The Contribution of Wild Mammals to the Epidemiology of Tuberculosis (*Mycobacterium bovis*) in New Zealand

A thesis presented
in partial fulfilment of the requirements for the degree of
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AMENDMENTS

Page	Paragraph	Line	Comment
iii	1	4	insert *study* after Castlepoint longitudinal
10	4	5	change *hypneumoniae* to *hyopneumoniae*
13	1	2	replace *suggests* with *suggest*
13	2	4	replace *varies* with *vary*
14	2	6	insert *oral* before *inoculation*
14	3	7	omit *that* following *reported*
15	1	1	replace *patch* with *patches*
15	1	3	insert *M. bovis* after *where*
20	2	10	omit the second *that*
21	2	4	replace *size range from* with *diameter range of*
42	1	3	insert *of* after favour
44	2	1	omit first *have*
46	2	6	omit *that*
69	2	7	omit *in*
74	1	3	replace second *as* by *than*
77	3	1	insert *the* after *in*
83	2	last	replace *yet* by *while*
101	3	last	delete *that*
102	1	5	replace *curious* with *inquisitive*
112	3	2	omit *are* after *always*
113	3	8	replace *enlargement* with *enlargements*
119	2	1	insert *Prior to this study (Chapter 7) the hedgehog had* at the start of sentence
121	3	5	replace *reports* with *instances*
126	2	3	insert *locations* after *several*
127	1	2	replace *mycobacteria* with *mycobacterial diseases*
139	1	8	replace *have* with *had*
158	2	5	insert *grossly* after *have*
178	1	1	insert *own offspring,* after *to*
185	1	13	insert *LTA positive* after *five*
212	2	5	replace *mature* with *older*
221			the tonsillar lesions depicted in Figures 6-1 and 6-2 represent normal crypt pathology found in both cattle and deer. Infection with *M. bovis* may be the cause of such lesions in some animals, but this cannot be determined grossly
301	3	2	detailed necropsies, consisting of post mortem examination, followed by isolation of *M. bovis* and/or the finding acid-fast organisms during histopathological examination, were considered to be the 'gold standard' by which the other diagnostic methods were evaluated
346	1	6	insert *subsequently* before *died*
352	5	4	replace *this* with *these*
352	5	6	replace *This data was* with *These data were*
408	1	2	insert *within a radius of* after *within*
414	3	11	replace *A latter two* with *Two later*
425	2	1	replace *challenges* with *challenge*
469	10	1	insert *in feral pigs* after *Mycobacterium bovis*

Note: Infected is used in the text to refer to an animal or tissue which contains viable pathogenic micro-organisms, whereas *diseased*, is used to denote a situation where organs or tissues have pathologic changes as a result of such infection. The adjective '*tuberculous*' specifically refers to pathologic conditions caused by tubercle bacilli.

"To question all things - never to turn away from any difficulties, to accept no doctrine either from ourselves or from other people without rigid scrutiny by negative criticism; letting no fallacy, or incoherence, or confusion of thought, step by unperceived; above all to insist upon having the meaning of a word clearly understood before using it, and the meaning of a proposition before assenting to it, these are the lessons we learn from the ancient dialecticians." - John Stuart Mill

Abstract

The objective of these studies was twofold. The primary aim was to gain a better understanding of the role of free-living mammalian species, other than possums, in the epidemiology of wildlife tuberculosis in New Zealand. The other objective was to continue the operation of the Castlepoint longitudinal so that hypotheses regarding the epidemiology of *M. bovis* infection in possums could be further refined and clarified.

Of the wild carnivores found in New Zealand, the disease persists at high prevalence only in ferrets, and is probably maintained principally by ingestion of tuberculous carrion. Although a moderate number of ferrets excrete *M. bovis* orally, there appears to be only minor intraspecific transmission by bite wounding. Although cats and stoats can also become infected through scavenging, they appear to be less susceptible to oral infection than ferrets. There is no substantial evidence to suggest that any of New Zealand's free-living carnivores are likely to be reservoir hosts of *M. bovis*.

Observational studies involving twelve domestic red deer suggested that cervids probably become infected through close inspection and investigation of moribund tuberculous possums, and that the likelihood of exposure to *M. bovis* was related to the curiosity and social ranking of the deer. Necropsies conducted principally on wild red deer and involving 152 animals provided evidence to suggest that significant bacillary excretion from infected deer was uncommon, and that only the few with advanced disease had the potential to be highly infectious. However, behavioural phenomena and disease characteristics preclude the ready transmission of disease amongst cohorts. There is now strong evidence to suggest that a high prevalence of tuberculosis infection in wild deer can only be maintained through contact with infected possums. However, deer may still be able to maintain the disease amongst themselves, albeit at a low prevalence, in the absence of infection in possums. This study also confirmed the importance of lymphoepithelial tissues, such as the oropharyngeal and nasopharyngeal tonsils, as primary sites for the establishment of *M. bovis* infection, and the subsequent excretion of organisms in deer.

The gross and histopathological appearance of the lesions found in six infected hedgehogs are described. It is likely that infection arose from the scavenging

behaviour of hedgehogs. The moderate prevalence (3.9%) of tuberculosis in these animals, combined with their small home ranges may allow them to be used successfully in wildlife surveys to pinpoint the locality in which tuberculous possums have died.

To gain an understanding of the potential role of wild pigs, goats, sheep, rabbits, hares, rats and mice in the dynamics of *Mycobacterium bovis* infection in free-ranging animals, numbers of these species were examined for evidence of infection. Of these, only the pig appears to have sufficient potential for intraspecific transmission to be of concern in tuberculosis control programmes. Sheep and goats appear to be simply spillover hosts, which may have a limited role in disease amplification following possible, but limited, intraspecific transmission. Rodents and lagomorphs are most unlikely to play any substantial role in the epidemiology of tuberculosis in New Zealand, under current circumstances.

A longitudinal study was established in 1989 to examine the disease behaviour in an infected possum population on a farm in the southern North Island of New Zealand, by trapping, using a fixed set of 295 traps for at least 3 days per month. Animals captured were examined at 2 monthly intervals for evidence of tuberculosis. During the first 5.5 years of this project over 900 individual possums were captured and tagged. Blood was collected from each possum examined, and the sera retained were stored frozen. Using these stored sera, three indirect ELISAs were evaluated as diagnostic tests for tuberculosis in possums. All ELISAs had low sensitivity when a cutoff selected to maximise the specificity was chosen. None of the ELISAs reliably detected possums infected with tuberculosis and they therefore have limited value for epidemiological studies. The lymphocyte transformation assays performed on blood taken from possums was estimated to have a sensitivity for detection of tuberculosis of approximately 80%, when the specificity was set at 99%. The lymphocyte transformation assay was the best of the *in vivo* tests evaluated, with the moderate sensitivity allowing it to be used with a degree of confidence to retrospectively diagnose disease, and aid the development of hypotheses regarding the epidemiology of tuberculosis in possums. The evaluated tests were applied retrospectively to sera and blood samples from possums from the Castlepoint longitudinal study. The additional data arising from these assays suggested that perhaps as few as one fifth of study site possums which had contact with *M. bovis*

had been previously detected as infected by clinical examination. A proportion of these test positive/examination negative animals may have been exhibiting resistance to *M. bovis* infection, and/or had resolved lesions or cryptic infection. Such animals may have formed a pool of possums in which future reactivation of tuberculosis was possible. The time from earliest evidence of infection till death, in those possums which showed clinical disease, varied from months to several years.

Cortisol assays performed on stored sera, and monitoring of trends in body weight, were used to investigate the role of stressful environmental phenomena in the epidemiology of tuberculosis in possums. Major stressful periods involving inadequate nutrition, heat, cold and moisture stress appear to precipitate severe tuberculosis outbreaks, which are believed to have their origins in the reactivation of subclinical/latent infection in the population. As the period of pre-clinical disease varies substantially, and can be as long as several years, this epidemic of tuberculosis takes several years to subside. Thereafter a small number of clinically diseased possums are likely to be restricted to "hot spots" conducive to transmission of *M. bovis.*

Isolates of *M. bovis* recovered from a variety of species, both wild and domestic, in the Castlepoint environs, and in particular the Castlepoint study site, were subjected to restriction endonuclease analysis to DNA fingerprint the strains present, and hence gain a better understanding of the inter- and intraspecific epidemiology of tuberculosis. The results do not challenge the accepted view of possums being the major reservoir hosts of tuberculosis in the Wairarapa. There was also no evidence to suggest that host adaptation of *M. bovis* has occurred, except in the case of possums, where they appear to be able to maintain clusters of individuals infected with particular restriction types, in microhabitats for at least 5 year periods. The occurrence of newly introduced restriction types has made possible new observations on the epidemiology of infection, including the documentation of the occurrence of latent infections, duration of primary progressive disease in newly infected possums (7-8 months), and the likely occurrence of post-primary reactivation of tuberculosis.

Acknowledgments

I was indeed fortunate to be offered, and able to take up the position of research officer in Professor Roger Morris's epidemiology group, as this step catapulted me headlong into a tough, but worthwhile struggle to enlarge my professional horizons, expand my interests in wildlife, and I hope, to make a significant contribution to solving the problem of bovine tuberculosis in New Zealand. I must first thank NSW Agriculture, for allowing me the opportunity to undertake these studies. To Roger Morris I am also truly grateful for this rare chance to conduct PhD studies under such fortunate circumstances. Under Roger's tutelage I have learned a great deal, and am wiser for the experience. To him I owe my thanks.

To Dirk Pfeiffer, my friend, mentor and co-supervisor, I am deeply indebted. He ably assisted the transition from raw novice with figures to one now competent to take on many statistical challenges. Dirk was often brimming with great ideas, enthusiasm and good cheer, when these were at low ebb in myself. He was prepared to listen to ideas in an impartial manner, despite them often being contrary to the "accepted wisdom" on tuberculosis. I miss his company and the daily debriefing chats we had on our bicycle rides home from the university.

My other supervisor, Associate Professor Peter Wilson, was instrumental in teaching me something of the husbandry and diseases of deer, topics of which I possessed complete ignorance before enrolling in the PhD studies. I thank Peter for his tuition, encouragement, friendship and prompt attention to my needs throughout my stay at Massey university.

To my colleagues, particularly Carola Sauter, and support staff, and especially those involved in the study of tuberculosis, I am indebted for their help and company on many occasions. There were many who contributed substantially in various ways, and these folk are acknowledged at the end of each chapter.

The management of the Castlepoint study site presented its share of difficulties, none insurmountable, but challenging none the less. Without the friendly co-operation of Ron Goile, manager of Waio station, and his partner, Donna Lewis, these problems may have been difficult to overcome. Their help was sincerely appreciated, as was their outstanding contribution to the longitudinal study.

To my family I am deeply indebted and sincerely appreciative of their support. They were separated from their father and husband for the first six months of the studies, dragged unwillingly across the Tasman to settle in a foreign urban environment and then uprooted again to return to Australia. Whilst in New Zealand they endured my prolonged absences, disappearances on weekends and late nights working on the thesis, with little complaint. I sincerely apologise for the neglect of family matters, and the time for which the PhD studies precluded me spending with my loved ones. I hope in the fullness of time that the completion of the studies will bring rewards which have a tangible benefit to my family.

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12/6/1997

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Table of Abbreviations and Units

Codes	Descriptions
%	Percentage
°C	Degrees celsius
µg	Microgram
µm	Micrometre
ACTH	Adrenocorticotrophic hormone
AFB	Acid-fast bacillus(i)
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
AVP	Arginine vasopressin
BALT	Bronchus-associated lymphoid tissue
B/A SI	Bovine to avian stimulation index
BCG	Bacille Calmette-Guérin
bcg^r	Bacille Calmette-Guérin resistant genotype
bcg^s	Bacille Calmette-Guérin susceptible genotype
BUN	Blood urea nitrogen
CALT	Conjunctiva-associated lymphoid tissue
CBG	Cortisol binding globulin
cfu	Colony forming unit
CI	Condition index
cm	Centimetre
CMI	Cell-mediated immunity
Con A	Concanavalin A
cpm	Counts per minute
CRH	Corticotrophin releasing hormone
CV	Coefficient of variation
Delta Con A	Concanavalin A stimulated, minus control counts in lymphocyte transformation assays
DF	Degrees of freedom
dl	Decilitre
DNA	Deoxyribonucleic acid
DR	Direct repeat elements
DSP	Deer slaughter premises
DTH	Delayed-type hypersensitivity
ELISA	Enzyme-linked immunosorbent assay
Eta squared	Approximation for the amount of variance explained by a term in a general linear model
F - value	Value of the F statistic in a general linear model
g	Gram
GALT	Gut-associated lymphoid tissue
GC	Glucocorticoid
GPH	Game packing house
H&E	Haematoxylin and Eosin stain
ha	Hectare
Hg	Mercury
HPA	Hypothalamo-pituitary-adrenal
HPF	High power microscope field
IFN-γ	Interferon gamma
Ig	Immunoglobulin

Table of abbreviations continued

Codes	Descriptions
IL	Interleukin
kg	Kilogram
km^2	Square kilometre
LH	Luteinising hormone
ln.	Lymph node
lnn.	Lymph nodes
LPF	Low power microscope field
LTA	Lymphocyte transformation assay
MAF	Ministry of Agriculture and Fisheries
MALT	Mucosa-associated lymphoid tissue
Max	Maximum
mg	Milligram
MHC	Major histocompatibility complex
Min	Minimum
mm	Millimetre
NK cell	Natural killer cell
ng	nanogram
NGL	No gross lesions
OR	Odds ratio
P or p	Probability
PAM	Pulmonary alveolar macrophage
PCR	Polymerase chain reaction
PGRS	Polymorphic GC-rich repetitive sequence
PHA	Phytohaemagglutinin
PIM	Pulmonary intravascular macrophage
pO_2	Oxygen tension
POMC	Proopiomelanocortin
PPD	Purified protein derivative
PWM	Poke weed mitogen
R	Correlation coefficient
R^2	Coefficient of determination
REA	Restriction endonuclease analysis
RNA	Ribonucleic acid
RFLP	Restriction fragment length polymorphism
s	second
SD	Standard deviation
SE	Standard error
SI	Stimulation index
SRBC	Sheep red blood cells
T_3	Triiodothyronine
T_4	Thyroxine
Tb	Tuberculosis
TbL	Tuberculous lesion
Th1	T - helper 1
Th2	T - helper 2
TNF	Tumour necrosis factor
ZN	Ziehl-Neelsen stain
95%CI	95% Confidence interval