



UNIVERSITI PUTRA MALAYSIA

***PREVALENCE AND PUBLIC HEALTH RISK OF TUBERCULOSIS AND
NONTUBERCULOUS MYCOBACTERIA IN CAPTIVE ASIAN
ELEPHANTS (*Elephas maximus* Linnaeus)
IN PENINSULAR MALAYSIA***

YUSUF YAKUBU

FPV 2015 16



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By

YUSUF YAKUBU

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

May 2015

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DEDICATION

Dedicated to my wife and children.



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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

PREVALENCE AND PUBLIC HEALTH RISK OF TUBERCULOSIS AND NONTUBERCULOUS MYCOBACTERIA IN CAPTIVE ASIAN ELEPHANTS (*Elephas maximus* Linnaeus) IN PENINSULAR MALAYSIA

By

YUSUF YAKUBU

May 2015

Chairperson: Assoc. Prof. Zunita Zakaria, PhD
Faculty: Veterinary Medicine

Tuberculosis (TB) remains a major public health problem worldwide with an estimated 9 million cases in 2013 and over 100 million deaths in the last century. The disease, caused by *Mycobacterium tuberculosis*, is estimated to infect one third of the world human population and was declared a global health emergency by the World Health Organization in 1993. *Mycobacterium tuberculosis* is an airborne multi-host pathogen capable of causing tuberculosis in humans and wide range of animal species. The disease has been reported among the captive Asian elephants (*Elephas maximus*) worldwide in the recent years with considerable conservation and public health threats. Infected elephants shed the pathogen in respiratory secretions thereby endangering other elephants and human in close contact. Few nontuberculous mycobacterium species (NTM) known to be opportunistic pathogens in humans have also been shown to cause fatal pulmonary disease in elephants. Nontuberculous mycobacterium infections in elephants have very poor prognosis and are often misdiagnosed for TB or other trivial respiratory problems. As effective treatment regimen for elephant TB and mycobacterial infection is yet to be established, the diseases continue to threaten the survivability of captive elephants and pose serious occupational hazard to humans especially elephant handlers and wildlife veterinarians. This study was conducted to investigate the epidemiology and public health hazards of tuberculosis and nontuberculous mycobacteria in captive Asian elephants in Peninsular Malaysia. The specific objectives were to detect and isolate *M. tuberculosis* and nontuberculous mycobacteria in trunk washes of captive Asian elephants, to determine the seroprevalence, incidence and risk factors for tuberculosis in captive Asian elephants, to determine prevalence and risk factors for tuberculosis among workers at elephant holding premises; and to investigate presence of novel virulence or survival adaptation genes in nontuberculous mycobacteria isolated from elephants.

In the study, the proportion of elephant positive on molecular detection of *M. tuberculosis* complex (MTBC) in trunk washes was (16/28) 27.59% (CI: 95%, 17.05 – 41.12). The MTBC detection rates by location were (2/2) 100% in facility D, (5/8) 65.50% in facility C, (4/9) 44.40% in facility E, (3/8) 37.50% in facility B and (2/31) 6.45% in facility A. Though *M. tuberculosis* could not be isolated, the proportion of elephants with nontuberculous mycobacteria isolates was (36/58) 62.07% (CI: 95%, 48.35-74.19). The isolation rates were (19/31) 61.29% in facility A, (4/8) 50.00% in facility B, (7/8) 87.50% in facility C, (1/2) 50.00% in facility D and (5/9) 55.56% in facility E. More than 70% of the NTM belong to three important NTM complexes, the *M. terrae* complex comprising *M. terrae*, *M. nonchromogenicum*, *M. arupense*, *M. hiberniae* and *M. longobardum* (31.48%); the *M. fortuitum* complex with *M. fortuitum*, *M. peregrinum*, *M. septicum* and *M. boenickei* (20.36%); and the *M. avium* complex (MAC) comprising *M. avium-intracellulare* and *M. colombiense* (20.37%). The less frequently isolated spp. were *M. asiaticum*, *M. agri*, *M. rutilum*, *M. mantenii*, *M. aurum*, *M. insubricum*, *M. wolinskyi*, *M. poriferae*, *M. flavescens*, and *M. gilvum* (27.75%).

The overall prevalence of elephant tuberculosis based on serological screening was (14/60) 23.33% (95% CI: 13.78–36.33). The prevalence elephant TB at first, second and third samplings were (10/51) 19.61%, (14/53) 26.42% and (9/37) 24.32% respectively. New cases of seroconversion were detected in two elephants resulting in an overall incidence of 2.6 cases per 100 elephant-years. Statistical analyses of putative risk factors showed that elephants with designated mahouts were at significantly higher risk of infection [$p = 0.022$, OR: 4.887 (95% CI: 1.258-18.248)]. Throughout the study, elephants in facility B and F were all seronegative.

The overall seroprevalence of latent tuberculosis infection (LTBI) among wildlife staff was (37/149) 24.80% (95% CI: 18.29-32.70). Staff in facility E had the highest prevalence of (6/12) 50% while facility A had the lowest (13/70) 18.57%. The prevalence of latent tuberculosis among staff in facility B, C, D and F were (5/21) 23.81%, (5/20) 25%, (6/18) 33.30% and (2/8) 25% respectively. Univariable logistic regression showed risk of infection to be significantly associated with facility E staff [$p = 0.024$, OR: 4.385 (95% CI: 1.217-15.801)], workers older than 30 years of age [$p = 0.007$, OR: 3.333 (95% CI: 1.351-8.227)], mahouts [$p = 0.034$, OR: 3.778 (95% CI: 1.076-13.259)] and foreigners [$p = 0.018$, OR: 3.313 (95% CI: 1.214-8.086)]. However, upon multivariable analysis only staff older than 30 years of age were significantly associated with the risk of LTBI [$p = 0.010$, OR: 3.315 (95% CI: 1.329-8.270)]. Correlation of tuberculosis among mahouts and elephants revealed minimal agreement with kappa value of 0.222.

Investigation of novel virulence and survival adaptation genes in nontuberculous mycobacterium species (NTM) revealed presence of glycoside hydrolase family 76 gene in *Mycobacterium fortuitum*. The gene encodes for glycoside hydrolase family 76 protein, which is a mannosidase used for nutrient acquisition through the hydrolysis of mannose sugar. It has

not been previously reported in *M. fortuitum* and phylogenetic analysis showed similarity of the gene with that of *M. tuberculosis* and *Mycobacterium smegmatis*.

The study has provided significant information on the presence of *M. tuberculosis* and NTM in trunk washes of captive elephants in Peninsular Malaysia. Latent TB infection and potentially associated risk factors in elephants and wildlife staff have also been identified. The impending conservation and public health hazards necessitate the need for control strategies such as screening and quarantine of newly-acquired elephants, isolation of infected elephants and early treatment of confirmed cases. Periodic occupational hazards and training program need to be organized for wildlife employees. They should be provided with personal protective equipment (PPE) such as N95 mask, goggles and disposable aprons when dealing with TB infected elephants. There is the need to also initiate pre-employment and annual tuberculin skin test (TST) in order to monitor the risk of infection among wildlife employees. From a global standpoint, health authorities need to recognize the public health risk of elephant TB and make its elimination an integral part of the WHO “STOP-TB partnership” programme.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**PREVALENS DAN RISIKO KESIHATAN AWAM TERHADAP
MIKOBAKTERIA TUBERKULOSIS DAN NONTUBERKULOSIS
PADA GAJAH ASIA (*Elephas maximus* Linnaeus) KURUNGAN
DI SEMENANJUNG MALAYSIA**

Oleh

YUSUF YAKUBU

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Tuberkulosis atau batuk kering (TB) masih menjadi masalah kesihatan awam yang utama di seluruh dunia dengan anggaran 9 juta kes pada tahun 2013 dan lebih 100 juta kematian dalam abad yang lalu. Penyakit ini disebabkan oleh *Mycobacterium tuberculosis*, yang dianggarkan menjangkiti satu pertiga daripada penduduk dunia dan kesihatan global telah diisytiharkan oleh Pertubuhan Kesihatan Sedunia pada tahun 1993. *Mycobacterium tuberculosis* adalah patogen bawaan udara berbilang hos yang boleh menyebabkan tuberkulosis pada manusia dan pelbagai spesies haiwan. Penyakit ini telah muncul semula di kalangan gajah Asia (*Elephas maximus*) kurungan di seluruh dunia pada tahun kebelakangan ini dan mengakibatkan ancaman yang besar dari segi pemuliharaan dan kesihatan awam. Gajah yang dijangkiti akan menyebarkan patogen melalui rembesan yang akan membahayakan gajah lain dan juga manusia yang mempunyai kontak yang rapat dengan gajah terjangkit. Beberapa spesies mikobakteria bukan tuberkulos (NTM) yang dikenali sebagai patogen oportunistik pada manusia juga telah terbukti boleh menyebabkan penyakit paru-paru yang membawa maut pada gajah. Jangkitan NTM dalam gajah mempunyai prognosis yang lemah dan sering disalah diagnos sebagai TB atau lain-lain masalah pernafasan yang remeh. Oleh kerana tiada lagi regimen rawatan yang berkesan untuk TB gajah dan jangkitan mikobakteria yang lain, penyakit ini terus mengancam kemandirian gajah dalam kaptif dan mengancam kesihatan manusia terutamanya pengendali gajah dan veterinar. Kajian ini telah dijalankan untuk menyiasat epidemiologi tuberkulosis dan jangkitan mikobakteria bukan tuberkulos pada gajah Asia (*Elephas maximus*) kurungan di Semenanjung Malaysia dan kesannya kepada kesihatan awam. Objektif khusus adalah untuk mengesan dan memencilkan bakteria *M. tuberculosis* dan mikobakteria nontuberkulos daripada air basuhan dalam belalai gajah kurungan, untuk menentukan seroprevalens, insiden dan faktor risiko tuberkulosis pada gajah Asia kurungan, untuk menentukan prevalens

dan faktor risiko tuberkulosis pada pengendali gajah; dan untuk menyiasat kehadiran gen virulen atau kemandirian yang novel dalam mikobakteria nontuberkulos yang dipencilkan daripada gajah.

Dalam kajian ini, jumlah gajah yang positif melalui pengesanan molekular kompleks mikobakteria dalam air basuhan belalai gajah adalah (16/58) 27.59% (CI: 95%, 17.05 – 41.12). Kadar pengesanan menurut lokasi adalah (2/2) 100% di fasiliti D, (5/8) 62.50% di fasiliti C, (4/9) 44.40% di fasiliti E, (3/8) 37.50% di fasiliti B dan (2/31) 6.45% di fasiliti A. Walaupun *M. tuberculosis* tidak boleh dipencilkan, jumlah pemencilan adalah (19/31) 61.29% di fasiliti A, (4/8) 50.00% di fasiliti B, (7/8) 87.50% di fasiliti C, (1/2) 50.00% di fasiliti D dan (5/9) 55.56% di fasiliti E. Lebih daripada 70% daripada NTM tergolong dalam tiga kompleks NTM yang penting iaitu kompleks *M. terrae* terdiri daripada *M. terrae*, *M. nonchromogenicum*, *M. arupense*, *M. hiberniae* dan *M. longobardum* (31.48%); kompleks *M. fortuitum* dengan *M. fortuitum*, *M. peregrinum*, *M. septicum* dan *M. boenickei* (20.36%); dan *M. avium* kompleks (MAC) yang terdiri daripada *M. avium-intracellulare* dan *M. colombiense* (20.37%). Spesis *M. asiaticum*, *M. pertaniam*, *M. rutilum*, *M. mantenii*, *M. aurum*, *M. insubricum*, *M. wolinskyi*, *M. poriferae*, *M. flavescens*, dan *M. gilvum* (27.75%) adalah spesis yang kurang kerap terpencil.

Seroprevalens keseluruhan tuberkulosis gajah adalah (14/60) 23.33% (95% CI: 13.78–36.33). Prevalens di persampelan pertama, kedua dan ketiga adalah masing-masing pada (10/51) 19.61%, (14/53) 26.42% dan (9/37) 24.32%. Insiden keseluruhan adalah 2.6 kes bagi setiap 100 gajah-tahun akibat daripada penukaran sero dua gajah di persampelan kedua. Dengan menggunakan analisis univariat dan analisis multivariat, didapati gajah yang mempunyai pengendali yang khas menunjukkan hubungan yang signifikan dengan risiko jangkitan [$p = 0.031$, OR: 3.778 (95% CI: 1.071-28.248)] dan [$p = 0.022$, OR: 4.887 (95% CI: 1.258-18.248)]. Semua gajah di fasiliti B dan F adalah seronegatif di sepanjang kajian ini.

Seroprevalens keseluruhan jangkitan tuberkulosis pendam (LTBI) di kalangan kakitangan hidupan liar adalah (37/149) 24.80% (95% CI: 18.29-32.70). Fasiliti E mempunyai prevalens tertinggi pada (6/12) 50%, manakala fasiliti A mempunyai prevalens yang paling rendah (13/70) 18.57%. Prevalens dalam fasiliti B, C, D dan F masing-masing adalah (5/21) 23.81%, (5/20) 25%, (6/18) 33.30% (2/8) dan 25%. Regresi logistik univariat menunjukkan risiko jangkitan yang signifikan dengan kakitangan di fasiliti E [$p = 0.024$, OR: 4.385 (95% CI: 1.217-15.801)], kakitangan yang lebih tua daripada 30 tahun [$p = 0.007$, OR: 3.333 (95% CI: pekerja asing [$p = 0.018$, OR: 3.313 (95% CI: 1.214-8.086)]. Walaubagaimanapun, hanya kakitangan yang lebih tua daripada 30 tahun sahaja didapati mempunyai kaitan signifikan dengan LTBI pada analisis multivariat [$p = 0.010$, OR: 3.315 (95% CI: 1.329-8.270)]. Korelasi tuberkulosis di kalangan kakitangan dan gajah menunjukkan kolerasi minimum dengan nilai kappa 0.222.

Penyiasatan kehadiran gen virulen dan kemandirian baharu dalam spesies mikobakteria nontuberkulos (NTM) mendedahkan kehadiran glycoside hydrolase family 76 gen dalam *Mycobacterium fortuitum*. Gen yang mengekod famili protein 76 glycoside hydrolase, yang merupakan mannosidase digunakan untuk pengambilalihan nutrien melalui hidrolisis gula mannose. Gen ini belum pernah dilaporkan sebelum ini dalam *M. fortuitum* dan analisis filogenetik menunjukkan persamaannya dengan gen *M. tuberculosis* dan *Mycobacterium smegmatis*.

Kajian ini menghasilkan maklumat yang signifikan ke atas kehadiran *M. tuberculosis* dan NTM dalam air basuhan belalai gajah dalam kurungan di Semenanjung Malaysia. Jangkitan TB terpendam dan faktor-faktor risiko pada gajah dan kakitangan hidupan liar juga telah dikenal pasti. Strategi kawalan seperti saringan dan kuarantin pada gajah yang baru, pengasingan gajah yang dijangkiti dan rawatan awal kes yang disahkan perlu bagi memastikan pemuliharaan dan kesihatan awam terjaga. Kesedaran terhadap bahaya dalam pekerjaan secara berkala dan program latihan perlu diatur untuk kakitangan hidupan liar. Mereka harus disediakan dengan peralatan perlindungan diri (PPE) seperti topeng N95, cermin mata dan apron pakai buang apabila mengendalikan gajah yang dijangkiti. Ujian pra-pekerjaan dan ujian kulit tuberkulin tahunan (TST) perlu bagi memantau risiko jangkitan di kalangan kakitangan hidupan liar. Di peringkat global, pihak berkuasa kesihatan perlu menyedari risiko tuberkulosis gajah terhadap kesihatan awam dan seterusnya menjadikannya sebahagian daripada program WHO "STOP-TB partnership".

ACKNOWLEDGEMENTS

In the name of Allah, the Most Gracious and the Most Merciful

All praise and gratitude due to Allah (SWT) for the strength, health and opportunity to embark and complete this study. I would like to express my special appreciation and deepest gratitude to my supervisor, Assoc. Prof. Dr. Zunita Zakaria, for her guidance, patience, support and providing me with an excellent research atmosphere. You have been a tremendous mentor and your research advice has been priceless. I would also like to thank my research principal investigator and a co-supervisor, Prof. Dr. Ong Bee Lee, for her aspiring guidance and invaluable constructive criticism. My appreciation to my committee members, Assoc. Prof. Dr. Latiffah Hassan, Assoc. Prof. Dr. A. R. Mutalib and Prof. Dr. Y. F. Ngeow for their insightful discussion, constructive comments, corrections and advice.

I would like to thank staff of the Department of Wildlife and National Parks (DWNP) Malaysia especially Dr. Firdaus Arif and staff of other private zoos that enrolled in the study. Not forgotten, staff of the Bacteriology Laboratory, Encik Azri Roslan, Cik Krishnamma Kuppusamy and Encik Moh'd Hafiz. Many thanks for your excellent technical assistance and understanding.

A special thanks to my mother and father for their unique spiritual support and encouragement. My deepest appreciation to my beloved wife, Ummukulthum and my kids, for their prayers, patience and sacrifices. Words cannot express how grateful I am.

Finally, sincere thanks to all my friends especially Sani Ismail, Mustapha Abatcha, Faruk Bande, Dauda Goni, Rabi'u Aliyu, Adamu Abdul, Aliyu Mahmuda, Ibrahim Jalo, Sadeeq, Bashir Garba, Abdulrasheed Bello, Ibrahim Maina and Nafiu Lawal, for their friendly advices and moral support. Thanks for the friendship and memories.

I certify that a Thesis Examination Committee has met on 20 May 2015 to conduct the final examination of Yusuf Yakubu on his thesis entitled "Prevalence and Public Health Risk of Tuberculosis and Nontuberculosis Mycobacteria in Captive Asian Elephants (*Elephas maximus* Linnaeus) 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.

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LIST OF ABBREVIATIONS

AFB	Acid-fast Bacilli
AIDS	Acquired Immunodeficiency Syndrome
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BCCDC	British Columbia Center for Disease Control
BCG	Bacillus Calmette-Guerin
BLAST	Basic Local Alignment Search Tool
BSL II	Biosafety level II
bTB	Bovine Tuberculosis
CAZy	Carbohydrate Active Enzymes
CDC	Centers for Disease control and Prevention
CDR	Crude Death Rate
CFP	Culture Filtrate Protein
CI	Confidence Interval
CITES	Convention on International Trade in Endangered Species
CO ₂	Carbondioxide
CPK	Creatine Phosphokinase
CR	Complement Receptors
dH ₂ O	Distilled water
DNA	Deoxyribonucleic Acid
DOTs	Directly Observed Therapy Short-course
DPP	Dual Platform Pathway
DWNP	Department of Wildlife and National Parks
E values	Expected value
<i>E. coli</i>	<i>Escherichia coli</i>
EDTA	Ethylene diaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
EMB	Ethambutol
<i>erp</i>	Exported repetitive protein
ESAT	Early Secretory Protein
F	Forward
<i>fbpA</i>	Fibronectin-binding protein
FDA	Food and Drug Administration
Fig.	Figure
g	Gram
GH_F76	Glycoside Hydrolase family 76
HAART	Highly Active Antireteroviral Therapy
HBCs	High Burden Countries
<i>hbhA</i>	Heparin-binding hemagglutinin
HIV	Human Immunodeficiency Virus
HPA	Hybridization Protection Assay
HPLC	High-performance Liquid Chromatography
<i>hsp</i>	Heat Shock Protein
IACUC	Institutional Animal Care and Use Committee
ID	Identity

IFN γ	Interferon Gamma
IL-2	Interleukin-2
INH	Isoniazid
IS	Insertion Sequence
IUCN	International Union for the Conservation of Nature
<i>KasB</i>	Beta-ketoacyl-acyl carrier
LJ	Lowenstein-Jensen
LTBI	Latent Tuberculosis Infection
M	Marker
MAC	<i>Mycobacterium avium</i> complex
MAP	<i>Mycobacterium avium</i> paratuberculosis
MAPIA	Multiantigen Print Immunoassay
MDGs	Millennium Development Goals
MDR	Multi Drug Resistant
mg	Milligram
MGIT	Mycobacteria Growth Indicator Tube
<i>mgtC</i>	Magnesium Transport P-type ATPase gene
MHC	Major Histocompatibility Complex
ml	Milliliter
mm	Millimeter
<i>ModA</i>	Molybdate-binding protein
MOTT	Mycobacteria other than Tuberculosis
MR	Mannose Receptors
MTBC	<i>Mycobacterium tuberculosis</i> complex
<i>M. tuberculosis</i>	<i>Mycobacterium tuberculosis</i>
NA	Not Applicable
NaOH	Sodium Hydroxide
NCBI	National Center for Biotechnology Information
No.	Number
NTM	Nontuberculous Mycobacteria
OADC	Oleic acid, Albumin, Dextrose and Catalase
OD	Optical Density
<i>OmpA</i>	Outer membrane protein A
OR	Odds Ratio
PANTA	Polymyxin B, Amphotericin B, Nalidixic acid, Trimethoprim and Azolocillin
PAS	Para-aminosalicylic Acid
PCR	Polymerase Chain Reaction
PFGE	Pulsed field gel electrophoresis
PM	Postmortem
PPD	Purified Protein Derivative
PPE	Personal Protective Equipment
PZA	Pyrazinamide
R	Reverse
Ref.	Reference
RFLP	Restriction Fragment Length Polymorphism
RIF	Rifampicin
RNA	Ribonucleic Acid
RNase	Ribonuclease

rpm	Revolutions per minute
rRNA	Ribosomal Ribonucleic Acid
rRNA	Ribosomal ribonucleic acid
SD	Standard Deviation
SPSS	Statistical Package for Social Sciences
TB	Tuberculosis
TBE	Tris-borate-EDTA
TE	Tris-EDTA
Th	T-helper
TMA	Transcription-mediated Amplification
TNF	Tumour Necrosis Factor
TST	Tuberculin Skin Test
UK	United Kingdom
USA	United States of America
USDA	United States Department of Agriculture
UV	Ultraviolet
V	Volt
WHO	World Health Organization
XDR	Extensively Drug-resistant
%	Percent
~	Approximately
=	Equal to
>	Greater than
≥	Greater or equal to
°C	Degree Celsius
µg	Microgram
µl	Microliter
µm	Micrometer
µM	Micromolar
<i>p</i>	Probability value

CHAPTER ONE

INTRODUCTION

With one third of the world human population infected and 2 to 3 million deaths every year, tuberculosis (TB) was declared a global health emergency by the World Health Organization (WHO) in 1993 (WHO, 2012; Raviglione, 2003). Over 100 million people died of the disease in the last century, making it second to HIV/AIDS as the greatest killer disease due to single infectious agent (WHO, 2013). Tuberculosis is an ancient disease caused by a group of genetically related bacteria known as the *Mycobacterium tuberculosis* complex (MTBC). They are primarily respiratory pathogens comprising *Mycobacterium tuberculosis*, *Mycobacterium bovis*, *Mycobacterium africanum*, *Mycobacterium canetti*, *Mycobacterium pinnipedii*, and *Mycobacterium microti* (Wirth et al., 2008). The most important member of the complex is *Mycobacterium tuberculosis*, a known human pathogen causing disease in wide range of animal species including non-human primates, rhinoceroses, tapirs, giraffes, buffaloes, psittacine birds, cattle, dogs and elephants (Stephens et al., 2013; Parsons et al., 2012; Romero et al., 2011; Kaewamatawong et al., 2010; Une and Mori, 2007a; Yoshikawa, 2006; Lewerin et al., 2005; Ocepek et al., 2005; Erwin et al., 2004; Montali et al., 2001).

Pandemic tuberculosis has been described as one of several factors responsible for mastodon (*Mammot americanum*) extinction following detection of tuberculous lesions in 52% of mastodon skeleton recovered in North America (Rothschild and Laub, 2006). The disease has been described in Asian elephants over 2000 years ago in the Indian Ayuverdic text (McGaughey, 1961; Iyer, 1937). In elephants, tuberculosis is caused by *Mycobacterium tuberculosis* which is believed to have been transmitted from human to domestic elephants used for religious, cultural and draught purposes (Mikota, 2007). It is an asymptomatic disease that has now reemerged among captive Asian elephants over the last century (Mikota and Maslow, 2011; Lewerin et al., 2005; Mikota et al., 2001; Montali et al., 2001). Infected elephants appear apparently healthy but intermittently shed the pathogen in respiratory secretions, vaginal discharges, urine and milk (Mikota, 2008), thereby contaminating the environment posing significant risk to other elephants, susceptible animals and humans in close proximity. TB transmission among elephants and between captive elephants and other susceptible animals has been reported to be associated with bacterial load, droplet size, length of exposure, immune status, proximity to infected animal and ventilation (Maslow, 1997). The occurrence of the disease in captive Asian elephants worldwide poses significant threat to the conservation of the wildlife species.

In the early 90s, over 100,000 wild Asian elephants were estimated to have lived in Southeast Asia (ECI, 2008). Due to illicit poaching, loss of habitat and scourge of diseases, the population has gradually fallen to between 50,000 to 35,000 with an estimated 16,000 in captivity (ECI, 2008) This prompted the International Union for the Conservation of Nature (IUCN) to enlist Asian elephants in the Red List of threatened species (Choudhury et al., 2008), while the Convention on International Trade in Endangered Species of the Wild Fauna and Flora also classified Asian elephants as most endangered species threatened with extinction (CITES, 2011). These wildlife are becoming increasingly endangered following the recent emergence of TB among the captive population. Hence there is need to determine the occurrence of the disease and develop effective measures toward its prevention and control.

Tuberculosis is a major public health problem worldwide especially in Africa and Southeast Asia (WHO, 2014a). Genetic profiling of *M. tuberculosis* isolated from captive elephants and their handlers in the United States and Nepal have proven inter-species transmission between elephants and humans (Paudel et al., 2014; Michalak et al., 1998). The zoonotic transmission has been reported to be associated with close contact with elephants, engaging in post mortem examination, undertaking medical procedures, administering treatment and participating in elephant pen sanitation (Murphree et al., 2011; Oh et al., 2002). Elephant handlers or mahouts, who are in frequent close contact with elephants are at higher risk of infection followed by zoo veterinarians and visitors (Mikota and Maslow, 2011). The significant health impact of the disease necessitates the need to determine the potential risk of transmission between captive elephants and their handlers.

In Malaysia, elephants are considered an endangered species and protected under the Wildlife Conservation Act 716 (Laws of Malaysia, 2010). There were an estimated 1223-1677 wild elephants and 62 captive elephants in Peninsular Malaysia (Saaban et al., 2011). Some of the captive elephants were imported from other Asian countries but majority were rescued from the wild and subsequently transferred to zoos and conservation centers for ecotourism (Saaban et al., 2011). TB has been reported among captive elephants in India, Sri Lanka, Nepal and Thailand (Verma-Kumar et al., 2012; Mikota and Maslow, 2011; Perera et al., 2011; Angkawanish et al., 2010). However, despite its conservation and public health threats, there is dearth of information on the occurrence of the disease and its associated risk factors in captive elephants and their handlers in Peninsular Malaysia.

Non-tuberculous mycobacterium species (NTM) also known as atypical mycobacteria or mycobacteria other than tuberculosis (MOTT) are environmental organisms found in the soil and water (Griffith, 2007). They are opportunistic pathogens capable of causing various disease conditions referred to as mycobacterioses, particularly in immunocompromised persons (Tortoli, 2009). The ubiquitous organisms affect multiple organ systems such as gastrointestinal, lymphatic and skeletal systems; and are transmitted via contact, ingestion and inhalation of aerosolized droplets (Cassidy et al.,

2009; Grubek-Jaworska et al., 2009). A significant number of NTM predominantly affect the respiratory system resulting into chronic pulmonary disorders such as hypersensitivity pneumonitis and bronchiectasis especially in the elderly and individuals with underlying pulmonary disease (Grubek-Jaworska et al., 2009; Tortoli, 2009; Fowler et al., 2006; Falkinham, 2003a). Gradual increase in disease susceptibility due to immune-suppression and inherent resistance of NTM to broad spectrum antibiotics has been associated with increase in proportion of mycobacterial infections in many developed and developing countries (Falkinham, 2003b).

NTM species have been isolated from elephant trunk washes with no associated clinical signs (Mikota, 2007). However few species such as *Mycobacterium elephantis* and *Mycobacterium szulgai* have been reported to cause disease in both elephants and humans (Heidarieh et al., 2011; Lacasse et al., 2007; Manalac and Bonilla, 2007; Tappe et al., 2004; Shojaei et al., 2000). Over 90% of NTM infections involve the respiratory system followed by the lymph nodes, skin, soft tissue and bones (Griffith et al., 2007; Falkinham, 1996). Pulmonary mycobacterial infections such as asthma, hypersensitive pneumonitis and bronchitis have been associated with exposure to water aerosols from showers, hot tubs/spas and swimming pools (Sood et al., 2007; Falkinham, 2003a). Elephants are naturally exposed to environmental organisms when feeding, dusting and hosing with the trunk (Schulte, 2006). They also spray aerosols containing droplet nuclei of ubiquitous organisms that could be inhaled by humans in close contact (Mikota and Maslow, 2011). In view of the risk of NTM infection in elephants and the hazard associated with human exposure to mycobacterial aerosols, it is important to investigate presence of these organisms in elephant respiratory secretions in order to understand the impending hazard faced by elephants and humans in close contact.

Over 500 proteins in *Mycobacterium* species have been described to be involved in various crucial cell wall processes such as virulence, detoxification and intermediary metabolism (Wolfe et al., 2010; Mawuenyega et al., 2005). A number of these proteins and their encoding genes have been reported in some nontuberculous mycobacteria (Weir et al., 2003). With the increase rate of pulmonary mycobacterioses in developed and developing countries (Falkinham, 2003b), it is important to investigate presence of unreported genes encoding for important proteins in potentially pathogenic NTM species. This will help in the understanding of their genetic make-up, clinical significance and developing effective treatment against the highly resistant organisms.

1.1 Hypotheses

The hypotheses of this study were:

1. Captive Asian elephants in Peninsular Malaysia shed *Mycobacterium tuberculosis* in respiratory secretions and harbor important non-tuberculous *Mycobacterium* species in their trunk.
2. Elephant handlers in Peninsular Malaysia have latent tuberculosis infection (LTBI).
3. A number of risk factors are associated with tuberculosis in captive Asian elephants and their handlers in Peninsular Malaysia.
4. There are unreported genes that play vital role in the survival and pathogenicity of some important NTM species.

1.2 Study objectives

The objectives of this study were:

1. To determine the presence of *Mycobacterium tuberculosis* and non-tuberculous *Mycobacterium* species in trunk washes of captive Asian elephants.
2. To estimate the prevalence, incidence and risk factors for tuberculosis in captive Asian elephants in Peninsular Malaysia.
3. To estimate the prevalence and risk factors for tuberculosis among workers in elephant holding premises in Peninsular Malaysia
4. To identify new genes involved in the virulence or survival adaptation of some important non-tuberculous *Mycobacterium* species.

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