JOHNE'S DISEASE IN A FARMED RED DEER HERD IN THE UK

P J Goddard, A R Fawcett,W A C McKelvey*,
D Buxton[1], H W Reid[1], A Greig[2] and A J Macdonald

Macaulay Land Use Research Institute, Craigiebuckler, Aberdeen, Scotland

ABSTRACT

An outbreak of Johne's disease in a herd of farmed red deer, comprising 500 breeding hinds and yearlings, was studied over a five-year period. Clinical findings and the results of post-mortem examinations are described. Serological, histopathological and cultural techniques were used to monitor progress of the disease. Delayed type hypersensitivity skin tests were also conducted. The clinical findings were generally confined to live weight loss, with some animals showing diarrhoea and a decline in coat quality with patchy alopecia. Gross post-mortem findings included an irregular thickening and reddening of the mucosal surfaces of the gut, particularly in the lower small intestine. Mesenteric lymph nodes were enlarged and often firm. Histopathological methods offered a sensitive and specific means of subsequently confirming infection in these animals. Results from the serological tests, however, showed that they were poor predictors of future clinical cases and animals harbouring mycobacteria. Skin tests showed low sensitivity and results were poorly correlated with serological results in seropositive individuals. A vaccination policy was instituted which was accompanied by a reduction in the number of clinical cases.

INTRODUCTION

Johne's disease (paratuberculosis) is a chronic wasting disease of ruminants which occurs world wide following infection by Mycobacterium paratuberculosis. It is characterised by loss of live weight and condition. Diarrhoea normally occurs in the latter stages of disease and post-mortem inspection shows granulomatous inflammation of the intestines and mesenteric lymph nodes. Emaciation results from the ensuing protein-losing enteropathy. There have been few reports of the disease in farmed deer (Gumbrell 1986; 1988; Jorgensen and Jorgensen 1987; McKelvey 1987; Power *et al.*, 1993).

This report describes the clinical, gross post-mortem, histopathological and serological findings over a 5-year period following an outbreak of Johne's disease during 1985 in a herd of farmed red deer. Johne's disease had not previously been diagnosed in the herd despite close monitoring of health since its establishment in 1970. Confirmation of the presence of Johne's disease prompted the adoption of a screening programme and slaughter policy. Vaccination was instituted during the course of the investigation as it was concluded that this afforded the best method of control as adjudged by previous work in cattle and goats (Wilesmith 1982; Saxegaard and Fodstadt 1985).

* Current address: SAC Veterinary Services, Bush Estate, Penicuik, Midlothian, Scotland
1: Moredun Research Institute, 408 Gilmerton Road, Edinburgh, Scotland
2: SAC Veterinary Services, Cleeve Gardens, Oakbank Road, Perth, Scotland

MATERIALS AND METHODS

Animals

Deer farming commenced in 1970 using calves from a variety of locations in Scotland uplifted from the wild at birth and subsequently hand-reared. This practice continued to a varying extent until 1980 after which there were no other intakes. Over the 5-year period described here, the stock comprised on average 300 red deer breeding hinds, 15 stags and up to 300 yearling calves which were finished to slaughter weight in approximately 16 months. The calves were weaned in September and housed during their first winter. Whilst there was no common grazing with other ruminants, goats had previously grazed some areas subsequently used by deer between 1982 and 1986.

Disease history

In April 1985 an 11-month-old animal started to lose live weight rapidly and developed diarrhoea. Faecal smears revealed the presence of large numbers of acid-fast organisms. The animal was destroyed and cultures from samples of faeces and intestinal mucosa were identified as M. paratuberculosis. During the following twelve months, 15 other yearlings developed symptoms and were slaughtered or died.

Post-mortem monitoring

Examinations were undertaken where possible on all animals which died or were destroyed following clinical diagnosis of disease, with histological study of the terminal ileum and associated mesenteric lymph nodes. Culture of faeces was attempted in most cases and involved seeding slopes of Dubos medium (with and without mycobactin) and incubating at 37^{0}C for up to twelve weeks. Identification was carried out by morphological examination of colonies and mycobactin dependence. In addition, an attempt was made to culture organisms from some lymph nodes. When culture was not attempted, confirmation of mycobacterial infection relied on the detection of typical macroscopic and histological changes.

Clinically healthy yearlings slaughtered from October 1986 until December 1989 had their mesenteric lymph nodes removed at the abattoir and processed for histological examination.

Clinical and laboratory tests

Serological screening commenced in March 1986 with blood samples collected every 4 to 6 months. Sera were subjected to the agar gel immuno-diffusion (AGID) test and the complement fixation test (CFT). Both tests used a bovine paratuberculosis antigen. Sera exhibiting precipitin lines in the immuno-diffusion test or any titre, however low, in the CFT was defined as seropositive. At the time of the first whole herd screening only, faeces samples were taken from the rectum of all animals and direct smears prepared and stained by Ziehl Neelsen's method for the presence of acid-fast bacilli.

In the 12 months before vaccination in 1987 and in 1988 all yearling deer were subjected to an intradermal delayed hypersensitivity (DTH) tuberculin test. Avian tuberculin (0.1 ml) was injected into a previously shaved area (10 cm X 12 cm) on the lateral aspect of the middle third of the neck; 72 hours later the site was inspected and reactions ranging from a diffuse plaque to nodular reactions of up to 14 mm thick were recorded as positive.

Vaccination
From 1987 all calves were vaccinated during the first 48 hours of life using the standard Weybridge, cattle, live vaccine against Johne's disease with 1.5 ml being injected subcutaneously towards the front of the brisket.

RESULTS

Clinical and post-mortem findings
Loss of live weight, one of the main clinical signs, was sometimes very rapid in affected animals. The other frequently observed sign, although not seen in every case, was increasingly soft, sometimes fluid, faeces.

In 1985 confirmation of clinical Johne's disease in sixteen of the 221 yearlings at risk (3 deaths and 13 culls) was made on gross lesions at post-mortem examination, histology and in three cases culture of the organism from faeces. All carcases were well below expected weight. On some there was faecal staining of the hind quarters and the coat was dry, often with patchy alopecia. The serosal surfaces of the intestines appeared normal but when opened showed irregular thickening with reddened areas of mucosa, predominantly in the lower small intestine, ileo-caecal valve, caecum and occasionally in the proximal colon; there was no ulceration. Sometimes clear peritoneal fluid was present. Most mesenteric lymph nodes were enlarged, firm and yellowish when sectioned. No evidence of caseation or calcification was found in any of the nodes examined. Histologically the mucosa and sub-mucosa of the gut were heavily infiltrated with macrophages full of acid-fast organisms. Mesenteric lymph node sections showed evidence of hyperplasia with acid-fast organisms packed into macrophages and free in the stroma of the gland.

During 1986 four further animals not found to be seropositive at any of the regular screenings, developed typical clinical signs and were confirmed as having Johne's disease following post-mortem and histological examination. (M. paratuberculosis was cultured from one animal.) In 1987 one 5-year-old animal, not detected at the regular screening times, developed signs of disease. It was shown to be positive by AGID and the CFT at that time, was culled and the mesenteric lymph node showed histological changes consistent with Johne's disease.

No animals exhibiting clinical signs attributable to Johne's disease were detected in either 1988 or 1989 and no animals died in these years

Histopathology of abattoir material
Histological examination of mesenteric lymph nodes from 59 clinically healthy yearlings, slaughtered at the local abattoir in 1986, showed that 24 animals (40.7%) had lesions consistent with mycobacterial infection. The presence of acid-fast bacilli was demonstrated in all these animals (Table 1). Of the mesenteric lymph nodes removed from 167 clinically healthy yearlings following routine slaughter in 1987, 64 animals (38.3%) contained lesions consistent with those seen in clinical cases of Johne's disease and, of these, acid-fast bacilli were identified in 36 animals (Table 1). The animals slaughtered in 1988 could not be identified individually but of 201 vaccinated yearlings slaughtered 32 animals (15.9%) showed lesions in the mesenteric lymph nodes consistent with those seen in clinical cases of Johne's

disease. Nine of these animals also harboured acid-fast bacilli (Table 1). Fifteen yearlings that had not been vaccinated were also slaughtered and 4 (26.7%) were found with both lesions and acid-fast bacilli. Mesenteric lymph nodes were recovered at slaughter in 1989 from 33 yearlings vaccinated in 1988 and 8 (24.2%) were found with lesions as defined above but no acid-fast bacilli were detected (Table 1).

TABLE 1 Prevalence of Johne's disease based on histological findings in the lymph nodes of slaughtered deer

YEAR	NO. EXAMINED	NO. POSITIVE (%)	
		With Lesions	With Organisms
1986	59	24 (40.7)	24 (40.7)
1987	167	64 (38.3)	36 (21.6)
1988	15	4 (26.7)	4 (26.7)
1988	201 (vaccinated)	32 (15.9)	9 (4.5)
1989	33 (vaccinated)	8 (24.2)	0 (0)

By pooling the results of lymph node histology (Table 2) and using the chi squared test there was a significantly greater likelihood of finding organisms in the lymph nodes of unvaccinated deer (chi squared = 47.08; $P < 0.01$).

TABLE 2 Pooled results from histological studies showing the effect of vaccination upon the presence of acid-fast organisms in mesenteric lymph nodes

	Vaccinated	Unvaccinated
With acid-fast bacilli	9	64
Without acid-fast bacilli	225	177

Faecal sampling

Microscopic examination of faeces samples from 452 animals in 1986 revealed 5 animals (1.1%) passing acid-fast bacilli; 4 were seropositive animals subsequently confirmed as having Johne's disease at post-mortem examination

Serological studies in relation to other findings

Serological screening was conducted on three occasions in 1986 (Table 3), with 3.3%, 3.5% and 3.4% animals seropositive in March, August and December respectively. Of the 35 animals culled, 18 were subjected to post-mortem examination, the results of which also appear in Table 3. Confirmation of disease was based upon gross lesions and histopathological changes. Results from March and August indicated that some seropositive animals subjected to post-mortem examination showed no sign of infection. As a consequence, in December only the 3 animals reacting to both the CFT and the AGID test were culled, two of which showed gross lesions and histological findings consistent with Johne's disease.

TABLE 3 Results of serological screening for Johne's disease antibody in unvaccinated deer, together with the results of post-mortem examinations

	1986			1987			1988		1989	
	Mar	Aug	Dec	Mar	Aug	Dec	Mar	Aug	Feb	Sep
No sampled	457	509	319	553	515	328	312	329	229	176
Seropositive CFT	3	0	3	6	10	3	ND	2	0	0
Seropositive AGID	11	18	11	11	0	3	6	2	0	0
Total seropositive	15	18	11	16	19	3	6	2	0	0
Mean age in years (of seropositive)	3	2	5	2	2	5	3	3	-	-
No examined post-mortem	10	5	3	5	1	1	0	0	0	0
No confirmed with Johne's disease	5	3	2	3	1	1	0	0	0	0
Mean age in years (of confirmed cases)	4	2	2	3	2	4	-	-	-	-

ND = not determined

Three serological screenings were made in 1987. Only animals which were seropositive on two successive occasions were culled: the results of the post-mortem examinations appear in the table.

In March 1988, 6 of 312 adult deer screened (1.9%) were shown to be seropositive for M. paratuberculosis (Table 3). In August one of the seropositive animals had also been positive at the previous sampling and was therefore culled, but no post-mortem examination was carried out. In 1989, serological screening of unvaccinated adult animals took place in February and September (Table 3) and no animals were found to be seropositive nor was

there any ill-health attributable to Johne's disease in either vaccinated or unvaccinated deer.

Of the 64 out of 167 clinically healthy animals examined in 1987 which showed lesions in their lymph nodes only two animals had been seropositive at screenings earlier in the year.

Intradermal hypersensitivity tests

Intradermal tests carried out in March 1987 revealed reactions in 55 (32.2%) of the 171 yearlings tested (Table 4). In August, 77 (60.2%) of 128 retested animals showed a reaction (20 of these animals had reacted also to the previous test). Of the deer which were skin tested, 10 (5.8%) and 7 (5.5%) in March and August respectively had positive serological reactions. Only 65% of the seropositive animals gave a positive skin reaction (Table 4). Only 8% of those with a positive skin reaction were seropositive.

TABLE 4 Intradermal hypersensitivity test and serological test results for groups of unvaccinated and vaccinated yearling deer

	No. tested	Skin test	Serology
			+ve
1987			
March	171	+ve 55	6
		-ve 116	4
August	128	+ve 77	5
		-ve 51	2
1988 Vaccinated animals			
April	114	+ve 98	84†
		-ve 16	10
August	91	+ve 84	22*
		-ve 7	0‡

† 4 animals not tested, * 10 animals not tested, ‡ 2 animals not tested serologically

The correlation between animals with lesions detected in abattoir specimens and positive skin test reactions was higher (Table 5); 17 animals out of 64 with lesions (26.6%) had a positive skin test reaction in either March or August (although only one of these animals with lesions had a positive skin reaction on both occasions). However, 56 animals (33.5% of the total examined) had a positive skin reaction yet did not have detectable changes at post-mortem examination. (No deer was found to be positive by serology, skin test and histopathology).

TABLE 5 **Results of histological examinations in 1987 in relation to delayed hypersensitivity skin tests.**

	No. tested		Skin test
1987	167	With lesions 64	+ve 17
			-ve 47
		Without lesions 103	+ve 56
			-ve 47

The effect of vaccination on the serological response and intradermal tests

The yearlings, which had been vaccinated as calves in June of the previous year (1987), were screened for antibody to M. paratuberculosis in April and August. The serological results showed that in April 86.9% of the yearlings were positive by AGID while 54% were positive by the CFT; 51% were positive to both tests. In August the proportions were lower with 24.2% and 12.8% positive to the AGID test and CFT respectively: 12.7% of the animals were positive to both tests.

The skin tests carried out on the vaccinated yearling stags in 1988 showed that 98 (86.0%) and 84 (92.3%) animals reacted in April and August 1988 respectively (Table 4) and 72 animals reacted on both occasions. Of these animals with positive skin tests, 84 (86%) and 22 (26%) respectively had antibody to M. paratuberculosis. The correlation between the two tests was much greater than in the previous year for unvaccinated animals, since 91% of seropositive animals had positive skin test reactions. Also, 58% of those with a positive skin reaction were also seropositive.

Progress of the disease outbreak

During the five years since the cessation of the routine herd screening programme, although occasional animals have been found to have lesions consistent with Johne's disease on routine post-mortem examination, there have been no further clinical cases. As vaccination is still being carried out and the proportion of unvaccinated animals in the herd declines (in 1994 only 49.6% of adult hinds remain unvaccinated), the value of serological testing for suspected clinical cases also diminishes.

DISCUSSION

The aim of the study reported was to control the disease. Consequently it was not possible to conduct a systematic survey into the diagnostic methods available, or to undertake a comparison of control strategies.

It is important to emphasise the distinction between clinical disease and infection with organisms, even when this leads to histological change, seroconversion or cell mediated immunity (or, in the case of Johne's disease, the presence of organisms in faeces). While a

considerable number of animals had evidence of exposure to mycobacteria only 21 animals developed clinical Johne's disease. (In only a relatively small number of cases was M. paratuberculosis definitively identified).

Both serological tests used a bovine M. paratuberculosis antigen and it is unlikely that either test could discriminate between organisms within the M. avium complex (which includes M. paratuberculosis). The advent of ELISA to detect antibodies to mycobacteria (Milner *et al.*, 1990) may allow enhanced serological specificity but ELISA for detection of antibodies to M. bovis has poor sensitivity (Fifis *et al.*, 1992). Thus serological discrimination is currently difficult. The introduction of a gamma-interferon assay which provides evidence of the CMI response may give a better indication of protective immunity against paratuberculosis.

The serological tests used resulted in a poor prediction of future clinical cases. Clinical cases of disease occurred without there being serological evidence of infection. The false positive results obtained may have reflected exposure to mycobacteria of the M. avium complex with the outcome of challenge varying with the balance between host immunity and infectious dose (Chiodini *et al.*, 1984). Thus the serological screening of the herd was of limited value in this study, since many animals harbouring mycobacteria remained undetected, at least with single sampling. The poor correlation between skin tests and serological results may reflect the evolution of the immune response since it has been suggested that there is an initial cell-mediated response followed by a humoral response (Milner *et al.*, 1990). Conversely, for vaccinated animals a serological response often accompanied a positive skin reaction. This concurrent positive result may be useful in discriminating between vaccinated and field-challenged animals. In vitro studies (Kerby, 1993) employing a lymphocyte stimulation test showed that, following vaccination, deer calves failed to mount a good initial IgG response and at 10 weeks there was only a variable CMI response, while in yearlings, although the CMI response was variable, the IgG response was much better.

Histological studies are able both to describe characteristic changes and identify acid-fast organisms although neither of these features are specific for M. paratuberculosis. Although not all animals were examined post-mortem, the high prevalence of changes in mesenteric lymph nodes was noteworthy, suggesting that currently histopathology offers the most sensitive and specific method available for confirmation of infection.

While the skin tests were not rigorously interpreted and were thus likely to have poor specificity, they also correlated poorly with the serological results in 1987 (with only 65% of the seropositive animals giving a skin response). Also there was a poor correlation between animals with lesions at post-mortem examination and previous positive skin reactions (27%). Thus the skin test showed poor sensitivity and would not prove valuable under a test and slaughter regime. As M. avium and M. paratuberculosis are antigenically closely related (Buergelt *et al.*, 1987) substitution of an M. paratuberculosis PPD would not improve the sensitivity. The ability of the skin tests to detect animals vaccinated the previous year was high (Table 4) and the concordance with a serological response in April 1988 was better.

Vaccination did not prevent the development of pathognomonic lymph node lesions indicating that field challenge continued though clinical cases no longer occurred. In cattle, Stuart (1965) showed that vaccination reduced the incidence of clinical disease but that vaccinated animals still harboured organisms. Vaccination may complicate the interpretation of

comparative intradermal tests for identification of animals infected with M. bovis. However, the precise change in reactivity to M. bovis PPD in stock vaccinated against M. paratuberculosis has not been determined although it is likely that some vaccinated deer would respond to bovine PPD. In deer, comparative intradermal tests have been reported to distinguish poorly between M. avium and M. bovis (Griffin and Cross, 1989) and Kerby (1993) demonstrated that lymphocytes from animals sensitized to M. paratuberculosis showed an in vitro cross reactivity to M. bovis PPD, although to a much lesser extent.

The source of the infection in this herd is uncertain since it had been closed for a number of years preceding the first diagnosis. No cases of Johne's disease were recorded in goats which used the grazing area prior to the confirmation of disease in the deer. The evidence from the histopathology studies suggests that mycobacterial infection was still occurring and although there have been no further clinical cases caution will need to be exercised when deciding if vaccination can be stopped.

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