UNIVERSITI PUTRA MALAYSIA

SEROPREVALENCE OF MELIOIDOSIS IN ANIMALS AND ENVIRONMENTAL FACTORS INFLUENCING PRESENCE OF BURKHOLDERIA PSEUDOMALLEI IN SMALL RUMINANT FARMS IN PENINSULAR MALAYSIA

HASSAN ISMAIL MUSA

FPV 2014 27

SEROPREVALENCE OF MELIOIDOSIS IN ANIMALS AND ENVIRONMENTAL FACTORS INFLUENCING PRESENCE OF *BURKHOLDERIA PSEUDOMALLEI* IN SMALL RUMINANT FARMS IN PENINSULAR MALAYSIA

By

HASSAN ISMAIL MUSA

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in the Fulfilment of the Requirements for the Degree of Doctor of Philosophy

December 2014

COPYRIGHT

DEDICATION

This work is dedicated to my late parents, Mallam Musa Sambo and Amina Adamu both of whom returned to their Creator and Lord (Allah SWT) during the period of this study.

Abstract of the thesis submitted to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

SEROPREVALENCE OF MELIOIDOSIS IN ANIMALS AND ENVIRONMENTAL FACTORS INFLUENCING PRESENCE OF *BURKHOLDERIA PSEUDOMALLEI* IN SMALL RUMINANT FARMS IN PENINSULAR MALAYSIA

By

HASSAN ISMAIL MUSA

December 2014

Chairman: Latiffah Hassan, DVM, PhD

Faculty: Veterinary Medicine

Melioidosis results in chronic debilities that reduce productivity and causes condemnation of carcasses of affected animals. There have been anecdotal reports of increase in the disease in animals in Malaysia in recent times. However, there are limited scientific information on the distribution and risk factors of the disease in animal populations as well as the factors in the environments of endemic areas that influence presence of the disease agent. A study was carried out to determine the distribution, risk factors and environmental factors that may influence the presence of the disease agent in small ruminant farms in Peninsular Malaysia. Dataset on the continuous surveillance of melioidosis in animals and another on rainfall and wind speed were both obtained for a period of 10 years. The data were summarized according to animal species, state, year and month of occurrence and relationships between disease prevalence and rainfall and wind speed were examined. The overall seroprevalence between the year 2000 and 2009 was 6.20% (95% CI; 6.02-6.37). The seroprevalence according to animal species showed that buffalo had the highest prevalence (28.18%, 95% CI; 19.04-39.54), followed by sheep (16.55%, 95% CI; 16.03 – 17.09), cattle (7.42%, 95% CI; 5.61-9.75), deer (6.45%, 95% CI; 4.50-9.17), pigs (5.88%, 95% CI; 1.05–26.98), rabbits (3.90%, 95% CI 2.90-4.90) and goats (2.19%, 95% CI; % 2.06-2.37). In terms of year of the study, the prevalence varied from 3.14% (95% CI; 2.84-3.46) in 2008 to 11.79% (95%CI; 10.6-13.09) in 2003. The prevalence for the year 2004 (7.46%, 95% CI; 6.73-8.26) was observed to be significantly higher while those for the years 2008 (3.14%, 95% CI; 2.84-3.46) and 2009 (3.64%, 95% CI; 3.27-4.04) were significantly lower when compared to the prevalence for the year 2000 (6.11%, 95% CI; 5.24-7.11). Correlation analysis showed a moderate, positive and statistically significant correlation between melioidosis prevalence and rainfall (r =0.58, 95% CI; 0.12-0.87, p=0.047) during the period under review. The apparently low seroprevalence among pigs (5.88%) and goats (2.19%) may partly be due to intensive farm management systems in which animals had less contact with contaminated soil and water while the relatively high seroprevalence in buffaloes may partly be due to the extensive management system

in which animals are at higher risk of exposure to the disease agent. The higher prevalence in 2000-2004 may be due to increased importations of animals and unfavorable weather conditions during this period. The study however observed that serosurveillance and data recording were not carried out consistently across states, year and animal or livestock species.

To investigate the farm level risk factors of melioidosis seropositivity, questionnaires were administered to the identified melioidosis negative and positive farms from four selected states (Pahang, Perak, Negeri Sembilan and Selangor). The characteristics and putative exposure to risk factors were compared between case and the control farms using Chi-square test and logistic regression analysis. In the logistic regression model, melioidosis–positive farms were significantly more likely to have; had bush clearing around farms (odds ratio [OR]; 6.61, 95% confidence interval [CI]; 1.12-38.84, $p<0.037$), *B. pseudomallei* present in the farm soil (OR; 6.23, 95% CI; 1.03-37.68, $p<0.046$), other species raised on farm (OR; 7.96, 95% CI; 1.14-55.99, $p<0.037$) and flooding or waterlogging condition (OR;11.95, 95% CI;1.39-102.6, $p=0.024$) compared to negative farms. Farms that treated the soil with lime (OR; 0.028, 95% CI; 0.003-0.29, $p=0.003$) were protected from the infection. Bush clearing may increase the risk of exposure to *B. pseudomallei* because it involves dispersal of large amount of dust into the atmosphere, brings up agents located at lower levels to the surface thereby increasing the risk of infection. Flood and or waterlogging and presence of agent in farm soil may increase the risk of contact between agent in soil and water with resident animals in the farm.

To investigate the physicochemical properties of soil and water that may influence occurrence of the agent in the farm environment, soil and water samples collected from small ruminant farms in the four states and cultured for *B. pseudomallei*. For the soil properties, comparisons of the *B. pseudomallei*-positive and negative soil samples using logistic regression model found that, when compared with *B. pseudomallei* negative soil samples, a positive sample was found to be significantly ($p<0.05$) more likely to have higher iron contents (OR; 1.009, 95% CI; 1.001-1.018, $p=0.034$), higher water contents (OR; 1.28, 95% CI; 1.052-1.546, $p=0.013$) and higher clay contents (OR; 1.54, 95% CI; 1.153-2.062, $p=0.004$). The significantly higher iron, water and clay contents of the *B. pseudomallei*-positive soil is consistent with the understanding that iron regulates expression of respiratory enzymes, soil water is essential for soil ecology and agent's biological processes and clay has excellent water and nutrient retention capabilities due to its surface area and chemical activity which favor survival of the agent in soil.

Investigation of the water properties found that, when compared with *B. pseudomallei* negative water samples, positive water samples were found to be significantly ($p<0.05$) more likely to have higher chemical oxygen demand (COD) (OR; 1.002, 95% CI; 1.000-1.004, $p=0.031$) and higher pH value (OR; 12.69, 95% CI; 2.67-60.34, $p=0.001$). This suggested that higher COD and higher water pH supported the survival of the agent in water samples.

In conclusion, melioidosis is widely distributed in Peninsular Malaysia and affects all livestock species regardless of the size, species, population size and breed. The disease occurrence was associated with several factors such as events/activities around farm such bush clearing, water logging/flooding and presence of other

species of animal in the farm. The farm soil iron, water and clay content as well chemical oxygen demand and pH of farm water supplies were the factors that influence the presence of *B. pseudomallei* in farm environments. This information may be of benefits when planning for control strategies against exposure to *B. pseudomallei* from soil or water source.

Abstrak tesis yang dikemukanan kepada senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

SEROPREVALENS MELIOIDOSIS DALAM HAIWAN DAN FAKTOR PERSEKITARAN YANG MEMPENGARUHI KEHADIRAN *BULKHOLDERIA PSEUDOMALLEI* DI LADANG RUMINAN KECIL DI SEMENANJUNG MALAYSIA

Oleh

HASSAN ISMAIL MUSA

Disember 2014

Pengerusi: Latiffah Hassan, DVM, PhD

Fakulti: Perubatan Veterinar

Melioidosis menyebabkan ketidakupayaan kronik yang mengurangkan produktiviti dan penolakan karkas haiwan. Terdapat laporan anekdot tentang peningkatan dalam penyakit ini pada haiwan di Malaysia sejak kebelakangan ini. Walau bagaimanapun, maklumat saintifik adalah terhad mengenai taburan dan faktor risiko penyakit dalam populasi haiwan dan juga faktor persekitaran di kawasan endemik yang mempengaruhi kewujudan organisma. Satu kajian telah dijalankan untuk menentukan taburan, faktor risiko dan faktor persekitaran yang mempengaruhi kehadiran agen penyakit ini di ladang ruminan kecil di Semenanjung Malaysia. Set data dari pengawasan dan pemantauan berterusan melioidosis pada haiwan dan data meteorologi hujan dan kelajuan angin diperolehi bagi tempoh 10 tahun. Data diringkaskan mengikut spesis haiwan, negeri, tahun dan bulan dan hubungan antara kelaziman penyakit, hujan dan kelajuan angin diperiksa. Seroprevalens keseluruhan antara tahun 2000 dan 2009 adalah 6.20% (95% CI; 6.02-6.37). Seroprevalens mengikut spesis haiwan menunjukkan bahawa kerbau mempunyai kadar reaktor tertinggi (28.18%, 95% CI; 19.04-39.54), diikuti dengan kambing biri-biri (16.55%, 95% CI; 16.03-17.09), lembu (7.42%, 95% CI; 5.61-9.75), rusa (6.45%, 95% CI; 4.50-9.17), babi (5.88%, 95% CI; 1.05-26.98), arnab (3.90%, 95% CI; 2.90-4.90) dan kambing (2.19%, 95% CI; 2.06-2.37). Dari segi tahun kajian, kelaziman yang berbeza dari 3.14% (95% CI; 2.84-3.46) pada tahun 2008 ke 11.79% (95% CI; 10.6-13.09) pada tahun 2003. Berbanding dengan kelaziman bagi tahun 2000 (6.11 %, 95% CI; 5.24-7.11), kadar yang lebih tinggi (7.46%, 95% CI; 6.73-8.26) diperhatikan pada tahun 2004 manakala kadar jauh lebih rendah dilihat pada tahun 2008 (3.14%, 95% CI; 2.84-3.46) dan 2009 (3.64%, 95% CI; 3.27-4.04). Analisis korelasi menunjukkan korelasi yang sederhana, positif dan bekeertian statistik antara kelaziman melioidosis dan hujan ($r = 0.58$, 95% CI;0.12-0.87, $p = 0.047$). Seroprevalens yang rendah kalangan di babi dan kambing berkemungkinan disebabkan oleh sistem pengurusan ladang intensif dimana haiwan mempunyai pendedahan kurang kepada tanah dan air yang tercemar manakala seroprevalens yang agak tinggi pada kerbau mungkin disebabkan oleh sistem pengurusan yang

ekstensif dimana haiwan berisiko lebih tinggi terdedah kepada agen penyakit. Kadar yang lebih tinggi dalam 2000-2004 disebabkan pengimportan haiwan dan keadaan cuaca yang buruk dalam tempoh ini. Kajian ini bagaimanapun mendapati bahawa pengawasan dan pengumpulan data tidak dilakukan secara sama rata di antara negeri, tahun dan haiwan atau spesies ternakan.

Untuk menyiasat faktor risiko melioidosis di ladang, borang soal selidik telah diberikan kepada ladang yang melioidosis negatif dan positif dari negeri terpilih (Pahang, Perak, Negeri Sembilan dan Selangor). Ciri dan faktor risiko penyakit dibandingkan antara ladang kes dan ladang kawalan menggunakan ujian c*hi-square* dan regresi logistik. Dalam model regresi logistik, ladang melioidosis positif nyata lebih cenderung; melakukan pembersihan belukar sekitar ladang (nisbah mungkin (OR);6.61, 95% selang keyakinan [CI]; 1.12-38.84, p <0.037), wujud *B. pseudomallei* pada tanah ladang (OR; 6.23, 95% CI; 1.03- 37.68, p <0.046), membela spesies lain di ladang (OR;7.96, 95% CI; 1.14-55.99, p <0.037) dan telah berlaku banjir atau genang air (OR;11.95, 95% CI;1.39-102.6, p = 0.024) berbanding ladang negatif. Ladang yang tanah dirawat dengan kapur (OR; 0.028, 95% CI;.0.003-0.29, p = 0.003) telah dilindungi dari jangkitan. Pembersihan belukar boleh meningkatkan risiko pendedahan kepada *B. pseudomallei* kerana ia menyebabkan penyebaran jumlah besar debu ke udara, dimana agen dari bawah tanah dibawa ke permukaan atas justeru meningkatkan risiko transmisi. Banjir atau genang air di tanah ladang juga akan meningkatkan risiko pendedahan antara agen di dalam tanah dan air kepada haiwan bermastautin di ladang.

Untuk menyiasat ciri fisikokimia tanah dan air yang boleh mempengaruhi kehadiran agen itu dalam persekitaran ladang, sampel tanah dan sampel air di ladang ruminan kecil telah diambil dan dikultur untuk *B. pseudomallei*. Perbandingan sampel tanah *B. pseudomallei* positif dan negatif menggunakan model regresi logistik mendapati bahawa, berbanding dengan *B. pseudomallei* sampel tanah yang negatif, sampel yang positif didapati bekeertian (p <0.05) lebih cenderung untuk mempunyai kandungan besi yang lebih tinggi (OR;1.009, 95% CI;1.001-1.018, p = 0.034), kandungan air yang lebih tinggi (OR;1.28, 95% CI; 1.052-1.546, p = 0.013) dan kandungan tanah liat yang lebih tinggi (OR;1.54, 95% CI;1.153-2.062, p = 0.004). Besi, kandungan tanah liat dan air jauh lebih tinggi pada tanah *B. pseudomallei* positif adalah konsisten dengan pemahaman yang besi mengawal ekspresi enzim pernafasan, air tanah adalah penting untuk ekologi tanah dan proses biologi agen dan tanah liat berkebolehan menampung air dan mengekal nutrien kerana kawasan permukaannya dan aktiviti kimia yang memihak kepada kemandirian agen itu di dalam tanah.

Penyiasatan sifat air mendapati bahawa, berbanding dengan *B. pseudomallei* sampel air negatif, sampel air yang positif bekeertian (p <0.05) lebih cenderung mempunyai permintaan oksigen kimia yang lebih tinggi (COD) (OR;1.002, 95% CI;1.000-1.004, p = 0.031) dan nilai pH yang lebih tinggi (OR;12.69, 95% CI; 2.67-60.34, p = 0.001). Ini mencadangkan bahawa COD yang lebih tinggi dan pH air yang lebih tinggi menyokong kemandirian agen itu dalam air.

Kesimpulannya, melioidosis tersebar secara meluas di Semenanjung Malaysia dan memberi kesan kepada semua spesis ternakan tanpa mengira saiz, saiz populasi, spesis dan baka. Berlakunya penyakit dikaitkan dengan beberapa faktor seperti berlakunya aktiviti di sekitar ladang seperti pembersihan belukar, genang air/banjir

dan kehadiran spesis haiwan lain di ladang. Kandungan besi, air, tanah liat, permintaan oksigen kimia dan pH bekalan air ladang merupakan faktor yang mempengaruhi kehadiran *B. pseudomallei* dalam persekitaran ladang. Maklumat dari kajian ini mungkin bermanafaat apabila merancang strategi kawalan pendedahan kepada *B. pseudomallei* dari tanah atau sumber air.

ACKNOWLEDGEMENTS

I wish to express my profound gratitude to Almighty Allah for giving me the opportunity and wisdom to undergo this study. I am most grateful to the Chairman of my PhD supervisory committee, Associate Professor Dr. Latiffah Binti Hassan for her continuous coaching, constructive criticisms, encouragements and support throughout the years of my study. She indeed endured the pains and burned the midnight candles reading, re-reading and revising to improve the quality of work. She made insightful comments, asked useful questions and offered invaluable advice. I am equally grateful to other members of the committee, namely Associate Professor Dr. Zunita Zakaria, Professor Dr. Zulkifli Hj. Shamsuddin, Professor Dr. Saleha Abdul Aziz and Dr. Chandrawathani Panchadcharam for their invaluable guidance, advice, and encouragements during the course of the study and preparation of this thesis. Without their patience, support and contribution, I wouldn‘t have accomplished the goal of finishing this thesis.

I am especially grateful to the Department of Veterinary Services Putrajaya and Veterinary Research Institute Ipoh, the Veterinary Services Departments in Negeri Sembilan, Pahang, Perak and Selangor and their district veterinary offices staff and owners of small ruminant farms in the four states for granting the permission to collect samples from their farms and for their valuable times during the interviews session. I am especially thankful to the Directors of the Veterinary Services in the four states for their assistance in granting permissions to visit and collect samples from farms in states under their jurisdictions. Special thanks to the DVS headquarters staff in the persons Dr. Azri Adzhar, Dr. Salina Bugis and late Dr. Roosevien Farida Nilawati Rachmat for their help and assistance during the course of the study.

I equally appreciate the help of Dr. Annas Saleh, Dr. Muhamad Salim Tahir, Encik Azwan Muhammad and Encik Hendri Eri for their kind assistance during the data collection. Thank you very much for facilitating communications with the farmers. Special thanks to the other staff who accompanied us to the field during our farm sampling, individuals like Encik Mohd Faizal Abdullah, Encik Amin Abd.Hamid, Puan Nazira Abu, Encik Mohd Helmy Mahroob, Encik Azri Samin, Encik Wan Azaman Wan Mahmud, Encik Hassan Gua Musang, and many others whom I may fail to mention for lack space.

To my colleagues and friends especially Dr. Muhammad Modu Bukar, Dr. Ibrahim Abdulazeez, Dr. Abdulrasheed Bello Aliyu, Dr. Mukhtar Salihu Anka, Dr. Lawan Adamu, Dr. Yusuf Iliyasu, Dr. Adamu Abubakar Yerima, Dr. Khumran Armiyau Mada, for their guidance from the inception to the completion of this study, thank you. They have been instrumental in helping me shape my thesis and providing me with insight into the joys (and stress) of academic research. I am also thankful to Encik Mohd Hafizudin Abdullah, Encik Hajar, Encik Mohd Azri Roslan and Cik Krishnamma Kuppusamy in the Bacteriology Laboratory, Department of Pathology and Microbiology, Faculty of Veterinary Medicine, UPM for their cooperation and technical assistance during the laboratory work. I am also grateful to Encik Azali Mohd Bin Sab, Puan Fouziah Sulaiman, Encik Dzulkifli Duaji and Hajji Jamil Bin Omar of the Department of Land management, Faculty of Agriculture, UPM for their technical assistance during processing of the soil samples. Special thanks also to Dr.

Fuad Matori of Aquatic Animal Health Section, Faculty of Veterinary Medicine, UPM for his technical assistance and advice in handling and processing of water samples. I am especially grateful to all the staff and management of the University of Maiduguri, Borno State, Nigeria for the patients and understanding during the period of my absence. Special thanks also goes to my Head of Department, Dr. Nuhu Bala Adamu and all members of academic, technical and administrative staff of the Department of Veterinary Public Health and Preventive Medicine, University of Maiduguri. To School of Graduate Studies, UPM, I say thank you so much for giving me the opportunity to come to Malaysia for my studies.

I am especially grateful to my late parents, Mallam Musa Sambo and Amina Musa for their love care, guidance, support and prayers. May Allah forgive all your shortcomings and admit you into Ajannatul Firddausi. My hearty appreciation goes to my lovely wife Dr. Salamatu Muhammad Tukur, whose dedication, love, tolerance and persistent confidence in me has taken the load off my shoulder. To my two sons Muhammad Hassan Musa and Tahir Hassan Musa, you are the sources of strength and perseverance. You motivated me when times were difficult and I felt like there was no light at the end of the tunnel.

Finally, I would like to thank everyone who contributed to the success of the thesis in one way or the other, whom due to lack of space and the forgetfulness of my human nature, I am unable to mention their names in persons. I sincerely apologized for my inadequacy as a human being.

I certify that a Thesis Examination Committee has met on 9 December 2014 to conduct the final examination of Hassan Ismail Musa on his thesis entitled "Seroprevalence of Melioidosis in Animals and Environmental Factors Influencing Presence of *Burkholderia pseudomallei* in Small Ruminant Farms in Peninsular Malaysia" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

Mohd Zamri b Saad, PhD
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Chairman)

Cheah Yoke Kqueen, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Internal Examiner)

Siti Khairani binti Bejo, PhD
Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Internal Examiner)

Ian Robertson, PhD
Professor
School of Veterinary and Biomedical Sciences
Murdoch University
(External Examiner)

ZULKARNAIN ZAINAL, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 26 February 2015

This thesis was submitted to the senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follows:

Latiffah Hassan, PhD
Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Chairman)

Zunita Zakaria, PhD
Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

Saleha Abdul Aziz, PhD
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

Zulkifli Hj. Shamsuddin, PhD
Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Chandrawathani Panchadcharam, PhD
Deputy Director
Veterinary Research Institute,
Ipoh
(Member)

BUJANG KIM HUAT, PhD
Professor and Dean
School of graduate Studies
Universiti Putra Malaysia

Date:

Declaration by graduate student

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any other institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and Innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software.

Signature: _____ Date: _____

Name and Matric No.: HassanIsmailMusa GS26737

Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

Signature: _____
Name of
Chairman of
Supervisory
Committee: _____

Signature: _____
Name of
Member of
Supervisory
Committee: _____

Signature: _____
Name of
Member of
Supervisory
Committee: _____

Signature: _____
Name of
Member of
Supervisory
Committee: _____

Signature: _____
Name of
Member of
Supervisory
Committee: _____

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectrophotometer
Arc GIS	Geographic information system (GIS) software
ASA	Ashdown's Selective agar
bp	base pairs
ASB	Ashdown's Selective Enrichment Broth
BHI	Brain heart infusion
CDC	Centers for Disease Control and Prevention
CEC	Cation Exchange Capacity
CFSPH	Center for Food Security and Public Health
CFT	Complement Fixation Test
CI	Confidence Interval
COD	Chemical oxygen demand
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
DO_2	Dissolved oxygen
DOC	Deoxycholate
DSM	Department of Statistics Malaysia
DVS	Department of Veterinary Services
ELISA	Enzyme-linked immunosorbent assay
ESRI	Economic and Social Research Institute
EU	European Union
FAO	Food and Agricultural organization of united nation
FeSOD	Iron superoxide dismutase
GDP	Gross Domestic Product
GIS	Geographical Information System
HMA	Hydroxymyristic acid
HPAI	Highly Pathogenic Avian Influenza
IFAT	Indirect immunofluorescent Technique
IgM	Immunoglobulin M
JHSPH	Johns Hopkins Bloomberg School of Public Health
JUPEM	Department of Survey and Mapping

LOI	Loss on Ignition
LPS	Lipopolysaccharide
MMD	Malaysian Meteorology Department
MNRE	Ministry of Natural Resources and Environment, Malaysia
MOA	Ministry of Agriculture
NAP-A	Norfloxacin, ampicillin and polymyxin B into Ashdown medium
OD	Optical density
OIE	International Organization for Animal Health
OR	Odds ratio
PEG	polyethylene glycol
PHB	Polyhdroxybutyrate
PCR	Polymerase chain reaction
PFGE	Pulsed field gel electrophoresis
PPE	Personal Protective Equipment
RM	Malaysian Ringgit
ROI	Reactive oxygen intermediate
rRNA	Ribosomal ribonucleic acid
SDO	Short-chain dehydrogenase/oxidoreductase
SNP	Single nucleotide polymorphism
SOM	Soil Organic Matter
T3SS	Type III secretion systems
T6SS	Type VI secretion systems
TBSS	Threonine basal salt solution
UPM	Universiti Putra Malaysia
VNBC	Viable but non culturable
VRI	Veterinary Research Institute
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

Melioidosis (Whitmore's disease, pseudoglanders) is an infectious disease caused by a bacterium, *Burkholderia pseudomallei* (*B. pseudomallei*). The name "melioidosis" is derived from the Greek words "melis" (distemper of asses) and "eidosis" (resembles glanders), which simply means "glanders-like illness" or "distemper of the asses" (Raja *et al.*, 2005, Stanton & Fletcher, 1932). The disease was first described by Alfred Whitmore and Krishnawaswami in Burma (Myanmar) in 1912 (Whitmore & Krishnaswami, 1912) but being a predominantly tropical disease (Limmathurotsakul & Peacock, 2011), it attracted little attention from the global community until the Vietnam wars when French and US personnel and military dogs were affected (Acha & Szyfres, 2001). In addition, the recognition of the disease as an emerging global problem (Dance, 2000b, Acha & Szyfres, 2001) and the classification of *B. pseudomallei* as category B bioterrorism agent (Meselson *et al.*, 2002) have both resulted in increased interest in the diseases. However, the fact that eradication of the disease is difficult with slow response to antibiotic treatments, the high chances of relapse of cases as well as the prolonged period between infection and manifestation of disease may affect control strategies against the disease (Currie *et al.*, 2000a).

The disease is endemic in Southeast Asia and northern Australia but has been reported in almost all continents of the world (Currie *et al.*, 2008) in places which including Britain, Europe (Dance *et al.*, 1992), Brazil, South America (Miralles *et al.*, 2004), South Africa (Van der Lugt & Henton, 1995) and Saudi Arabia in the Middle East (Barbour *et al.*, 1997) among others. The clinical course and presentation of the disease varies in different species of animals where the outcome of infection varies from mild to severe illness, including death (Inglis & Sousa, 2009, Sprague & Neubauer, 2004). The disease is considered to be an emerging infectious disease in both humans and animals partly due to the increased global animal and fomite transportation as well as the increase in its recognition as a result of increased awareness, better laboratory facilities and increasing incidence (Dance, 2000b). The continuous migration of people and transport of animals around the world as well the global increase in tourism were thought to increase the risk of spread of melioidosis worldwide (Sprague & Neubauer, 2004). Over the last few years, the disease was reported to have emerged as a major public health problem in the endemic regions (Inglis & Sousa, 2009).

Soil and water constitute the two major reservoirs of *B. pseudomallei* in the environment and understanding the dynamics of these environmental reservoirs is important in planning control measures against the disease. However, the physicochemical properties of these reservoirs that may influence presence of *B. pseudomallei* in soil and water from the environments of the endemic regions are poorly understood (Rattanavong *et al.*, 2011, Dance, 2000a). Studies on survivability of *B. pseudomallei* in these reservoirs showed a complex relationship between the organism and environmental factors such as soil types, farming activities, environmental disturbance and water parameters among other factors (Palasatien *et*

al., 2008, Currie *et al*., 2008, Inglis & Sagripanti, 2006). In general, changes in environmental factors and farming practices, increased in numbers susceptible hosts resulting from better management of chronic diseases were said to be some of the reasons responsible for the expansion in melioidosis endemic areas and environmental presence of its agent (Currie *et al*., 2008, Raja *et al*., 2005, Dance, 2000a). Furthermore, increased incidence of occurrence of the agent in soil has been shown to be associated with increased incidence of melioidosis cases in the same area (Palasatien *et al*., 2008). In essence, since melioidosis is mostly considered to be an environmental disease, climate and biogeochemical functions of *B. pseudomallei* in its normal inanimate habitats might be an important factor in the geographical distribution of the disease (Inglis *et al.*, 2001). Consequently, soil types, its pH and composition, environmental disturbances (excavation, ploughing etc), rainfall, humidity, UV radiation, temperature, other vegetation, and the use of fertilizers are some of the factors suggested to influence the environmental presence of the agent (Cheng & Currie, 2005).

In Malaysia, first documented cases of the disease was described in 1913 by Stanton and Fletcher (1932) and since then cases have continued to be reported in both humans and animals from both the Peninsular and East Malaysia (Puthucheary, 2009, Little, 1979, Strauss *et al.*, 1969a). Even though the disease is endemic in Malaysia, anecdotal reports in recent times showed an increase in cases of the disease in animals. In addition, the sustained advocacies and calls for the inclusion of the disease on the list of notifiable diseases in Malaysia due to its threat to public health (Kamaruddin, 2013, Jayaram, 2005) also underscores the increasing levels of importance of the disease in human population in the country. However, information on the epidemiology of the disease in local animals and the factors contributing to the occurrence of its agent in the environment is scanty. This is in view of the fact that melioidosis in small ruminants result in chronic debilitating disease that causes reduced productivity of the animals and condemnation of carcasses in the abattoir (Choy *et al.*, 2000, Ketterer *et al.*, 1986). This study aimed at improving the understanding of melioidosis in animals in Peninsular Malaysia through investigation of its distribution in animal populations and factors influencing presence of *B. pseudomallei* in water and soil samples from animal farm environments.

The research set out to answer the following questions:
What is the seroprevalence of melioidosis among livestock in Malaysia between the year 2000 and 2009?

What are the factors or farm practices that may play significant roles in seropositivity of small ruminants against melioidosis test in Peninsular Malaysia?

Is there any relationship between occurrence of melioidosis cases in animals and the amount of rainfall recorded during the period of the study?

What are the physicochemical properties of soil that could influence the presence of *B. pseudomallei* in soil from small ruminants' farms in Peninsular Malaysia?

What are the physicochemical properties of water that could influence the presence of *B. pseudomallei* in water from small ruminant farms in Peninsular Malaysia?

The three major hypotheses of the research were:

1 .Occurrence of melioidosis in animals during the last decade is related to the intensity and variations of rainfall and wind speed recorded during the period of the study.
2. There is a difference in isolation rates of *Burkholderia pseudomallei* between small ruminant farms with seropositive and those with seronegative animals.
3. Some physicochemical properties of soil and water influence presence of *B. pseudomallei* in soil and water in small ruminant farm environment in Peninsular Malaysia.

The specific objectives of the study were:

1. To determine the seroprevalence of melioidosis in Peninsular Malaysian animal population between the year 2000 and 2009 as well as to examine the relationships between melioidosis seroprevalence in animals and the amount of rainfall and wind speed recorded during the period under review.
2. To determine factors (risks) in small ruminant farms that may influence serological status of animals against *Burkholderia pseudomallei* in Peninsular Malaysia.
3. To examine the physicochemical properties of soil that may influence the presence of *B. pseudomallei* in soil from small ruminant farms from Peninsular Malaysia.
4. To examine the physicochemical properties of water that may influence presence of *B. pseudomallei* in water from small ruminant farms from Peninsular Malaysia.

REFERENCES

Ab Karim, B., (2007) Situational Analysis of Melioidosis In Terengganu, Malaysia. Available at: http://www.elitepco.com.tw/tephinet2007taiwan/images/PresentationDownload/OralPresentation_PDF/OP58.pdf.

Abdulllah, F., V. C. Chai, K. Anuar and T. S. Tan, (2004) *An Overview On The Growth and Development Of The Malaysian Construction Industry, Skudai, Johor, Malaysia*.

Abram, D. and N. Gibbons, (1961) The effect of chlorides of monovalent cations, urea, detergents, and heat on morphology and the turbidity of suspensions of red halophilic bacteria. *Canadian Journal of Microbiology,* 7, 741-750.

Acha, P. N. and B. Szyfres, (2001) Zoonoses. Bacterioses listed M (Melioidosis). *Zoonoses and Communicable Diseases Common to Man and Animals. Third Edition,* 1, 184-190.

Afzan, T. A., (2009) Pahang melioidosis registry. *Medical Journal of Malaysia,* 64, 27-30.

Agus, M. R., (2002) The role of state and market in the Malaysian housing sector. *Journal of Housing and the Built Environment,* 17, 49-67.

Ahmad, N., R. Hashim and A. Mohd Noor, (2013) The In Vitro Antibiotic Susceptibility of Malaysian Isolates of *Burkholderia pseudomallei*. *International Journal of Microbiology,* 2013, 121845.

Ahmad, T. and C. Suntharalingam, (2009) Transformation and Economic Growth of the Malaysian Agricultural Sector. *Economic and Technology Management Review,* 4, 1-10.

Amornrit, W., (2012) Elevated intracellular levels of iron in host cells promotes *Burkholderia pseudomallei* infection. *Asian Biomedicine,* 6, 465-471.

Anuntagool, N., P. Intachote, V. Wuthiekanun, N. J. White and S. Sirisinha, (1998) Lipopolysaccharide from nonvirulent Ara+ *Burkholderia pseudomallei* isolates is immunologically indistinguishable from lipopolysaccharide from virulent Ara− clinical isolates. *Clinical and Diagnostic Laboratory Immunology,* 5, 225-229.

Ashbolt, N. J., (2004) Microbial contamination of drinking water and disease outcomes in developing regions. *Toxicology,* 198, 229-238.

Athan, E., A. M. Allworth, C. Engler, I. Bastian and A. C. Cheng, (2005) Melioidosis in tsunami survivors. *Emerging Infectious Diseases,* 11, 1638-1639.

Baker, A., M. Mayo, L. Owens, G. Burgess, R. Norton, W. J. H. McBride, B. J. Currie and J. Warner, (2013) Biogeography of *Burkholderia pseudomallei* in the Torres Strait Islands of Northern Australia. *Journal of Clinical Microbiology*, 51, 2520-2525.

Baker, A., D. Tahani, C. Gardiner, K. L. Bristow, A. R. Greenhill and J. Warner, (2011) Groundwater seeps facilitate exposure to *Burkholderia pseudomallei*. *Applied and Environmental Microbiology*, 77, 7243-7246.

Barbour, E., N. Nabbut, S. Hamadeh and H. Al-Nakhli, (1997) Bacterial identity and characteristics in healthy and unhealthy respiratory tracts of sheep and calves. *Veterinary Research Communications*, 21, 421-430.

Barnes, P. F., M. D. Appleman and M. M. Cosgrove, (1986) A case of melioidosis originating in North America. *The American Review of Respiratory Disease*, 134, 170-171.

Batchelor, B. I. F., J. Paul, S. Trakulsomboon, M. Mgongo and D.A.B. Dance, (1994) Melioidosis survey in Kenya. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 88, 181.

Bharumshah, A. Z. and Z. A. Mohamed, (1993) Demand for meat in Malaysia: An application of the almost ideal demand system analysis. *Pertanika Journal of Social Sciences & Humanities*, 1, 91-99.

Booth, I., (1985) Regulation of cytoplasmic pH in bacteria. *Microbiological Reviews*, 49, 359-378.

Borgherini, G., P. Poubeau, F. Paganin, S. Picot, A. Michault, F. Thibault and C. A. Berod, (2006) Melioidosis: an imported case from Madagascar. *Journal of Travel Medicine*, 13, 318-320.

Bot, A. and J. Benites, (2005) *The importance of soil organic matter: key to drought-resistant soil and sustained food and production*. FAO. 21-124.

Braid, M. D., L. M. Daniels and C. L. Kitts, (2003) Removal of PCR inhibitors from soil DNA by chemical flocculation. *Journal of Microbiological Methods*, 52, 389-393.

Brent, A. J., P. C. Matthews, D. A. Dance, T. L. Pitt and R. Handy, (2007) Misdiagnosing melioidosis. *Emerging Infectious Diseases*, 13, 349.

Brook, M., B. Currie and P. Desmarchelier, (1997) Isolation and identification of *Burkholderia pseudomallei* from soil using selective culture techniques and the polymerase chain reaction. *Journal of Applied Microbiology*, 82, 589-596.

Brown, L. D., T. T. Cai and A. DasGupta, (2001) Interval estimation for a binomial proportion. *Statistical Science*, 101-117.

Burtnick, M. N., P. J. Brett, S. V. Harding, S. A. Ngugi, W. J. Ribot, N. Chantratita, A. Scorpio, T. S. Milne, R. E. Dean, D. L. Fritz, S. J. Peacock, J. L. Prior, T. P. Atkins and D. DeShazer, (2011) The Cluster 1 Type VI Secretion System Is a Major Virulence Determinant in *Burkholderia pseudomallei*. *Infection and Immunity,* 79, 1512-1525.

CDC, (2012) Melioidosis. Center for Disease Control and Prevention, Atlanta. Available online at: http://www.cdc.gov/melioidosis/. Accessed on January 26.

Chantratita, N., D. A. Rholl, B. Sim, V. Wuthiekanun, D. Limmathurotsakul, P. Amornchai, A. Thanwisai, H. H. Chua, W. F. Ooi and M. T. Holden, (2011) Antimicrobial resistance to ceftazidime involving loss of penicillin-binding protein 3 in *Burkholderia pseudomallei*. *Proceedings of the National Academy of Sciences,* 108, 17165-17170.

Chantratita, N., V. Wuthiekanun, K. Boonbumrung, R. Tiyawisutsri, M. Vesaratchavest, D. Limmathurotsakul, W. Chierakul, S. Wongratanacheewin, S. Pukritiyakamee and N. J. White, (2007) Biological relevance of colony morphology and phenotypic switching by *Burkholderia pseudomallei*. *Journal of Bacteriology,* 189, 807-817.

Chen, Y., S. Chen, C. Kao and Y. Chen, (2003) Effects of soil pH, temperature and water content on the growth of *Burkholderia pseudomallei*. *Folia Microbiologica,* 48, 253-256.

Chen, Y. L., Y. C. Yen, C. Y. Yang, M. S. Lee, C. K. Ho, K. D. Mena, P. Y. Wang and P. S. Chen, (2014) The concentrations of ambient *Burkholderia Pseudomallei* during Typhoon Season in Endemic Area of Melioidosis in Taiwan. *PLoS Neglected Tropical Diseases,* 8, e2877.

Chen, Y. S., H. H. Lin, J. J. Mu, C. S. Chiang, C. H. Chen, L. M. Buu, Y. E. Lin and Y. L. Chen, (2010) Distribution of melioidosis cases and viable *Burkholderia pseudomallei* in soil: evidence for emerging melioidosis in Taiwan. *Journal of Clinical Microbiology,* 48, 1432-1434.

Cheng, A. C. and B. J. Currie, (2005) Melioidosis: epidemiology, pathophysiology, and management. *Clinical Microbiology Reviews,* 18, 383.

Cheng, A. C., S. P. Jacups, D. Gal, M. Mayo and B. J. Currie, (2006a) Extreme weather events and environmental contamination are associated with case-clusters of melioidosis in the Northern Territory of Australia. *International Journal of Epidemiology,* 35, 323-329.

Cheng, A. C., S. J. Peacock, D. Limmathurotsakul, G. Wongsuvan, W. Chierakul, P. Amornchai, N. Getchalarat, W. Chaowagul, N. J. White and N. P. J. Day, (2006b) Prospective evaluation of a rapid immunochromogenic cassette test for the diagnosis of melioidosis in northeast Thailand. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 100, 64-67.

Chierakul, W., A. Rajanuwong, V. Wuthiekanun, N. Teerawattanasook, M. Gasiprong, A. Simpson, W. Chaowagul and N. J. White, (2004) The changing pattern of bloodstream infections associated with the rise in HIV prevalence in northeastern Thailand. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 98, 678-686.

Chierakul, W., W. Winothai, C. Wattanawaitunechai, V. Wuthiekanun, T. Rugtaengan, J. Rattanalertnavee, P. Jitpratoom, W. Chaowagul, P. Singhasivanon, N. J. White, N. P. Day and S. J. Peacock, (2005) Melioidosis in 6 tsunami survivors in southern Thailand. *Clinical Infectious Diseases,* 41, 982-990.

Chiou, C. T., D. E. Kile, T. I. Brinton, R. L. Malcolm, J. A. Leenheer and P. MacCarthy, (1987) A comparison of water solubility enhancements of organic solutes by aquatic humic materials and commercial humic acids. *Environmental Science & Technology,* 21, 1231-1234.

Cho, M.-H., Y.-W. Shin, J.-H. Chun, K.-J. Hong, B.-K. Na, G.-e. Rhie, B.-L. Seong and C.-K. Yoo, (2012) Expression and characterization of an iron-containing superoxide dismutase from *Burkholderia pseudomallei*. *Journal of Microbiology,* 50, 1029-1033.

Choy, J. L., M. Mayo, A. Janmaat and B. J. Currie, (2000) Animal melioidosis in Australia. *Acta Tropica,* 74, 153-158.

Christenson, B., Z. Fuxench, J. Morales, R. Suarez-Villamil and L. Souchet, (2003) Severe community-acquired pneumonia and sepsis caused by *Burkholderia pseudomallei* associated with flooding in Puerto Rico. *Boletin de la Asociacion Médica de la Puerto Rico,* 95, 17-20.

Chua, K., K. See, K. Thong and S. Puthucheary, (2010) DNA fingerprinting of human isolates of *Burkholderia pseudomallei* from different geographical regions of Malaysia. *Tropical Biomedicine,* 27, 517-524.

Chung, K. M., D. W. Chou, C. H. Chen and B. M. Cheung, (2007) Lymphocytic pleural effusion in acute melioidosis. *Journal of the Formosan Medical Association,* 106, 874-877.

Coenye, T. and P. Vandamme, (2003) Diversity and significance of *Burkholderia* species occupying diverse ecological niches. *Environmental Microbiology,* 5, 719-729.

Corkeron, M., R. Norton and P. Nelson, (2010) Spatial analysis of melioidosis distribution in a suburban area. *Epidemiology and Infection,* 138, 1346-1352.

Cottew, G., (1950) Melioidosis in Sheep in Queensland. A Description of the Causal Organism. *Australian Journal of Experimental Biology and Medical Science,* 28, 677-683.

Courtois, S., Å. Frostegård, P. Göransson, G. Depret, P. Jeannin and P. Simonet, (2001) Quantification of bacterial subgroups in soil: comparison of DNA extracted directly from soil or from cells previously released by density gradient centrifugation. *Environmental Microbiology,* 3, 431-439.

Cruz-Migoni, A., G. M. Hautbergue, P. J. Artymiuk, P. J. Baker, M. Bokori-Brown, C.-T. Chang, M. J. Dickman, A. Essex-Lopresti, S. V. Harding and N. M. Mahadi, (2011) A *Burkholderia pseudomallei* toxin inhibits helicase activity of translation factor eIF4A. *Science,* 334, 821-824.

Cuadros, J., H. Gil, J. De Miguel, G. Marabé, T. A. P. Gómez-Herruz, B. Lobo, R. Marcos and P. Anda, (2011) Melioidosis imported from West Africa to Europe. *The American Journal of Tropical Medicine and Hygiene,* 85, 282-284.

Cullinane, M., L. Gong, X. Li, N. Lazar-Adler, T. Tra, E. Wolvetang, M. Prescott, J. D. Boyce, R. J. Devenish and B. Adler, (2008) Stimulation of autophagy suppresses the intracellular survival of *Burkholderia pseudomallei* in mammalian cell lines. *Autophagy,* 4, 744-753.

Currie, B. J., (2001) Environmental change, global warming and infectious diseases in Northern Australia. *Environmental Health,* 1, 35.

Currie, B. J., (2003) Melioidosis: an important cause of pneumonia in residents of and travellers returned from endemic regions. *European Respiratory Journal,* 22, 542-550.

Currie, B. J., (2008) Advances and remaining uncertainties in the epidemiology of *Burkholderia pseudomallei* and melioidosis. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 102, 225-227.

Currie, B. J., D. A. Dance and A. C. Cheng, (2008) The global distribution of *Burkholderia pseudomallei* and melioidosis: an update. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 102 Suppl 1, S1-4.

Currie, B. J., D. A. Fisher, N. M. Anstey and S. P. Jacups, (2000a) Melioidosis: Acute and chronic disease, relapse and re-activation. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 94, 301-304.

Currie, B. J., D. A. Fisher, D. M. Howard and J. N. Burrow, (2000b) Neurological melioidosis. *Acta Tropica,* 74, 145-151.

Currie, B. J., D. A. Fisher, D. M. Howard, J. N. Burrow, D. Lo, S. Selva-nayagam, N. M. Anstey, S. E. Huffam, P. L. Snelling, , P. J. Marks, D. P. Stephens, G. D. Lum, S. P. Jacups and V. L. Krause, (2000c) Endemic melioidosis in tropical northern Australia: A 10-year prospective study and review of the literature. *Clinical Infectious Diseases,* 31, 981-986.

Currie, B. J., D. A. Fisher, D. M. Howard, J. N. Burrow, S. Selvanayagam, P. L. Snelling, N. M. Anstey and M. J. Mayo, (2000d) The epidemiology of melioidosis in Australia and Papua New Guinea. *Acta Tropica,* 74, 121-127.

Currie, B. J. and S. P. Jacups, (2003) Intensity of rainfall and severity of melioidosis, Australia. *Emerging Infectious Diseases,* 9, 1538.

Currie, B. J., S. P. Jacups, A. C. Cheng, D. A. Fisher, N. M. Anstey, S. E. Huffam and V. L. Krause, (2004) Melioidosis epidemiology and risk factors from a prospective whole-population study in northern Australia. *Tropical Medicine and International Health,* 9, 1167-1174.

Currie, B. J., M. Mayo, N. M. Anstey, P. Donohoe, A. Haase and D. J. Kemp, (2001) A cluster of melioidosis cases from an endemic region is clonal and is linked to the water supply using molecular typing of *Burkholderia pseudomallei* isolates. *The American Journal of Tropical Medicine and Hygiene,* 65, 177-179.

Currie, B. J., L. Ward and A. C. Cheng, (2010) The epidemiology and clinical spectrum of melioidosis: 540 cases from the 20 year Darwin prospective study. *PLoS Neglected Tropical Diseases,* 4, e900.

Cuzzubbo, A. J., V. Chenthamarakshan, J. Vadivelu, S. D. Puthucheary, D. Rowland and P. L. Devine, (2000) Evaluation of a new commercially available immunoglobulin M and immunoglobulin G immunochromatographic test for diagnosis of melioidosis infection. *Journal of Clinical Microbiology,* 38, 1670-1671.

Da Silva, A. M. M. and L. B. Sacomani, (2001) Using chemical and physical parameters to define the quality of pardo river water (Botucatu-SP-Brazil). *Water Research,* 35, 1609-1616.

Dabisch, P., J. Yeager, J. Kline, K. Klinedinst, A. Welsch and M. L. Pitt, (2012) Comparison of the efficiency of sampling devices for aerosolized *Burkholderia pseudomallei*. *Inhalation Toxicology,* 24, 247-254.

Dai, D., Y. S. Chen, P. S. Chen and Y. L. Chen, (2012) Case cluster shifting and contaminant source as determinants of melioidosis in Taiwan. *Tropical Medicine and International Health,* 17, 1005-1013.

Dance, D., (2014) Treatment and prophylaxis of melioidosis. *International Journal of Antimicrobial Agents,* article in press, 310-318.

Dance, D. A., (1991) Melioidosis: the tip of the iceberg? *Clinical Microbiology Reviews,* 4, 52-60.

Dance, D. A., C. King, H. Aucken, C. D. Knott, P. G. West and T. L. Pitt, (1992) An outbreak of melioidosis in imported primates in Britain. *Veterinary Record,* 130, 525-529.

Dance, D. A. B., (2000a) Ecology of *Burkholderia pseudomallei* and the interactions between environmental *Burkholderia* spp. and human–animal hosts. *Acta Tropica,* 74, 159-168.

Dance, D. A. B., (2000b) Melioidosis as an emerging global problem. *Acta Tropica,* 74, 115-119.

Dance, D. A. B., (2005) Melioidosis and Glanders as Possible Biological Weapons. In: I. W. Fong, K. Alibek and I. W. Fong (eds.), *Bioterrorism and infectious agents: a new dilemma for the 21st century.* Springer. 99-145.

Dejsirilert, S., E. Kondo, D. Chiewsilp and K. Kanai, (1991) Growth and survival of Pseudomonas pseudomallei in acidic environments. *Japanese Journal of Medical Science and Biology,* 44, 63-74.

Deni, S. M., A. Jemain and K. Ibrahim, (2008) The spatial distribution of wet and dry spells over Peninsular Malaysia. *Theoretical and Applied Climatology,* 94, 163-173.

Department of Statistics Malaysia, (2012) 2011 Economic Census: livestock. In: M. Department of Statistics (ed.), *Economic Census.* Department of Statistics, Malaysia, Federal Government Administrative Centre, 62514, Putrajaya.

DeShazer, D., (2007) Virulence of clinical and environmental isolates of *Burkholderia oklahomensis* and *Burkholderia thailandensis* in hamsters and mice. *FEMS Microbiology Letters,* 277, 64-69.

Deuble, M., C. Aquilina and R. Norton, (2013) Neurologic Melioidosis. *The American Journal of Tropical Medicine and Hygiene,* 89, 535-539.

Devendra, C., (1993) *Sustainable animal production from small farm systems in South-East Asia*. Food & Agriculture Organisation. Animal Production & Health, 106.

Devendra, C. and D. Thomas, (2002) Crop–animal systems in Asia: importance of livestock and characterisation of agro-ecological zones. *Agricultural Systems,* 71, 5-15.

Dobson, A., (2004) Population dynamics of pathogens with multiple host species. *The American Naturalist,* 164 Supplementary 5, S64-78.

Dodin, A., (1992) Naissance, vie... et assoupissement d'une maladie infectieuse: la melioidose. *Annal de Insttitute Pasteur,* 3, 267-270.

Dodin, A. and M. Galimand, (1986) Naissance, vie et recul d'une maladie infectieuse, la mdlioidose en pays tempere. *Archives Institute Pasteur Tunis 63*, 69-73.

DOSM, (2012) Selected Indicators for Agriculture, Crops and Livestock, Malaysia, 2006-2010. In: M. Department of Statistics (ed.). http://www.statistics.gov.my/.../Summary_Indikator_Terpilih_Pertanian_Tanaman_Dan_Ternakan_malaysia_2006-2010.

Draper, A., M. Mayo, G. Harrington, D. Karp, D. Yinfoo, L. Ward, A. Haslem, B. Currie and M. Kaestli, (2010) Association of the melioidosis agent *Burkholderia pseudomallei* with water parameters in rural water supplies in Northern Australia. *Applied and Environmental Microbiology,* 76, 5305-5307.

DVS, (2011a) Arahan Prosedur Tetap Veterinar Malaysia Penyiasatan Penyakit Dan Kajian Epidemiologi. Jabatan Perkhidmatan Veterinar, Kementerian Pertanian Dan Industri Asas Tani Malaysia, pp46.

DVS, (2011b) Livestock Population. *Agro-Food Statistics.* Department of Veterinary Services, Ministry of Agriulture and Agro-Based Industry Malaysia.

El-Tarabily, K. A., G. E. S. J. Hardy, K. Sivasithamparam and I. D. Kurtböke, (1996) Microbiological differences between limed and unlimed soils and their relationship with cavity spot disease of carrots (*Daucus carota* L.) caused by *Pythium coloratum* in Western Australia. *Plant and Soil,* 183, 279-290.

England, L. S., H. Lee and J. T. Trevors, (1993) Bacterial survival in soil: effect of clays and protozoa. *Soil Biology and Biochemistry,* 25, 525-531.

Fatimah, I., B. O. Ikede and R. A. Mutalib, (1984) Granulomatous orchitis and periorchitis caused by *Pseudomonas pseudomallei* in a goat. *Veterinary Record,* 114, 67-68.

Forster, J., (1995) Soil physical analysis. *Methods in Applied Soil Microbiology and Biochemistry,* 105-106.

Galyov, E. E., P. J. Brett and D. DeShazer, (2010) Molecular insights into *Burkholderia pseudomallei* and *Burkholderia mallei* pathogenesis. *Annual Review of Microbiology,* 64, 495-517.

Garkaklis, M. J., J. Bradley and R. Wooller, (2003) The relationship between animal foraging and nutrient patchiness in south-west Australian woodland soils. *Soil Research,* 41, 665-673.

Gibney, K. B., A. C. Cheng and B. J. Currie, (2008) Cutaneous Melioidosis in the Tropical Top End of Australia: A Prospective Study and Review of the Literature. *Clinical Infectious Diseases,* 47, 603-609.

Gilad, J., (2007) *Burkholderia mallei* and *Burkholderia pseudomallei*: the causative micro-organisms of glanders and melioidosis. *Recent Patents on Antiinfective Drug Discovery,* 2, 233-241.

Goodyear, A., H. Bielefeldt-Ohmann, H. Schweizer and S. Dow, (2012) Persistent gastric colonization with *Burkholderia pseudomallei* and dissemination from the gastrointestinal tract following mucosal inoculation of mice. *PloS one,* 7, e37324.

Goodyear, A., L. Strange, D. A. Rholl, J. Silisouk, D. A. Dance, H. P. Schweizer and S. Dow, (2013) An improved selective culture medium enhances the isolation of *Burkholderia pseudomallei* from contaminated specimens. *The American Journal of Tropical Medicine and Hygiene,* 89, 973-982.

Gudmundsdottir, T., H. Asgeirsson, H. S. Hardarson and A. S. Thorisdottir,(2014) [Melioidosis, first four cases in Iceland]. *Laeknabladid,* 100, 85-89.

Hamad, M. A., C. R. Austin, A. L. Stewart, M. Higgins, A. Vázquez-Torres and M. I. Voskuil, (2011) Adaptation and antibiotic tolerance of anaerobic *Burkholderia pseudomallei*. *Antimicrobial Agents and Chemotherapy,* 55, 3313-3323.

Hampton, V., M. Kaestli, M. Mayo, J. L. Choy, G. Harrington, L. Richardson, S. Benedict, R. Noske, S. T. Garnett, D. Godoy, B. G. Spratt and B. J. Currie, (2011) Melioidosis in birds and *Burkholderia pseudomallei* dispersal, Australia. *Emerging Infectious Diseases,* 17, 1310-1312.

Harland, D. N., K. Chu, A. Haque, M. Nelson, N. J. Walker, M. Sarkar-Tyson, T. P. Atkins, B. Moore, K. A. Brown and G. Bancroft, (2007) Identification of a LolC homologue in *Burkholderia pseudomallei*, a novel protective antigen for melioidosis. *Infection and Immunity*, 75, 4173-4180.

Harley, V., D. Dance, G. Tovey, M. McCrossan and B. Drasar, (1997) An ultrastructural study of the phagocytosis of *Burkholderia pseudomallei*. *Microbios,* 94, 35-45.

Hassan, M. R., S. P. Pani, N. P. Peng, K. Voralu, N. Vijayalakshmi, R. Mehanderkar, N. A. Aziz and E. Michael, (2010) Incidence, risk factors and clinical epidemiology of melioidosis: a complex socio-ecological emerging infectious disease in the Alor Setar region of Kedah, Malaysia. *BMC Infectious Diseases,* 10, 302.

Hayden, H. S., R. Lim, M. J. Brittnacher, E. H. Sims, E. R. Ramage, C. Fong, Z. Wu, E. Crist, J. Chang, Y. Zhou, M. Radey, L. Rohmer, E. Haugen, W. Gillett, V. Wuthiekanun, S. J. Peacock, R. Kaul, S. I. Miller, C. Manoil and M. A. Jacobs, (2012) Evolution of *Burkholderia pseudomallei* in recurrent melioidosis. *PloS One,* 7, e36507.

Hicks, C., R. Kinoshita and P. Ladds, (2000) Pathology of melioidosis in captive marine mammals. *Australian Veterinary Journal,* 78, 193-195.

Hisham, A. B., C. N. A. Shaharom, M. Marzukhi, R. Norli, O. Fatimah, K. Kee, O. Arbaiah, M. M. Yusof and A. Adam, (2009) Spectrum of flood-related diseases encountered during flood disaster in Johore, Malaysia. *Malaysian Journal of Community Health,* 16, 16-26.

Holden, M. T. G., R. W. Titball, S. J. Peacock, A. M. Cerdeño-Tárraga, T. Atkins, L. C. Crossman, T. Pitt, C. Churcher, K. Mungall, S. D. Bentley, M. Sebaihia, N. R. Thomson, N. Bason, I. R. Beacham, K. Brooks, K. a. Brown, N. F. Brown, G. L. Challis, I. Cherevach, T. Chillingworth, A. Cronin, B. Crossett, P. Davis, D. DeShazer, T. Feltwell, A. Fraser, Z. Hance, H. Hauser, S. Holroyd, K. Jagels, K. E. Keith, M. Maddison, S. Moule, C. Price, M. a. Quail, E. Rabbinowitsch, K. Rutherford, M. Sanders, M. Simmonds, S. Songsivilai, K. Stevens, S. Tumapa, M. Vesaratchavest, S. Whitehead, C. Yeats, B. G. Barrell, P. C. F. Oyston and J. Parkhill, (2004) Genomic plasticity of the causative agent of melioidosis, *Burkholderia pseudomallei*. *Proceedings of the National Academy of Sciences of the United States of America,* 101, 14240-14245.

How, H.-S., K.-H. Ng, H.-B. Yeo, H.-P. Tee and A. Shah, (2005) Pediatric melioidosis in Pahang, Malaysia. *Journal of Microbiology, Immunology, and Infection,* 38, 314-319.

Howard, K. and T. J. J. Inglis, (2003) The effect of free chlorine on *Burkholderia pseudomallei* in potable water. *Water Research,* 37, 4425-4432.

Howard, K. and T. J. J. Inglis, (2005) Disinfection of *Burkholderia pseudomallei* in potable water. *Water Research,* 39, 1085-1092.

Hugh-Jones, M. E., W. T. Hubbert and H. V. Hagstad, (2008) *Zoonoses: Recognition, Control, and Prevention*. John Wiley & Sons. Pp79-120.

Iliukhin, V., T. Senina, N. Plekhanova, V. Antonov, L. Merinova and I. Seimova, (2002) B*urkholderia thailandensis*: biological properties, identification and taxonomy]. *Molekuliarnaia Genetika, Mikrobiologiia I Virusologiia*, 7.

Inglis, T., S. Garrow, M. Henderson, A. Clair, J. Sampson, L. O'Reilly and B. Cameron, (2000a) *Burkholderia pseudomallei* traced to water treatment plant in Australia. *Emerging Infectious Diseases,* 6, 56.

Inglis, T. J., A. Merritt, G. Chidlow, M. Aravena-Roman and G. Harnett, (2005) Comparison of diagnostic laboratory methods for identification of *Burkholderia pseudomallei*. *Journal of Clinical Microbiology,* 43, 2201-2206.

Inglis, T. J., D. B. Rolim and J. L. Rodriguez, (2006) Clinical guideline for diagnosis and management of melioidosis. *Revista do Instituto de Medicina Tropical de São Paulo,* 48, 1-4.

Inglis, T. J. and J.-L. Sagripanti, (2006) Environmental factors that affect the survival and persistence of *Burkholderia pseudomallei*. *Applied and Environmental Microbiology,* 72, 6865-6875.

Inglis, T. J. J., M. Aravena-Roman, S. Ching, K. Croft, V. Wuthiekanun and B. J. Mee, (2003a) Cellular fatty acid profile distinguishes *Burkholderia pseudomallei* from avirulent *Burkholderia thailandensis*. *Journal of Clinical Microbiology,* 41, 4812-4814.

Inglis, T. J. J., N. F. Foster, D. Gal, K. Powell, M. Mayo, R. Norton and B. J. Currie, (2004) Preliminary report on the northern Australian melioidosis environmental surveillance project. *Epidemiology and Infection,* 132, 813-820.

Inglis, T. J. J., A. Levy, A. J. Merritt, M. Hodge, R. McDonald and D. E. Woods, (2009) Melioidosis Risk in a Tropical Industrial Environment. *The American Journal of Tropical Medicine and Hygiene,* 80, 78-84.

Inglis, T. J. J., B. J. Mee and B. J. Chang, (2001) The environmental microbiology of melioidosis. *Reviews in Medical Microbiology,* 12, 13-20.

Inglis, T. J. J., P. Rigby, T. A. Robertson, N. S. Dutton, M. Henderson and B. J. Chang, (2000b) Interaction between *Burkholderia pseudomallei* and *Acanthamoeba* species results in coiling phagocytosis, endamebic bacterial survival, and escape. *Infection and Immunity,* 68, 1681-1686.

Inglis, T. J. J., T. Robertson, D. E. Woods, N. Dutton and B. J. Chang, (2003b) Flagellum-Mediated Adhesion by *Burkholderia pseudomallei* Precedes Invasion of *Acanthamoeba astronyxis*. *Infection and Immunity,* 71, 2280-2282.

Inglis, T. J. J. and A. Q. Sousa, (2009) The public health implications of melioidosis. *Brazilian Journal of Infectious Diseases,* 13, 59-66.

Ishida, A., S. H. Law and Y. Aita, (2003) Changes in food consumption expenditure in Malaysia. *Agribusiness,* 19, 61-76.

Ismail, G., R. Mohamed, S. Rohana, H. Sharifah and N. Embi, (1991) Antibody to *Pseudomonas pseudomallei* exotoxin in sheep exposed to natural infection. *Veterinary Microbiology,* 27, 277-282.

Jabbar, Z. and B. J. Currie, (2013) Melioidosis and the kidney. *Nephrology (Carlton),* 18, 169-175.

Jayaram, M., (2005) Melioidosis- should it be a notifiable disase in Malaysia? *Medical Journal of Malaysia,* 60, 531.

Juma, N. G., (1993) Interrelationships between soil structure/texture, soil biota/soil organic matter and crop production. *Geoderma,* 57, 3-30.

Jurkevitch, E., Y. Hadar and Y. Chen, (1986) The remedy of lime-induced chlorosis in peanuts by pseudomonas sp. siderophores. *Journal of Plant Nutrition*, 9, 535-545.

Kaestli, M., M. Mayo, G. Harrington, L. Ward, F. Watt, J. V. Hill, A. C. Cheng and B. J. Currie, (2009) Landscape changes influence the occurrence of the melioidosis bacterium *Burkholderia pseudomallei* in soil in northern Australia. *PLoS Neglected Tropical Diseases*, 3, e364.

Kaestli, M., M. Mayo, G. Harrington, F. Watt, J. Hill, D. Gal and B. Currie, (2007) Sensitive and specific molecular detection of *Burkholderia pseudomallei*, the causative agent of melioidosis, in the soil of tropical northern Australia. *Applied and Environmental Microbiology*, 73, 6891-6897.

Kaestli, M., M. Schmid, M. Mayo, M. Rothballer, G. Harrington, L. Richardson, A. Hill, J. Hill, A. Tuanyok and P. Keim, (2012) Out of the ground: aerial and exotic habitats of the melioidosis bacterium *Burkholderia pseudomallei* in grasses in Australia. *Environmental Microbiology*, 14, 2058-2070.

Kamaruddin, S. B., (2013) Call to categorise melioidosis as notifiable disease. *UKM News Portal*. UKM, Malaysia., http://www.ukm.my/news/index.php/research-news/1454-call-to-categorise-melioidosis-a-notifiable-disease.html.

Kamjumphol, W., S. Chareonsudjai, P. Chareonsudjai, S. Wongratanacheewin and S. Taweechaisupapong, (2013) Environmental factors affecting *Burkholderia pseudomallei* biofilm formation. *Southeast Asian Journal of Tropical Medicine and Public Health*, 44, 72-81.

Kanai, K., E. Kondo, S. Dejsirilert and P. Naigowit, (1994) Growth and survival of *Pseudomonas pseudomallei* in acidic environment with possible relation to the ecology and epidemiology of melioidosis. Pp26-38. *In* Puthucheary S.D. & Malik, Y.A. (Eds) Melioidosis: prevailing problems and future directions. Malaysian Society of Infectious Diseases and Chemotherapy, Kuala Lumpur, Malaysia.

Ketterer, P. J., W. R. Webster, J. Shield, R. J. Arthur, P. J. Blackall and A. D. Thomas, (1986) Melioidosis in intensive piggeries in south eastern Queensland. *Australian Veterinary Journal*, 63, 146-149.

Kung, C.-T., C.-J. Li, S.-F. Ko and C.-H. Lee, (2013) A melioidosis patient presenting with brainstem signs in the emergency department. *The Journal of Emergency Medicine*, 44, e9-e12.

Lazar Adler, N. R., B. Govan, M. Cullinane, M. Harper, B. Adler and J. D. Boyce, (2009) The molecular and cellular basis of pathogenesis in melioidosis: How does *Burkholderia pseudomallei* cause disease? *FEMS Microbiology Reviews*, 33, 1079-1099.

Lehtola, M., I. Miettinen, M. Keinänen, T. Kekki, O. Laine, A. Hirvonen, T. Vartiainen and P. Martikainen, (2004) Microbiology, chemistry and biofilm development in a pilot drinking water distribution system with copper and plastic pipes. *Water Research,* 38, 3769-3779.

Lengwehasatit, I., A. Nuchtas, S. Tungpradabkul, S. Sirisinha and P. Utaisincharoen, (2008) Involvement of *B. pseudomallei* RpoS in apoptotic cell death in mouse macrophages. *Microbial Pathogenesis,* 44, 238-245.

Lévesque, M. J., S. L. Boissière, J. C. Thomas, R. Beaudet, R. Villemur and S. L. Boissière, (1997) Rapid method for detecting Desulfitobacterium frappieri strain PCP-1 in soil by the polymerase chain reaction. *Applied Microbiology and Biotechnology,* 47, 719-725.

Li, L., Z. Lu and O. Han, (1994) [Epidemiology of melioidosis in China]. *Zhonghua liu xing bing xue za zhi= Zhonghua liuxingbingxue zazhi,* 15, 292-295.

Lim, K. and V. Chong, (2010) Radiological manifestations of melioidosis. *Clinical Radiology,* 65, 66-72.

Limmathurotsakul, D., K. Jamsen, A. Arayawichanont, J. A. Simpson, L. J. White, S. J. Lee, V. Wuthiekanun, N. Chantratita, A. Cheng and N. P. Day, (2010a) Defining the true sensitivity of culture for the diagnosis of melioidosis using Bayesian latent class models. *PloS One,* 5, e12485.

Limmathurotsakul, D., M. Kanoksil, V. Wuthiekanun, R. Kitphati, N. P. Day and S. J. Peacock, (2013) Activities of daily living associated with acquisition of melioidosis in northeast Thailand: a matched case-control study. *PLoS Neglected Tropical Diseases,* 7, e2072.

Limmathurotsakul, D. and S. J. Peacock, (2011) Melioidosis: a clinical overview. *British Medical Bulletin,* 99, 125-139.

Limmathurotsakul, D., G. Wongsuvan, D. Aanensen, S. Ngamwilai, N. Saiprom, P. Rongkard, J. Thaipadungpanit, M. Kanoksil, N. Chantratita, N. Day and S. Peacock, (2014) Melioidosis Caused by *Burkholderia pseudomallei* in Drinking Water, Thailand, 2012. *Emerging Infectious Diseases,* 20, 265-268.

Limmathurotsakul, D., V. Wuthiekanun, P. Amornchai, G. Wongsuwan, N. P. Day and S. J. Peacock, (2012) Effectiveness of a simplified method for isolation of *Burkholderia pseudomallei* from soil. *Applied and Environmental Microbiology,* 78, 876-877.

Limmathurotsakul, D., V. Wuthiekanun, N. Chantratita, G. Wongsuvan, P. Amornchai, N. P. J. Day and S. J. Peacock, (2010b) *Burkholderia pseudomallei* is spatially distributed in soil in northeast Thailand. *PLoS Neglected Tropical Diseases,* 4, e694.

Lipsitz, R., S. Garges, R. Aurigemma, P. Baccam, D. D. Blaney, A. C. Cheng, B. J. Currie, D. Dance, J. E. Gee and J. Larsen, (2012) Workshop on Treatment of and Postexposure Prophylaxis for *Burkholderia pseudomallei* and *B. mallei* Infection, 2010. *Emerging Infectious Diseases*, 18, e2.

Little, P., (1979) A Malaysian Experience with Animal Disease. *The Canadian Veterinary Journal*, 20, 13.

LuČnsdorf, H., R. Erb, W. Abraham and K. Timmis, (2000) Clay hutches': a novel interaction between bacteria and clay minerals. *Environmental Microbiology*, 2, 161ą168.

Lugt, J. J. and M. Henton, (1995) Melioidosis in a goat. *Journal of the South African Veterinary Association*, 66, 71-73.

Maharjan, B., N. Chantratita, M. Vesaratchavest, A. Cheng, V. Wuthiekanun, W. Chierakul, W. Chaowagul, N. P. J. Day and S. J. Peacock, (2005) Recurrent melioidosis in patients in northeast Thailand is frequently due to reinfection rather than relapse. *Journal of Clinical Microbiology*, 43, 6032-6034.

Malczewski, A. B., K. M. Oman, R. E. Norton and N. Ketheesan, (2005) Clinical presentation of melioidosis in Queensland, Australia. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 99, 856-860.

Marshall, K., (1975) Clay mineralogy in relation to survival of soil bacteria. *Annual Review of Phytopathology*, 13, 357-373.

Mayo, M., M. Kaesti, G. Harrington, A. C. Cheng, L. Ward, D. Karp, P. Jolly, D. Godoy, B. G. Spratt and B. J. Currie, (2011) *Burkholderia pseudomallei* in unchlorinated domestic bore water, tropical northern Australia. *Emerging Infectious Diseases*, 17, 1283-1285.

McCormick, J. B., D. J. Sexton, J. G. McMurray, E. Carey, P. Hayes and R. A. Feldman, (1975) Human-to-human transmission of *Pseudomonas pseudomallei*. *Annals of Internal Medicine*, 83, 512-513.

McRobb, E., M. Kaestli, M. Mayo, E. P. Price, D. S. Sarovich, D. Godoy, B. G. Spratt and B. J. Currie, (2013) Melioidosis from contaminated bore water and successful UV sterilization. *The American Journal of Tropical Medicine and Hygiene*, 89, 367-368.

Meselson, M., J. Guillemin and M. Hugh-Jones, (2002) Public health assessment of potential biological terrorism agents. *Emerging Infectious Diseases*, 8, 225.

Meumann, E. M., A. C. Cheng, L. Ward and B. J. Currie, (2012) Clinical features and epidemiology of melioidosis pneumonia: results from a 21-year study and review of the literature. *Clinical Infectious Diseases*, 54, 362-369.

Millan, J. M., M. Mayo, D. Gal, A. Janmaat and B. J. Currie, (2007) Clinical variation in melioidosis in pigs with clonal infection following possible environmental contamination from bore water. *The Veterinary Journal*, 174, 200-202.

Miller, W. and D. Miller, (1987) A micro- pipette method for soil mechanical analysis. *Communications in Soil Science & Plant Analysis*, 18, 1-15.

Miralles, I. S., M. d. C. A. Maciel, M. R. F. Angelo, M. M. Gondini, L. H. F. Frota, C. M. F. d. Reis and E. Hofer, (2004) *Burkholderia pseudomallei*: a case report of a human infection in Ceará, Brazil. *Revista do Instituto de Medicina Tropical de São Paulo*, 46, 51-54.

MNRE, (2011) Malaysia Second National Communication to the UNFCC (NC2). Ministry of Natural Resources and Environment, Malaysia, Putrajaya, pp145, Putrajaya.

Moore, R., A. Tuanyok and D. Woods, (2008) Survival of *Burkholderia pseudomallei* in water. *BMC Research Notes*, 1, 11.

Moran, G. J., (2002) Threats in bioterrorism II: CDC category B and C agents. *Emergency Medicine Clinics of North America*, 20, 311-330.

Moule, M. G., C. M. Hemsley, Q. Seet, J. A. Guerra-Assuncao, J. Lim, M. Sarkar-Tyson, T. G. Clark, P. B. Tan, R. W. Titball, J. Cuccui and B. W. Wren, (2014) Genome-wide saturation mutagenesis of *Burkholderia pseudomallei* K96243 predicts essential genes and novel targets for antimicrobial development. *MBio*, 5, e00926-00913.

Mu, J. J., P. Y. Cheng, Y. S. Chen, P. S. Chen and Y. L. Chen, (2014) The occurrence of melioidosis is related to different climatic conditions in distinct topographical areas of Taiwan. *Epidemiology and Infection*, 142, 415-423.

Munckhof, W. J., M. J. Mayo, I. Scott and B. J. Currie, (2001) Fatal human melioidosis acquired in a subtropical Australian city. *The American Journal of Tropical Medicine and Hygiene*, 65, 325-328.

Na-ngam, N., S. Angkititakul, P. Noimay and V. Thamlikitkul, (2004) The effect of quicklime (calcium oxide) as an inhibitor of *Burkholderia pseudomallei*. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 98, 337-341.

Nachiangmai, N., P. Patamasucon, B. Tipayamonthein, A. Kongpon and S. Nakaviroj, (1985) *Pseudomonas pseudomallei* in southern Thailand. *The Southeast Asian Journal of Tropical Medicine and Public Health*, 16, 83-87.

Nandi, T., C. Ong, A. P. Singh, J. Boddey, T. Atkins, M. Sarkar-Tyson, A. E. Essex-Lopresti, H. H. Chua, T. Pearson and J. F. Kreisberg, (2010) A genomic survey of positive selection in *Burkholderia pseudomallei* provides insights into the evolution of accidental virulence. *PLoS Pathogens*, 6, e1000845.

Neamnak, J., S. Tabunhan, S. Wongwajana, S. Wongratanacheewin, Y. Chamgramol, C. Pairojkul, K. Faksri and W. Namwat, (2014) Comparison of Stress Adaptation and Survival Rate Between *Burkholderia pseudomallei* with Mutant and Wild Type bfmR. *Southeast Asian Journal of Tropical Medicine and Public Health,* 45, 346-351.

Nelson, M., J. L. Prior, M. S. Lever, H. E. Jones, T. P. Atkins and R. W. Titball, (2004) Evaluation of lipopolysaccharide and capsular polysaccharide as subunit vaccines against experimental melioidosis. *Journal of Medical Microbiology,* 53, 1177-1182.

Nguyen, B., (1956) Apropos d'une epizootie porcine de melioidose dans une province meridionale du Vietnam. *Bulletin de la Société de Pathologie Exotique,* 49, 25.

Nigg, C., (1963) Serologic studies on subclinical melioidosis. *The Journal of Immunology,* 91, 18.

OIE, (2004) Bovine Brucellosis, Chapter 2.3.1. *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* OIE, Paris.

Olds, R. and F. Lewis, (1954) Melioidosis in goats. *Australian Veterinary Journal,* 30, 253-261.

Olds, R. and F. Lewis, (1955) Melioidosis in a pig. *Australian Veterinary Journal,* 31, 273-274.

Omar, A. R., (1963) Pathology of Melioidosis in Pigs, Goats and a Horse. *Journal of Comparative Pathology and Therapeutics,* 73, 359-372.

Omar, A. R., M. Anwar and P. G. Joseph, (1970) Cerebral melioidosis with hydrocephalus in a buffalo calf. *Kajian Veterinaire,* 2, 215-217.

Ouadah, A., M. Zahedi and R. Perumal, (2007) Animal melioidosis surveillance in Sabah. *The Internet Journal of Veterinary Medicine,* 2.

Palasatien, S., R. Lertsirivorakul, P. Royros, S. Wongratanacheewin and R. W. Sermswan, (2008) Soil physicochemical properties related to the presence of *Burkholderia pseudomallei*. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 102 Suppl 1, S5-9.

Parkes, J., R. Henzell and G. Pickles, (1996) *Managing vertebrate pests: feral goats*. Australian Government Publishing Service Canberra.45-78.

Parry, C. M., V. Wuthiekanun, N. T. T. Hoa, T. S. Diep, P. Van Loc, B. A. Wills, J. Wain, T. T. Hien, N. J. White and J. J. Farrar, (1999) Melioidosis in Southern Vietnam: clinical surveillance and environmental sampling. *Clinical Infectious Diseases,* 29, 1323-1326.

Partida-Martinez, L. P. and C. Hertweck, (2005) Pathogenic fungus harbours endosymbiotic bacteria for toxin production. *Nature*, 437, 884-888.

Partoutomo, S., P. Ronohardjo, A. Wilson and P. Stevenson, (1985) Review of diseases in Indonesia affecting draught power in domestic animals. in Proceedings of the ACIAR Proceedings Series, 1985, p. 140-146.

Paul, E. A., (2006) Part 2: The soil Habitat. In: E. A. Paul (ed.), *Soil Microbiology, Ecology and Biochemistry.* Academic press.

Payne, S., (1993) Iron acquisition in microbial pathogenesis. *TRENDS in Microbiology*, 1, 66-69.

Peacock, S. J., (2006) Melioidosis. *Current Opinion in Infectious Diseases*, 19, 421-428.

Peacock, S. J., G. Chieng, A. C. Cheng, D. A. B. Dance, P. Amornchai, G. Wongsuvan, N. Teerawattanasook, W. Chierakul, N. P. J. Day and V. Wuthiekanun, (2005) Comparison of Ashdown's Medium, *Burkholderia cepacia* medium, and *Burkholderia pseudomallei* selective agar for clinical isolation of *Burkholderia pseudomallei*. *Journal of Clinical Microbiology*, 43, 5359-5361.

Peacock, S. J., D. Limmathurotsakul, Y. Lubell, G. C. Koh, L. J. White, N. P. Day and R. W. Titball, (2012) Melioidosis vaccines: a systematic review and appraisal of the potential to exploit biodefense vaccines for public health purposes. *PLoS Neglected Tropical Diseases*, 6, e1488.

Peacock, S. J., H. P. Schweizer, D. A. Dance, T. L. Smith, J. E. Gee, V. Wuthiekanun, D. DeShazer, I. Steinmetz, P. Tan and B. J. Currie, (2008) Management of accidental laboratory exposure to *Burkholderia pseudomallei* and *B. mallei*. *Emerging Infectious Diseases*, 14, e2.

Perkin-Elmer, (1964) *Analytical methods for atomic absorption spectrophotometry*. PerkinElmer Incoporated, Shelton, CT, USA. 9-75.

Pingali, P. L., (1997) From subsistence to commercial production systems: the transformation of Asian agriculture. *American Journal of Agricultural Economics*, 79, 628-634.

Pitt, T. L., (1995) *Pseudomonas, Burkholderia* and related genera In: W. J. Haussler and M. Sussman (eds.), *Topley and Wilson's microbiology and microbial infections.* Arnod, London, United Kingdom.

Podin, Y., D. S. Sarovich, E. P. Price, M. Kaestli, M. Mayo, K. Hii, N. HieUng, S. Wong, I. Wong, J. Wong, A. Mohan, M. Ooi, T. Fam, J. Wong, A. Tuanyok, P. Keim, P. M. Giffard and B. J. Currie, (2014) *Burkholderia pseudomallei* isolates from sarawak, malaysian borneo, are predominantly susceptible to aminoglycosides and macrolides. *Antimicrobial Agents and Chemotherapy*, 58, 162-166.

Praveen, S., (2009) Analysis of Micronutrients in Soil by Using AA 800 Atomic Absorption Spectrophotometer. *PerkinElmer Global Application Center Mumbai, India*.

Pumirat, P., U. Boonyuen, M. Vanaporn, P. Pinweha, S. Tandhavanant, S. Korbsrisate and N. Chantratita, (2014) The role of short-chain dehydrogenase/oxidoreductase, induced by salt stress, on host interaction of *B. pseudomallei*. *BMC Microbiology,* 14, 1.

Pumirat, P., P. Saetun, S. Sinchaikul, S.-T. T. Chen, S. Korbsrisate and V. Thongboonkerd, (2009) Altered secretome of *Burkholderia pseudomallei* induced by salt stress. *Biochimica et Biophysica Acta,* 1794, 898-904.

Pumpuang, A., N. Chantratita, C. Wikraiphat, N. Saiprom, N. Day, S. Peacock and V. Wuthiekanun, (2011) Survival of *Burkholderia pseudomallei* in distilled water for 16 years. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 105, 598-600.

Puthucheary, S., N. Parasakthi and M. Lee, (1992) Septicaemic melioidosis: a review of 50 cases from Malaysia. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 86, 683-685.

Puthucheary, S. D., (2009) Melioidosis in Malaysia. *The Medical Journal of Malaysia,* 64, 266-274.

Raja, N., M. Ahmed and N. Singh, (2005) Melioidosis: an emerging infectious disease. *Journal of Postgraduate Medicine,* 51, 140.

Raja, N. S., (2008) Cases of melioidosis in a university teaching hospital in Malaysia. *Journal of Microbiology, Immunology, and Infection. Wei Mian Yu Gan Ran Za Zhi,* 41, 174-179.

Ralph, A., J. McBride and B. J. Currie, (2004) Transmission of *Burkholderia pseudomallei* via breast milk in northern Australia. *The Pediatric Infectious Disease Journal,* 23, 1169.

Ranjard, L. and A. Richaume, (2001) Quantitative and qualitative microscale distribution of bacteria in soil. *Research in Microbiology,* 152, 707-716.

Rattanavong, S., V. Wuthiekanun, S. Langla, P. Amornchai, J. Sirisouk, R. Phetsouvanh, C. E. Moore, S. J. Peacock, Y. Buisson and P. N. Newton, (2011) Randomized soil survey of the distribution of *Burkholderia pseudomallei* in rice fields in Laos. *Applied and Environmental Microbiology,* 77, 532-536.

Rholl, D. A., K. M. Papp-Wallace, A. P. Tomaras, M. L. Vasil, R. A. Bonomo and H. P. Schweizer, (2011) Molecular investigations of PenA-mediated β-lactam resistance in *Burkholderia pseudomallei*. *Frontiers in Microbiology,* 2.

Riedel, K., R. Renneberg, M. Kühn and F. Scheller, (1988) A fast estimation of biochemical oxygen demand using microbial sensors. *Applied Microbiology and Biotechnology*, 28, 316-318.

Robert, M. and C. Chenu, (1992) Interactions between soil minerals and microorganisms. In: B. J. M. Stotzky G. (ed.), *Soil Biochemistry*. Marcel Dekker Inc, New York, Hong Kong.

Robertson, J., A. Levy, J.-L. Sagripanti and T. J. Inglis, (2010) The survival of *Burkholderia pseudomallei* in liquid media. *The American Journal of Tropical Medicine and Hygiene,* 82, 88-94.

Roesnita, B., S. T. Tay, S. D. Puthucheary and I. C. Sam, (2012) Diagnostic use of *Burkholderia pseudomallei* selective media in a low prevalence setting. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 106, 131-133.

Roman, M. R., A. L. Gauzens, W. K. Rhinehart and J. R. White, (1993) Effects of low oxygen waters on Chesapeake Bay zooplankton. *Limnology and Oceanography,* 38, 1603-1614.

Sagripanti, J. L., A. Levy, J. Robertson, A. Merritt and T. J. J. Inglis, (2009) Inactivation of virulent *Burkholderia pseudomallei* by sunlight. *Photochemistry and Photobiology,* 85, 978-986.

Salehi, M., O. H. Beni, H. B. Harchegani, I. E. Borujeni and H. Motaghian, (2011) Refining soil organic matter determination by loss-on-ignition. *Pedosphere,* 21, 473-482.

Sam, I. and S. Puthucheary, (2006) Melioidosis in children from Kuala Lumpur, Malaysia. *Annals of Tropical Paediatrics: International Child Health,* 26, 219-224.

Sam, I. C. and S. D. Puthucheary, (2007) Melioidosis and rainfall in Kuala Lumpur, Malaysia. *Journal of Infection,* 54, 519-520.

Sánchez, E., M. F. Colmenarejo, J. Vicente, A. Rubio, M. G. García, L. Travieso and R. Borja, (2007) Use of the water quality index and dissolved oxygen deficit as simple indicators of watersheds pollution. *Ecological Indicators,* 7, 315-328.

Saonanon, P., S. Tirakunwichcha and W. Chierakul, (2013) Case Report of Orbital Cellulitis and Necrotizing Fasciitis From Melioidosis. *Ophthalmic Plastic and Reconstructive Surgery*, 29, e81-e84.

Sarkar-Tyson, M., S. J. Smither, S. V. Harding, T. P. Atkins and R. W. Titball, (2009) Protective efficacy of heat-inactivated *B. thailandensis*, *B. mallei* or *B. pseudomallei* against experimental melioidosis and glanders. *Vaccine,* 27, 4447-4451.

Sarkar-Tyson, M. and R. W. Titball, (2010) Progress toward development of vaccines against melioidosis: a review. *Clinical Therapeutics,* 32, 1437-1445.

Schneegurt, M. A., S. Y. Dore and C. F. Kulpa, Jr., (2003) Direct extraction of DNA from soils for studies in microbial ecology. *Current Issues in Molecular Biology,* 5, 1-8.

Schulte, E. E., (2004) Soil and Applied Iron. *Understanding Plant Nutrients.* Cooperative Extension Publications, University of Wisconsin.

Schulte, E. E. and K. A. Kelling, (1999) Soil and applied copper. *Understanding Plant Nutrients.* Cooperative Extensions Publications, University of Wisconsin.

Schwarz, S., T. E. West, F. Boyer, W.-C. Chiang, M. A. Carl, R. D. Hood, L. Rohmer, T. Tolker-Nielsen, S. J. Skerrett and J. D. Mougous, (2010) Burkholderia type VI secretion systems have distinct roles in eukaryotic and bacterial cell interactions. *PLoS Pathogens,* 6, e1001068.

Schweizer, H. P., (2012) Mechanisms of antibiotic resistance in *Burkholderia pseudomallei*: implications for treatment of melioidosis. *Future Microbiology,* 7, 1389-1399.

Schwertmann, U., (1993) Relations between iron oxides, soil color, and soil formation. *Soil Color,* 51-69.

Sergent, E. S. G., (2014) Epitools epidemiological calculators. AusVet Animal Health Services and Australian Biosecurity Cooperative Research Centre for Emerging Infectious Disease. Available at: http://epitools.ausvet.com.au.

Shams, A. M., L. J. Rose, L. Hodges and M. J. Arduino, (2007) Survival of *Burkholderia pseudomallei* on Environmental Surfaces. *Applied and Environmental Microbiology,* 73, 8001-8004.

Shan, G., W. Jin, E. K. H. Lam and X. Xing, (2008) Purification of total DNA extracted from activated sludge. *Journal of Environmental Sciences,* 20, 80-87.

Shanta, C., (1960) A note on the isolationof Pf. whitmori from the large intestines of a goat. *Journal of the Malayan Veterinary Medical Association,* 3, 35-36.

Sheikh-Omar, A. R. and H. Muda, (1986) Melioidosis in Sika deer (*Cervus nippon nippon*). *Australian Veterinary Journal,* 63, 168.

Siripanthong, S., S. Teerapantuwat, W. Prugsanusak, Y. Suputtamongkol, P. Viriyasithavat, W. Chaowagul, D. Dance and N. White, (1991) Corneal ulcer caused by *Pseudomonas pseudomallei*: report of three cases. *Review of Infectious Diseases,* 13, 335-337.

Smith, M. D., V. Wuthiekanun, A. L. Walsh and N. J. White, (1995) Quantitative recovery of *Burkholderia pseudomallei* from soil in Thailand. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 89, 488-490.

Soffler, C., A. M. Bosco-Lauth, T. A. Aboellail, A. J. Marolf and R. A. Bowen, (2014) Pathogenesis of percutaneous infection of goats with *Burkholderia pseudomallei*: clinical, pathologic, and immunological responses in chronic melioidosis. *International Journal of Experimental Pathology,* 95, 101-119.

Sommanustweechai, A., T. Kasantikul, W. Somsa, S. Wongratanacheewin, R. W. Sermswan, P. Kongmakee, W. Thomas, S. Kamolnorranath, B. Siriaroonrat, M. Bush and W. Banlunara, (2013) Environmental management procedures following fatal melioidosis in a captive chimpanzee (Pan troglodytes). *Jounal of Zoo and Wildlife Medicine,* 44, 475-479.

Song, Y., C. Xie, Y. M. Ong, Y. H. Gan and K. L. Chua, (2005) The BpsIR quorum-sensing system of *Burkholderia pseudomallei*. *Journal of Bacteriology,* 187, 785-790.

Sprague, L. D. and H. Neubauer, (2004) Melioidosis in animals: a review on epizootiology, diagnosis and clinical presentation. *Journal of Veterinary Medicine. B: Infectious Diseases and Veterinary Public Health,* 51, 305-320.

Srikawkheaw, N. and O.-a. Lawhavinit, (2007) Detection of antibodies against melioidosis from animal sera in Thailand by indirect haemagglutination test. *Kasetsart Journal: Nature Science,* 41, 81-85.

Srikitjakarn, L., A. Sirimalaisuwan, R. Khattiya, K. Krueasukhon and M. Mekaprateep, (2002) Seroprevalence of melioidosis in dairy cattle in Chiang Mai Province, northern Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health,* 33, 739-741.

St Arnaud, M. and A. Elsen, (2005) Interaction of arbuscular mycorrhizal fungi with soil-borne pathogens and non-pathogenic rhizosphere micro-organisms. *In-vitro Culture of Mycorrhizas*, 217-231.

Stach, J. E. M., S. Bathe, J. P. Clapp and R. G. Burns, (2001) PCR- SSCP comparison of 16S rDNA sequence diversity in soil DNA obtained using different isolation and purification methods. *FEMS Microbiology Ecology,* 36, 139-151.

Stanton, A. and W. Fletcher, (1932) *Melioidosis: studies from the institute of medical research, Federated Malay States*. John Bale & Sons and Danielson Ltd, London.

Stanton, A. T. and W. Fletcher, (1921) *Melioidosis; a new disease of the tropics,* p. 1 p.l., 2 p. F. M. S. government printing officce, Kuala Lumpur.

Stanton, A. T. and W. Fletcher, (1925) Melioidosis and its relation to glanders. *Journal of Hygiene (London),* 23, 347-368.

Stevens, M. P., M. W. Wood, L. A. Taylor, P. Monaghan, P. Hawes, P. W. Jones, T. S. Wallis and E. E. Galyov, (2002) An Inv/Mxi-Spa-like type III protein secretion system in *Burkholderia pseudomallei* modulates intracellular behaviour of the pathogen. *Molecular Microbiology,* 46, 649-659.

Stevens, R. and R. Laughlin, (1996) Effects of lime and nitrogen fertilizer on two sward types over a 10-year period. *Journal of Agricultural Science-Cambridge,* 127, 451-462.

Stotzky, G., (1997) Soil as an environment for microbial life. *Modern soil microbiology. Dekker, New York,* 1-20.

Strauss, J. M., A. D. Alexander, G. Rapmund, E. Gan and A. E. Dorsey, (1969a) Melioidosis in Malaysia: III. Antibodies to *Pseudomonas pseudomallei* in the human population. *The American Journal of Tropical Medicine and Hygiene,* 18, 703-707.

Strauss, J. M., M. G. Groves, M. Mariappan and D. W. Ellison, (1969b) Melioidosis in Malaysia. II. Distribution of *Pseudomonas pseudomallei* in soil and surface water. *The American Journal of Tropical Medicine and Hygiene,* 18, 698-702.

Su, H. P., H. W. Yang, Y. L. Chen, T. L. Ferng, Y. L. Chou, T. C. Chung, C. H. Chen, C. S. Chiang, M. M. Kuan, H. H. Lin and Y. S. Chen, (2007) Prevalence of melioidosis in the Er-Ren River Basin, Taiwan: implications for transmission. *Journal of Clinical Microbiology,* 45, 2599-2603.

Suputtamongkol, Y., W. Chaowagul, P. Chetchotisakd, N. Lertpatanasuwun, S. Intaranongpai, T. Ruchutrakool, D. Budhsarawong, P. Mootsikapun, V. Wuthiekanun and N. Teerawatasook, (1999) Risk factors for melioidosis and bacteremic melioidosis. *Clinical Infectious Diseases,* 29, 408-413.

Suputtamongkol, Y., A. Hall, D. Dance, W. Chaowagul, A. Rajchanuvong, M. Smith and N. White, (1994) The epidemiology of melioidosis in Ubon Ratchatani, northeast Thailand. *International Journal of Epidemiology,* 23, 1082-1090.

Surin, U., (2012) Monthly Rainfall and Severity of Melioidosis in Nakhon Phanom, Northeastern Thailand. *Journal of Health Science,* 17, 363-375.

Sylvia, D. M., J. J. Fuhrmann, P. Hartel and D. A. Zuberer, (2005) *Principles and applications of soil microbiology*. Pearson Prentice Hall New Jersey. 342-476.

Thatrimontrichai, A. and G. Maneenil, (2012) Neonatal melioidosis: systematic review of the literature. *The Pediatric Infectious Disease Journal,* 31, 1195-1197.

Thibault, F. M., E. Valade and D. R. Vidal, (2004) Identification and Discrimination of *Burkholderia pseudomallei, B. mallei,* and *B. thailandensis* by Real-Time PCR Targeting Type III Secretion System Genes. *Journal of Clinical Microbiology,* 42, 5871-5874.

Thin, R., M. Brown, J. Stewart and C. Garrett, (1970) Melioidosis: a report of ten cases. *Quarterly Journal of Medicine,* 39, 115-127.

Thin, R., M. Groves, G. Rapmund and M. Mariappan, (1971) *Pseudomonas pseudomallei* in the surface water of Singapore. *Singapore Medical Journal,* 12, 181-182.

Thomas, A., J. Forbes-Faulkner, T. D'arcy, J. Norton and D. Hoffmann, (1990) Experimental infection of normal and immunosuppressed pigs with *Pseudomonas pseudomallei*. *Australian Veterinary Journal,* 67, 43-46.

Thomas, A., J. Forbes-Faulkner and M. Parker, (1979) Isolation of *Pseudomonas pseudomallei* from clay layers at defined depths. *American Journal of Epidemiology,* 110, 515.

Thomas, A. and J. C. Forbes-Faulkner, (1981) persistence of *Pseudomonas pseudomallei* in soil. *Australian Veterinary Journal,* 57, 535-536.

Thomas, A. D., (1981) Prevalence of melioidosis in animals in northern Queensland. *Australian Veterinary Journal,* 57, 146-148.

Thomas, A. D., G. A. Spinks, T. L. D'Arcy, J. H. Norton and K. F. Trueman, (1988) Evaluation of four serological tests for the diagnosis of caprine melioidosis. *Australian Veterinary Journal,* 65, 261-264.

Tong, S., S. Yang, Z. Lu and W. He, (1996) Laboratory investigation of ecological factors influencing the environmental presence of *Burkholderia pseudomallei*. *Microbiology and Immunology,* 40, 451-453.

Trung, T. T., A. Hetzer, E. Topfstedt, A. Göhler, D. Limmathurotsakul, V. Wuthiekanun, S. J. Peacock and I. Steinmetz, (2011) Improved culture-based detection and quantification of *Burkholderia pseudomallei* from soil. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 105, 346-351.

Tuanyok, A., H. S. Kim, W. C. Nierman, Y. Yu, J. Dunbar, R. A. Moore, P. Baker, M. Tom, J. M. Ling and D. E. Woods, (2005) Genome- wide expression analysis of iron regulation in *Burkholderia pseudomallei* and *Burkholderia mallei* using DNA microarrays. *FEMS Microbiology Letters,* 252, 327-335.

Tumapa, S., M. T. Holden, M. Vesaratchavest, V. Wuthiekanun, D. Limmathurotsakul, W. Chierakul, E. J. Feil, B. J. Currie, N. P. Day and W. C. Nierman, (2008) *Burkholderia pseudomallei* genome plasticity associated with genomic island variation. *BMC Genomics,* 9, 190.

Ulrich, R. L., D. DeShazer, E. E. Brueggemann, H. B. Hines, P. C. Oyston and J. A. Jeddeloh, (2004) Role of quorum sensing in the pathogenicity of *Burkholderia pseudomallei*. *Journal of Medical Microbiology,* 53, 1053-1064.

Vadivelu, J., S. Puthucheary, G. Gendeh and N. Parasakthi, (1995) Serodiagnosis of melioidosis in Malaysia. *Singapore Medical Journal,* 36, 299-302.

Vadivelu, J. and S. D. Puthucheary, (2000) Diagnostic and prognostic value of an immunofluorescent assay for melioidosis. *The American Journal of Tropical Medicine and Hygiene,* 62, 297-300.

van der Kooij, D., H. Veenendaal and W. Scheffer, (2005) Biofilm formation and multiplication of *Legionella* in a model warm water system with pipes of copper, stainless steel and cross-linked polyethylene. *Water Research,* 39, 2789-2798.

Van der Lugt, J. and M. Henton, (1995) Melioidosis in a goat. *Journal of the South African Veterinary Association,* 66, 71-73.

Vanaporn Wuthiekanum, M. D. S. a. N. J. W., (1995) Survival of *Burkholderia pseudomallei* in the absence of nutrients. *Transcation of the Royal Society of Tropical Medicine and Hygiene,* 89, 491.

Vidyalakshmi, K., S. Lipika, S. Vishal, S. Damodar and M. Chakrapani, (2012) Emerging clinico-epidemiological trends in melioidosis: analysis of 95 cases from western coastal India. *International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases*. 34-145.

Vongphayloth, K., S. Rattanavong, C. Moore, R. Phetsouvanh, V. Wuthiekanun, A. Sengdouangphachanh, P. Phouminh, P. Newton and Y. Buisson, (2012) *Burkholderia pseudomallei* detection in surface water in southern Laos using Moore's swabs. *The American Journal of Tropical Medicine and Hygiene,* 86, 872-877.

Vorachit, M., K. Lam, P. Jayanetra and J. Costerton, (1993) Resistance of *Pseudomonas pseudomallei* growing as a biofilm on silastic discs to ceftazidime and co-trimoxazole. *Antimicrobial Agents and Chemotherapy,* 37, 2000-2002.

Walsh, A. L., M. D. Smith, V. Wuthiekanun, Y. Suputtamongkol, V. Desakorn, W. Chaowagul and N. J. White, (1994) Immunofluorescence microscopy for the rapid diagnosis of melioidosis. *Journal of Clinical Pathology,* 47, 377-379.

Wang-ngarm, S., S. Chareonsudjai and P. Chareonsudjai, (2014) Physicochemical factors affecting the growth of *Burkholderia pseudomallei* in soil microcosm. *The American Journal of Tropical Medicine and Hygiene,* 90, 480-485.

Weightman, B. A., (2011) *Dragons and tigers: a geography of South, East, and Southeast Asia*. Wiley. com. 312-436.

White, N. J., (2003) Melioidosis. *The Lancet,* 24, 311-1722.

Whitmore, A. and C. Krishnaswami, (1912) An account of the discovery of a hitherto undescribed infective disease occurring among the population of Rangoon. *Indian Medical Gazette,* 47, 262-267.

Wiersinga, W. J., B. J. Currie and S. J. Peacock, (2012) Medica progress: Melioidosis. *New England Journal of Medicine,* 367, 1035-1044.

Wiersinga, W. J., T. van der Poll, N. J. White, N. P. Day and S. J. Peacock, (2006) Melioidosis: insights into the pathogenicity of *Burkholderia pseudomallei*. *Nature Reviews. Microbiology,* 4, 272-282.

Wuthiekanun, V., W. Chierakul, J. Rattanalertnavee, S. Langa, D. Sirodom, C. Wattanawaitunechai, W. Winothai, N. J. White, N. Day and S. J. Peacock, (2006) Serological evidence for increased human exposure to *Burkholderia pseudomallei* following the tsunami in southern Thailand. *Journal of Clinical Microbiology,* 44, 239-240.

Wuthiekanun, V., V. Desakorn, G. Wongsuvan, P. Amornchai, A. C. Cheng, B. Maharjan, D. Limmathurotsakul, W. Chierakul, N. J. White, N. P. J. Day and S. J. Peacock, (2005) Rapid immunofluorescence microscopy for Diagnosis of Melioidosis. *Clinical and Diagnostic Laboratory Immunology,* 12, 555-556.

Wuthiekanun, V., M. D. Smith, D. A. B. Dance and N. J. White, (1995a) Isolation of *Pseudomonas pseudomallei* from soil in north-eastern Thailand. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 89, 41-43.

Wuthiekanun, V., M. D. Smith and N. J. White, (1995b) Survival of *Burkholderia pseudomallei* in the absence of nutrients. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 89, 491.

Yabuuchi, E., Y. Kosako, H. Oyaizu, I. Yano, H. Hotta, Y. Hashimoto, T. Ezaki and M. Arakawa, (1992) Proposal of *Burkholderia* gen. nov. and transfer of seven species of the genus *Pseudomonas* homology group II to the new genus, with the type species *Burkholderia cepacia* (Palleroni and Holmes 1981) comb. nov. *Microbiology and Immunology,* 36, 1251-1275.

Yee, K., M. Lee, C. Chua and S. Puthucheary, (1988) Melioidosis, the great mimicker: a report of 10 cases from Malaysia. *The Journal of Tropical Medicine and Hygiene,* 91, 249-254.

Zanetti, F., G. De Luca and S. Stampi, (2000) Recovery of *Burkholderia pseudomallei* and *B. cepacia* from drinking water. *International Journal of Food Microbiology,* 59, 67-72.

Zgurskaya, H. I. and H. Nikaido, (2000) Multidrug resistance mechanisms: drug efflux across two membranes. *Molecular Microbiology*, 37, 219-225.