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

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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
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.
ACKNOWLEDGEMENTS

All the work was undertaken with approval obtained from the Animal Ethics Committees at Massey University, AgResearch (Wallaceville) and Landcare Research (Christchurch), for the research and experiments described in the thesis.

There are many people whose collective efforts have helped make this thesis possible. I have listed most of them in alphabetical order.

Scientists, without whose help and willingness to collaborate, field and experimental studies would not have been possible, include: Bryce Buddle (AgResearch, Wallaceville), for collaborative experimental M. bovis studies, and professional critique; Jim Coleman (Landcare Research, Lincoln), for collaborative field studies, discussion, advice, and encouragement; Phil Cowan (Landcare Research, Palmerston North), for the use of cage traps and cages with nesting-boxes; Geoff de Lisle (AgResearch, Wallaceville), for the provision of BCG for my experimental studies; and Dave McMurray (visiting scientist to AgResearch, Wallaceville), for collaborative experimental studies with M. bovis. I am also extremely grateful to Duncan Hedderley (Massey University), for assistance with statistical analyses.

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<table>
<thead>
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<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AFOs</td>
<td>acid fast organisms</td>
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<tr>
<td>Anon.</td>
<td>Anonymous</td>
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<td>BCG</td>
<td>Bacille Calmette-Guerin</td>
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<tr>
<td>°C</td>
<td>degrees centigrade/Celsius</td>
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<tr>
<td>cfu</td>
<td>colony forming units</td>
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<td>carbon dioxide</td>
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<td>gastrointestinal tract</td>
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<tr>
<td>H&amp;E</td>
<td>haematoxylin and eosin</td>
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<td>I/D</td>
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<td>I/M</td>
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<td>I/N</td>
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<td>I/T</td>
<td>intra-tracheal</td>
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<td>LSD</td>
<td>Least Significant Difference test</td>
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<td>MAFF</td>
<td>Ministry of Agriculture, Fisheries and Food</td>
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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