Diagnosis and prevention of mycobacterial disease

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Summary

All deer species are susceptible to infection by three major species of mycobacteria; *M. bovis, M. paratuberculosis* and *M. avium* intracellular complex (MAIC). While *M. bovis* causes tuberculosis and *M. paratuberculosis* produces Johne's disease, MAIC cause infection but rarely produce clinical disease in deer. While susceptibility to infection is influenced by genotypic factors, the prevalence of mycobacterial infection and the severity of disease is exacerbated by phenotypic stressors which reduce innate resistance. Mycobacterial infection is difficult to diagnose by direct isolation of the infectious organism from clinical specimens obtained from living animals. Immunodiagnosis using the intradermal skin test which measures cell-mediated immunity has been used for over a century. While cheap and easy to apply, the intradermal skin test has limited sensitivity in diagnosing mycobacterial infection in every infected animal [False(-)] and poor specificity in excluding non-infected animals [False(+)]. A recently developed laboratory blood test for tuberculosis (BTB) provides new precision for diagnosis of mycobacterial infection in deer. It has shown that antibody-mediated assays can identify immunopathogenic pathways associated with active infection and disease. Separate pathways of cell-mediated immunity may be monitored as an indicator of protective immunity. Diagnosis of mycobacterial infection is optimised when both antibody- and cell-mediated immunity are assessed in combination.

Mycobacterioses in deer

The three major species of mycobacteria which infect deer are *Mycobacterium bovis* (*M. bovis*), *M. avium intracellular complex* (MAIC) and *M. paratuberculosis*. Whereas *M. bovis* causes tuberculosis in deer and is considered as the primary mycobacterial pathogen, *M. avium* and *M. paratuberculosis* produce sporadic and almost indistinguishable diseases in deer, with the gut being the most likely organ to be affected.

M. avium intracellular complex (MAIC) infections

M. avium (MAIC) is the most common saprophytic mycobacterium affecting deer world-wide. While MAIC bacteria rarely produce disease or pathology in deer, they are isolated from up to 5% of lesions considered to be *typical of tuberculosis* (G.W. de Lisle, pers.comm.). Certain breeds of deer appear to have an increased susceptibility to MAIC. In Britain, Munro (1986) noted that fallow deer (*Dama dama)* and sika deer (*Cervus nippon*) are more susceptible to *M.avium* infection than red deer (*Cervus elaphus*). A similar trend is evident in New Zealand where fallow deer are more likely to present with lesions due to *M. avium* (de Lisle & Havill, 1985). MAIC bacteria have also been isolated from axis deer (*Axis axis*) in zoological parks in England (Jones *et al.*, 1976). While disease due to *M. avium* is usually encountered as caseo-calcified mesenteric lesions, it may produce purulent lesions and involve haematogenous spread to the liver and lungs, with miliary lesions in the lungs and a fatal septicaemia (Nyage, 1989). Unless deer are subjected to extreme stress, it is uncommon to find severe pathology resulting from MAIC infection.

While MAIC causes *opportunistic* infection which rarely produces disease in farmed deer, indirect problems result from infection. Deer exposed to high levels of MAIC, through ingestion of food, soil or water contaminated with the MAIC organisms from the faeces of birds, develop immunological sensitization to common mycobacterial antigens and may produce False(+) reactivity, when skin-tested for tuberculosis. In New Zealand there are some herds which have high levels of non-specific skin-test reactivity due to MAIC. Skin-test reactions to the single mid-cervical skin test (MCT) may occur at a prevalence of between 5-50% in these herds. Similarly, high levels of non-specific skin-test reactivity are found in farmed deer in the UK and Europe (Clifton-Hadley & Wilesmith, 1991). In New Zealand there is seasonal variation in the incidence of False(+) MCT reactions caused by MAIC. Reactions are highest during spring, amongst animals exposed to MAIC present in winter feed or in stags during the rut when wallowing in mud pools may result in unduly high exposure to saprophytic mycobacteria present in soil and water. Studies in our laboratory also show that there are higher levels of sensitization in fallow deer farmed in Southern parts of the United States, such as South Texas and Florida (J.F.T. Griffin, unpublished data). de Lisle & Havill (1985) reported 35 isolates of MAIC from New Zealand farmed deer during the period, 1979-1983.

M. paratuberculosis infections

Johne's disease was first described in roe deer in 1905, six years before the discovery of *M. paratuberculosis*, the causative organism. It manifests as a chronic granulomatous enterocolitis, with marked thickening of the colon and rectum (Nyage, 1989). Infection may develop over a period of years before chronic scouring occurs. A significant outbreak of Johne's disease was recorded in a Scottish red deer herd where the infection remained intractable to management or diagnosis (McKelvey, 1987). Vaccination of young stock was used successfully to arrest the spread of disease. Johne's disease has also been reported in wild deer in the United States, including fallow and axis deer (Reiman *et al.*, 1979), white-tailed deer (Chiodini & van Kruiningen, 1983) and elk (Jessup *et al.*, 1981). Serological evidence of exposure to *M. paratuberculosis* has been demonstrated in 2.5% of 954 wild white-tailed deer sampled in Ohio (Shulaw *et al.*, 1986). Reports of isolation of *M. paratuberculosis* from farmed deer in New Zealand have been on the increase since the first recorded case in 1979 (Gumbrell, 1987), with ten cases of Johne's disease recorded between 1979 and 1986. A recent report (Staples, 1994) showed that the number of New Zealand deer herds with confirmed Johne's disease increased from 8 in 1990 to 29 in 1993.

Not only is Johne's disease insidious in its onset, but it is impossible to treat and extremely difficult to diagnose. Culture of the bacterium may be difficult to achieve even when acid-fast organisms are visible in the intestinal mucosa during histological examination (Williams *et al.*, 1985). The intestines may be slightly thickened or oedematous, especially in the regions of the small intestine and caecum (Hutton, 1992). This contrasts with *M. avium* infection, where there is more extensive thickening and corrugation of the intestinal mucosa. As MAIC and *M. paratuberculosis* infection in deer may produce almost identical pathology, microbial culture is required for confirmatory diagnosis. *M. paratuberculosis* and *M.avium* share common antigens so it may not be possible to distinguish between these species immunologically (Camphausen *et al.*, 1988).

M. bovis infection

Infection caused by *M. bovis* may produce tuberculous lesions in wild or farmed deer. Species of wild deer affected by tuberculosis include fallow deer (Quinn & Towar, 1963), axis deer (Jones *et al.*, 1976), sika deer (Dodd, 1984), roe deer (*Capreolus capreolus*) (Gunning, 1985) and red deer (de Lisle & Havill, 1985). Tuberculosis infection caused by *M. bovis* was first identified in farmed deer in New Zealand in 1978 (Beatson *et al.*, 1984). Since then tuberculosis has been diagnosed in captive deer in England, Denmark, Sweden, Hungary, Malaysia, Taiwan, China, Australia, U.S. and Canada (Griffin & Buchan, 1993). The disease usually presents, in wild deer,

as a lymphadenitis involving one or more lymph nodes draining the nasopharynx (*retropharyngeal*), lung (*mediastinal*) or gut (*mesenteric*). Animals may harbour severe multiple lesions without any clinical evidence of disease. When an infected animal presents with clinical signs, it will invariably die within a few weeks (Griffin, 1988). However, when tuberculosis is found in a well managed deer herd it will usually only affect a low percentage (<5%) of animals with one or more lesions involving the head, thorax and abdomen. Usually farmed animals present with one or two lymphatic lesions in most especially the retropharyngeal lymph node (Beatson *et al.*, 1984). Therefore, detailed *post mortem* examination is required to identify lesions.

Generalised tuberculosis with severe pathology is often found in the first infected herd of farmed deer discovered within a country. Although this can invoke an emotive response, it is not usually representative of most of the tuberculosis found within infected herds. The reason for this is that the initial diagnosis usually occurs in animals which have been subjected to translocation or transport and encounter adaptational problems post-capture, coupled with the stressors associated with the establishment of a new deer farming unit. As the severity and spread of Tb is influenced markedly by stress (Griffin, 1989), it is likely that the first herd to be diagnosed in any country will have been exposed to phenotypic modifiers which exacerbate infection and cause widespread disease with severe pathology. Of all the known infectious diseases, tuberculosis is the best biological barometer for the underlying health and well-being of individuals. When tuberculosis occurs in a healthy population of deer it should be relatively easy to diagnose, treat or eradicate. Providing deer are well managed and not exposed to any severe stressors, there is no evidence that they are any more genotypically susceptible to tuberculosis than cattle. However, within an infected herd, age (0-6 months old), adverse climate, inadequate nutrition and inappropriate handling or transport may increase individual animal's susceptibility to tuberculosis. Pseudovertical transmission, from the dam to the offspring, is the most likely route of transmission for tuberculosis. Not only are young animals more susceptible to infection but should they become infected, diagnosis is difficult because they may not develop any visible lesions or detectable sensitization to immunodiagnostic tests, even though they may harbour large numbers of infectious organisms within their lymph nodes (Griffin & Buchan, 1993).

Isolation of *M. bovis* by microbiological culture can be achieved from more than 90% of tissue specimens containing lesions '*typical of tuberculosis*' (Table 1), obtained from deer at slaughter. While this is relevant for tuberculosis diagnosis and facilitates slaughter surveillance for *post mortem* diagnosis, it is imperative to have other diagnostic tests which can diagnose tuberculosis prior to slaughter, as it is extremely difficult to isolate *M. bovis* from mucosal secretions, and impossible to access pathological lesions or biopsy material *ante mortem* for

Table 1. Correlation between histological diagnosis of tuberculosis (Tb) in post mortem specimens and recovery of M. bovis by culture.

Culture Status	Histopathological Status		
	Lesion Typical of Tb	Lesion Atypical for Tb	No Visible lesions (NVL)
M. bovis (+)	118	0	9
M. bovis (-)	9	17	118
Predictive Value	92.9% (+)	100% (-)	92.9% (-)

microbial culture. Rather than rely on the direct isolation of the infectious organism, immunodiagnostic tests measure facets of the host immune response to the infectious organism and are used as indirect markers of mycobacterial infections.

Immunodiagnostic test for mycobacterial infections

Mid-cervical skin test (MCT)

Intradermal injection of extracts (purified protein derivative - PPD) obtained from individual species of mycobacteria, *M. bovis* (PPD-B), *M. avium* (PPD-A) and *M. paratuberculosis* (PPD-J), have been used for over a century to detect immune sensitization in animals and humans harbouring mycobacterial infections. The single mid-cervical skin test (MCT), using a single intradermal injection of PPD-B, adapted from conventional tests in cattle, was first used in deer by Beatson *et al.* (1984). A positive test is recorded if there is any visible or palpable thickening of the skin at the test site, 72 hours post-injection. After applying the MCT in 29 separate testing episodes (3620 tests) to 433 animals in an infected herd, Beatson *et al.* (1984) identified 82 *M. bovis*-infected MCT(+) animals. However, 43 out of 326 (13%) of the remaining animals, which were repeatedly MCT(-), had tuberculosis lesions at slaughter. Beatson *et al.* (1984) claimed that while the MCT was capable of identifying a tuberculosis-infected herd, it could not be used with confidence to identify every tuberculosis-infected animal within a herd because of its limited sensitivity.

Carter *et al.* (1984) evaluated the performance of MCT in 72 deer experimentally infected with virulent *M. bovis*. MCT had a sensitivity of 86% (36/44) when all visible or palpable skin test reactions were taken as positive. While the MCT has acceptable sensitivity to diagnose Tb in deer, it has insufficient specificity to exclude the significant but variable number of animals with non-specific sensitization due to exposure of deer to MAIC organisms. When MCT is used as the sole diagnostic test, for tuberculosis in farmed deer, there is unacceptable wastage of False(+) animals, 80-90% of which are not infected with *M. bovis* (Carter, 1990).

Comparative Cervical Skin Test (CCT)

To resolve the problems of skin-test specificity the performance of a comparative cervical test (CCT) was evaluated in deer experimentally infected with virulent *M. bovis* (Carter *et al.*, 1985; Corrin *et al.*, 1993). While their results show a sensitivity of 91.4% for CCT, they do not correlate with the performance of CCT for tuberculosis diagnosis in deer naturally infected with *M. bovis*. Under field conditions CCT is subject to many variables (test application, reading and interpretation) which may compromise its performance. CCT used under field conditions in deer has reported sensitivity values which vary between 31% and 80% (Stuart *et al.*, 1988; Griffith, 1989; Griffin & Buchan, 1993). Concerns about its variable performance under field conditions in New Zealand has led to the recommendation (Wilson, 1986) that CCT should not be used in herds known or suspected to be infected with *M. bovis*, or in herds without a test history which excludes tuberculosis. The sensitivity of CCT is also significantly reduced by prior application of MCT (Corrin *et al.*, 1993). A test interval of 90 days is recommended if CCT is to be applied to deer previously tested by MCT.

Laboratory tests for mycobacterial infection

The blood test for tuberculosis in deer - BTB

A multifaceted test blood test for tuberculosis (BTB) has been developed in our laboratory (Griffin & Cross, 1986; 1989) which measures two independent but complementary aspects of immunity and inflammatory proteins (IFP) in deer blood. The lymphocyte transformation (LT) reaction, which measures the relative cellular reactivity to purified protein derivative (PPD), *M. bovis* (PPD-B) and *M. avium* (PPD-A), is an *in vitro* analogue of the comparative skin test (CCT) reaction. Antibody levels of PPD-B and PPD-A and a *M. bovis*-specific protein (MPB-70) are also monitored using an ELISA assay (Griffin & Buchan, 1989). Earlier studies which evaluated ELISA tests in deer showed that the test was effective in diagnosing severe tuberculosis infection (de Lisle *et al.*, 1984).

Test performance values calculated from a number of different datasets over the past eight years show sensitivity values for BTB of >95% and specificity values >98.5% (Griffin & Buchan, 1993). Summary findings on the sensitivity of ST and laboratory tests for 345 animals, taken from 54 *M. bovis* infected herds throughout New Zealand are given in Fig. 1. Tuberculosis was diagnosed histologically and animals were segregated into groups with varying levels of disease. These results show that while the MCT was adequate at diagnosing disease overall (82%), it was less sensitive (67%) in diagnosing severely diseased animals. By contrast, BTB was equally sensitive across the disease spectrum (95%).

Data on the performance of BTB has also been obtained from 103 tuberculous deer, where isolation of *M. bovis* on culture was used as the *gold standard* for diagnosis in each animal. These results (Fig. 2) show that either the LT, ELISA or the combined BTB test are very effective in diagnosing *M. bovis* infection in deer. LT had a sensitivity of 88.1%, ELISA, 85.0% and the combined BTB test [(LT(+) or ELISA(+)] 95.1%. Not only did this test have very high levels of sensitivity but no seriously diseased animals were found among the 4.9% BTB(-) animals which were cleared by the test.

The complementary performance of tests, which measure both cellular immunity (LT or MCT) and antibody (ELISA), is confirmed by data in Fig. 3 which combines results from MCT and ELISA in 102 *M. bovis*(+) deer. These results show that, while the MCT alone was 82.4% sensitive and the ELISA was 85.3% sensitive, the combined [MCT(+) or ELISA(+)] composite results gave a sensitivity of 95.0%. It should be noted that the ELISA results are influenced by application of MCT and blood samples were obtained 14 days post-MCT, when ELISA values are higher than when blood is taken prior to MCT.

Results given in Fig. 4 show the specificity of LT, ELISA and BTB to exclude *M. bovis* reactivity in 217 disease-free animals from nine New Zealand deer herds. The specificity of values were: LT, 98%, ELISA, 100% and BTB, 98%.

Taken together the results shown in Figs. 1, 2 and 4 show that the BTB can be used effectively as an ancillary test to clarify the status of MCT(+) animals, to either diagnose *M. bovis* infection or identify non-specific sensitization in False(+) MCT reactors. As a composite test using multiple antigens, it retains high levels of both sensitivity and specificity. The ability of the ELISA test to detect False(-) MCT non-reactor animals (Fig. 3) means that this test may be used in series, 14 days post-MCT, to identify MCT(-) tuberculous animals in herds with significant levels of *M. bovis* infection.

Immunodiagnostic laboratory tests for *M.paratuberculosis*

Data obtained, using LT (Williams *et al.*, 1985) or ELISA (Shulaw *et al.*, 1986) to diagnose *M.paratuberculosis* in deer, have shown promising results when compared with alternative tests, such as faecal sampling and microbial culture. No further development of these techniques has been reported to confirm the potential of these laboratory-based immunodiagnostic tests for Johne's disease in deer. When we carry out BTB tests on animals with suspected Johne's disease we find strong LT reactivity to PPD-A and PPD-J, compared with reactivity to PPD-B. However, it is not possible to distinguish between the LT responses of animals with suspected Johne's disease or MAIC infection. In contrast, animals with disease have much higher levels of ELISA reactivity to PPD-A or PPD-J than MAIC-infected deer.

Protective immunity to *M. bovis* in deer

During the course of our development of the BTB test for Tb diagnosis in deer we noted that LT and ELISA reactivity to *M. bovis* antigens provided different predictive values, for disease, in individual animals. ELISA(+) reactivity alone was a strong positive predictive indicator of disease caused by *M. bovis*, where the level of ELISA reactivity increased proportionally along with disease severity (Griffin *et al.*, 1991). Animals, which were LT(+)/ELISA(+), had a greater than 80% chance of harbouring tuberculosis lesions while animals which were LT(+)/ELISA (-) had a less then 20% chance of being diseased. This prompted us to ask the question; "Do some deer develop protective immunity following

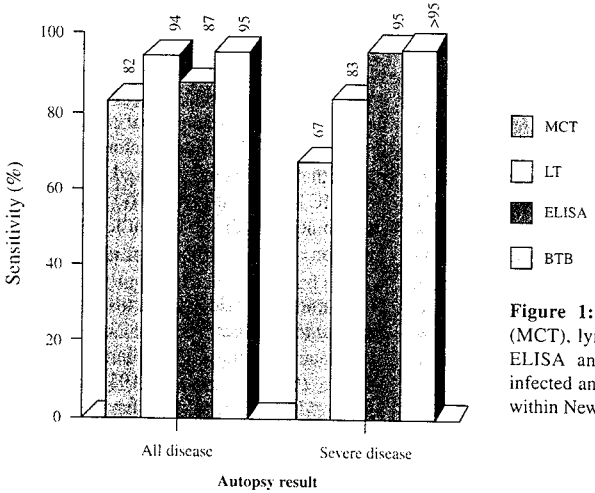

Figure 1: Sensitivity (%) of skin test (MCT), lymphocyte transformation (LT), ELISA and BTB in 345 Tuberculosis-infected animals taken from 54 deer herds within New Zealand.

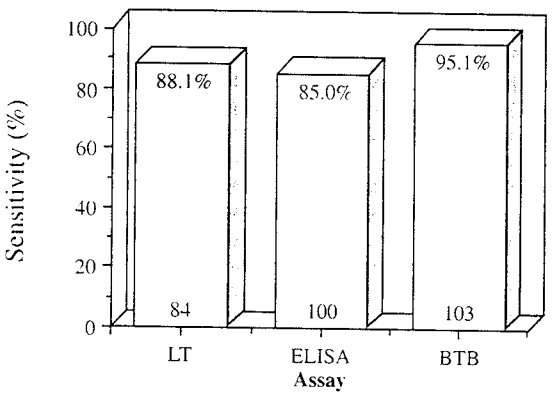

Figure 2: Sensitivity (%) of LT, ELISA and BTB in 103 *M. bovis*(+) deer.

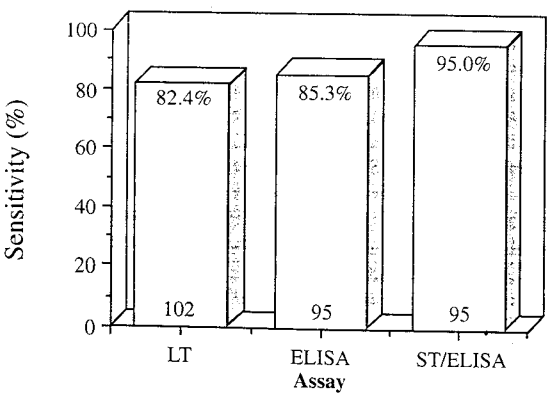

Figure 3: Sensitivity(%) of MCT and ELISA, alone and when combined, in 102 *M. bovis*(+) deer.

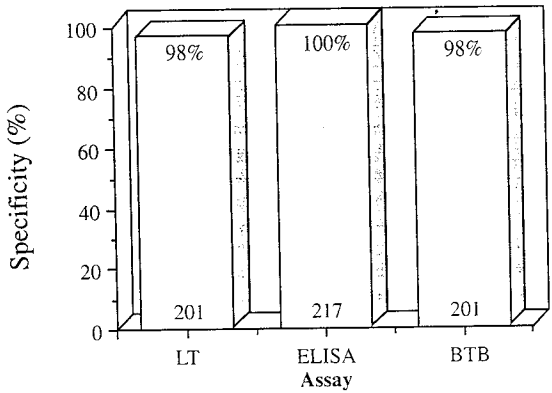

Figure 4: Specificity (%) of LT, ELISA and BTB in 217 disease-free deer from nine deer herds.

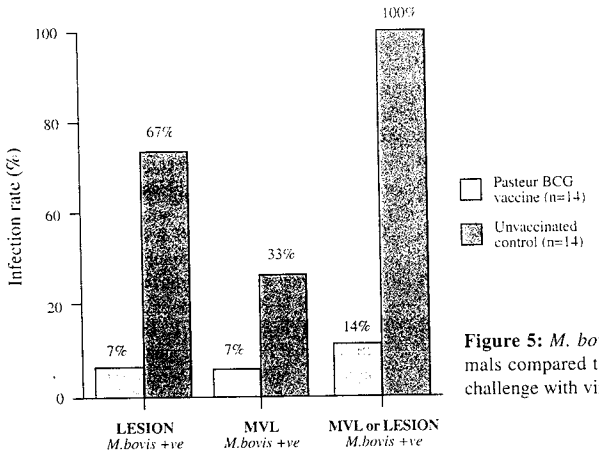

Figure 5: *M. bovis* infection in vaccinated animals compared to non-vaccinated controls after challenge with virulent *M. bovis*.

infection with *M. bovis*?" (Griffin *et al.*, 1988; Griffin & Buchan, 1993). To further develop this concept we have used tuberculosis vaccines to chart pathways of immune reactivity and possibly identify responses which could discriminate between disease-related immune reactivity and protective immunity (Griffin *et al.*, 1993).

Over the past four years we have carried out a number of experiments which have monitored immune parameters in deer vaccinated with conventional tuberculosis (BCG) vaccines (Griffin *et al.*, 1993). These included the injection of animals with live BCG in saline, or live BCG in an oil adjuvant, or killed BCG in oil. A spectrum of immune reactivity, which ranged from disease-like LT(+)/ELISA(+) reactions, to LT(+) reactivity without any antibody, was observed with killed BCG in oil and live BCG in saline, respectively (Griffin *et al.*, 1993). While the former response is compatible with disease-specific diagnostic reactivity, it remains to be established if the latter was protective.

Studies, just completed, which used live BCG in saline to vaccinate animals, have attempted to evaluate the efficacy of this vaccine in protecting animals against experimental challenge, with virulent *M. bovis*, by the intratonsillar route (Mackintosh *et al.*, 1993). The results, given in Fig. 5, show that 100% (6 animals) of the control unvaccinated animals had *M. bovis* recovered fol-

lowing virulent challenge. Two out of 14 vaccinated animals (14%) had *M. bovis* (+) cultures following challenge and one of these animals had no detectable lesions while the other had a single retropharyngeal lesion. These preliminary data show that vaccination may provide protection against *M. bovis* infection, although further studies are warranted to refine the deer vaccination protocols. Studies are currently underway to identify optimal doses of vaccines and delivery systems which would allow BCG, or a new generation tuberculosis vaccine, to be used to protect deer or other wildlife at risk from infection with *M. bovis*.

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