

**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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## ABSTRACT

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 *Rickettsia*-specific citrate synthase gene (*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 *Rickettsia* spp. and whether infection was associated with clinical signs.

Ectoparasite collection produced three genera of ticks (*Ixodes*, *Amblyomma* and *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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