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THE EPIDEMIOLOGY OF MELIOIDOSIS IN PAPUA NEW GUINEA

A Thesis submitted by
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in December, 2004

for the degree of Doctor of Philosophy in
the discipline of Microbiology and Immunology
of the School of Biomedical Science at
James Cook University, Townsville
DECLARATION

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references given.

J M Warner
December 2004

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STATEMENT ON THE CONTRIBUTION OF OTHERS

I acknowledge the help and support of Mr Daniel Gal, Mr Mark Mayo and Prof. Bart Currie in the preparation of the macro restriction digest gels. Also, the help of Dr Catriona McElene in the preparation of the PCR gels. Finally I acknowledge the help of Dr Bryant Allen for access to the PNGRIS and preparation of biogeographical maps.

J M Warner
December 2004
ACKNOWLEDGMENTS

This project has been much more than a study to fulfill the requirements of a PhD, more a fulfilment of ideas and seeds of inspiration planted through my career over the years by many mentors. I thank Roderick Hughes who over 20 years ago trained me in bacteriology but, perhaps more importantly, also started me thinking about the world around me and my part in it; the late Peter Hunt, a veteran medical technologist and leader of the MLT program at the then Riverina College of Advanced Education whose stories of working in the developing world set the vision and inspired me; Mark Stewart senior microbiologist at Mona Vale Hospital who taught me the importance of quality; the late Ian Mogg for his encouragement to fulfil my dreams.

In PNG I thank Dr Graham and Pat Tucker for their inspiration and encouragement during my first “tour” in 1992. The Asia Pacific Christian Mission for enabling me to work in PNG and the opportunity for a life changing experience. My expatriate friends in PNG, Dr David and Ali Learoyd, Keith and Rose Pauley and Keith and Norma Briggs. I particularly thank Dr Wayne Melrose who introduced me to PNG and has provided me with support ever since.

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To my practical helpers at JCU Drs Cat McElene, Brad Cullen, Ray Layton for their help with molecular biology. To Dr Jan Smith and Ruth Campbell for their ELISA support. The School of Biomedical Science technicians, past and present for putting up with my thieving and disorganised prac sessions. Thanks for the haircuts Helen! At Menzies School of Health Research Professor Bart Currie, Mark Mayo and Daniel Gal for their help with molecular epidemiology. At the Australian National University, Dr Bryant Allen for access to the PNG Resource Information System and preparation of the maps. I look forward to further collaboration. To Clement and staff of the microbiology laboratory at Port Moresby, many thanks for help during the Port Moresby based study.

Funding for this work was provided by BHP Community Trust and the Asian Pacific Foundation, many thanks for their interest in a “boutique infectious disease” of the developing world.

I present this work for Papua New Guinean scientists and clinicians and include details of cases so they are documented for publication. In their description I hope they may trigger awareness when similar cases are encountered in the future.

Finally my extended and personally PNG family. To my friends and adopted relatives at Balimo I say gae kabigibega dae waelabega dima. Without the support of both staff of Balimo Health Centre and the community of Balimo very little of this work would have been possible. To my special friend and wabeya kabeya Daniel Pelowa, no way to repay your enormous contribution, many thanks wabeya, gae
kabigibega - this is as much yours as mine. To my Australian-based family and friends, thanks for sticking by me at a time of acute selfishness.

But to my long suffering wife, [REDACTED] and our other important project of the last five years, our son [REDACTED]. To them I dedicate this work in the memory of the sacrifices they both have endured during their support of me as I have attempted to complete this work. I am coming home now!
ABSTRACT

Melioidosis has only been sporadically reported in PNG and its contribution to the disease burden of Papua New Guineans has been questioned. The rural district of Balimo, located within the Aramia flood plain of the Western province, was chosen to test the hypothesis that melioidosis is under recognised in rural PNG due to a lack of clinical awareness and a poorly resourced laboratory sector. A prospective clinical screening program conducted at Balimo Health Centre revealed melioidosis as the cause of a previously recognised fatal febrile illness affecting children. The implementation of diagnosis and treatment protocols reduced the apparent case fatality rates from 100% to 45%. Although case numbers were small, features of melioidosis in this community include childhood predilection (average age 12-years), a lack of traditional co-morbidity and regional clustering.

Simple methods of isolate identification were tested against gold standards of phenotypic and genotypic techniques and found to be sensitive and sustainable.

An IHA serological study of 747 children demonstrated a correlation between sero-reactivity and clinical incidence. Furthermore, selective culture of 374 soil samples taken from the environment within this region revealed autochthonous *B. pseudomallei* from village communities demonstrated to be melioidosis endemic. Of the 191 samples taken from areas within these villages where children play, 3.7% were found to harbour the organism. DNA macro restriction analysis demonstrated clonality between clinical and environmental strains further substantiating the hypothesis that a driver of childhood predilection is behaviour typical of children which encourages exposure to *B. pseudomallei* from permanently saturated soil and/or water, most likely through preexisting abrasions or pernasal inoculation.

A lack of genetic diversity of *B. pseudomallei* revealed by DNA macro restriction analysis is a feature. This may represent recent importation or the comfortable niche of environment - host cycling of this virulent saprophyte. This is in contrast to the diversity demonstrated in the analysis of the avirulent PNG derived *B. thailandensis*. 
In a geographical analysis of the Balimo region, the environmental attributes of low altitude (<600 m), inundation and extent of inundation and hydraquents as the predominate soil type are typical of this melioidosis implicated region. The subsequent mapping of PNG in terms of these attributes revealed only isolated regions which share these features. If the rare reports of melioidosis elsewhere in PNG is an accurate reflection of the national burden of the disease, these environmental attributes may represent important biogeographical boundaries for melioidosis in PNG. These data may serve in the remote sensing of melioidosis in PNG and throughout the Pacific-Australasian region.

To further substantiate the importance of these geographic boundaries, an indirect IgG ELISA-based sero-epidemiological assay was developed using antigen derived from PNG 

\textit{B. pseudomallei} and used on samples taken from individuals from 16 regions throughout PNG. The assay was able to detect sero-reactivity that was dependent on region which varied according to degrees of melioidosis prevalence. The true sero-prevalence ranged from 0 - 55%, demonstrating significant spatial sero-clustering. Further, when regions were classified into risk-localities based on sero-reactivity, a correlation was revealed between regions determined high-risk by population sero-reactivity and biogeography.

A prospective study in Port Moresby where 3561 samples were selectively screened for 

\textit{B. pseudomallei} demonstrated melioidosis to be endemic in the empirically diagnosed tuberculosis (TB) patient cohort and patients presenting with sepsis associated type 2 diabetes, although the incidence is low.

In demonstrating endemic melioidosis in rural PNG for the first time, it is hoped this work will contribute to decreasing the fatality rates of pneumonia and sepsis in this rural subsistence community and may aid in the uncovering of the submerged iceberg that is melioidosis within this region.
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<th>Definition</th>
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<td>&lt;</td>
<td>less than</td>
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<tr>
<td>&gt;</td>
<td>greater than</td>
</tr>
<tr>
<td>ACD</td>
<td>acid citrate dextrose</td>
</tr>
<tr>
<td>AFB</td>
<td>acid fast bacilli</td>
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<tr>
<td>ALP</td>
<td>alkaline phosphatase</td>
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<tr>
<td>ALT</td>
<td>analine transaminase</td>
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<td>ANOVA</td>
<td>analysis of variance</td>
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<td>ARA</td>
<td>arabinose</td>
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<tr>
<td>ASH</td>
<td>Ashdown agar</td>
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<tr>
<td>ASHEB</td>
<td>Ashdown environmental selective broth</td>
</tr>
<tr>
<td>AST</td>
<td>aspartate transaminase</td>
</tr>
<tr>
<td>ATCC</td>
<td>American Type Culture Collection</td>
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<tr>
<td>B. thailandensis</td>
<td><em>Burkholderia thailandensis</em></td>
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<tr>
<td>B. cepacia</td>
<td><em>Burkholderia cepacia</em></td>
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<tr>
<td>B. mallei</td>
<td><em>Burkholderia mallei</em></td>
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<tr>
<td>B. pseudomallei</td>
<td><em>Burkholderia pseudomallei</em></td>
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<tr>
<td>BD</td>
<td>Becton Dickinson</td>
</tr>
<tr>
<td>bp</td>
<td>base pair</td>
</tr>
<tr>
<td>CF</td>
<td>cystic fibrosis</td>
</tr>
<tr>
<td>cfu</td>
<td>colony forming unit</td>
</tr>
<tr>
<td>CHEF</td>
<td>contour-clamped homogenous electric field</td>
</tr>
<tr>
<td>CI</td>
<td>confidence limit</td>
</tr>
<tr>
<td>cm</td>
<td>centimeter</td>
</tr>
<tr>
<td>CMI</td>
<td>cell mediate immunity</td>
</tr>
<tr>
<td>CPHL</td>
<td>Central Public Health Laboratory</td>
</tr>
<tr>
<td>CTAB</td>
<td>hexadecyltrimethy ammonium bromide</td>
</tr>
<tr>
<td>df</td>
<td>degrees of freedom</td>
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<tr>
<td>dl</td>
<td>decilitre</td>
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<tr>
<td>DM</td>
<td>diabetes mellitus</td>
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<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
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<tr>
<td>EDTA</td>
<td>ethyl diamine tetra acetic acid</td>
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<tr>
<td>ELISA</td>
<td>enzyme linked immunosorbant assay</td>
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<tr>
<td>ESP</td>
<td>East Sepik province</td>
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<tr>
<td>fl</td>
<td>femtolitres</td>
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<tr>
<td>g</td>
<td>gram</td>
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<tr>
<td>g</td>
<td>gravity</td>
</tr>
<tr>
<td>G-CSF</td>
<td>granulocytic colony stimulating factor</td>
</tr>
<tr>
<td>GASP</td>
<td>growth advantage in stationary phase</td>
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<tr>
<td>GIS</td>
<td>geographic information system</td>
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<tr>
<td>GP</td>
<td>soil from garden place</td>
</tr>
<tr>
<td>GPS</td>
<td>global positioning system</td>
</tr>
<tr>
<td>Hb</td>
<td>haemoglobin</td>
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<tr>
<td>HLA</td>
<td>human leukocyte antigen</td>
</tr>
<tr>
<td>hr</td>
<td>hour(s)</td>
</tr>
<tr>
<td>ICT</td>
<td>immuno chromatography test</td>
</tr>
<tr>
<td>IFA</td>
<td>immuno fluorescent assay</td>
</tr>
</tbody>
</table>
IgG immunoglobulin G
IgM immunoglobulin M
IHA immunohaemoagglutination
IMI intra muscular injection
IV intra venous
kb kilobase
kg kilogram
km kilometer
l litre
Lat latitude
Long longitude
LPS lipopolysaccharide
m meter
Mb megabase
MCV mean cell volume
mg milligram
min minute
ml millilitre
MLA Medical Laboratory Assistant
MLT Medical Laboratory Technology
MLST multi locus sequencing typing
mm millimeter
mM millimolar
mmol millimoles
MPN most probably number
N. fowleri Naegleria fowleri
NA nucleic acid(s)
NCCLS National Committee of Clinical Laboratory Standards
NCTC National Collection of Type Cultures
N–PtC soil from the body of village (not points of land) frequented by children
NPV negative predictive value
NT not tested
p probability
P. fluorescens Pseudomonas fluorescens
PaLMS Pacific Laboratory Medical Services
PCR polymerase chain reaction
PFGE pulse field gel electrophoresis
PNG Papua New Guinea
PNGRIS Papua New Guinea Resource Information System
POM Port Moresby
POMGH Port Moresby General Hospital
POMGHP Port Moresby General Hospital Pathology
PPV positive predictive value
pt point
PtC soil from points of land frequented by children
PUO pyrexia of unknown origin
QID quarter in die (four times a day)
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>RAPD</td>
<td>random amplified polymorphic DNA</td>
</tr>
<tr>
<td>RMU</td>
<td>Resource Mapping Unit</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
</tr>
<tr>
<td>RT</td>
<td>room temperature</td>
</tr>
<tr>
<td>S. aureus</td>
<td><em>Staphylococcus aureus</em></td>
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<tr>
<td>SBps</td>
<td>suspected <em>B. pseudomallei</em></td>
</tr>
<tr>
<td>SD</td>
<td>standard deviation</td>
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<tr>
<td>SDS</td>
<td>sodium dodecyl sulfate</td>
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<tr>
<td>SNH</td>
<td>soil from near or under houses</td>
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<tr>
<td>spp</td>
<td>species</td>
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<tr>
<td>TB</td>
<td>tuberculosis</td>
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<tr>
<td>TBE</td>
<td>tris boric acid EDTA</td>
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<td>typical <em>B. pseudomallei</em></td>
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<td>TE</td>
<td>tris-EDTA</td>
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<tr>
<td>TP</td>
<td>true prevalence</td>
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<td>type III secretion</td>
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<td>Unevangelised Fields Mission</td>
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<td>United Kingdom</td>
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<tr>
<td>URT</td>
<td>upper respiratory tract infection</td>
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<td>UV</td>
<td>ultra violet</td>
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<td>vs.</td>
<td>versus</td>
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<td>white blood count</td>
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<tr>
<td>WCC</td>
<td>white cell count</td>
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<tr>
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<td>soil adjacent to wells</td>
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<tr>
<td>°C</td>
<td>degrees Celsius</td>
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<tr>
<td>µl</td>
<td>micro litre</td>
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<tr>
<td>µm</td>
<td>micro metre</td>
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