ASPECTS OF THE PATHOGENESIS OF
OVINE JOHNE’S DISEASE

by

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Declaration

Apart from the help acknowledged this thesis represents the unaided work of the author. The investigations presented here have not been presented for any other degree or diploma at any other university.

All research reported in this thesis which involves animals has been approved by the Elizabeth Macarthur Agricultural Institute’s Animal Ethics Committee.

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Summary

Johne’s disease is a chronic enteropathy of ruminants caused by infection with \textit{M. a. paratuberculosis}. Ovine Johne’s disease in Australia is an emerging disease and the National Ovine Johne’s Control and Evaluation Program, established in 1998, identified further research into ovine Johne’s disease in the Australian context as a priority. It is against this background that this PhD program was undertaken.

Alternative methods to end-point titration in liquid media for estimation of viable numbers of Australian ovine strains of \textit{M. a. paratuberculosis} were evaluated. A simple technique using cumulative growth indices in Bactec vials that was applicable to isolates from a variety of sources was developed. This allowed quantification of the loss in numbers which occurs during the routine decontamination procedures necessary for the isolation of \textit{M. a. paratuberculosis} from faeces and tissues. Storage of cultured suspensions at -80°C was shown to result in minimal loss of viable numbers. These techniques will facilitate future experimental infections and the efficient determination of numbers of \textit{M. a. paratuberculosis} in clinical and environmental samples.

In controlled pen trials, lambs were given low oral doses (10^7 to 10^8 viable organisms) of ovine strain \textit{M. a. paratuberculosis}. Successful infection was demonstrated by culture of tissues 2 to 4 months after the first dose. There were no associated lesions, but skin testing detected 66% of culture-positive lambs with 100% specificity. Significantly, this was the first demonstration of infection with ovine \textit{M. a. paratuberculosis} in Australian Merino sheep at low doses likely to be representative of natural infection. In follow-up field trials, culture of tissues collected at necropsy was shown to be the most sensitive method for the detection of early infection in flocks of sheep after natural exposure to ovine strains of \textit{M. a. paratuberculosis}. Antemortem diagnostic tests (skin testing, IFN-\gamma and faecal culture) were shown to have low sensitivity at this early stage of naturally acquired disease. These findings suggested that groups of naive sheep, used as tracer animals and tested by culture of tissues at slaughter after 6 months exposure, might be useful to assess pasture infectivity in disease control programs.
Immunoperoxidase labelling (for CD4, CD8, TCR-γδ, WC1, CD1b, IFN-γ, CD45R, CD56, lysozyme and *M. a. paratuberculosis*) was used to investigate changes in cell mediated immune effector cell populations in the intestine and associated lymph nodes in these early infections. Increased numbers of CD4⁺, TCR-γδ⁺ and WC1⁺ cells were demonstrated in the infected lambs, while a decrease in cells expressing CD1b was shown.

Polymorphisms at loci in a number of genes associated with immune function (NRAMP, MHC complex, IFN-γ, lysozyme, leukaemia inhibiting factor) were examined in two independent flocks of Merino sheep, each with a high prevalence of Johne’s disease. Possible associations of NRAMP and MHC alleles with resistance to Johne’s disease were detected.

**Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
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<tbody>
<tr>
<td>AFB</td>
<td>acid-fast bacilli</td>
</tr>
<tr>
<td>AGID</td>
<td>agar gel immunodiffusion test</td>
</tr>
<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>APC</td>
<td>antigen presenting cell(s)</td>
</tr>
<tr>
<td>BHI</td>
<td>brain heart infusion broth</td>
</tr>
<tr>
<td>BSA</td>
<td>bovine serum albumin</td>
</tr>
<tr>
<td>CFU</td>
<td>colony forming units</td>
</tr>
<tr>
<td>CGI</td>
<td>cumulative growth index/indices</td>
</tr>
<tr>
<td>CMI</td>
<td>cell-mediated immunity</td>
</tr>
<tr>
<td>DTH</td>
<td>delayed type hypersensitivity</td>
</tr>
<tr>
<td>EIA</td>
<td>enzyme immunoassay</td>
</tr>
<tr>
<td>ELISA</td>
<td>enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>EMAI</td>
<td>Elizabeth Macarthur Agricultural Institute</td>
</tr>
<tr>
<td>GI</td>
<td>growth index/indices</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>granulocyte macrophage colony stimulating factor</td>
</tr>
<tr>
<td>H&amp;E</td>
<td>haematoxylin and eosin</td>
</tr>
<tr>
<td>HPC</td>
<td>hexadecylpyridinium chloride</td>
</tr>
<tr>
<td>ICV</td>
<td>ileocaecal valve</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>gamma interferon</td>
</tr>
<tr>
<td>IL</td>
<td>interleukin</td>
</tr>
<tr>
<td>IPX</td>
<td>immunoperoxidase</td>
</tr>
</tbody>
</table>
IV intravenous(ly)
KW Kruskal-Wallis
LAM lipoarabinomannan
mAb monoclonal antibody
*M. a. paratuberculosis* *Mycobacterium avium* subsp. *paratuberculosis*
MB methylene blue (0.02% w/v)
MHC major histocompatibility complex
MLN mesenteric lymph node(s)
mRNA messenger RNA
NO nitric oxide
OD optical density
OJD ovine Johne’s disease
PCR polymerase chain reaction
PBMC peripheral blood mononuclear cell(s)
PBS phosphate buffered saline
PBST phosphate buffered saline containing 0.1% Tween-80
PP Peyer’s patch(es)
PPD purified protein derivative
REA restriction endonuclease analysis
SC subcutaneous(ly)
TCR T cell receptor
TI terminal ileum/ileal
TNF tumour necrosis factor
VAN vancomycin (100 µg/mL), nalidixic acid (100 µg/mL) and amphotericin B (50 µg/mL)
ZN Ziehl-Neelsen
# Table of contents

Acknowledgments ........................................................................................................ ii

Declaration ..................................................................................................................... iv

Summary ......................................................................................................................... v

Abbreviations ................................................................................................................. vi

Table of contents .......................................................................................................... viii

## Chapter 1. Introduction and literature review ..............................................................

1

### Introduction

........................................................................................................................................ 1

### Aetiology

........................................................................................................................................ 2

### Host and geographic range

........................................................................................................................................ 3

### Epidemiology

........................................................................................................................................ 5

  Transmission of infection between animals

  *Faecal excretion and oral infection*

  *Infection via the milk*

  *Congenital infection*

  *Venereal transmission*

  *Embryo transfer*

  Age and susceptibility to infection

  Introduction and development of infection in a herd or flock

  Strains of *M. a. paratuberculosis* – host specificity

### Pathology and clinical signs

........................................................................................................................................... 11
Clinical findings
Gross lesions
Histopathological lesions

**Diagnosis of *M. avium* subsp. *paratuberculosis* infection**

Detection of *Mycobacterium avium* subsp. *paratuberculosis*

- **Culture**
- **Molecular techniques**
- **Stained smears**
  - **Immunoperoxidase labelling of histological sections**

Detection of host immune responses

- **Humoral immune responses**
  - **Cell Mediated Immune responses**

Pathology – biopsies from live animals

**Control**

- **Vaccination**
- **Other management options**
  - **Establishing and maintaining herds free of infection**
  - **Culling of infected animals**
  - **Management of young stock**
  - **Selection of resistant animals**

Treatment of clinically affected animals

**Pathogenesis**

- **Overview**

Entry to the host - route of infection

Penetration of the mucosal barrier
Survival in macrophages
........................................................................................................... 28
Host immunological responses
........................................................................................................... 30

Innate immune responses

Specific adaptive immune responses
Progression to clinical disease
........................................................................................................... 34

The Th1 to Th2 shift in the immune response

Factors triggering clinical disease
Development of clinical signs
Humoral antibodies to M. a. paratuberculosis
Recovery
.................................................................................................................................
38

Chapter 2. Development of methods for the enumeration of ovine strains of
Mycobacterium avium subsp. paratuberculosis
.................................................................................................................. 40

Summary
General introduction
GENERAL METHODS
......................................................................................................................
............. 43

2.1. Evaluation of direct counts, spectrophotometry and colony counts on
solid media as alternatives to MPN estimation for the enumeration of M.
a. paratuberculosis (ovine strain)
....................................................................................................................... 45

Introduction and aims
Methods
Results
Discussion

2.2. The effect of 0.1% Tween-80 in serial dilutions used to provide MPN
estimates for M. a. paratuberculosis (ovine strain)
....................................................................................................................... 54

Introduction and aims
Methods
Results
Discussion

2.3. Use of cumulative growth indices in Bactec vials for the enumeration of ovine strains of \textit{M. a. paratuberculosis} .................................................. 57

Introduction and aims
Methods
Results
Discussion

2.4. The effect of storage 4 oC, -20 oC or -80 oC on the survival of cultured \textit{M. a. paratuberculosis} in PBST suspensions ........................................ 70

Introduction and aims
Methods
Results
Discussion

2.5. The effect of decontamination protocols on the numbers of \textit{M. a. paratuberculosis} of ovine origin isolated from tissues and faeces ................................. 75

General discussion ............................................................................................................ 83

Chapter 3. Experimental infection of weaner lambs with \textit{M. avium} subsp. \textit{paratuberculosis} – pilot study in housed sheep ................................................. 85

Summary
Introduction and aims
Methods ................................................................................................................................
86
Experimental design
Animals
Inocula
Clinical and necropsy sampling
\textit{M. a. paratuberculosis} isolation
Histopathology
Serology for antibodies to \textit{M. a. paratuberculosis}
Intradermal testing for delayed hypersensitivity
Gamma interferon assay
Results ................................................................................................................................
89
Quantification of \textit{M. a. paratuberculosis} dose
Culture of tissues for \textit{M. a. paratuberculosis}
Faecal culture
Gross pathology
Histopathology
Serology for antibodies to \textit{M. a. paratuberculosis}
Intradermal testing for delayed hypersensitivity
IFN-\(\gamma\) assay
Chapter 4. Early detection of natural infection of sheep with *Mycobacterium avium* subsp. *paratuberculosis* .......................................................... 97

**Summary**

Introduction and aims

Methods

Experimental design

Intradermal testing for delayed hypersensitivity (DTH)

Gamma interferon (IFN-γ) assay

Necropsy sampling

*M. a. paratuberculosis* isolation

Histopathology

**Results**

Overview of findings on Farm H

Overview of findings on Farm A

Culture of *M. a. paratuberculosis*

Intradermal testing for delayed hypersensitivity

IFN-γ

Histopathology

**Discussion**

...................................................................................................................... 109

Chapter 5. Cell mediated immune effector cell populations in early *Mycobacterium avium* subsp. *paratuberculosis* infection in sheep ............ 115

**Summary**

Introduction and aims

Methods

Animals and experimental design

Antibodies for immunoperoxidase labelling

Immunoperoxidase labelling

Microscopic examination

Statistical analysis

**Results**

CD4

CD8

TCR-γδ

WC1

CD1b
Chapter 6. A preliminary study of possible genetic influences on the susceptibility of sheep to Johne's disease ................................................................. 140
  Summary
  Introduction and aims
  Methods
  .......................................................................................................................... 142
  Experimental flocks
  Assessment of clinical signs
  Serology for antibodies to M. a. paratuberculosis
  Intradermal testing for delayed hypersensitivity
  Necropsy sampling
  M. a. paratuberculosis isolation
  Histopathology
  Phenotypic classification of sheep
  Candidate genes
  DNA extraction
  PCR
  Statistical analysis
  Results
  .......................................................................................................................... 146
  NRAMP alleles
  MHC complex
  Lysozyme alleles
  IFN-γ alleles
  Leukaemia inhibiting factor alleles
  Discussion
  .......................................................................................................................... 154

Chapter 7. General discussion and conclusions .................................................. 159

References .......................................................................................................... 167

Appendices to Chapter 3
  ......................................................................................................................... 193
  Appendix 3a. Log_{10} direct counts of M. a. paratuberculosis compared to Log_{10} MPN estimates of viable organisms in suspensions used to prepare experimental inocula
Appendix 3b. Intended and actual doses of *M. a. paratuberculosis*
Appendix 3c. Culture results, tissues collected at necropsy
Appendix 3d. Quantitative culture results, tissues collected at necropsy
Appendix 3e. Faecal culture results from fortnightly samplings
Appendix 3f. Faecal culture results from daily collections
Appendix 3g. Parachek ELISA results
Appendix 3h. Skin test results
Appendix 3i. Gamma-interferon results, OD (Avian PPD) - OD (PBS)

Appendices to Chapter 4
................................................................................................. 205
Appendix 4a. Tracer weaner field evaluation. Farm H, Year 1
Appendix 4b. Tracer weaner field evaluation. Farm H, Year 2
Appendix 4c. Tracer weaner field evaluation. Farm A

Appendices to Chapter 6
................................................................................................. 219
Appendix 6a. Summary of phenotypic assessment and genotype of individual sheep from Flock A
Appendix 6b. Summary of phenotypic assessment and genotype of individual sheep from Flock B