Rickettsiales and rickettsial diseases in Australia

Leonard Heinz Izzard

Bachelor of Applied Science (Honours)

This thesis is presented for the degree of Doctor of Philosophy of Murdoch University

2010
Declaration

I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary institution.

…………………………

Leonard Heinz Izzard
Abstract

Currently, there are 12 known *Rickettsiales* species in Australia. However, research into the diversity and range of these agents in Australia is still far from complete.

A sero-epidemiological study was undertaken around the city of Launceston in Tasmania, Australia to determine the level of exposure to spotted fever group (SFG) rickettsiae among the local cat and dog population. The study showed that over 50% of the dogs and cats tested were positive for SFG rickettsiae antibodies. However, no correlation was observed between the animals’ health and seropositivity at the time of testing.

*Ixodes tasmani* ticks collected from Tasmanian devils in Tasmania were tested for the presence of SFG and typhus group (TG) rickettsiae using a specific real-time PCR (qPCR), and 55% were found to be positive. The *gltA*, *rompA*, *rompB* and *sca4* genes were then sequenced. Using the current criteria this new rickettsia qualified as a *Candidatus* species, and was named *Candidatus* Rickettsia tasmanensis, after the location from which it was first detected.

Soft ticks of the species *Argas dewae* were collected from bat roosting boxes north of Melbourne. Of the ten ticks collected, seven (70%) were positive for SFG rickettsiae using the qPCR mentioned above. An isolate was obtained using cell culture isolation methods and the *rrs*, *gltA*, *rompA*, *rompB* and *sca4* genes were sequenced. Using the current criteria this new rickettsia qualified as
a novel species, and was tentatively named *Rickettsia argasii* sp. nov. after the tick genus from which it was isolated.

Four family members and their neighbour living in metropolitan Victoria became ill after exposure to a flea-infested kitten. Initial serological analysis indicated a typhus group (TG) rickettsial infection. However, testing of fleas from the group of cats in Lara, Victoria, where the kitten originated, revealed the presence of *R. felis*, the agent of cat flea typhus. This was the first case of human infection with *R. felis* in Australia and the first detection of *R. felis* in fleas in Victoria.

A tourist returning to Australia from the United Arab Emirates was diagnosed with a scrub typhus group (STG) rickettsial infection and the agent was isolated from their blood. Analysis of the *rrs* and *47kDa* genes showed significant divergence compared to all available strains of *Orientia tsutsugamushi*. Due to the degree of genetic divergence and the geographically unique origin of this isolate it was considered to be a new species, which has been tentatively named *Orientia chuto*, with ‘chuto’ being Japanese for ‘Middle East’.

Dogs in central and northern Australia were tested for *Anaplasma platys* using a specifically designed real-time PCR (qPCR) assay. Of the 68 dogs tested, 27 (40%) were positive for *A. platys* DNA, including six dogs from Western Australia. This was the first report of *A. platys* in Western Australia.

These studies offer an insight into the range and diversity of Rickettsiales and rickettsial diseases previously unrecognised in Australia.
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Acknowledgements

I would first like to thank my supervisors A/Prof. John Stenos and A/Prof. Stephen Graves from the Australian Rickettsial Reference Laboratory, and Prof. Stan Fenwick from the School of Veterinary and Biomedical Science, Murdoch University, for their patience and support over the previous few years. I would especially like to thank John and Stephen for offering their experience and ideas when I was problem solving and for reviewing my drafts over the years. I would also like to thank the Australian Rickettsial Reference Laboratory and Murdoch University for their financial support throughout my candidature.

Within the Australian Rickettsial Reference Laboratory I would first like to thank Chelsea Nguyen for teaching me serological and culture methods and Michelle Lockhart for her support in assisting me to brainstorm. Furthermore, I would like to thank all of the staff at the Australian Rickettsial Reference Laboratory for their support and friendship.

I would also like to thank collaborators and colleagues who have assisted me with most of my chapters. These include for chapter three, Helen Owen for providing the cat control sera. For chapter 4, I would like to thank Ian Norton, his colleagues, and Dydee Mann for collecting ticks from sites in Tasmania. I would also like to thank Ian Beveridge for his assistance with tick speciation. For chapter five, I would like to thank Lisa Evans for supplying me with the initial tick samples and contacts, and Natasha Schedvin, Robert Bender, Marissa Izzard and Stephen Weste for assisting me with collecting ticks. For chapter six,
I would like to thank Molly Williams and Julian Kelly for bringing this interesting case to my attention and for supplying me with the case study. I would also like to thank Aminul Islam for his assistance with attempted isolation. For Chapter seven, I would like to thank Andrew Fuller for bringing this case to my attention and supplying the case study. As well, I would like to thank Stuart Blacksell, Daniel Paris, Al Richards, Nuntipa Aukkanit, and Ju Jiang for their assistance with sequencing various genes and Chelsea Nguyen for her assistance in culturing this isolate. Finally, for chapter eight, I would like to thank Graeme Brown for supplying me with dog blood samples used in this study.

On a personal note I would like to thank all of my family including, Mum and Dad, Debbie, Steve, David, Sue and my sisters Jo and Bianca. They may not have always known what my projects entailed, nevertheless they always supported me and had faith that I would succeed.

Most of all I would like to thank my new wife Marissa Izzard (née McCaw) for her support throughout not only my PhD but my entire University education. She has been my rock when I was lost for direction and was always there to show me the positives when I was feeling negative. I would also like to thank her for helping to support me financially over my schooling life and for looking at more drafts than I can remember.
Preface

The work in this thesis is my own research. Collaborating authors mentioned in chapters 3 were mainly responsible for sample collection and manuscript revision prior to submission, while in chapter 4, collaborating authors were primarily responsible for manuscript revision prior to submission. The contributions of all other parties are mentioned in the acknowledgments section of this thesis.

Original manuscripts


Izzard L, Stenos J, Fenwick S, Graves S. Rickettsiales and rickettsial diseases in Australia. In Preparation for submission to Annals of the ACTM.

Original published abstracts


Izzard L, Fuller A, Blacksell S, Paris D, Aukkanit N, Graves S, Fenwick S, Nguyen C, Day N, Stenos J. Isolation of a highly variant Orientia sp. from a patient returning from Dubai – **Poster presentation at the 5th International Conference on Rickettsiae and Rickettsial Diseases, Marseille, France (2008)**

Izzard L, Williams M, Kelly J, Graves S, Fenwick S, Stenos J. First cases of *Rickettsia felis* (cat flea typhus) in Australia - **Poster presentation at Murdoch University WA (2009)**

**Co-authored manuscripts**


Co-authored published abstracts


Abbreviations

AG – Ancestral group

AGRF – The Australian Genomic Research Facility

ASF – Australian spotted fever

ATF – Australia tick fever

BHQ-1 – Black hole quencher-1

Bp – Base pairs

CCFM – Cell culture freezing media

CF – Complement Fixation

C_i – Threshold cycle

DNA – Deoxyribonucleic acid

dNTP – Deoxyribonucleic triphosphate

EDTA – Ethylenediaminetetraacetic Acid

ELISA – Enzyme-linked immuno sorbent assay

FISF – Flinders island spotted fever

FITC – Fluorescein isothiocyanate

gltA – Citrate synthase gene

HEPES – 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
IFA – Immunofluorescent assay
kDa – Kilodalton
NCBI – National Centre for Biotechnology Information
NSW – New South Wales
NTC – No template control
OPNP – Organ Pipes National Park
PBMC – Peripheral Blood Mononuclear Cells
PBS – Phosphate buffered saline
PCR – Polymerase chain reaction
QLD – Queensland
qPCR – Real-time PCR
QTT – Queensland tick typhus
RBC – Red blood cell
RNA – Ribonucleic acid
rompA – Rickettsial outer membrane protein A gene
rompB – Rickettsial outer membrane protein B gene
RPM – Revolutions per minute
rRNA – Ribosomal RNA.
rrs – 16S ribosomal RNA gene
sca4 – The rickettsial gene D / PS–120 gene

SDS – Sodium dodecyl sulphate

SFG – Spotted fever group

TAE – Tris-acetate-EDTA

TG – Typhus group

Tm – Melting temperature

TRG – Transitional group