

An Epidemiological and Serological Study of *Rickettsia* in Western Australia

Presented By

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Submitted in total fulfilment of the requirement of the degree of

Doctor of Philosophy

School of Veterinary and Biomedical Sciences,
Division of Health Sciences,
Murdoch University

November 2011

Declaration

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

This thesis is less than 100,000 words in length, inclusive of tables, figures, bibliography and appendices, and it complies with the stipulations set out for the degree of Doctor of Philosophy by Murdoch University.

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Abstract

The study was aimed at investigating Western Australian rickettsiae, delving deeper into the epidemiology of a recently described rickettsia, *Rickettsia gravesii*, and any other rickettsiae lurking in the Western Australian bush. Prior to the discovery of *R. gravesii*, only *Rickettsia typhi* was known to be endemic to Western Australia. With the addition of *R. gravesii* and another novel rickettsia named candidatus “*Rickettsia antechini*”, understanding and investigation of the potential effects these organisms may have on human and animal health becomes paramount. This research adds to the limited information available in the literature pertaining to Australian rickettsial organisms.

Serotyping of *R. gravesii* in mice demonstrated distinct specificity differences between *R. gravesii*, *Rickettsia massiliae*, *Rickettsia australis* and *Rickettsia honei*. *R. massiliae* was chosen due to *R. gravesii*'s very close genotypic similarities to the *R. massiliae* sub-group. *R. australis* and *R. honei* were chosen for the possibility of them sharing a similar geographical distribution with *R. gravesii*.

Collection of ectoparasites, in particular ticks, from humans and larger mammals in south-west Western Australian bush was performed to investigate the prevalence of *R. gravesii* in tick populations and also to investigate any other rickettsial organisms that may be present. No other rickettsiae were uncovered except for *R. gravesii* among ixodid ticks collected. Four tick species collected during the course of the study harboured *R. gravesii* with prevalences of up to 15% in *Amblyomma albolimbatum* (n=45), 75% in *Amblyomma triguttatum* (n=187), 51% in *Ixodes australiensis* (n=63) and 25% in *Ixodes feicalis* (n=8). No rickettsial DNA was detected in *Haemaphysalis*

longicornis, though only a single specimen was found. Transovarial transmission of *R. gravesii* in *A. triguttatum* ticks from infected females to viable larvae was observed by PCR. Detection of *R. gravesii* in the salivary glands, ovaries and gut suggests the potential of horizontal, transovarial and transtadial transmission.

Feral pigs (n=148) trapped within the water-catchment areas for Perth were tested for SFG rickettsiae and a prevalence of 49% was observed by microimmunofluorescence at titres of 1:128 and higher. A baseline control group made up of domestic farmed pigs (n=67) was used to determine the cut-off titre (1:128) where no non-specific antigen-antibody detectable by microscopy was observed.

Sero-prevalence in humans was also investigated. Volunteers that are exposed to ticks as a result of occupational and recreational activities were recruited over a 3 year period. Sera were collected and a questionnaire asking about participants' bush activities was filled out. Two occupational groups were involved in the study, workers from Barrow Island (n=67) and Whiteman Park (n=12). The recreational group was made up of members of the Western Australian Rogaining Association (n=104). The control group used in the study was made up of volunteers among the staff and students of Murdoch University (n=60). Rickettsial prevalence was observed at higher levels in the occupational groups than recreational group participants; 44.8% in Barrow Island workers, 50% in Whiteman Park staff, 23.4% in rogainers and 1.7% in the control group.

Analysis of the questionnaires filled out by participants from the human sero-prevalence study demonstrated a significant association between bush activity and exposure to rickettsial organisms and their vectors. A follow-up of volunteers 10-14

months after the first serum collection from Whiteman Park staff (n=8) and among the rogainers (n=62) showed no significant rates of seroconversion or seroreversion.

Odds ratio analysis (adjusted for age and gender) based on questionnaire results and sero-prevalence of rickettsial antibodies in the different interest groups and control cohort showed that the recreational group had an odds ratio of 13.70 (95% CI=1.73-108.49). Odds ratio for the occupational group is 38.81 (95% CI=5.02-300.34). This shows a significant risk for people performing activities in the bush for extended periods of time, with occupational activities posing the highest risk, potentially due to the longer hours compared to recreational activities in general.

The presence of *R. gravesii* in Western Australia and the data reported in this study underscores the lack of information available to public health authorities with regards to rickettsial organisms and their hosts. Even though no official reports of debilitating spotted fever infections have been reported in WA in the literature thus far, the potential of it happening cannot be disregarded. Increased awareness of rickettsial organisms and infection need to be instilled among public health officials, doctors and veterinarians. Other animal populations and parts of Western Australia not covered in this study need to be further investigated.

Acknowledgements

First and foremost, I would like to acknowledge and express my deep appreciation towards my three supervisors. Professor Stan Fenwick, whose ideas and enthusiasm had inspired me from the beginning and sticking by me from start to finish. Professor Cassandra Berry, who had sparked my interest in immunology and all things infectious. Professor Ian Robertson, who spent countless hours at the start introducing epidemiological research to me.

I am eternally grateful to Professor Stephen Graves, A/Professor John Stenos and Ms Chelsea Nguyen of the Australian Rickettsial Reference Laboratory, VIC, who in no small part had given this study the needed resource support in the form of laboratory space, culturing facilities, rickettsial strains and validation of my results. They have stuck by me from start to finish.

My sincerest gratitude to Professor Angus Cook of the University of Western Australia and Dr John Dyer of Fremantle Hospital had given me numerous crucial advice and assistance on the direction of my research into the clinical and epidemiological aspect of Rickettsiology.

I am extremely grateful to Professor Pierre-Edouard Fournier of the Unite des Rickettsies, Marseille, for the time and effort he had put in to introduce me to the field of Rickettsiology from the early stages of my research.

Dr Stuart Blacksell of the Wellcome Trust were kind enough to lend their expertise and host a significant portion of my experiments in their BSL3 laboratory and animal

holding facilities at Mahidol University in Bangkok, Thailand, for which I am most appreciative.

My sincerest gratitude to Dr Peter Adams and Andrew Li for the collection of feral pig and tick samples from the bush and allowing me to use them. Also the rest of the Trailer Trash, an international group of “rejects” thrown out into a miserable corner of campus, which fortuitously is a couple of steps from the best and cheapest food on campus. Thank you, for all the light banter, international food, friendship and support you shared with me.

I am extremely grateful to the many study volunteers who helped by contributing their blood and time to allow the completion of a significant portion of my thesis. My sincerest thanks to Dr Helen Owen for allowing me the use of her questionnaire data from Barrow Island employees who volunteered for her research.

To Dr Bong Sze How, his friendship and support kept me sane throughout the duration of my candidature. Thank you for being an awesome friend and the brother I never had.

Last but not least, I would like to express my gratitude to my mother (Rosiah Mardi), and sisters (Fawziah Abdad and Sa’adiyah Abdad), for their undying support and sacrifice. My sincerest apologies and regret for missing out on so many celebrative occasions and not being on site during moments of tragedy.

In Memoriam

Embarak Moxsin Abdad

1952-2009

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List of Abbreviations

AF	Astrakhan Fever
ARC	Australian Research Council
ARRL	Australian Rickettsial Reference Laboratory
ATBF	African Tick Bite Fever
CBD	Central Business District
CD4	Cluster of Differentiation 4
CD8	Cluster of Differentiation 8
CI	Confidence Interval
CPE	Cytopathic Effect
DEBONEL	<i>Derma</i> centor Borne Necrosis Erythema Lymphadenopathy
DNA	Deoxyribonucleic Acid
EDTA	Ethylenediaminetetra-Acetic Acid
ELISA	Enzyme Linked Immunosorbent Assay
FBSF	Flea-Borne Spotted Fever/Cat Flea Typhus
GTPase	Guanosine Triphosphatase
HVE	Healthy Volunteer Effect
ID ₅₀	Infectious Dose 50%
IFA	Immunofluorescent Assay
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL-1	Interleukin-1
IL-8	Interleukin-8
IL-12	Interleukin-12
INF	Interferon

INF- α	Interferon Alpha
INF- β	Interferon Beta
INF- γ	Interferon Gamma
ISF	Israeli Spotted Fever
ITT	Indian Tick Typhus
LAR	Lymphangitis-Associated Rickettsiosis
LD ₅₀	Lethal Dose 50%
MCP-1	Monocyte Chemotactic Protein-1
MIF	Microimmunofluorescence
MSF	Mediterranean Spotted Fever
NHMRC	National Health and Medical Research Council
NK	Natural Killer
OD	Optical Density
OmpA	Outer Membrane Protein A
OmpB	Outer Membrane Protein B
OR	Odds Ratio
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
RMSF	Rocky Mountain Spotted Fever
RNA	Ribonucleic Acid
rRNA	Ribosomal Ribonucleic Acid
SD ₅₀	Seroconversion Dose 50%
SFG	Spotted Fever Group
SPD	Specificity Difference
STG	Scrub Typhus Group
STT	Siberian Tick Typhus
TCID ₅₀	Tissue Culture Infectious Dose 50%

TG	Typhus Group
TIBOLA	Tick-Borne Lymphadenopathy
TNF- α	Tumour Necrosis Factor Alpha
TRG	Transitional Group
TMB	Tetramethylbenzidine
WA	Western Australia
WARA	Western Australia Rogaining Association
WASP	Wiskott-Aldrich Syndrome Protein
WHO	World Health Organisation
WP	Whiteman Park

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