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**PATHOGENESIS OF TUBERCULOSIS**  
**IN THE BRUSHTAIL POSSUM,**  
***TRICHOSURUS VULPECULA***



Hand-coloured steel engraving by W Jardine, 1843

A thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy at Massey University, Palmerston North, New Zealand

**Michèle Mary Cooke**

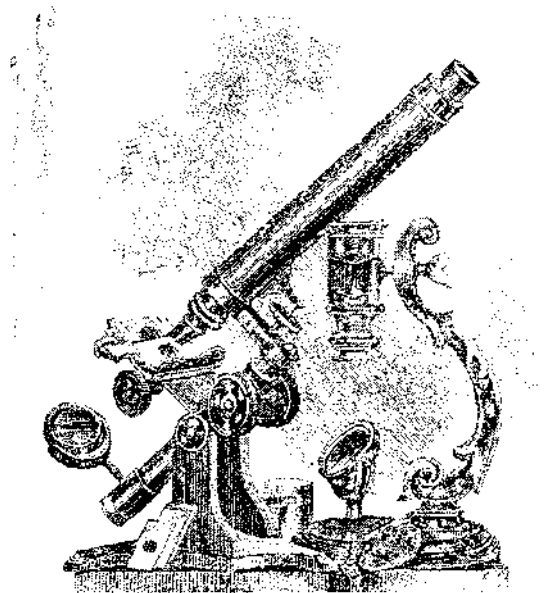
**2000**

“Too much reliance on the absence of macroscopic lesions has always constituted a source of error in pathological analysis.”

**Innes (1949)**

“Not invisible but unnoticed, Watson. You did not know where to look, and so you missed all that was important”.

**Sherlock Holmes**



Hogg J. The Microscope- its History, Construction, and Applications  
Herbert Ingram & Co., London, 1855

## ABSTRACT

The brushtail possum, *Trichosurus vulpecula*, is the main wildlife reservoir of *Mycobacterium bovis* infection for domestic species such as cattle and deer in New Zealand. Tuberculosis control and eradication are dependent on knowledge and understanding of the pathogenesis of the disease in this pest species, regardless of whether eradication or control of the disease by vaccination is contemplated.

Early field studies of tuberculosis in wild possums detected infected animals usually with advanced disease, and showed the two most common sites for macroscopic lesions were the respiratory tract and superficial lymph nodes. A comprehensive pathological study of the nature and distribution of lesions of naturally occurring tuberculosis which involved 117 non-terminally ill and 20 terminally ill possums was undertaken. Significantly more males (62%) than females (38%) were affected by the disease, and this is probably related to differences in behaviour. In non-terminally ill possums, the two most common sites for macroscopic lesions were the superficial lymph nodes (75%) and respiratory tract (69%). However, microscopic assessment of the distribution of total lesions disclosed 93% of lesions in superficial lymph nodes compared with 79% in the respiratory tract, indicating that the former are a predilection site for the establishment and development of lesions. This distribution raised the possibility that infection may occur via the percutaneous as well as the respiratory route. It was found that the disease disseminates early and rapidly via blood and lymph, and acid fast organisms increase in number in concert with increasing size and development of lesions.

In order to understand why lesions are common in the respiratory tract, a morphological study of the lung of the normal possum was undertaken. It revealed that the lung of the possum lacks a conventional mucociliary apparatus, a prime defence mechanism of the proximal airways against inhaled particles. However, this may be compensated for by the presence of Clara cells, which were abundant throughout the bronchial tree. Additionally, the lung appeared to be adequately supplied with mucosal associated lymphoid tissue. The lung of the possum may therefore be more susceptible to the deposition of particles larger than droplet nuclei into the airways than some other species.

Experimental respiratory infection with *M. bovis* involving the inoculation of 33 possums with 20-100 colony forming units (cfu) by the endo-bronchial route, and aerosol infection of 20 possums with 10-20 organisms, were completed over 4 and 5 week periods (respectively). This

allowed the study of the nature and development of pulmonary tuberculosis in possums, and comparison with the natural disease. Macroscopic lesions were largely confined to the respiratory tract, and at the microscopic level, there was a paucity of lesions in superficial lymph nodes, suggesting that in the natural disease percutaneous infection may be responsible for lesions in these nodes. A progression of lesion development from granulomatous through pyogranulomatous to large caseating lesions was observed. Rapid haematogenous and lymphatic spread occurred early in the experimentally induced disease. These findings confirm that the possum is highly susceptible to infection with *M. bovis*, and suggest that only an extremely small number of tubercle bacilli may be required to initiate the disease.

The results of experimental intra-dermal (I/D) inoculation of  $5 \times 10^6$  cfu of BCG injected into the dorsal midline of the neck of 38 possums were followed over a 4 week period. This produced evidence that infection through the skin is associated with lesions in superficial lymph nodes. Although overall 76% of experimental possums had lesions in superficial nodes, few animals (21%) had lesions in the lower respiratory tract. The phenomenon of lesion resolution restricts the use of BCG to the study of early lesion development, however it avoids problems with overwhelming disease encountered in experiments using *M. bovis*. Further work using a very low dose of *M. bovis* via the percutaneous route will be necessary to understand whether the I/D route of infection operates simultaneously or sequentially with infection via the respiratory tract.

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## LIST OF ABBREVIATIONS

AFOs	acid fast organisms
Anon.	Anonymous
BCG	Bacille Calmette-Guérin
°C	degrees centigrade/Celsius
cfu	colony forming units
cm	centimetre(s)
CO <sub>2</sub>	carbon dioxide
DNA	deoxyribonucleic acid
E/B	endo-bronchial
ed(s)	editor(s)
Edtn.	Edition
g	gram(s)
<b>g</b>	centrifugal force
G	gauge
GIT	gastrointestinal tract
h	hour(s)
H&E	haematoxylin and eosin
I/D	intra-dermal
I/M	intramuscular
I/N	intra-nasal
I/P	intra-peritoneal
I/T	intra-tracheal
I/V	intravenous
kg	kilogram(s)
L	litre(s)
lbs	pounds
LSD	Least Significant Difference test
MAFF	Ministry of Agriculture, Fisheries and Food
mg	milligram(s)
min.	minute(s)
mL	millilitre(s)
mm	millimetre(s)
No.	Number

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NVL	no visible lesions
P	Page
PALS	periarteriolar lymphoid sheath(s)
PBS	phosphate buffered saline
PCR	polymerase chain reaction
pers. comm.	personal communication
pers. obs.	personal observation
p.i.	post-inoculation
Pp	Pages
S/C	subcutaneous
spp.	species
TEM	transmission electron microscopy
µm	micrometre(s) (micron(s))
UV	ultraviolet
ZN	Ziehl-Neelsen