The epidemiology of tuberculosis in wild red deer in New Zealand

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Introduction

Tuberculosis was first noted in wild red deer on the West Coast of the South Island in 1956, and since then *M. bovis* has been isolated from numerous wild deer. The disease is widespread in wild deer populations, and has been seen mostly in animals from the Wairarapa, Central North Island endemic area and the West Coast, but a few have also come from Fiordland, Otago and Southland. It is noteworthy that areas such as Taranaki and Northland, which do not have significant wild deer populations, also do not have tuberculous possum populations.

It has been speculated that the wild deer of New Zealand may have been infected for a long period, and were responsible for the initial introduction and establishment of tuberculosis in possum populations in what are now regarded as endemic areas (Morris and Pfeiffer, 1995). This hypothesis has been given recent support by a number of instances where farmed deer appear to have been responsible for the introduction of tuberculosis to possum populations. The determination of whether wild deer are likely to maintain infection in their own populations independently of outside sources, will be important to tuberculosis control efforts of the future, particularly if the eradication of the disease from possum populations becomes feasible.

The aims of this study were to investigate the epidemiology of the disease in free-ranging populations, and to acquire a better understanding of the pathogenesis of tuberculosis in deer. This project also investigated the likely routes of transmission between deer, and attempted to determine whether the requirements for deer to be considered as reservoir hosts of tuberculosis were fulfilled. Greater detail of this research is presented in Lugton (1997).

Materials and Methods

Origin of deer

Whole deer or parts thereof were gathered from five sources (Table I). The majority of the wild deer were recovered by helicopter shooting over the Hauhungaroa Range, to the west of Lake Taupo, in the Central North Island endemic area. Other wild deer were shot by ground hunters from around the Castlepoint district in the Wairarapa endemic area. Both of these are areas in which tuberculous possums are regularly found. To supplement the data from the wild deer, samples were also collected from skin test positive farmed deer killed at Deer Slaughter Premises (DSPs). Data from the

necropsy examination of the farmed deer used in the natural tuberculosis transmission trial (Lugton *et al.*, 1997) were also used.

Data collection

Information collected on each deer at the time of necropsy included: Date; area of origin; whether wild or farmed; sex; estimated age; portions of carcass available for examination; presence and type of tonsillar crypt lesions; presence of small (up to 8mm) grey homogeneous pulmonary nodules; presence of lungworm (*Dictyocaulus viviparus*) in the major bronchi; description of lesions; provisional diagnosis. The majority of the wild deer had their age determined by teeth eruption and examination of cementum annuli. The ages of the remaining wild deer were estimated from the body size and antler growth.

Table I. Description of the deer examined and prevalence of *M. bovis* infection, including area of origin and portions examined

Origin and date	Type[b]	Parts examined[a]					Total deer	Numbe infecte	Prevalence (95% CI)
		HTAB	*HT*	*HT*	*HT*	*H*			
Hauhungaroas 1994-1995[c]	W	40	26	4	6		76	25	0 33 (0 22 - 0 44)
Castlepoint[d] 1993-1995	W		7		21	2	30	9	0 30 (0 14 - 0 46)
Deer trial July 1994[e]	F	7	2		1		10	5	0 50 (0 19 - 0 81)
Feilding DSP Feb-March	F	24					24	0	0
Mamaku DSP March 1995	F	22					22	19	0 86 (0 72 - 1 0)

a HTAB = head, thoracic contents, abdominal contents and body; HTA = head, thoracic contents and abdominal contents; HTB = head, thoracic contents and body; HT = head and thoracic contents; H = head only

b W = wild; F = farmed - skin test positive

c 52 deer recovered between March and June 1994, and 24 between October and December 1995

d deer recovered between November 1993 and December 1995

e animals from the Castlepoint trial reported in Lugton *et al.*, (1997), including two that were shot following escape from the trial site

Sample collection and examination

A detailed necropsy was performed on each deer examined. Tissues were handled in a similar manner for all deer, with technique variation dependent upon the parts of carcasses available, and gun shot damage. Swabs were used to gather material from the nasal cavity; the transected trachea; and the oropharynx. The pharyngeal sample was taken from the area surrounding the oropharyngeal tonsils, but the tonsillar fossae were deliberately avoided. A faecal sample was extracted from the rectum, and a urine sample was removed from the bladder. All major lymph nodes were laid out on pre-

labelled paper sheets and sliced at 2 mm intervals. Lungs initially had the major bronchi opened and examined for the presence of lungworm, and were then sliced to a 2 cm thickness to facilitate thorough palpation and visual examination. Four tissue pools of approximately 5 to 8 g each were retained from each deer for mycobacterial culture. These comprised a sub-sample of both the oropharyngeal (palatine) tonsils (including any visible lesions), the nasopharyngeal tonsil (adenoid), a sub-sample of both medial retropharyngeal lymph nodes, and a combined sample of the caudal mediastinal, left tracheobronchial and the cranial tracheobronchial (apical) lymph nodes. Other suspicious lesions were kept for culture, if typical lesions were not included in the other tissue pools already retained. Isolation of *M. bovis* from faeces, urine, and the nasal, tonsillar and tracheal swabs was attempted if the animal was tuberculous.

Tissues removed for bacteriology were stored in sterile plastic containers, and together with swabs and other samples, stored at -84°C. Samples were later submitted to the AgResearch tuberculosis laboratory, Wallaceville, for culture. Animals or tissues from which *M. bovis* were isolated were termed 'infected', and animals or tissues showing tuberculous lesions were termed 'diseased'. From selected cases, fixed tissues were held for later histopathological examination.

Unconditional logistic regression, was used to examine the relationship between a range of independent covariates and dichotomous dependent variables (Table II). Where appropriate, 95% confidence limits have been presented with results.

Table II. Summary of variables used for logistic regression analyses. Highlighted covariates were removed by preliminary univariate screening

		Covariates	
Dependent variable	N	*Categorical*	*Continuous*
Infection status of wild deer	106	sex, age (3 levels)[a], sex*age	
Presence of tonsillar crypt lesions	153	sex, infection status, sex*infection status	age, sex*age
Presence of grey pulmonary nodules	90	sex, infection status, lungworm presence, sex*infection, sex*lungworm	age
Presence of typical tuberculous lesions	58	sex	age, sex*age

a age was incorporated as a categorical variable, with three classes, i.e. weaners, yearlings and mature animals (mature and aged classes combined).

Results

Between November 1993 and February 1996, 162 red deer were examined (Table I). The class of deer, sex and age distribution are presented in Table III. The youngest deer was estimated to be 4 months of age, and the oldest deer 10.3 years of age.

Table III. Class, age and sex distribution of deer examined

	Farmed			Wild		
Age	*female*	*male*	*total*	*female*	*male*	*total*
Weaners ≤12 m			0	6	12	18
Yearlings 13 - 23 m	13	16	29	6	23	29
Mature 24 - 48 m	13	11	24	11	30	41
Aged ≥49 m	3	0	3	12	6	18
Total	29	27	56	35	71	106

Infection status and relationships

Mycobacterium bovis was isolated from 58 (35.8%) of the deer examined. The combined prevalence for all wild deer from the two locations was 0.32 (0.23 - 0.41).

Table IV. Summary of gross and bacteriological findings in 58 infected deer

Site	No examined	No typical gross lesions [a]	Proportion with typical gross lesions	No cultured	No culture positive	Proportion culture positive	Proportion lesion -ve /culture +ve
OP Tonsil	56	1	0 018	56	34	0 607	0 971
NP Tonsil	53	0	0	53	4	0 075	1 00
Medial retro-pharyngeal lnn	55	12	0 218	55	21	0 382	0 429
Head-associated[b]	58	13	0 224	58	39	0 672	0 666
Respiratory lnn	58	17	0 310	46	22	0 478	
Lung[c]	58	15	0 259	7	4	0 571	
Total thoracic	58	20	0 345	52	25	0 481	
Mesenteric lnn	47	14	0 298	5	4	0 800	
Peripheral lnn	52	4	0 077	7	2	0 286	

a characterised by purulent/caseous appearance at the centre of lesions, which can be grossly differentiated from most other pathological conditions
b includes all head-associated lymph nodes and tonsillar sites
c does not include pleuritis or grey pulmonary nodules

There was a significant trend for increasing prevalence of infection as the wild deer became older ($p = 0.005$). The age prevalence of infection is summarised in Table. However, there was little difference in prevalence between the mature and aged classes, suggesting that the trend was non-linear.

Table V. Chi^2 analysis for trend examining the relationship between age class and infection status in 106 wild red deer

Age class	Number without infection	Number with infection	Prevalence	Odds ratio
Weaners	17	1	0 056	1 0
Yearlings	21	8	0 276	6 5
Mature	24	17	0 415	12 0
Aged	10	8	0 444	13 6

$_{}^{2}{}_{3} = 7.92$, p = 0.005

Neither sex, nor the interaction term of sex and age had any significant effect on the probability of infection. Age was the only influential independent variable, with the odds of infection in yearling deer 6.5 times as great as weaners, and the odds of infection in mature animals 12.5 times as great as weaners.

The presence of typical gross lesions in infected deer was found to be significantly associated with the age of the deer, the prevalence of such lesions decreasing as the age increased. Although there was not a significant statistical association between sex and the presence of typical lesions, there was an indication that infected males were two to three times as likely as females to be found with lesions (p = 0.18). The four deer judged to have the most severe gross lesions, either because of size and/or number of sites involved, were all males with estimated ages of 1.3 (farmed deer), 3.3, 3.83, and 4.4 (wild deer) years old.

Sites of infection

Tonsillar crypt lesions usually ranged in appearance from small foci of translucent mucopurulent liquid through to firm yellow caseous deposits in the crypt lumen although occasional white granular lesions were seen. Crypt debris and exudate typically caused little crypt distension and it was uncommon for a lesion to be over 3 mm in diameter. Vegetable matter, including grass seeds were commonly found in the crypts, often encrusted in a yellow caseous coating. Two deer had a purulent tonsillitis with a typical tuberculous appearance, both with tonsillar lesions over 1 cm in diameter. Only one of these purulent lesions was tuberculous and this came from a cachectic wild deer (Case 2730) with very advanced terminal tuberculosis and numerous large lesions. The other purulent tonsillar lesion came from an uninfected deer, and was the only gross lesion visible in the portions of the carcass examined. From the gross descriptions recorded at necropsy it was not possible to ascribe any particular tonsillar lesion type to the possible presence of *M. bovis* infection in the tonsil, and indeed 16 of 34 deer with oropharyngeal tonsillar *M. bovis* isolates showed no gross lesions in the oropharyngeal tonsil whatsoever. Seven deer had oropharyngeal tonsillar tissues examined histologically. Significant lesions were found in Deer 2730 only. These tonsils showed a granulomatous tonsillitis in which numerous giant cells, some containing AFB, were present. The only covariate shown to have a significant association with tonsillar infection was the deer infection status, with infected animals being twice as likely as *M. bovis*-free animals to have lesions found in the crypts. Analysis of the relationship between infection of the oropharyngeal tonsil or the medial

retropharyngeal lymph nodes, and the presence of tonsillar crypt lesions showed a significant relationship ($\chi^2_1 = 4.47$, $p = 0.035$, odds ratio $0.99<2.23<5.06$).

There were four deer in which *M. bovis* was isolated from the nasopharyngeal tonsil. None showed gross lesions, and one was obtained from Deer 2730, from which isolates of *M. bovis* were obtained from multiple sites, including the nasal cavity. Of the 21 medial retropharyngeal lymph nodes from which *M. bovis* was isolated, there were 12 (57.1%) which showed gross lesions.

Isolates of *M. bovis* were obtained from thoracic sites in 25 of 52 (48.1%) animals which had samples of their thoracic viscera cultured. Lesions were present in 17 of 22 (77.3%) respiratory node pools from which *M. bovis* was isolated. Typical lung tubercles from five deer were submitted for culture. Four of the five furnished isolates of *M. bovis* (the fifth was a tiny 2 mm diameter lesion). Typical pulmonary lesions from seven other deer were not submitted for culture. Thirteen deer also had small grey homogeneous pulmonary nodules (often multiple), submitted for culture. From these, *M. bovis* was recovered in two cases.

Mycobacterium bovis was not recovered from nine of 11 (81.8%) of the pooled respiratory nodes submitted for culture from animals which had typical tuberculous lung lesions (tubercles or pleuritis). From seven of 22 (31.8%) cases from which *M. bovis* was isolated from pooled bronchial nodes, there was an absence of gross tuberculous lung lesions. Small grey pulmonary nodules were significantly associated with the presence of lungworm in the major bronchi. Animals with lungworm were three times as likely to have the nodules present as those without.

Of the 26 deer with *M. bovis* isolated from the thoracic contents, there were 11 which showed evidence of tuberculous pleuritis, either active or inactive. Five of these cases were wild deer. In every case *M. bovis* was isolated from the respiratory lymph node pool. In nine cases the pleuritis was associated with the presence of subpleural lung tubercles. Active cases were characterised by the presence of fleshy hemispherical translucent pulmonary outgrowths up to 1 cm in diameter ("grapes"). In three cases which were of apparently longer duration, the pleuritis had resolved and adhesions had formed between the visceral and parietal pleura in the mid to cranial thorax. Ten of the 11 deer with pleuritis were males, and although this relationship with sex was not statistically significant (Fisher's exact 2-tailed test, $p = 0.178$, odds ratio $0.58<6.67<336.7$), it was suggestive that males were more likely to develop pleuritis.

Of the 98 deer with peripheral nodes examined there were eight with suspicious lesions which were submitted for culture. From these, isolates of *M. bovis* were recovered from four deer. Deer 2730 had multiple gross lesions. Of the other deer with infected body nodes, one showed a mandibular lesion and also had an infected oropharyngeal tonsil, another had an infected popliteal lymph node and also had an infected oropharyngeal tonsil, and the remaining one showed an infected popliteal lymph node and also had a tuberculous respiratory lymph node.

Gross versus bacteriological findings

Tuberculosis was provisionally diagnosed at necropsy by the identification of typical lesions. Sixteen of the 58 (27.6%) culture positive deer showed no typical gross tuberculous lesions. In these 16 deer, isolates of *M. bovis* were recovered from the oropharyngeal tonsil in 14 cases (which were associated with infection of the medial retropharyngeal lymph node in four instances, and an infected grey pulmonary nodule in one case), and the nasopharyngeal tonsil in two cases (with one of these animals also having an infected medial retropharyngeal lymph node). Seventy two deer were provisionally diagnosed as tuberculosis-free at necropsy. From these animals *M. bovis* was isolated in nine cases (which comprise the majority of those mentioned above).

Association between lesion sites

Culture-positive oropharyngeal tonsils occurred concurrently with infected medial retropharyngeal lymph nodes in 17 of 33 cases with infected medial retropharyngeal lymph nodes (where corresponding records were available). There was a positive and significant association between infection in the medial retropharyngeal lymph nodes and the oropharyngeal tonsil ($\chi^2_1 = 6.21$, $p = 0.013$), with those infected in the oropharyngeal tonsil 4.8 (1.2 - 21.3) times as likely to have concurrent infection in the medial retropharyngeal lymph nodes as those without. In the four animals with nasopharyngeal tonsil infection, two were also infected in the oropharyngeal tonsil, but there was no significant association between infection at these two tonsillar sites (Fisher's exact 2-tailed test, $p = 1.0$). However, three of the deer infected in the nasopharyngeal tonsil also had infection of the medial retropharyngeal lymph nodes. The association between infection at these sites was found not to be significant (Fisher's exact 2-tailed test, $p = 0.28$, odds ratio $0.37<5.17<279.8$), but although limited by low power, the odds ratio of 5.17 was suggestive of a relationship.

In nine of 23 deer with thoracic isolates of *M. bovis* there was concurrent infection present in the oropharyngeal tonsil or medial retropharyngeal lymph nodes. This association was highly significant ($\chi^2_1 = 13.7$, $p < 0.001$), with the odds of infection in the thoracic viscera being 12.4 (2.4 - 72.1) times as high in animals with oropharyngeal tonsil or medial retropharyngeal lymph node infection, as those without infection in these head sites. Seven of the 13 deer with mesenteric lesions also had isolates of *M. bovis* from either the medial retropharyngeal lymph node or the oropharyngeal tonsil. Although the association between these head sites and mesenteric lesions was not significant (Fisher's exact 2-tailed test, $p = 0.29$; odds ratio $0.50<2.38<10.87$), it was weakly suggestive of a relationship between orally acquired infection and lesions associated with the intestinal tract.

Excretion site sampling

Fifty three of the 58 infected deer had swab samples taken from the trachea, nasal cavity and the pharynx. *Mycobacterium bovis* was recovered from four pharyngeal swabs (7.5%), and in each of these cases the oropharyngeal tonsil was found to be infected. Deer 2730 was also excreting bacilli from the medial retropharyngeal lymph node via a suppurating sinus to the skin of the ventral neck. Mycobacterium bovis was also recovered from one nasal and one tracheal swab (1.9%) from Deer 2730. No

other deer had isolates recovered from nasal or tracheal swabs. The faeces of 46 infected deer were submitted for culture, but only from the faeces of Deer 2730 was *M. bovis* recovered (prevalence = 2.2%). From none of 36 urine samples submitted for culture was *M. bovis* isolated, despite a sample from Deer 2730 being included.

Discussion

The prevalence of tuberculosis (30 and 33%) in the two groups of wild deer was similar, despite the animals coming from populations a great distance apart. The prevalence was similar to that reported from earlier studies in the Central North Island endemic area. The prevalence is much higher than that which has been reported in free-ranging deer overseas. In Britain, tuberculous wild and extensively managed deer have been found in the apparent absence of infected wildlife, but the prevalence of disease has been below 0.2%. In areas where tuberculosis is endemic in badger (*Meles meles*) populations, British and Irish reports suggest that the prevalence of disease in deer may approach 5%. A recent outbreak occurred in a group of "managed" wild white-tailed deer (*Odocoileus virginianus*) in Michigan, in an area in which tuberculosis has been eradicated from cattle for many years. This suggests that the deer have been able to maintain the disease amongst themselves for a prolonged period in the absence of other hosts. However, in both Switzerland and Hawaii, where a low prevalence of tuberculosis in deer was known to occur whilst infected cattle herds were present, it is now believed that the wild deer are free of tuberculosis following elimination of the disease in livestock.

The point prevalence of tuberculosis in possums of the Hauhungaroa Ranges has been shown to vary, over a number of years, between 0.4 and 4.7%. The area surrounding Castlepoint is similar, in that tuberculous possums have been trapped there since the late 1970s, they are widespread and present throughout the year. The high prevalence of tuberculosis found in the wild deer of New Zealand may be due to sharing the environment with infected possums. This notion is given support by Nugent and Proffitt (1994) who found a high prevalence of disease in deer, associated with a focus of infection in possums on the forest-pasture margin, but with the tuberculosis prevalence in deer decreasing rapidly as the distance from the tuberculous possums increased.

In the central North Island endemic area, despite many wild deer being inspected at Game Packing Houses (GPH), no tuberculous deer have come from areas without infection being already present in possums (K. Paterson, pers. comm.). However, a low prevalence of infection with tuberculosis in wild deer has been found in the apparent absence of infection in possums e.g. Timahanga and Waikaka. Despite the absence of tuberculous possums or other wildlife hosts, it appears that tuberculosis may remain endemic, at a low prevalence, in some deer populations. The fact that the disease has apparently disappeared from deer in some countries, in the absence of other infected hosts suggests that wild deer alone are poor reservoir hosts of tuberculosis, and that the maintenance of disease may be density dependent.

The low prevalence of infection in deer less than 13 months of age suggests that despite approximately 40% of their mothers being infected, few of the offspring acquire infection from their dam. In farmed deer there are a number of reports suggesting that the prevalence of disease during outbreaks is lower in weaners than in older animals (Griffin 1988; Atkinson, 1993; Paterson, 1993; Whiting and Tessaro, 1994), suggesting that there may be some behavioural characteristics operating which reduce the risk of infection in deer calves, despite having a close association with infected dams. It is possible that these younger deer may be too timid to approach possums, as has been observed with some older animals. In addition the various routes of pseudo-vertical transmission are apparently not influential in deer.

The apparent high incidence of disease in yearling and mature deer suggests that most infection occurs in deer between one and four years of age. This supports the hypothesis that interspecific infection is acquired by older and bolder animals investigating sick tuberculous possums (Sauter and Morris, 1995a; Sauter and Morris 1995b). At present deer population densities at both Castlepoint and in the Hauhungaroa Ranges, deer seldom form groups of more than a few animals, so the opportunity for intraspecific transmission is limited. The results suggest that transmission within these groups is not common, which is consistent with the low transmission rates usually observed in in-contact controls in experimental studies, and the low prevalence of tuberculosis in wild deer without contact with tuberculous possums.

Disease transmission by deer is likely to be associated with discharging sinuses from tuberculous deer in which the disease is under poor immunological control. Most severe outbreaks of disease in deer, have reported the presence of draining sinuses (Beatson *et al.* 1984; Robinson *et al.* 1989; Atkinson, 1993; Whiting and Tessaro, 1994; Mackintosh and Griffin, 1994). Coughing and associated generation of infectious aerosols is not likely to be the major route of transmission, as coughing is not a prominent sign in deer with pulmonary infection, as it is in cattle. Discharges from sinuses are likely to contain large numbers of bacilli which can potentially infect cohorts by direct contact and subsequent carriage of the bacilli onto the oral or nasal lymphoepithelial tissues. The victimisation of weak members of mobs of farmed deer, particularly by biting, will provide one effective transmission pathway. However, despite the occurrence of severe disease and suppurating sinuses in wild deer, the likelihood of transmission is reduced by the sickening deer parting company with others prior to death. Helicopter shooters have reported that animals in poor condition, affected by severe tuberculosis are commonly located away from other deer.

The recovery rate of *M. bovis* from the nasal, pharyngeal and tracheal swabs, urine and faeces was low, indicating that the majority of infected deer excrete few bacilli, whereas the small number with severe disease excrete far higher numbers of bacilli. The isolation of *M. bovis* from three pharyngeal swabs taken from deer without severe disease, but with oropharyngeal tonsil infections, and the similar recovery from swabs of tonsillar fossae of the two deer reported by Lugton *et al.* (1997), strongly suggests that excretion from infected lymphoepithelial tissues is common. As well as being capable of taking up mycobacteria, the lymphoepithelium also seems capable of shedding organisms (Figure 1). Nasal isolates of *M. bovis* have been recovered using sensitive cultural techniques, from a number of cattle, both naturally and experimentally

infected, but without gross lesions in the lungs or upper respiratory tract (Neill *et al.*, 1988a; Neill *et al.*, 1988b; Neill *et al.*, 1989). Although the reports did not suggest a source of the organisms, it is quite possible that they may have been shed by the nasopharyngeal tonsils, which were not examined.

The significant increase in the number of infected deer, showing no typical gross lesions, corresponding with an increase in age suggests that infected animals may be capable of resolving lesions over time. Lesion resolution is a distinct possibility in this species where most animals show only evidence of single gross lesions at slaughter (Hathaway *et al.*, 1994), and in which lesions are not characterised by extensive fibrosis and mineralisation. Lesion regression/resolution has been observed in a number of other species, and there is no reason to believe that tissue repair processes in tuberculosis, where a protective immune response has been generated, will be different from other similar disease resolution processes.

The suggestion from the data that male deer are more likely to show gross tuberculous lesions, including pleuritis, and develop severe disease has not previously been reported, but is in accord with observations in many other species in which males have been found to be less immunologically competent. However, there is no compelling evidence in the literature to suggest that males are more susceptible to tuberculosis *per se*. It is more likely that, as most of the deer were killed during the period in which the males were in hard antler, that stress effects from intraspecific aggression, gathering and maintaining a group of hinds, and in some cases malnutrition may have played a role. Deer have been shown to be sensitive to the effects of stress and may respond by developing elevated circulating glucocorticoids, which are likely to increase the susceptibility of deer to a variety of diseases including tuberculosis (Thomson *et al.*, 1994; Thomson and Griffin, 1995).

Oropharyngeal tonsils were the most commonly infected site in the deer, but with crypt lesions being found in only half of the infected animals. The efferent drainage from the oropharyngeal tonsil is believed to pass directly to the medial retropharyngeal lymph nodes, and accounts for the strong association between infection in the oropharyngeal tonsil and these nodes. This is the first report of the distribution of tuberculous lesions in any animal, which has identified the oropharyngeal tonsil as the most commonly involved site. In deer the medial retropharyngeal lymph nodes have been previously recognised as the most common site for lesions. Recently Mackintosh *et al.* (1995) have established an intratonsillar infection model which has been shown to closely mimic the natural disease. In these studies as few as 8 cfu have been instilled into tonsillar crypts, and produced disease in 50% of the inoculated animals. Taken together these data suggest that the most common portal of entry of *M. bovis* into deer may be via the oropharyngeal tonsil, with only small numbers of organisms needed to establish infection in the tonsillar crypts. The oropharyngeal tonsil, nasopharyngeal tonsil and Peyer's patches are lymphoepithelial tissues, in which the epithelium overlying the lymphoid follicles, contains specialised M-cells which are capable of endocytosing adherent mycobacteria, and presenting these bacilli to underlying dendritic cells and macrophages (Fujimura, 1986; Momotani *et al.*, 1988). In this way mycobacteria and other antigens are presented for processing by the immunocytes underlying or within the epithelium (Figure 1).

From the tonsil, bacilli disseminate to other sites, principally the draining medial retropharyngeal lymph nodes. However, in contrast to other studies, the medial retropharyngeal lymph nodes showed a low prevalence of infection, and a high proportion showed no gross lesions, despite the presence of *M. bovis* in the nodes or in adjacent tonsillar tissues. This suggests that infection by possums may involve low numbers of organisms, that lesion development is less likely to occur, or that with a long duration of infection there is time for lesion regression and either destruction or induction of dormancy in bacilli.

Mackintosh and Griffin (1994) reported that gross tuberculous lesions of the tonsils were uncommon despite inoculation of *M. bovis* directly into the tonsillar crypts. The observations of this study also suggest that at least half of the deer with oropharyngeal tonsil infection show no gross lesions of the tonsil. However, the significant association between oropharyngeal tonsillar lesions and the infection status of the animals suggests that at least a proportion of the tonsillar lesions will be tuberculous in origin. Little can be said about the types of gross lesions which are caused by tuberculosis infection of the tonsil, except that they are common, occupy the crypts and take a variety of forms which are grossly indistinguishable from lesions caused by a variety of bacteria or reactions to foreign bodies.

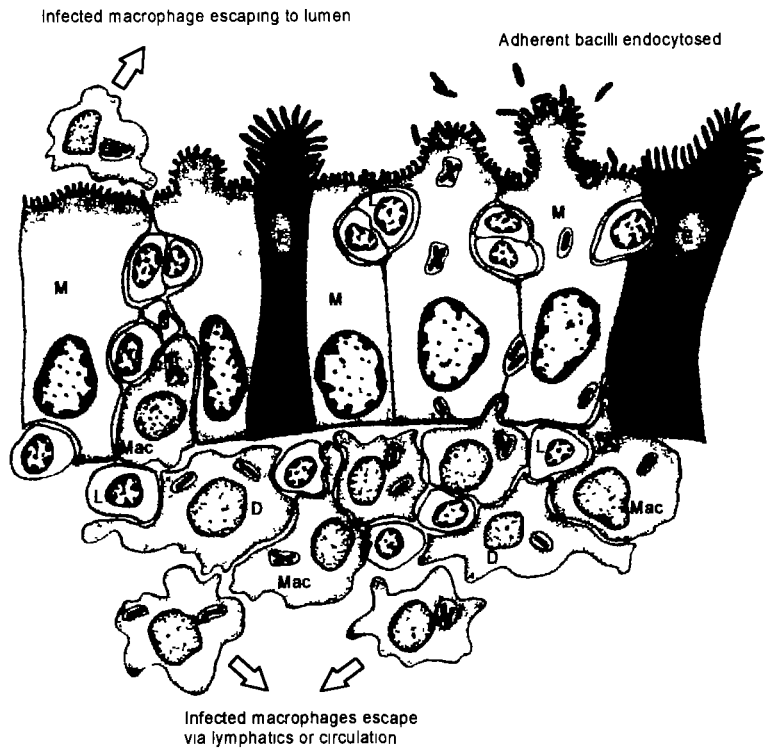

Figure 1 Schematic representation of a section of lymphoepithelial tissue (Peyer's patch) Mycobacteria free in the lumen adhere to microfolds and are endodocytosed by the M-cells (M), which present bacilli to underlying dendritic cells (D) and macrophages (Mac) These, and accompanying lymphocytes (L), lie in the dome area above the lymphoid follicles Macrophage traffic may carry the bacilli to other sites in the body, or back through the lymphoepithelium to the mucosal surface Normal epithelial cells (E) appear to take no part in the processing of particulate antigens

The observation that infection is often found in tonsils in which there are no visible lesions suggests that they, in common with other lymphoepithelial sites, such as the

nasopharyngeal tonsil or Peyer's patch, are immunologically privileged sites and usually remain free of grossly visible inflammatory responses when infected with mycobacteria, unless subjected to challenge from large numbers of bacilli. Immunological tolerance may hinder clearance of bacilli and allow development of a carrier state.

The nasopharyngeal tonsil was found to be infected in four cases, in three of which it was likely to have been the primary site of infection. This is the first time infection of this site with mycobacteria has been reported in any species. Although no significant relationship with medial retropharyngeal lymph node infection was found it is likely that one does exist, as the efferent drainage from the caudal nasopharyngeal area is believed to pass to the medial retropharyngeal lymph nodes. The nasopharyngeal tonsil is situated in the roof of the nasopharynx, such that antigenic material trapped after inhalation into the nasal cavity, is likely to be swept by mucociliary action over the surface of the organ, prior to swallowing directly from the nasopharynx. The nasopharyngeal tonsil is likely to be the only mucosal site in the respiratory system of ruminants where particulate antigens can be taken up and presented to immunocytes. The small number of isolates from this tonsillar tissue suggests that infection caused by aspiration of large airborne particles containing *M. bovis* which are likely to lodge in the nasal cavity, is uncommon in deer.

The significant association between head and thoracic infected sites suggests that after primary tonsillar infection that there is rapid haematogenous dissemination to the lungs. Calves fed contaminated milk have been shown to develop lesions in the lungs, suggesting that early post-primary haematogenous dissemination from lymphoepithelial tissues to sites of predilection is common. Dissemination of mycobacteria from lymphoepithelial sites, via the bloodstream, may occur immediately following infection if the dose of bacilli overwhelms the local phagocytic defence mechanisms, or may occur within weeks of the initial infection as bacillary numbers rise and some escape the lesion in migrating macrophages. The lungs have been found to contain a large population of intravascular macrophages capable of engulfing bacilli, and are also known to sequester large numbers of circulating leucocytes and to retain damaged (infected) cells, both mechanisms which can lead to the development of pulmonary disease.

The presence of grey subpleural pulmonary nodules was significantly related to the presence of lungworm only, but their occurrence presents a diagnostic dilemma as these can be caused by both lungworm and *M. bovis* infection. Isolates of *M. bovis* were recovered from 2 of 13 of these lesions submitted for culture, but of five examined histologically, all resembled typical lymphoid hyperplastic nodules. Similar subpleural lesions, composed of hyperplastic lymphoid tissue have been reported in cattle, where they are associated with previous exposure and acquired immunity to lungworm. In a study of thoracic pathology in Scottish red deer, lesions associated with *Dictyocaulus* spp. infection were found commonly in both wild and farmed deer (Munro and Hunter, 1985). Lymphoid tissue had formed in peribronchiolar, interlobular and subpleural sites, which was in accord with the findings of this study, and other experimental infections with *Dictyocaulus viviparus* in deer. In retrospect, it is believed that careful macroscopic examination should be able to differentiate the small grey homogeneous nodules into those with a granulomatous appearance and

those with a lymphoid follicular appearance. If the lesions are mature lymphoid nodules, the follicles they contain will give the cut surface a granular appearance, which is not apparent in the granulomatous tuberculous nodule. When the centre of a nodule is necrotic a tuberculous granuloma should always be suspected, but they can be quite small and nestled in amongst the parenchyma, and thus difficult to find. These small tuberculous pulmonary lesions are not routinely looked for at DSP nor GPH inspections, and their undetected presence will lower the sensitivity of inspection procedures as there will not always be corresponding gross lesions in the respiratory lymph nodes.

Tuberculous pleuritis is believed to be associated with small foci of sub-pleural pulmonary infection, and escape of bacilli to the pleural cavity. This seemed to be true in the current series of animals where in nine of the 11 cases small sub-pleural tubercles were found. Adhesions, the end result of tuberculous pleuritis, were uncommon and restricted to the cranial and intermediate lung lobes where there is less movement to disrupt their formation. Although adhesions resulting from caudal thoracic trauma and fractured ribs have been found in up to 2.2% of slaughtered farmed deer, none of this nature were found in the deer examined.

In each case where there was involvement of a peripheral body node, infection was found at another site. The oropharyngeal tonsil was also involved in two cases, the respiratory lymph nodes in another case, and generalised disease in another. This suggests that early post-primary bacillaemia, as well as allowing spread to the lungs, may also allow lesions to develop, in what appear to be peripheral predilection sites, such as the popliteal and caudal cervical lymph nodes (Hathaway *et al.*, 1994). Although skin wounding/contamination, with subsequent infection of the draining lymph node has been hypothesised as a cause of peripheral nodal lesions (Mackintosh and Griffin, 1994), there is no field evidence available to support such a notion. Although a significant association between head site infection and bowel-associated lesions was not established in our data, a significant association was found by Hathaway *et al.* (1994) when examining the slaughter records of 668 lesioned tuberculous deer. This suggests that head-associated infection and bowel infection occur at approximately the same time. From the present data, there was no indication that a significant association between infection in thoracic sites and the bowel-associated sites existed, which suggests that lung and bowel infection do not occur simultaneously, and that pulmonary lesions do not commonly result in sufficient excretion of bacilli to result in bowel-associated lesions.

The medial retropharyngeal node is the most common site of tuberculous lesions, outside of the thorax, in cattle (Francis, 1958; Crews, 1991; Neill, 1994). This suggests that the acquisition of infection by the tonsil may be one of the more important routes of natural infection in both cattle and deer. The inapparent infection of tonsils may also be a significant contributor to the occurrence of non-lesioned cattle and deer reactors (to intradermal or blood tests for tuberculosis) examined at slaughterhouses. This is a particularly common phenomenon in deer where 79.9% of reactors to intradermal testing fail to show lesions at abattoir inspection (Hathaway *et al.*, 1994). This high percentage of NGL reactors has typically been attributed to high levels of non-specific reactivity. However, the results of this research suggest that the proportion of truly infected deer which show no gross lesions can be considerable.

Conclusions

This study has shown the importance of lymphoepithelial tissues as primary sites for the establishment of tuberculosis infection, and for the subsequent excretion of organisms. These sites, such as the oropharyngeal tonsil and nasopharyngeal tonsil usually remain free of gross lesions, or if they do develop lesions they are impossible to classify as tuberculous from gross appearance. The overall impression of *M. bovis* infection of wild red deer, is one where the disease process is kept under control by appropriate cell-mediated immune responses, and with little bacillary excretion from most animals. The findings of this study are in agreement with those of Griffin and Buchan (1994) who concluded that deer are not innately highly susceptible to *M. bovis* infection as has been previously suggested. However, as with farmed deer, stress may precipitate serious disease, particularly in males during sexually active periods.

Wild deer should be principally considered as spillover and amplifying hosts for tuberculosis, as with all other susceptible wild mammals in New Zealand, with the exception of the possum (Figure 2). Most infection seems to be acquired by bold and inquisitive individuals which closely, and perhaps vigorously, investigate moribund tuberculous possums. However, they have the potential for a low level of disease maintenance which will increase in importance as possum tuberculosis control methods become more successful. Both tuberculous farmed and wild deer are capable of introducing disease into sympatric possum populations causing the development of new disease foci. Their role in this regard must not be overlooked. Indeed, the original infection of possums in endemic areas may have developed from the molestation of possums by tuberculous deer. Possums may also have contracted tuberculosis through carcase feeding when protein deficient, in areas where drought may have been devastating the countryside, and where wild deer were at high density and perishing. One such situation in the Wairarapa in the late 1940's, has been reported by Thomas *et al.* (1993), where starving possums abroad in daylight, were seen to feed on the carcasses of dead deer. In this circumstance, when densities of deer were high and suffering from environmental stress, the prevalence of tuberculosis may have been substantial, and sufficient to transmit infection to possums. Possums are now well recognised to incorporate flesh in their diet, and are frequently captured in cages or traps with meat lures.

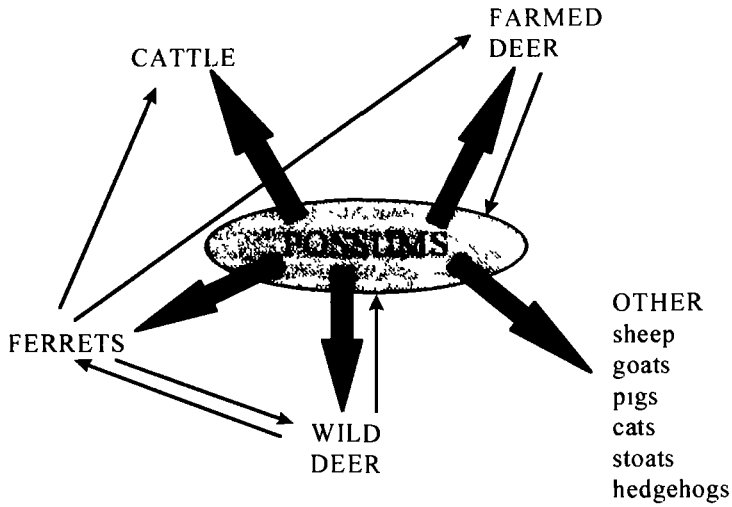

Figure 2. Species currently involved in the epidemiology of *Mycobacterium bovis* infection in New Zealand Arrows indicate the magnitude and direction of the principal transmission pathways

Given that red deer may be reservoir hosts of *M. bovis* it would seem prudent to continue investigations into the epidemiology of tuberculosis in cervids. Clarification of the role of other species of wild cervids in New Zealand is required. Isolated *M. bovis* infected deer populations, from areas in which tuberculosis is not endemic, need to be found, to accurately establish the prevalence of infection and the host status of wild deer. Uninfected red deer populations, outside of endemic areas also need to be identified to satisfactorily test the hypothesis that infected possums are responsible for maintaining a high prevalence of infection in wild deer.

Acknowledgments

The assistance of Steve Gamble, Ben Dellow, Noel Hutchins (Environment Waikato) and their associates who recovered deer by helicopter from the Hauhungaroa Range was greatly appreciated. Thanks also to Ron Goile, Alan Schofield, Gert Vermeer and others who provided carcasses from around the Castlepoint area; and to the staff and management of the Feilding and Mamaku DSPs, and the GPH at Rotorua, for allowing specimen collection during carcass inspection. Assistance with the necropsy of carcasses was cheerfully provided at times by Peter Caley, Peter Berben (Landcare Research, Massey University) and by Charlie Lambie (MAF Quality Management, Turangi). Gary Wobeser (University of Saskatchewan, Saskatoon, Saskatchewan) also assisted with a number of necropsies and the histological examination of a range of specimens. Jacqui Whitford (Landcare Research, Lincoln) also assisted by ageing deer by teeth sectioning. Mycobacteriology was performed by Geoff de Lisle, and Gary Yates, AgResearch, Wallaceville.

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