DETECTION AND CHARACTERIZATION OF
RICKETTSIAE IN WESTERN AUSTRALIA

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This thesis is presented for the degree of Doctor of Philosophy of Murdoch University, 2007.
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 education institution

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The aim of this study was to address the shortfall in current, in-depth knowledge of Western Australian rickettsiae. Historically, murine typhus had been extensively reported and, more recently, serological studies and a small number of diagnosed cases indicated that spotted fever group rickettsiae were also present in the State, however no attempts had been made to isolate or characterize these rickettsiae.

To facilitate investigation, ectoparasites (principally ticks) were opportunistically collected from across the State, with an emphasis on native and feral animals and people. All ectoparasites were screened for rickettsial infection using a polymerase chain reaction incorporating \textit{Rickettsia}-specific citrate synthase gene (\textit{gltA}) primers. Preliminary sequencing was performed on representative PCR-positive samples from each geographical location, vertebrate host and ectoparasite in order to identify and characterize the infecting rickettsia. Isolation in cell culture and further genotypic characterization was then performed. Finally, a serosurvey and questionnaire were implemented in one of the study areas to determine whether people were being infected with a \textit{Rickettsia} spp. and whether infection was associated with clinical signs.

Ectoparasite collection produced three genera of ticks (\textit{Ixodes}, \textit{Amblyomma} and \textit{Haemaphysalis}) from native animals, feral pigs and people, primarily from the southwest of Western Australia and Barrow Island in the Pilbara region. Ticks from a number of sources were shown to be infected with rickettsiae by the PCR, including feral pigs, people, bobtail lizards, kangaroos, bandicoots, burrowing bettongs,
common brushtail possums and yellow-footed antechinus. Genotypic characterization of positive amplicons from ticks revealed the presence of two novel spotted fever group rickettsiae. *Rickettsia gravesii* sp. nov., named in honour of Dr Stephen Graves, was identified extensively throughout the southwest of the State and on Barrow Island in *Ixodes, Amblyomma* and *Haemaphysalis* spp. ticks from multiple hosts. *Candidatus* “Rickettsia antechini” was detected in *Ixodes* spp. only from yellow-footed antechinus in Dwellingup. In addition, a novel *Bartonella* spp. (*Bartonella* sp. strain Mu1) was also detected from *Acanthopsylla jordani* fleas collected from yellow-footed antechinus in Dwellingup.

*Rickettsia gravesii* sp. nov. is most closely related to the *Rickettsia massiliae* subgroup of the spotted fever group and to *R. rhipicephali* in particular. Sequence similarities between this novel species and the subgroup were 99.7%, 98.4%, 95.8% and 97.4% based on its 16S rRNA, *gltA*, *ompA* and *ompB* genes respectively. *Candidatus* “Rickettsia antechini” also demonstrated a close relationship to the *R. massiliae* subgroup (99.4%, 94.8% and 97.1% sequence similarity based on its *gltA*, *ompA* and *ompB* genes respectively). The two novel Western Australian species demonstrated 98.4%, 96.3% and 96.7% sequence similarity to each other based on *gltA*, *ompA* and *ompB* genes respectively indicating separate species. The novel *Bartonella* spp. (*Bartonella* sp. strain Mu1) detected in fleas collected from yellow-footed antechinus in Dwellingup demonstrated greatest *gltA* gene sequence similarity to *Bartonella* strain 40 at 86.1%.

Results from the serosurvey and questionnaire-based investigation into the zoonotic importance of *R. gravesii* sp. nov. on Barrow Island supported the results of the tick
study and suggested that a tick-borne rickettsia(e) was infecting people on the island. However, a significant association between seroconversion and a history of symptoms consistent with a rickettsiosis was not found, and it is possible therefore, that *R. gravesii* sp. nov. produces only asymptomatic infections.

Future work on rickettsiae in Western Australia will involve phenotypic characterization of the novel species, further investigation of their epidemiology and pathogenicity and an ongoing search for additional undiscovered species.
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I had a lot of help. Thank you to my principal supervisor, Associate Professor Stan Fenwick for obtaining funding, recruiting collaborators and for his infectious enthusiasm for the project. Thanks also to my Murdoch co-supervisors, Associate Professor Ian Robertson and Associate Professor Phillip Clark. Ian agreed to help despite being a self-confessed molecular-biology sceptic, his presence on my supervisory panel was a constant source of reassurance and thanks to Phil especially for his perceptive and often amusing editing of my thesis. Thanks also to Dr Angus Cook, School of Population Health, UWA who guided me closely through the analysis of the questionnaire and Yazid Abdad, my fellow PhD candidate who kindly allowed me to use his serology results in my analysis.

I am also eternally thankful to everyone at The Australian Rickettsial Reference Laboratory. Thanks to my co-supervisor Dr John Stenos, colleague Dr Nathan Unsworth and Chelsea Nyugen for their extensive knowledge of all things rickettsial. John did a fantastic job of interstate supervising and Nathan performed much of the initial culturing of the Western Australia rickettsiae while trying to pass on some of his formidable skills to me. Thank you also to Dr Stephen Graves, the laboratory’s director, who is such a knowledgeable and willing collaborator and who provided such a catchy name for our novel rickettsia. Thank you to Gorgon, in particular Russell Langdon for their interest in the project and for contributing a great deal towards its funding.

Thank you to my wonderful friend Patchara Phuektes who introduced me to the foreign world of the laboratory with unlimited patience. Thank you to Russ Hobbs for helping me identify my ectoparasite samples and also to Dr Andrew Mikosa, Dr Peter Spencer and Dr Ryan Jeffries who kindly helped me with the phylogenetic component
of my project. Dr Peter Adams also provided a lot of helpful advice. Thank you to Dr Liam O’Connor, Dr Pierre-Edouard Fournier and Professor Didier Raoult for allowing me to spend time in their laboratories.

Scientists from the Department of Environmental Conservation (formerly CALM) especially Keith Morris and Tony Start, Kanyana Wildlife Rehabilitation Centre, Damien Cancilla, Roberta Bencini, Harriet Mills and John Drummond were kind enough to provide me with samples and thank you to Rod Armistead, Maggie Lilith and Felicity Donaldson for letting me come on some very entertaining fieldtrips as well.

I’m fairly certain that I couldn’t have gotten through this without all the moral support and welcome distractions provided by my lovely friends Peter Wai’in and Maggie Lilith. Thank you also for the companionship provided by my office mates: Kim, Danka, Celia, Jordan and Nico, who usually let me win the war over the air conditioner controls.

Thank you to my extra-curricular friends Karen, Emma, Leigh, Emma and Russell, Megs and everyone in the UQ veterinary pathology department for not having the first idea about what rickettsiae are and thank you to Judy and Jan for looking after me while I was working in Victoria.

The largest chunk of my gratitude must go to my family for their unflagging support, especially my Mum who can always lessen the high drama that seems to accompany all my life’s endeavours.