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

Presented By

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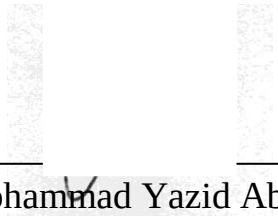
Declaration

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 fecialis* (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.

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In Memoriam

Embarak Moksin 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	*Dermacentor* 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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