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**THE EPIDEMIOLOGY OF MELIOIDOSIS IN  
PAPUA NEW GUINEA**

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A Thesis submitted by  
Jeffrey Mitchell WARNER B.App.Sci (MLS) (CSU)  
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 Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references given.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**

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December 2004

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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.

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## 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 hydroquents 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 *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 *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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## ABBREVIATIONS

<	less than
>	greater than
ACD	acid citrate dextrose
AFB	acid fast bacilli
ALP	alkaline phosphatase
ALT	alanine transaminase
ANOVA	analysis of variance
ARA	arabinose
ASH	Ashdown agar
ASHEB	Ashdown environmental selective broth
AST	aspartate transaminase
ATCC	American Type Culture Collection
<i>B. thailandensis</i>	<i>Burkholderia thailandensis</i>
<i>B. cepacia</i>	<i>Burkholderia cepacia</i>
<i>B. mallei</i>	<i>Burkholderia mallei</i>
<i>B. pseudomallei</i>	<i>Burkholderia pseudomallei</i>
BD	Becton Dickinson
bp	base pair
CF	cystic fibrosis
cfu	colony forming unit
CHEF	contour-clamped homogenous electric field
CI	confidence limit
cm	centimeter
CMI	cell mediate immunity
CPHL	Central Public Health Laboratory
CTAB	hexadecyltrimethyl ammonium bromide
df	degrees of freedom
dl	decilitre
DM	diabetes mellitus
DNA	deoxyribonucleic acid
EDTA	ethyl diamine tetra acetic acid
ELISA	enzyme linked immunosorbant assay
ESP	East Sepik province
fl	femtolitres
g	gram
<i>g</i>	gravity
G-CSF	granulocytic colony stimulating factor
GASP	growth advantage in stationary phase
GIS	geographic information system
GP	soil from garden place
GPS	global positioning system
Hb	haemoglobin
HLA	human leukocyte antigen
hr	hour(s)
ICT	immuno chromatography test
IFA	immuno fluorescent assay

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
<i>N. fowleri</i>	<i>Naegleria fowleri</i>
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
<i>P. fluorescens</i>	<i>Pseudomonas fluorescens</i>
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	<i>quarter in die</i> (four times a day)

RAPD	random amplified polymorphic DNA
RMU	Resource Mapping Unit
RNA	ribonucleic acid
RT	room temperature
<i>S. aureus</i>	<i>Staphylococcus aureus</i>
SBps	suspected <i>B. pseudomallei</i>
SD	standard deviation
SDS	sodium dodecyl sulfate
SNH	soil from near or under houses
<i>spp</i>	species
TB	tuberculosis
TBE	tris boric acid EDTA
TBps	typical <i>B. pseudomallei</i>
TE	tris-EDTA
TP	true prevalence
TSA	tryptone soya agar
TTS	type III secretion
UFM	Unevangelised Fields Mission
UK	United Kingdom
URT	upper respiratory tract infection
UV	ultra violet
vs.	versus
WBC	white blood count
WCC	white cell count
WELLS	soil adjacent to wells
°C	degrees Celsius
μl	micro litre
μm	micro metre