor signifies a deer which is deemed to be tuberculous (and must be identified accordingly) and a Reaction signifies the presence of a suspected non-specific swelling. The revised Tb test report (AgL 202) takes this distinction into account.

## 13. SALE AND CERTIFICATION OF NON-SPECIFIC REACTORS

Non-specific reactor deer can be freely sold. A herd diagnosed by the CCT as having only non-specific reactors is designated "clear" status and can become accredited in the same way as a CT negative herd. It is vitally important that buyers are aware of the status of the deer that they purchase as it is likely that the non-specific reactor will react again when tested on the next property. Accurate certification using the form AgL 202 is therefore essential. As more non-specific reactors are traded it will become even more essential for veterinarians to view the certificates in their trace-backs pertaining to herds which produce CT reactions.

There is currently a stigma and suspicion against non-specifcic reactors in the marketplace. Veterinarians must regard non-specific reactors positively ... they are not infected with M.bovis, that is the essence. As veterinarians we have a responsibility to engender a positive attitude toward such deer since our aim must be to encourage testing, not to discourage it for reasons which are incidental to the purpose of controlling tuberculosis in the national deer herd.

## 14. CONCLUSION

Non-specificity to the CT in deer is widely recognised and a means has been established whereby this phenomenon can be investigated. This has been a significant advance in the control of tuberculosis in the national deer herd since the farmer can now be assured that his/her losses due to non-specificity will be absolutely minimal.

The investigation of non-specificity requires a high degree of professional competence and understanding. It is important that the profession adopts a positive attitude to non-specific reactors to avoid prejudice within the industry against herds with this problem.

If a veterinarian has difficulty in assessing a herd status, or if advice is desired to assist the analysis of the problem herd, the Deer Branch and/or Animal Health Division staff are willing to assist.

## 15. REFERENCES

Beatson, N.S.; Hutton, J.B. (1981). Tuberculosis in farmed deer. Proc. Deer Seminar for Vets. NZVA Queenstown. 143-151.

Beatson, N.S.; Hutton, J.B.; de Lisle, G.W. (1984). Tuberculosis: Test and Slaughter. Proc Deer Course for Vets. Deer Branch NZVA No. 1 p 18-27.

Brooks, H.V. (1984). Pathology of Tuberculosis in Deer. Proc Deer course for Vets. Deer Branch NZVA No.1 p 13-17.

Carter, C.E.; Corrin, K.C.; de Lisle, G.W. (1984). Evaluation of the tuberculin test in deer. Proc Deer Course for Vets. Deer Branch NZVA Course No.1 p 1-8.

Carter, G.E.; Corrin, K.C.; de Lisle, G.W.; Kissling, R.C. (1985). Comparative Cervical test in deer. Proc Deer Course for Vets. Deer Branch NZVA Course No.2. p 80-87.

Carter et.al. (1986). The comparative cervical test in deer. This proceedings.

Griffin, F (1986). In-vitro tests for tuberculosis in deer. This proceedings.

Hunter, J.W. (1984). Tuberculin testing in deer: A Veterinary Practitioner's view. Proc Deer Course for Vets. Deer Branch NZVA No.1 p 28-33.

de Lisle, G.W.; Corrin, K.C.; Carter, C.E. (1984). Ancillary tests for detecting tuberculosis in deer. Proc Deer Course for Vets. Deer Branch Course No.1 p 9-12.

de Lisle, G.W.; Carter, C.E.; Corrin, K.C. (1985). Experimental Mycobacterium bovis infection in Red Deer. In Biology of Deer Production. Eds. Fennessy, P.F.; Drew, K.R. Bull 22 Roy.Soc.NZ. 151-153.

de Lisle, G.W.; Havill, P.F. (1985). Mycobacteria isolated from deer in New Zealand from 1970-1983. NZ.Vet.J 33: 138-140.

Livingstone, P.G. (1980). The evaluation of tuberculin tests in a tuberculous farmed red deer (Cervus Elaphus) herd in New Zealand. Masterate thesis, University of California.

Sutton, P.J.; de Lisle, G.W.; Wall, E.P. (1985) Serology of Tuberculosis in Red Deer. In Biology of Deer Production. Eds. Fennessy, P.F.; Drew, K.R. Bull 22 Roy.Soc.NZ. p 155-158.