# Paratuberculosis and Avian Tuberculosis in Red Deer in New Zealand: Clinical Syndromes and Diagnostic Tests

**C.G. Mackintosh[1], M.P. Reichel[2], J.F.T. Griffin[3], H. Montgomery[4], and G.W. de Lisle[5]**

*[1]AgResearch Invermay, Private Bag 50034, Mosgiel, New Zealand;*
*[2] National Centre for Disease Investigation, Wallaceville, Upper Hutt, New Zealand;*
*[3]University of Otago, Dunedin, New Zealand;*
*[4]Agriquality NZ, Invermay, Private Bag 50035, Mosgiel;*
*[5]AgResearch Wallaceville, Upper Hutt, New Zealand*

## Abstract

Paratuberculosis (caused by *Mycobacterium avium* subsp. *paratuberculosis*) and to a lesser extent avian tuberculosis (caused by *Mycobacterium avium* subsp. *avium*), are emerging as problems in red deer (*Cervus elaphus*) farmed in New Zealand. To date, the majority of reported cases have been subclinically affected animals detected at slaughter, usually with lesions in mesenteric lymph nodes. These lesions are a nuisance to the venison industry and result in financial loss to the farmers due to their gross and histological similarity to lesions caused by *Mycobacterium bovis*. Recently however, there have been a small number of outbreaks of severe clinical disease and fatalities in 8 to 15 month old deer. Studies were undertaken to determine which of the currently available tests were the most useful for diagnosing clinical paratuberculosis and detecting subclinically infected red deer. These tests were evaluated in a herd of red deer that had a severe outbreak of clinical paratuberculosis in yearling animals. An agar gel diffusion test (GD) and an Enzyme-linked Immunosorbent Assay (ELISA) were useful in confirming disease in clinically affected animals, although they could not differentiate between infections due to *M. a. paratuberculosis* and *M. avium*. Neither a GD test, various ELISA tests, a complement fixation test, a leukocyte transformation (LeT) test nor a comparative skin test were able to accurately diagnose subclinical paratuberculosis in deer.

## Introduction

The farming of deer in New Zealand started 25 years ago and today there are approximately 5,000 farms with over 1.5 million deer, of which 90% are red deer (*Cervus elaphus*). Farmed deer appear to be relatively healthy but are susceptible to the normal range of ruminant diseases (Mackintosh, 1998). The first bacteriologically confirmed case of paratuberculosis (or Johne's disease) in a red deer in New Zealand was in 1986 (Gumbrell, 1986). It occurred in a 3-year-old hind that was born on the property in question. It lost weight and had diarrhoea over a 2-month period. At necropsy it was emaciated and showed thickening of the ileum and lesions in the mesenteric lymph nodes. It had microscopic lesions typical of paratuberculosis in the intestines and draining lymph nodes and *M. a. paratuberculosis* was isolated from fresh material.

Five previous cases of paratuberculosis were suspected between 1979 and 1985 but none were confirmed by culture. Since then the numbers of individual cases and affected farms have risen markedly. In the last 6 years the numbers of newly infected herds (defined as herds in which *M. a. paratuberculosis* was identified by culture or IS*900* PCR from deer for the first time) were 4, 7, 22, 27, 46, 40 and 26 (first 10 months) from 1992 to 1998 respectively. The overwhelming majority of the infected animals were discovered in apparently healthy deer at meat inspection as having lesions resembling those caused by *M. bovis*, but recently there has been an increase in clinically affected animals. Another alarming development has been the increasing number of multiple cases of paratuberculosis on deer farms. Previously, clinical paratuberculosis

tended to be single sporadic cases in deer 2 years or older. However, in the last year there have been over 7 outbreaks and all have involved rising yearlings.

The number of cases detected in deer at slaughter suggests that many clinical cases on farms are going undiagnosed or unreported. It is important for the deer industry to determine the true prevalence of paratuberculosis in New Zealand farmed deer so that appropriate control strategies can be put in place.

Infections with *M. a. avium* in farmed deer have caused a few problems to the industry. The widespread reactivity of deer to avian antigens in skin tests and lymphocyte transformation tests suggest that a large number of farmed deer are exposed to *M. a. avium*. Cross-reaction to the bovine skin test necessitates a secondary test, either the comparative skin test or the Blood Test for Tuberculosis (Griffin et al., 1994) which comprises a lymphocyte transformation (LT) test plus antibody, to resolve the cause of the sensitisation.

A small proportion of clinically normal deer also develop lesions in the mesenteric lymph nodes due to *M. a. avium* infection and these cause problems at meat inspection because of their similarity to lesions caused by bovine tuberculosis and paratuberculosis. There have also been a few isolated outbreaks of disease due to *M. a. avium* infection, which are indistinguishable from paratuberculosis clinically, or by gross or histopathological examination of lesions (Mackintosh et al., 1997).

There are a number of tests developed to diagnose clinical and subclinical infection with *M. a. paratuberculosis* in cattle and sheep but few have been evaluated for use in red deer (de Lisle et al., 1996; Manning et al., 1998). This paper describes the clinical syndromes associated with paratuberculosis and avian tuberculosis in deer and presents results from studies to determine which of the currently available tests are the most useful for diagnosing clinical and subclinical paratuberculosis and avian tuberculosis.

## Clinical syndromes of Paratuberculosis and Avian Tuberculosis in Red Deer

*Paratuberculosis*. Sporadic cases of clinical paratuberculosis, characterised by chronic granulomatous enteritis, have occurred in all ages and classes of deer. Affected adult deer typically lose weight over a period of a few months and the majority develop diarrhoea. There is usually low morbidity (< 1%) but high mortality (~100%) with little or no response to symptomatic treatment. This is very similar to paratuberculosis in cattle and sheep, although the latter rarely develop diarrhoea. However, recently there have been at least seven outbreaks of disease in young red deer. Initially, 5-10 % of the 6 to 9 month-old-deer "failed to thrive", had low growth rates or were in poor condition. In spring they failed to lose their winter coats or had a patchy or "moth-eaten" appearance. They developed diarrhoea and became soiled with green faecal material around the tail, hindquarters and hocks, and they lost weight. The disease course was from days to months but it appears that the younger the animal, the quicker the progression to emaciation and death. In these outbreaks, deaths occur in young red deer aged 8 to 20 months with mortality rates up to 12%, compared with 2 to 4 years and mortality rates rarely above 5% in cattle and sheep. The differential diagnosis includes yersiniosis (in weaners in winter), abomasal parasites, fading elk/wapiti syndrome, bovine tuberculosis, avian tuberculosis and chronic MCF (Mackintosh, 1998).

Necropsy examination of paratuberculosis cases typically reveals enlarged jejunal and ileo-caecal lymph nodes, often with white or cream caseous lesions. There may not be gross thickening of the terminal ileum, but there are often prominent lymphatic drainage vessels from the jejunum to the adjacent lymph nodes, sometimes with small abscesses. There is usually no fat in the omentum, which is often oedematous and may be adherent to the affected jejunum and lymph nodes in severe cases. Histopathological examination of lesions typically reveals extensive areas of invasion of affected lymph nodes by macrophages, often with foci of calcification and/or caseation and numerous small acid-fast organisms (AFOs) present in the macrophages. The ileo-caecal valve may show loss of villus structure with mixed cellular infiltrate and contain numerous AFOs, but it appears that lesions are more commonly found further up the ileum and/or jejunum.

Subclinical paratuberculosis infection in deer is often detected at slaughter and typically a single tuberculoid lesion is detected in the jejunal lymph node without any macroscopic evidence of enteric lesions. Occasionally there is a generalised lymphadenitis with normal lymph node constituents replaced by bizarre giant cells. This condition is sometimes misdiagnosed as a lymphoid neoplasm.

There is little known of the epidemiology of paratuberculosis as it relates specifically to deer. It appears that they are susceptible to cattle and sheep strain of *M. a. paratuberculosis* (de Lisle and Collins, 1993) and it is presumed that the faecal-oral route of infection is the most common.

*Avian tuberculosis*. Clinically the syndrome is very similar to paratuberculosis in young deer with diarrhoea, faecal staining, low serum albumin and total protein, and loss of condition. Necropsy and histopathological examinations also reveal lesions indistinguishable from those of bovine tuberculosis and paratuberculosis. All the cases involving outbreaks of *M. a. avium* in young deer have occurred in groups of animals kept in feedlots or indoors in winter where there is obvious exposure to feed or water contaminated by bird faeces.

## Investigation of an Outbreak of Clinical Paratuberculosis in Young Red Deer

*Herd history*. A deer farm in the South Island of New Zealand experienced an outbreak of clinical paratuberculosis between September 1997 and March 1998 in which 32 out of a mob of 300 nine to fifteen-month-old red deer died. The herd comprised approximately 400 breeding hinds, 15 breeding stags, 55 velveting stags, 300 rising yearlings bred on the property and 230 rising yearlings bought in as weaners. All the yearlings that died of paratuberculosis were bred on the farm, whereas none were lost from the 230 animals purchased between May and June, when 5-6 months old, from four other farms. There had been a few sporadic cases of clinical paratuberculosis in the adult hinds over the previous two or three years. Paratuberculosis is endemic in the sheep flocks in adjoining paddocks on the farm, which have been progressively fenced off and incorporated into the deer farm over the past 10 years. *M. a. paratuberculosis* was isolated from both deer and sheep on the farm and both isolates have identical insertion sequence polymorphisms for IS1311-HINF1 (Lambeth, pers. comm.).

*Investigation of tests in red deer with clinical paratuberculosis*. Serum samples were taken from 9 clinically affected yearling deer that were subsequently killed, necropsied and confirmed as paratuberculosis cases. The GD, ELISA-W and CFT tests were carried out at the National Centre for Disease Investigation, Wallaceville. GD and CFT assays were performed as described previously (Hilbink et al., 1994), while culture methods and the ELISA-W are described by Reichel et al., 1999.

The GD was positive in all 9 confirmed cases of paratuberculosis compared with 4 positive ELISA-W and 2 positive and 2 suspicious CFT results.

*Investigation of tests for detecting subclinical paratuberculosis in red deer*. A study was undertaken on adult hinds in the herd that had experienced the outbreak of paratuberculosis in the yearlings described above. For practical purposes it was decided to test all the deer that could be handled in one day, which turned out to be 300 deer in three mobs, and a subgroup of 131 hinds was selected for further testing. Cell-mediated immune responses were assessed with a comparative cervical skin test (CCT) and a leukocyte transformation test (LeT) on heparinised blood samples taken at the time of the CCT. Humoral immune responses were assessed with GD and ELISA-W tests conducted by the Wallaceville Laboratory (as above) and with an ELISA-D conducted by the Deer Research Laboratory (see below) on blood samples taken at the time of the CCT and also 15 days later. Not all tests were carried out on all animals. Infection status was assessed by faecal culture or examination at slaughter; necropsy and culture of tissues were completed in selected animals.

*CCT*: The animals were restrained manually and two areas on the neck were closely clipped, the double skin thickness was measured and then 0.1 ml of 0.5 mg/ml avian PPD was injected intradermally into the upper patch and 0.1 ml of 1 mg/ml bovine PPD was injected intradermally into the lower patch. The skin thickness was measured again 72 hours later. The avian (A) minus bovine (B) skin test differences were calculated.

*LeT*: Eight ml heparinised blood samples were spun at 800g for 10 minutes to sediment the blood cells. A 0.5 ml aliquot of the buffy coat was resuspended in 4.5 ml of RPMI 1640 (Gibco). Cells were dispensed in 0.1ml volumes in round-bottom sterile microtitre trays (Nunc). The following were added in 0.05ml amounts to quadruplicate wells: *M. a. avium* culture supernatant, *M. a. avium* purified protein derivative (PPDA), *M. bovis* PPDB, *M. a. paratuberculosis* PPDJ, Concanavalin A and RPMI. Cells were cultured for 5 days at 37 C in a humidified 5% $CO_2$ atmosphere. Then 0.05 µCi 3H thymidine was added and incubated for a further 18 hours. Residual red blood cells were lysed by freezing at -20 C, harvested and mean levels of radioactivity determined as counts per minute (cpms).

*ELISA-D:* These were carried out by coating Maxisorp Immunoplates with 50 µl of antigen in carbonate buffer (2.93g of $NaHCO_3$ and 1.59g of $Na_2CO_3$/ litre $H_20$, pH 9.6). The antigens used were: PPD-A, PPD-B and PPD-J at a concentration of 12.5 µg/ml, and MpB 70, at a concentration of 2 µg/ml. After the plates had been incubated at 4 C overnight, unbound antigen was removed by washing plates six times in phosphate buffer (pH 7.4) containing 0.05% Tween 20 (wash buffer). Fifty µl of test serum, previously diluted 1/100 in wash buffer, was added to separate wells for each antigen. Following a 1 hr incubation at 37 C, plates were washed a further six times in wash buffer, prior to the addition of 50 µl of horseradish peroxidase (HRPO) conjugated swine anti-goat IgG, previously diluted 1/6000 in wash buffer. After 30 minutes incubation at 37 C, plates were thoroughly washed in wash buffer before the addition of 100 µl of substrate solution (equal volumes of 2.1 % citric acid and 2.84% $Na_2HPO_4$ in distilled water, 0.1% $H_2O_2$ plus 0.4 mg/ml of orthophenylenediamine dihydrochloride (OPD). Plates were incubated for 20 min at room temperature in the dark. The reaction was stopped by the addition of 50 µl of 2 M $H_2SO_4$ and the absorbance was measured at 490 nm using an automated microplate reader.

*Slaughter:* Forty-four deer that were positive on one or more tests (ELISA-W, ELISA-D, GD, LeT, and CCT) or were faecal culture positive plus 10 test-negative deer were slaughtered in June at a Deer Slaughter Plant and the viscera were examined for lesions. Samples were taken of suspect lesions for histopathological examination and culture and the ileo-caecal valve (ICV) and ileo-caecal lymph nodes (ICLN) were taken from all animals for culture.

*Results*. CCT: Of the 300 hinds tested, 61% had an A-B skin test difference of <4 mm and 33% had an A-B skin test difference of 4-7 mm, while 17% had a difference of 8mm or greater (see Table 1).

LeT: Of the 131 hinds tested, 55 were negative, 49 gave a low (A/J) response, 17 gave a moderate (A+ and/or J+) response, and 10 gave a high (A++ or +++/J++ or +++) response to avian (A) or johnin (J) PPD.

ELISA-W and GD: Of the 300 deer tested there were 25 positives and 1 weak positive to the ELISA-W and 6 positive and 3 suspects to the GD test at the time of the first CCT. There was poor correlation between ELISA-W and GD results with only one animal positive for both. There were fewer positives to both the ELISA-W and GD from blood samples taken 15 days after the CCT and only one animal was GD positive both times (see Table 1).

ELISA-D: Of the 131 hinds tested 13 were positive, 5 were suspects and 113 were negative. There was no correlation among ELISA-D, ELISA-W or GD assays.

| Test | No. tested | Results | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| CCT | 300 | (A-B) skin test difference | | | | | | | | |
| | | mm | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8+ |
| | | no. | 30 | 56 | 51 | 47 | 40 | 28 | 24 | 7 | 17 |
| LeT | 131 | Neg | | A/J | | A+/J+ | | A++ or +++/J++ or +++ | | | |
| | | 55 | | 49 | | 17 | | 10 | | | |
| ELISA-W | 300 | Neg | | Weak Positive | | Positive | | | | | |
| | | 274 | | 1 | | 25 | | | | | |
| GD | 300 | Neg | | Suspicious | | Positive | | | | | |
| | | 291 | | 3 | | 6 | | | | | |
| ELISA-D | 131 | Neg | | Suspicious | | Positive | | | | | |
| | | 113 | | 5 | | 13 | | | | | |
| Faecal culture | 66 | 7 positive cultures | | | | | | | | | |

Table 1. Results of CCT, LeT, ELISA-W, GD and ELISA-D tests and faecal cultures conducted on apparently normal red deer hinds.

Slaughter: Forty-four test-positive and 10 test-negative deer were slaughtered. Three of the 6 GD-positive animals, two of the 26 LeT A-positive animals and one of the 2 faecal culture-positive animals had lesions. Of these 6 animals with suspect lesions found at slaughter, 3 were culture-positive. The ileo-caecal valve plus attached ileo-caecal lymph nodes from the 54 deer were cultured for *M. a. paratuberculosis*. Table 2 summarises all the culture results for the slaughtered animals grouped according to their test results. All test groups had 44 to 86% culture-positive with the GD test positive group having the highest % culture-positive, but none of these results were significantly different from each other or from the group of culls which were negative by all tests. It was subsequently shown that two of these culls had positive faecal cultures but only one of them had a culture-positive ICV/ICLN sample.

| Test group | No. | Culture-positive | % positive |
|---|---|---|---|
| ELISA-W pos/weak pos | 16* | 9 | 56 |
| GD positive (suspicious) | 7 (1)* | 6 (0) | 86 |
| ELISA-D positive | 9 | 4 | 44 |
| LeT A | 26 | 14 | 54 |
| Culls | 10 | 6 | 60 |
| Overall | 55* | 33 | 60 |

* 54 deer killed but one animal was positive to both GD and ELISA

Table 2. Success rates of culture of ileo-caecal valves and lymph nodes for the deer grouped according to their test results plus a group of culls that were negative to all tests.

These results suggest that all the tests had relatively low sensitivity for detecting subclinical paratuberculosis in red deer but the GD had the highest positive predictive value. There was no evidence of a "boosting" effect by skin testing on serological test values. There appears to be considerable cross-reactivity in CMI tests between *M. a. paratuberculosis* and *M. avium* infections. Culture of faeces appeared to be the most sensitive ante mortem method of detecting subclinical infections.

## Investigation of an Outbreak of Avian Tuberculosis in Young Red Deer

*Clinical syndrome and investigation.* An outbreak of avian tuberculosis occurred in 80 eight month old red weaner stags in a feeding trial at Invermay. The animals were kept indoors for a period of 3 weeks after weaning in autumn and then divided up into small groups and kept in a feed-lot situation either indoors or outdoors over the winter. Five deer were clinically affected and showed chronic weight loss, diarrhoea and emaciation. All five were positive to GD and CFT tests and necropsy examination showed gross lesions ranging from small yellow foci (3 x 3 mm) on the terminal ileum and a 5 x 5 x 10 mm lesion in the ileo-caecal lymph node to severe extensive abscessation of the entire mesenteric lymph node chain, oedematous mesenteries and severe thickening of ileum and caecum.

Histopathological examination showed the following typical range of features: a) the mesenteric lymph nodes showed large foci of caseation and calcification surrounded by epithelioid cells, neutrophils, mononuclear and very occasionally giant cells. Large numbers of acid-fast organisms (AFOs) were usually seen at the edge or throughout the necrotic areas b) ileum: three of the five deer were examined and two had severe changes with obliteration of the mucosa and serosa with marked granulomatous reaction. Submucosal follicles appeared depleted and in many areas the submucosa was packed with epithelioid cells, mononuclear cells and neutrophils, and large numbers of AFOs were present. The caecum from one deer had diffuse colitis but no AFOs were seen.

The pyogranulomatous reaction in the lymph nodes and ileum are indistinguishable from that observed in deer with paratuberculosis. There was a noticeable degree of variation in the severity of the lesions among the clinically affected animals. The isolates from this outbreak were identified as *M. a. avium* on the basis of growth characteristics and biochemical tests. A PCR test based on the insertion sequence IS*900* for *M. a. paratuberculosis* was negative.

*Investigation of subclinical avian tuberculosis.* When the rest of the group were slaughtered at 9 months of age, 35 had no grossly visible lesions and 40 had mesenteric lymph node lesions ranging from mild to severe. Analysis of weight gains over the preceding 6 months showed that growth rates were significantly reduced in subclinically affected animals with visceral lymph node lesions. Blood samples taken prior to slaughter were tested by Wallaceville and the Deer Laboratory with the GD, ELISA-W, CFT and LeT assays used for detecting paratuberculosis.

| Test | End-Point | Sensitivity |
|---|---|---|
| ELISA-W | positive | 72.5% |
| CFT | deer end-point ≥ 4/8 | 22.5% |
| CFT | cattle end-point ≥ 4/4 | 57.5% |
| GD | pos end-point | 55% |
| GD | Pos + weak pos + sus | 65% |
| LeT | positive | 100% |

Table 3. Sensitivity of ELISA-W, CFT, GD and LeT paratuberculosis tests for detecting mesenteric lymph node lesions in red deer due to *M. a. avium*.

All the animals had moderate to high avian LeT reactivity suggesting a high level of exposure to *M. a. avium*. Overall the ELISA-W was the most sensitive of the antibody assays (72.5%) but had the poorest specificity (62.2%) (see Table 3). The specificity figures are based on the results of the tests on the 35 animals that had discernable macroscopic lesions. The CFT, when using the normal deer endpoint of 4/8, had poor sensitivity (22.5%) but was 100% specific. When the CFT was read at the normal cattle endpoint ( 4/4) the sensitivity rose to 57.5% and specificity fell to 97.2%. The GD using the "pos" endpoint was 55% sensitive and 100% specific. If the GD endpoint included "pos", "weak pos" and "suspicious" the sensitivity rose to 65% and specificity fell to 94.6%. These results demonstrate the high degree of cross-reactivity seen in deer affected by disease due to *M. a. avium* and *M. a. paratuberculosis* infections. This presents problems for the veterinarian trying to make a diagnosis and is important because the epidemiology of these two infections is very different.

## Discussion

Paratuberculosis has been reported sporadically in a wide range of wild, park and captive ruminants including white-tailed deer, roe deer, red deer, fallow deer, sika deer, Tule elk, moose, aodad, mouflon, camel, bighorn sheep, reindeer, gnu, water buffalo, yak and llama. (Temple et al., 1979; Jessup et al., 1981; Pacetti et al., 1994). Since the mid 1980s paratuberculosis has emerged as a problem in red and fallow deer on farms in the United Kingdom (Gilmour, 1988; Fawcett et al., 1995;), Germany (Commichau, 1982), New Zealand (Gumbrell, 1986; de Lisle and Collins, 1993; Mackintosh and de Lisle, 1998), Canada (Starke, 1991), Ireland (Power et al., 1993), USA (Manning et al., 1998), Argentina ( Mereb et al., 1994) and France (Pingard A, pers. comm.). Of major concern is the emergence of the disease in yearling animals, especially when the occurrence of outbreaks involves over 10% of this age group. The occurrence of lesions in mesenteric lymph nodes is also a nuisance to the industry and results in losses to the farmers (Campbell, 1995).

Avian tuberculosis occasionally causes clinical disease in wild deer (Hopkinson and M$^c$Diarmid, 1964; Blaxter et al., 1974) and has been reported in farmed deer in the UK (Blaxter et al., 1974; Reid HW, 1994; Otler et al., 1995) and New Zealand (de Lisle and Havill, 1985; Mackintosh et al., 1997). Lesions in retropharyngeal, mesenteric and ileo-caecal lymph nodes due to *Mycobacterium avium-intracellulare*-complex (MAIC) organisms are commonly found in clinically normal farmed deer at slaughter in New Zealand (de Lisle and Havill, 1985; de Lisle et al., 1995) and Ireland (Quigley et al., 1997).

The diagnosis of clinical paratuberculosis and the detection of subclinically affected animals for control programmes are dependent on the development of sensitive and specific tests. The tests used for diagnosing paratuberculosis in cattle and sheep include the GD, CFT, ELISA, LT, gamma interferon tests, skin tests, culture and PCR. Although these tests have been extensively evaluated in cattle and sheep there is little published data on the use of diagnostic tests for paratuberculosis in deer (Manning et al., 1998). Our study undertook to determine which of the currently available tests developed for cattle and sheep were the most useful for diagnosing subclinical and clinical paratuberculosis in red deer. Although there was excellent co-operation from the owners of the animals in these studies there were limitations associated with access to animals, restrictions on the number of deer that could be killed and financial constraints.

The initial study showed that the GD is the most sensitive for confirming clinical paratuberculosis in deer. The second study showed that none of the available immunological tests are effective for detecting subclinically infected deer although of the tests the GD appears to have the highest predictive value and be the most useful. Its value is likely to be in establishing that a given herd is infected, rather than clearing subclinically infected animals from an infected herd. None of the tests were able to differentiate disease due to *M. a. paratuberculosis* from a similar condition caused by *M. a. avium* using currently available antigens. A diagnostic test that could distinguish between paratuberculosis and avian tuberculosis would be useful because the epidemiology of the two conditions is probably quite different.

There does not appear to be any "boosting" effect of antibody levels by the CCT. This result is unexpected because the phenomenon occurs with tuberculosis in deer and cattle (Griffin et al., 1994). It suggests that either subclinically infected deer do not have significant B-cell responses until late in disease, or early B-cell responses to paratuberculosis are not boosted by intradermal injections of avian or bovine PPD. Conceptually, cellular immunity based assays are much more likely to detect subclinical infection.

The low correlation between test positive results from individual antibody assays was noteworthy. GD-positive, culture-positive animals were predominantly negative to the ELISA and vice-versa suggesting that these two assays are detecting antibody to different arrays of antigens. This highlights a need for the identification of new antigens to improve assay performance. There is also evidence that there is variation between different batches of PPDA and PPDJ that can lead to assay variability in sensitivity and specificity (Griffin, unpub.).

Faecal cultures (n=66) were effective at detecting 7 subclinically infected deer. None of these had positive serological tests, only four had low to moderate LT results and one had a skin test A-B of >4 mm. Of the 2 culls which were faecal culture-positive only one was ileo-caecal culture positive, suggesting that the ileo-caecal valve area in deer may not be the only site infected by *M. a. paratuberculosis*. The advantage with faecal culture is that it is likely to detect the animals that are responsible for the most contamination of the environment, irrespective of the immune response such as antibody or cellular reactivity, avian tuberculosis cross-reactivity or the stage of the disease. The organism can be positively identified as *M. a. paratuberculosis* and the strain can be typed if desired.

This study highlights the deficiencies of the currently available serological and cell-mediated assays for accurately diagnosing clinical and subclinical paratuberculosis in red deer. To enable practical control programmes to be implemented there is an urgent need for more sensitive immunological tests incorporating antigens which can differentiate between infections with *M. a. paratuberculosis* and other species of Mycobacteria .

## Acknowledgments

We thank all the laboratory workers and field workers who assisted with this study at AgResearch Invermay, AgResearch Wallaceville, Central Animal Health Laboratory, University of Otago and Agriquality NZ, Invermay.

## References

Blaxter, K.L., Kay, N.B., Sharman, G.A.M., Cunningham, J.M.M., Hamilton, W.J., 1974. Report : Farming the red deer. Dept Ag. and Fish for Scotland, HMSO, Edinburgh.

Campbell, A., 1995. The inconvenience of lymph node gross lesions (non-*M. bovis*) at post mortem inspection of deer. Deer Branch, New Zealand Vet Assoc. Proceedings 12, 87-95.

Commichau, C., 1982. Demonstration of paratuberculosis in fallow deer (*Dama dama*) Berliner-und-Munchener-Tierarztliche-Wochenschrift 95, 248-250.

de Lisle, G.W., Collins, D.M., 1993. Johne's disease in farmed deer. Surveillance 20,18-19.

de Lisle, G.W., Havill, P.F., 1985. Mycobacteria isolated from deer in New Zealand from 1970-1983. N. Z. Vet. J. 33, 138-140.

de Lisle, G.W., Joyce, M.A., Yates, G.F., Wards, B.J., Hoyle, F.P., 1995. *Mycobacterium avium* infection in a farmed deer herd. N. Z. Vet. J. 43, 1-3.

de Lisle, G.W., Wilson, C.A., Yates, G.F., Wards, B.J., Collins, D.M., 1996. The post-mortem diagnosis of paratuberculosis in farmed deer. Proc. Fifth Int. Colloq. On Paratuberculosis, Madison, U.S.A., pp 175-179.

Fawcett, A.R., Goddard, P.J., M'Kelvey, W.A.C., Buxton, D., Reid, H.W., Greig, A., Macdonald, A.J., 1995. Johne's disease in a herd of farmed red deer. Vet. Rec. 136, 165-169.

Gilmour, N.J.L., 1988. Paratuberculosis. In: Reid, H.W. (Eds.), The Management and Health of Farmed Deer. Kluwer Academic Publishers for the Commission of the European Communities, pp. 113-122.

Griffin, J.F.T., Cross, J.P., Chinn, D.N., Rodgers, C.R., Buchan, G.S. 1994. Diagnosis of tuberculosis due to *Mycobacterium bovis* in New Zealand red deer (*Cervus elaphus*) using a composite blood test and antibody assays. N. Z. Vet. J. 42, 173-179.

Gumbrell, R.C., 1986. Johne's disease in deer. Surveillance 13, 15-16.

Hilbink, F., West, D.M., de Lisle, G.W., Kittelberger, R., Hosie, B.D., Hutton, J., Cooke, M.M., Penrose, M., 1994. Comparison of a complement fixation test, a gel diffusion test and two absorbed and unabsorbed ELISAs for the diagnosis of paratuberculosis in sheep. Vet. Microbiol. 41, 107-116.

Hopkinson, F., M'Diarmid, A., 1964. Tuberculosis in a free-living red deer (*Cervus elaphus*) in Scotland. Vet. Rec. 76, 1521-1522.

Jessup, D.A., Abbas, B., Behymer, D., Gogan, P., 1981. Paratuberculosis in Tule Elk in California. J. Am. Vet. Med. Assoc. 179, 1252-1254.

Mackintosh, C.G., 1998. Deer health and disease. Acta Veterinaria Hungarica 46, 381-394.

Mackintosh, C.G., de Lisle, G.W., 1998. Johne's disease : The current situation in New Zealand deer. Deer Branch, NZVA Proceedings 15, 175-182.

Mackintosh, C.G., Webster, J., Corson, I., Masters, B., Pearse, A.J., Littlejohn, R.,1997. Outbreak of avian tuberculosis in red deer. Deer Branch, NZVA, Proceedings 14, 243-250.

Manning, E.J.B., Steinberg, H., Rossow, K., Ruth, G.R., Collins, M.T., 1998. Epizootic of a paratuberculosis outbreak in farmed elk (*Cervus elaphus*). J. Am. Vet. Med. Assoc. 213, 1320-1322.

Mereb, G.C., Bedotti, D.O., Suarez, V.H., Busetti, M.R., Moreira, A.R., Lorenzo, R.M., 1994. Paratuberculosis in red deer. Veterinaria Argentina 11, 107-112.

Otler, A., Munro, R., Palmer, N., 1995. Mycobacterial meningitis as a cause of ataxia and weight loss in a deer. Vet. Rec. 136, 180.

Pacetti, A., Belletti, G., Gabbi, M., Mutinelli, F., Genchi, C., 1994. Paratuberculosis in red deer. Obiettivi-e-Documenti-Veterinari 15, 67-70.

Power, S.B., Haagsma, J., Smyth, D.P., 1993. Paratuberculosis in farmed red deer (*Cervus elaphus*) in Ireland. Vet. Rec. 132, 213-216.

Quigley, F.C., Costello, E., Flynn, O., Gogarty, A., M°Guirk, J., Murphy, A., Egan, J., 1997. Isolation of mycobacteria from the lymph node lesions in deer. Vet. Rec. 141, 516-518.

Reichel, M.P., Kittelberger, R., Penrose, M.E., Meynell, R.M., Cousins, D., Ellis, T., Mutharia, L.M., Edward, S.E.A., Johns, A.H., de Lisle, G.W., 1999. Comparison of serological tests and faecal culture for the detection of *Mycobacterium avium* subsp. *paratuberculosis* infection in cattle and analysis of the antigens involved. Vet. Microbiol. (in press).

Reid, H.W., 1994. Mycobacterial infections. In: Alexander, T.L., Buxton, D.(Eds.).Management and diseases of deer (Second Ed.). Veterinary Deer Society Publication, London, pp. 93-101.

Starke, R.K.A., 1991. Paratuberculosis (Johne's disease) in a captive wapiti. In : Renecker, L.A., Hudson, R.J. (Eds.). Wildlife Production : Conservation and sustainable development. AFES misc. Pub. 91-6, University of Alaska, Fairbanks, Alaska, pp. 435-436.

Temple, R.M.S., Muscoplat, C.C., Thoen, C.O., Himes, E.M., Johnson, D.W., 1979. Observations on diagnostic tests for paratuberculosis in a deer herd. J. Am. Vet. Med. Assoc. 175, 914-915.