Update on Investigations into Diagnostic Tests for Johne's Disease in Red Deer

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Abstract

Johne's disease (JD) is emerging as a problem on deer farms in New Zealand. Two studies were conducted on a deer farm that had recently experienced an outbreak of clinical JD in yearlings. The first study showed that the gel diffusion (GD) test was the most reliable test for confirming JD in clinically affected deer.

The results of the second study suggest that none of the currently available tests, including GD, CFT, ELISA, CCT and LeT, were sufficiently sensitive for detecting subclinical paratuberculosis in red deer to be useful for detecting subclinical cases and therefor for control of JD. There was no evidence that skin testing with avian tuberculin produced a "boosting" effect on serological test values. There appears to be considerable cross-reactivity in CMI tests between *M paratuberculosis* and *M avium* infections. Culture of faeces appeared to be the most sensitive *ante mortem* method of detecting subclinical infections.

Introduction

Johne's disease (JD), also known as 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, JD 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; Mackintosh et al., 1999), 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 the occurrence of outbreaks involving over 10% of this age group. The occurrence of lesions in mesenteric lymph nodes is a nuisance to the meat industry and results in losses to farmers (Campbell, 1995).

The development or refinement of diagnostic tests for JD in deer is an essential step towards rational control. This paper reports on an evaluation of serological and cell-mediated JD tests on deer in an infected herd.

Herd History

A deer farm in the South Island of New Zealand experienced an outbreak of clinical JD between September 1997 and March 1998 in which 32 of a mob of 300 9- to 15-month-old red deer died (Mackintosh and de Lisle, 1998). The herd comprised approximately 400 breeding hinds, 15 breeding stags, 55 velveting stags, 300 rising yearlings bred on the farm and 230 rising yearlings bought in as weaners. All the yearlings which died of JD were bred

on the farm, whereas there were no deaths among the 230 animals which were purchased between May and June, when 5-6 months old, from four other farms. There had been a few sporadic cases of clinical JD in the adult hinds over the previous 2 or 3 years. JD is endemic in the sheep flocks in paddocks adjoining the farm, which have been progressively fenced off and incorporated into the deer farm over the past 10 years. *M paratuberculosis* was isolated from both deer and sheep on the farm and both isolates had identical insertion sequence polymorphisms for IS1311-HINF1 (Lambeth M., pers. comm.).

Study 1: Diagnosis of clinical JD

As reported previously (Mackintosh and de Lisle, 1998), serum samples were taken from nine clinically affected yearling deer, which were subsequently killed, necropsied and confirmed as JD cases. The gel diffusion (GD), ELISA-W and complement fixation (CFT) tests were carried out at the National Centre for Disease Investigation, Wallaceville. The GD test was positive in all nine confirmed cases of JD compared with four positive ELISA-W and two positive and two suspicious CFT results.

Study 2: Investigation of tests for detecting subclinical paratuberculosis in red deer

Method:

A study was undertaken on adult hinds in the herd that had experienced the above outbreak of JD in the yearlings. 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 and with an ELISA-D conducted by the Deer Research Laboratory on blood samples taken at the time of the CCT and also 15 days later. Not all tests were carried out on all animals (see Table 1). Infection status was assessed by faecal culture or by slaughter, necropsy and culture in 54 selected animals. These comprised 44 deer that were positive to one or more tests (ELISA-W, ELISA-D, GD, LeT, and CCT) or were faecal culture positive plus 10 test-negative deer. They were slaughtered in June (18 months of age) 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, 10% had an A-B skin test difference of 0mm, 51% had 1-3 mm, 33% had 4-7 mm, and 6% 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) tuberculin.

ELISA-W and GD:

- Of the 300 deer tested there were 25 positive and one weak positive to the ELISA-W and six positive and three suspicious 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 animals positive 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.

ELISA-D:

- Of the 131 hinds tested, 13 were positive, five were suspicious and 113 were negative.

There was no correlation between ELISA-D, ELISA-W or GD assays.

Slaughter:

- Three of the six GD positive animals, two of the 26 LeT A positive animals and one of the two faecal culture-positive animals had lesions. Of these six animals with suspect lesions found at slaughter, three were culture-positive.
- The ileo-caecal valve plus attached ileo-caecal lymph nodes from the 54 deer slaughtered in the DSP were cultured for *M. paratuberculosis*. A summary of all the culture results for the slaughtered animals grouped according to their test results is presented in Table 2. All test groups had 44 to 86% culture-positive with the GD test having the highest percentage culture-positive, but none of these results were significantly different from each other or from the group of culls which were all negative to all the 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. The GD had the highest positive predictive value.
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Table 1. **Results of CCT, LeT, ELISA-W, GD and ELISA-D tests and faecal cultures conducted on apparently normal red deer hinds.**

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 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.**

Test group	No	Culture positive	% positive
ELISA-W positive/weak positive	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

Discussion

The diagnosis of clinical JD 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 JD in cattle and sheep include the GD, CFT, ELISA, lymphocyte transformation (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 their for JD in deer (Goddard et al., 1994; 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 JD in red deer. Although there was excellent co-operation from the owner of the animals in these studies there were limitations associated with access to deer, restrictions on the number of deer that could be killed and financial constraints.

The first study (Mackintosh and de Lisle, 1998) showed that the GD was the most sensitive for confirming clinical JD in deer. The second study showed that none of the available immunological tests were effective for detecting subclinically infected deer, although the GD test 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. A previous paper (Mackintosh et al., 1997) showed that none of the tests was able to differentiate disease due to *M. paratuberculosis* from a similar condition caused by *M. avium*. A diagnostic test that could distinguish between JD and avian tuberculosis would be useful because the epidemiology of the two conditions is probably quite different.

The CCT does not appear to cause any "boosting" of antibody levels. This result is unexpected because the phenomenon occurs with Tb 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 *M. paratuberculosis* are not boosted by intradermal injections of avian or bovine PPD. Conceptually, cell-based assays are much more likely to detect subclinical infection

The low correlation between the serological tests was noteworthy. GD positive culture positive animals were predominantly negative to the ELISA and vice-versa suggesting that these two assays are detecting 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 avian PPD and Johne's PPD, which can lead to assay variability in sensitivity and specificity (Griffin, unpub.).

Faecal cultures (n=66) were effective in detecting seven subclinically infected deer, none of which had positive serological tests, only four had low to moderate LeT results and one had a skin test A-B of >4 mm. Of the two culls that 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 paratuberculosis* The advantage of 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 Tb cross-reactivity or the stage of the disease, and the organism can be positively identified as *M paratuberculosis* and the strain can be typed if desired.

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

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