The application of immunological assays for the monitoring and diagnosis of selected infectious diseases, with particular emphasis on neosporosis

by

## Michael P Reichel

Submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

at the

University of Technology, Sydney

2002

Dedicated to Kristin, Robin and Tim: for their patience
SAs for Parasitologists: or Lies, Damned Lies and ELISAs"
(P. Venkatesan and D. Wakelin, Parasitology Today 9, 228-232)

## **CERTIFICATE OF AUTHORSHIP & ORIGINALITY**

I certify that the work in this thesis has not been previously submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged in the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

Signed

# **TABLE OF CONTENTS**

CERTI	FICATE OF AUTHORSHIP & ORIGINALITY	iii
TABLE	OF CONTENTS	iv
ACKNO	OWLEDGMENTS	vi
ABSTR	ACT	vii
1.	INTRODUCTION	8
1.1.	General considerations about immunological assays (validation, performance characteristics).	8
1.2.	Paratuberculosis (Johne's disease)(Mycobacterium avium spp. paratuberculosis)	13
1.3.	Brucellosis (Brucella ovis)	30
1.4.	Enzootic Bovine Leukosis (Bovine leukaemia virus)	39
1.5.	Discussion and Conclusions	47
1.6.1. 1.6.2. 1.6.3. 1.6.4. 1.6.5.	Neosporosis (Neospora caninum)  Identification  Presence and disease in dogs  Discussion and Conclusions  Presence and disease in cattle  N. caninum in Australia and New Zealand	51 63 65
1.7. 1.7.1. 1.7.2. 1.7.3.	Serological diagnosis of neosporosis Introduction The situation in New Zealand Discussion and Conclusions	72 79
1.8.1. 1.8.2. 1.8.3. 1.8.4.	Sero-epidemiology Sero-prevalence Discussion and Conclusions Case studies and Transmission patterns Discussion and Conclusions	99 105 106
2.	CONCLUSIONS	147
3.	CONFERENCE CONTRIBUTIONS	149
4.	BIBLIOGRAPHY	153
5	APPENDICES	175

## **AUTHOR'S PUBLICATIONS CITED IN THIS THESIS**

Comparison of serological tests, immunoblot and faecal culture for the detection of <i>M. avium</i> spp.
paratuberculosis infection in cattle and analysis of the antigens involved
Performance of an enzyme-linked immunosorbent assay for the diagnosis of Brucella ovis infection in
rams
Evaluation of electrophoretic immunoblotting for Brucella ovis infection in deer using ram and deer
serum
Evaluation of alternative methods for the detection of Bovine Leukaemia virus in cattle
Prevalence of serum antibodies to <i>Neospora caninum</i> in different canid populations53
Neosporosis in a pup
Neospora caninum infections in Australia and New Zealand
Progress in the Serodiagnosis of <i>Neospora caninum</i> Infections in Cattle
The diagnosis of <i>Neospora</i> abortions in cattle
An analysis of the performance characteristics of serological tests for the diagnosis of <i>Neospora caninum</i>
infection in cattle87
Prevalence of Neospora antibodies in New Zealand dairy cattle and dogs
Prevalence of <i>Neospora</i> antibodies in beef cattle in New Zealand
Serology of a <i>Neospora</i> abortion outbreak on a dairy farm in New Zealand: A case study108
Bovine neosporosis: comparison of serological methods using outbreak sera from a dairy herd in New
Zealand
A longitudinal study of <i>Neospora caninum</i> infection on a dairy farm in New Zealand123
Control options for <i>Neospora caninum</i> infections in cattle – current state of knowledge

#### ACKNOWLEDGMENTS

Thanks go to a number of collaborators, both in New Zealand and Australia but also further afield, without whom the body of work that is presented in this thesis could not have been accomplished. It is thus totally appropriate that a large number of the papers presented here are collaborative ones, with the important contributors mentioned as co-authors. It has been extremely enjoyable and educational for me to be working with them and to be able to unravel a New Zealand piece of the Neospora story with them.

Thanks are also due to Prof John Ellis who has been a willing collaborator on a number of recent papers and been a constant source of encouragement in this sometimes-wearisome course towards the final thesis. I am particularly grateful to him for maintaining the contact across the globe, via Email and telephone.

I am grateful to Drs JE Peel and T Friedel for valuable comments on a number of the manuscripts and to Profs SK Hietala, A Hemphill and C Wilks for critically reviewing the thesis.

Lastly thanks to my family, Kristin, Robin and Tim for putting up with my spending family time on yet another dissertation.

#### **ABSTRACT**

The 16 publications presented in this thesis summarise the author's contribution to seroepidemiological approaches for the diagnosis and monitoring of animal diseases of importance to New Zealand.

The first four publications not only contribute to the above in relation to three important animal pathogens, namely Brucella ovis, Mycobacterium avium spp. paratuberculosis and Bovine Leukaemia Virus but also give an insight into more general consideration associated with the optimisation and validation of serological assays, namely regarding the definition and choice of gold standard reference sera, the determination of the cutoff threshold and discrimination between negative and positive reference populations. Two further publications deal with the establishment and validation of serological assays for the diagnosis of *Neospora caninum* infection and abortion in New Zealand. Then, baseline data were obtained for the sero-prevalence of the infection in dog and cattle populations in New Zealand. Three case studies provided initially information about the kinetics of serological responses after a N caninum abortion outbreak, and information about the usefulness of herd-based techniques rather than individual cowbased abortion diagnoses. A further study provided some early information about the mode of transmission seemingly predominating in New Zealand, which tends to be mainly via post-natal infection, in contrast to evidence provided by overseas researchers. A final case study, a longitudinal study of serological and other responses over a period of three years also provided data on the production effects of N caninum. The dissertation is completed by a number of reviews on sero-diagnosis of N caninum infection, its presence in Australasia and suggests finally control options, based on the present state of knowledge.