UNIVERSITI PUTRA MALAYSIA

EPIDEMIOLOGICAL STUDY ON Mycobacterium tuberculosis COMPLEX AND Mycobacterium avium COMPLEX IN FREE-RANGE AND CAPTIVE WILDLIFE IN MALAYSIA

LEKKO YUSUF MADAKI

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By

LEKKO YUSUF MADAKI

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

January 2022

DEDICATION

This thesis is dedicated to God Almighty, the giver of life who has given me knowledge, good health and life to pursue this degree. To my parents late Mr Mamman Lekko and Mrs Maryamu Lekko, my brothers, sisters, wife and daughter.

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

EPIDEMIOLOGICAL STUDY ON *Mycobacterium tuberculosis* COMPLEX AND *Mycobacterium avium* COMPLEX IN FREE-RANGE AND CAPTIVE WILDLIFE IN MALAYSIA

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January 2022

Chairman : Azlan Che' Amat, PhD
Faculty : Veterinary Medicine

Mycobacterium tuberculosis complex (MTBC) and *Mycobacterium avium* complex (MAC) are the causative for chronic diseases affecting domestic animals, wildlife and humans. Globally, the role of maintenance host for MTBC and MAC is known for some wildlife species. However, the epidemiology in the local free-ranged and captive wildlife in Malaysia is still undiscovered. Therefore, the aim of this study was to get the insight of these important mycobacteria among wildlife in Malaysia by a selected antemortem and postmortem diagnostic methods namely humoral and cell mediated immunity tests, macroscopic TB-like lesions (TBLL) and antigen detection by culture and molecular. Lungs, lymphoid tissues and blood were collected from carcasses of free-ranged wild boar (n=30) and long-tailed macaque (n=42) within the wildlife-human conflict (WHC) area in Selangor. Trunk washed and blood from living captive Asian elephants (n=21) were sampled in National Elephant Conservation Centre (NECC), Pahang, and n=12 living captive non-human primates (NHPs) were collected from the zoological parks in Melaka includes blood, pharyngeal swab and comparative tuberculin palpebral skin test.

Results showed that MTBC seropositive rate in wild boar was 16.7% (7.3-33.5 at 95% CI) and 10% (3.5-25.6 at 95% CI) using established an in-house ELISA bPPD and commercial DPP® VetTB kit, respectively; while for wild macaques and Asian elephant were seronegative. Wild boar gross TBLL of tonsils, submandibular LN, and lungs, spleen, kidney, liver, and mesenteric lymph nodes (LN) showed prevalence of 30% (9/30) at 95% CI. Multiple nodular lesions with necrotic-miliary and cavitation were found in submandibular LN, tonsil, lung, kidney and liver, while single nodular lesion was observed at the mediastinal LN, spleen and mesenteric LN. Conventional PCR from submandibular LN of wild boar with TBLL showed 75% (9/12) detection for *M. bovis* (95% CI: 46.77-91.11) and 100% were positive for MAC. In wild macaques, PCR showed 33.3% (10/30) detection for MAC and all PCR negative against MTBC. For captive NHPs, only two Orang utans (16.7%) were reacted to tuberculin test and both

seropositive against MTBC, while other NHPs were seronegative. PCR from all blood and pharyngeal swabs were negative for MTBC, however all samples (100%) were PCR positive for MAC. PCR of trunk washes and blood samples in Asian elephants were 95% (20/21) and 23% (5/21) positive for MAC respectively, but all negative against MTBC. The direct use of commercial IDEXX® *M. bovis* ELISA kit was not able to detect antibodies against MTBC from wild boar serum samples, this could be due to the non-species specific of the conjugates in the ELISA kits. However, the modified protocol using both goat anti-pig IgG and Recombinant Protein-G as conjugates in the commercial kit improves MTBC detection at 5/30 (16.7%) of wild boar serum samples with 62.5% and 88.2% sensitivity and specificity respectively. The establishment of modified protocol of an in-house ELISA aPPD against MAC antibodies in primates using different conjugates namely Protein-G and anti-monkey IgG at different dilutions were able to detect at rate 25% to 100% and 16% to 100%, respectively.

In conclusion, our initial studies showed a natural TB infection exists in free-ranging wild boar in Selangor and their potential as maintenance host should be further studied as it may help the understanding of TB epidemiology among livestock, wildlife and human. A significant detection of MAC among free-ranged wild macaques, captive Asian elephant and captive non-human primates indicate the important to review its control measures as it might cause health issues and may complicate the TB diagnosis. Implementing a combination of several diagnostic methods for MTBC and MAC detection and improvement of antibody rapid test would have enhanced the effectiveness of TB surveillance programs.

Keywords: tuberculosis, *Mycobacterium tuberculosis* complex, *M. avium* complex, wildlife, captive, wild boar, macaques, primates, elephant, serology, tuberculin skin test, molecular antigen detection, Malaysia

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

KAJIAN EPIDEMIOLOGI KE ATAS *Mycobacterium tuberculosis* KOMPLEKS DAN *Mycobacterium avium* KOMPLEKS TERHADAP RANGKAIAN-BEBAS DAN KONSERVASI HAIWAN LIAR DI MALAYSIA

Oleh

LEKKO YUSUF MADAKI

Januari 2022

Pengerusi : Azlan Che' Amat, PhD
Fakulti : Perubatan Veterinar

Mycobacterium tuberculosis kompleks (MTBC) dan *Mycobacterium avium* kompleks (MAC) menyebabkan penyakit kronik yang kepada haiwan domestik, hidupan liar dan manusia. Di peringkat global, peranan reservoir atau pembawa untuk MTBC dan MAC telah diketahui berlaku pada sebilangan spesies hidupan liar. Namun epidemiologi terhadap hidupan liar bebas dan dalam kurungan masih belum diketahui. Oleh itu, objektif kajian ini adalah untuk mendapatkan maklumat awal berkenaan mikobakteria penting terhadap hidupan liar di Malaysia melalui kaedah diagnostik antemortem dan postmortem iaitu ujian humoral dan imuniti pengantara sel, makroskopik lesi seperti TB (TBLL) dan pengesanan antigen melalui kultur dan molekular. Tisu peparu dan limfoid dan darah dimabil daripada karkas babi hutan liar (n=30) dan kera liar (n=42) di dalam kawasan konflik hidupan liar dan manusia (WHC) di Selangor. Sampel daripada cucian belalai gajah dan darah gajah Asia *Elephas maximus* hidup di dalam kurungan (n=21) di Pusat Konservasi Gajah Asia (NECC) di Pahang, manakala n=12 primat bukan manusia (NHP) hidup dari taman-taman zoo di Melaka iaitu mengambil sampel darah, calitan tekak dan melakukan ujian tuberkulin perbandingan palpebral mata.

Keputusan menunjukkan kadar pengesanan serologi terhadap MTBC pada babi hutan liar adalah 16.7% (7.3-33.5 pada 95% CI) menggunakan *in-house* ELISA bPPD IgG yang telah dibangunkan dan 10% (3.5-25.6 pada 95% CI) oleh ujian DPP® VetTB kit komersial, sementara untuk kera liar dan gajah Asia kurungan adalah seronegatif. Pemeriksaan TBLL kasar terhadap babi hutan liar iaitu tonsil, kelenjar submandibular, kelenjar tracheobronchial dan paru-paru, limpa, ginjal, hati, dan kelenjar mesenterik menunjukkan prevalen 30% (9/30) pada 95% CI. Lesi nodular berganda yang nekrotik-miliari dengan peronggaan ditemui pada kelenjar submandibular, tonsil, paru-paru, ginjal dan hati sementara lesi nodular tunggal pada kelenjar mediastinal, limpa dan kelenjar mesenterik. PCR konvensional terhadap tisu kelenjar submandibular dan trakeobronkial dengan TBLL menunjukkan 75% (9/12) sampel positif untuk *M. bovis*

(95% CI: 46.77-91.11) dan semua sampel (100%) positif untuk MAC. Untuk kera liar, 33.3% (10/30) positif PCR untuk MAC tetapi semua negatif PCR untuk MTBC. Untuk NHP di dalam kurungan, hanya dua Orang utan (16.7%) bertindak balas terhadap ujian tuberkulin dan kedua-duanya positif secara serologi terhadap MTBC, manakala NHP yang lain seronegatif. Reaksi PCR sampel darah semua NHP adalah negatif untuk MTBC, namun semua sampel daripada sampel darah dan calitan farinks masing-masing positif (100%) PCR untuk MAC. Untuk gajah Asia dalam kurungan, sampel cucian belalai dan darah positif PCR terhadap MAC iaitu masing-masing 95% (20/21) dan 23% (5/21), namun semua sampel negatif terhadap MTBC. Penggunaan secara terus kit komersial ELISA IDEXX® *M. bovis* dalam mengesan antibodi terhadap MTBC terhadap serum babi liar adalah negatif, ini mungkin kerana penggunaan konjugat yang tidak spesifik terhadap spesies. Walaubagaimanapun, modifikasi protokol menggunakan konjugat anti-babi IgG kambing dan Rekombinan Protein-G dalam kit IDEXX® *M. bovis* meningkatkan pengesanan antibodi MTBC di dalam serum babi hutan iaitu 5/30 (16.7%) dengan masing-masing sensitiviti 62.5% dan spesifisiti 88.2%. Penghasilan protokol baru *in-house* ELISA aPPD yang diubahsuai untuk pengesanan antibodi MAC dalam primat menggunakan konjugat iaitu Protein-G: dan anti-monyet pada kepekatan berbeza mampu untuk mengesan antibodi iaitu masing-masing pada kadar 25%-100% dan 16%-100%.

Kesimpulannya, hasil awal kajian ini menunjukkan wujud jangkitan TB semulajadi dalam populasi babi hutan liar di Selangor dan potensi babi hutan sebagai hos utama TB perlu kajian lanjut kerana ia dapat membantu memahami epidemiologi TB di antara haiwan ternakan, hidupan liar dan manusia. Penemuan ketara MAC terhadap kera liar, primat dan gajah Asia di dalam kurungan menunjukkan keperluan untuk menilai langkah kawalan penyakit kerana ianya boleh menyebabkan isu kesihatan dan merumitkan diagnosis TB. Melaksanakan kombinasi kepelbagaian kaedah diagnostik bagi pengesanan MTBC dan MAC dan penambahbaikan ujian pantas serologi mampu untuk mempertingkatkan keberkesanan program surveilan TB

Kata kunci: tuberkulosis, *Mycobacterium tuberculosis* kompleks, *M. avium* kompleks, hidupan liar, kurungan, babi hutan, kera, primat, gajah, serologi, ujian kulit tuberkulin, pengesanan antigen molekular, Malaysia

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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 were as follows:

Azlan bin Che' Amat, PhD
Senior Lecturer
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Chairman)

Ooi Peck Toung, PhD
Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

Sharina binti Omar, PhD
Senior Lecturer
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

ZALILAH MOHD SHARIFF, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 10 November 2022

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Signature: _____
Name of Chairman of Supervisory Committee: Dr. Azlan bin Che' Amat

Signature: _____
Name of Member of Supervisory Committee: Associate Professor Dr. Ooi Peck Toung

Signature: _____
Name of Member of Supervisory Committee: Dr. Sharina binti Omar

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LIST OF ABBREVIATIONS

AFB	Acid-fast bacilli
aPPD	avian Purified protein derivative
bPPD	bovine Purified protein derivative
CFP-10	Culture filtrate protein
CFT	Skin of caudal fold
CPC	Cetylperidinum chloride
DNA	Deoxyribonucleic acid
DTH	Delayed type hypersensitivity response
ELISA	Enzyme-linked immunosorbent assay
ESAT-6	Early secretory antigenic target
FGM	Fast growers
HE	Hematoxylin & Eosin stain
H^2SO4	Sulfuric acids
HCL	Hydrochloric acids
HIV	Human immunodeficiency virus
IFN-γ	Interferon gamma
IGRA	Interferon γ release assay
MAA	M. *avium* subsp. *avium*
MAC	*Mycobacterium avium* complex
MAH	*M. avium* subsp. *hominissuis*
MAP	*M. avium* subsp. *paratuberculosis*
MAPIA	Multi-antigen print immunoassay
MAS	*M. avium* subsp. *silvaticum*
MDR	Multiple drug resistant
MPB70	Mobility protein of bovis 70

MPB83	Mobility protein of bovis 83
MTBC	*Mycobacterium tuberculosis* Complex
NAOH	Sodium hydroxide
NECs	Necrosis associated extracellular clusters
NTM	Non-tuberculous mycobacteria
OADC	Oleic acid, albumin dextrose and catalase
OD	Optical density
OTF	Officially Tuberculosis Free
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
SCIT	Single comparative intradermal tuberculin test
Se	Sensitivity
SEA	Southeast Asia
SGM	Slow growers
SIT	Single intradermal tuberculin test
Sp	Specificity
TB	Tuberculosis
TMB	Tetramethylbenzidine
UPM	Universiti Putra Malaysia
WHO	World Health Organization
WLHI	Wildlife–livestock–human interface
XDR	Extensively Drug-Resistant
ZN	Ziehl-Neelsen

CHAPTER 1

GENERAL INTRODUCTION

1.1 Background of the study

Tuberculosis (TB) is a globally important reemerging disease that affects livestock, wildlife animals and zoonotic TB in human. The disease is chronic in nature and can infect multi host species and the causative agent is *Mycobacterium tuberculosis* complex (MTBC) (Waters *et al*., 2016; Miller *et al*., 2019). The said disease causes serious burden where it gives a negative impact to the economy of some developing countries that relies on livestock industry (Michel *et al*., 2010; Chambers, 2013). Among human population, World Health Organization (WHO) stated that the probability of being infected by TB (mainly caused by *M. tuberculosis*) is 33.3% worldwide and it is estimated that 9.6 million people are infected with 1.5 million deaths in 2014 (WHO, 2015; Thoen *et al*., 2016). In recent data, 2017, it was recorded about 10 million new TB cases with 1.3 million deaths were recorded where human TB still remains as one of the 10 major diseases that causes death worldwide. It was also stated that a total allocation of funding about US$6.9 billion were used for TB disease control programs (WHO, 2017; Good *et al*., 2018; Kohl *et al*., 2018). The endemic countries that recorded mortality due to TB in 2020 were Africa (74%), followed by South-East Asia (18%), Eastern Mediterranean (5%), Western Pacific (2%) and Europe (1%) (WHO, 2020).

In livestock animals, TB control is mainly by adapting the test and slaughter policy (Amanfu, 2006). For pig industry, TB disease or outbreak gives out serious concerned and worries about economic losses in production (Bailey *et al*., 2013). Bovine TB infection of cattle and other domestic ruminants, is one of the primary limitations of livestock farming. If tested positive (antemortem), a loss of profit which is mainly due to the culling of the animal and the need to immobilize the rest of the herd (Pérez-Morote et al., 2020). For conservation areas, misunderstanding or conflicts among hunters, farmers and ecologists due to TB disease could lead to serious impacts on the health of the wild animals and protection programmes in wildlife sanctuaries (Gortázar *et al*., 2010). Therefore, TB surveillance and control programmes are important for public health, conservations and economy aspects. In advanced and developed nations, regulations are mainly on intensive test and slaughter policy, movement control and total culling of the herd (Brooks-Pollock *et al*., 2014). In order to have total TB eradication, there are challenges which will be very difficult task to be implemented if the role played by all reservoir hosts (livestock and wildlife) are poorly understood. (O'Reilly & Daborn, 1995; Fitzgerald & Kaneene, 2013; Gortazar & Cowan, 2013).

In order to overcome these challenges, a holistic approach is required to have integrated TB control program among the livestock, wildlife population and human communities (Palmer *et al*., 2012; Palmer, 2013; Palmer *et al*., 2014; Gortázar *et al*., 2015; Gortázar *et al*., 2017). Control programmes can be difficult in places or areas of host community where no proper livestock movement strategies were in place (Acevedo *et al*., 2013).

This host community consists of domestic livestock and wildlife animals. The domestic host livestock species are cattle, sheep, goats and pigs (Gortazar *et al*., 2011), while the wildlife animals are the reservoir species which may act as the maintenance hosts or spill over host (Palmer, 2013). Environmental contamination due to animal excretion in water and soil may contribute towards the shedding and maintaining MTBC in the environment and this may complicate the epidemiology (Ghodbane *et al*., 2014; Walter *et al*., 2014). There are several factors that had contributed to the MTBC and other important mycobacterial maintenance that poses a greater risk to disease control. For now, cattle is well known as a maintenance host and the target host in terms of disease control. While human is a target host for zoonotic TB and certain wildlife are known to be the maintenance host that serve as source of infection to other animals and humans.

There are three subspecies of native wild boar in southeast Asia (SEA), (Groves & Grubb, 1993; Che-Amat & Ong, 2018), these include 'Indian races' (*Sus scrofa cristatus*) ranged in Myanmar and Thailand, 'Eastern races' (*Sus scrofa moupinensis*) in Vietnam and Indonesia, and banded pig (*Sus scrofa vittatus*) ranged from Indonesia to Malaysia. Global data showed that Eurasian wild boar (*Sus scrofa*), may act as reservoir hosts, or amplifier host or 'dead-end hosts' (Gortázar *et al*., 2012; Miller & Sweeney, 2013; Nugent *et al*., 2015; Gortázar *et al*., 2016). They are causing spillover to livestock animals (cattle, goats, pigs) and contributing to the spread and persistence of disease (Gortazar *et al*., 2005; Corner, 2006; Amanfu, 2006; Pavlik, 2006; Naranjo *et al*., 2008; García-Jiménez *et al*., 2013). Despite the abundance of free-ranged wild boar in Malaysia, to date, there is no published data about wild boar TB, MTBC and MAC detection in Malaysia (Che-Amat & Ong, 2018). Several reports had justified that Eurasian wild boar are implicated in the epidemiology of tuberculosis (Gortázar *et al*., 2012). Remarkably, wild boars shared similar molecular type of MTBC with about 54% of MTBC antigens spoligotypes that is closely related to human types, which may cause zoonotic risk to hunters, wildlife personnel and bushmeat consumers (Che-Amat & Ong, 2018).

Nevertheless, several SEA countries had reported cases of TB wildlife such as in elephants (in Thailand) where four Asian elephants were reported being infected with *M. tuberculosis* (Angkawanish *et al*., 2010). Activities that bring people and elephants together such as tourist attraction and religious festivals provide an avenue for interspecies transmission. In Nepal, *M. tuberculosis* was isolated from five captive elephants that died between 2009 and 2013 with the evidence of granulomatous lesions in the lungs (Paudel *et al*., 2020). Captive elephants in Nepal are used for tourist safaris, patrolling and elephants bathing, and other activities that bring them in contact with humans thereby providing avenue for TB transmission among elephants and humans. Serological screening in Asian elephants in range countries and elephant's sanctuaries are carried out using serology (STAT –PAK and DPP). A study carried out in India shows that 45 out of 300 captive Asian elephants tested positive to STAT-PAK and 25.4% of samples were from temples where there had contact with humans (Paudel *et al*., 2020). In Malaysia, serological surveillance was conducted in captive Asian elephants between 2013 and 2016. The results showed that 10/49 (20.4%) and 14/60 (23.3%) was *M. tuberculosis* positive by PCR in trunk washed samples (Ong *et al*., 2013; Yakubu *et al*., 2016). Thus, the data showed that MTBC, specifically *M. tuberculosis*

are prevalent among Asian elephant, however their role as a maintenance host need further investigation.

In non-human primates (NHP), several evidences were showing that this species was detected with MTBC. Buccal swab samples collected from three SEA countries of Thailand, Indonesia and Singapore showed that free ranging monkeys, in zoos and temple monkeys were infected with TB. The PCR amplification using IS6110 results showed 84 (31.9%) of the macaques were infected with *M. tuberculosis*. *Mycobacterium tuberculosis* is endemic in human populations in some SEA countries, providing an avenue for zoonotic transmission between human and NHPs. There is limited published studies available on the interactions of NHPs and people, though range overlap is common, giving the chance for cross species transmission to NHPs (Che-Amat & Ong, 2018).

1.2 Problem Statement

Tuberculosis is a reemerging multi host infectious disease (Gortázar, *et al*., 2008) which infects about 60 different wild animals globally (de Lisle *et al*., 2002; Barnett *et al*., 2013; Amato *et al*., 2018). The increased incidence of mycobacterial infections among wild boars have been reported (Naranjo *et al*., 2008) where it causes major sporadic deaths among wild boars (Segalés *et al*., 2005). The majority of the wild boar piglets succumb to this infection during the first 6 month of age (Che' Amat *et al*., 2015). The data of wild boar TB in Asian, SEA nations including in Malaysia, are scarce and under reported.

Mortalities due to TB in SEA were reported in wild and captive Asian elephants in Sri Lanka (Perera *et al*., 2014), India (Zachariah *et al*., 2017; Chandranaik *et al*., 2017; Paudel *et al*., 2020), Nepal (Paudel *et al*., 2018) and Thailand (Angkawanish *et al*., 2010). In Malaysia, there were only preliminary data of TB in captive elephant had been conducted in 2013-2016 (Ong *et al*., 2013; Yakubu *et al*., 2016) but no study and report on TB infection in wild elephant.

Despite the strong measures implemented for controlling TB in NHPs, outbreaks of TB continue to occur in well-known colonies, with potential serious consequences as results of zoonotic exposures and animal losses (Min *et al*., 2013). Nonetheless, TB screening or surveillance is not a common practice in zoological primates and free-ranged primates in Malaysia and data are not available. Initial data need to be generated and veterinary personnel in the zoological and wildlife department has to conduct the screening on a routine basis.

Test and slaughter control policy for TB had caused unaccountable losses for domestic livestock animals such as in cattle, sheep, goats and pig (Crawshaw *et al*., 2008; Hope & Villarreal-Ramos, 2008; Quintas *et al*., 2010; Bezos *et al*., 2012; Crawshaw *et al*.,

2013; Napp *et al*., 2013). For Malaysia, the prevalence of bovine TB in cattle population showed an increasing trend where from 147 (28%) cases reported in 2016 had increased to 378 (39%) cases in the year 2017 (Chandrawathani *et al*., 2018). Despite the endemic and prevalent TB among cattle in Malaysia, the role of wildlife for TB and other important mycobacterial infection in disease transmission to livestock, human and wildlife (inter-species) need to be investigated.

Malaysia has a number of biodiversity areas with abundance of wildlife species and encroachment of these wildlife into farms, plantation areas and human settlement, which exposed risk of disease transmission to one another. The incidence of bovine TB among cattle farms particularly in Selangor had continuously being detected despite the implementation of test and cull strategy in accordance to the state authority. According to the Ministry of Health of Malaysia, human TB caused by *M. tuberculosis* incidence had increased by 8% from year 2015 to 2017. With the increasing number of urban macaques, the roles of these free-ranged wild animals causing human TB should not be neglected and should be investigated. *Mycobacterium avium* complex (MAC) is known to inhibit variety of sources, which include soil, water, foodstuffs, livestock and wild animals causing different kind of disease in humans, mammals and birds that cause tuberculosis-like diseases in humans and birds, lymphadenitis in humans and mammals, and chronic enteric disease in ruminants. The prevalence of MAC has recently increased around the world where it is responsible for 47% of cases NTM infections. Since relevant authorities do not typically monitor MAC infections relevant epidemiological and surveillance data are not readily available (Shin *et al*.,2020).

1.3 Justification of the study

Currently, there is a huge gap of knowledge and little information on the wildlife disease, zoonotic potential in its potential hot-spot region in Malaysia. Information on zoonotic bacteria from free-ranged wild macaques and wild boars in Malaysia is not well described. Current knowledge on the prevalence and the role played by wildlife species in the transmission of animal TB and important mycobacteria in Southeast Asia (SEA) is generally scanty and not known yet. Their role as reservoir and/or spill over host need to be investigated since they have close contact with multispecies livestock (cattle, buffalo and small ruminants) under the current farm practice in Malaysia. Wild boar and wild macaques are abundance and over the years the encroachment into human area known as wildlife-livestock-human interface are increasing. While other wildlife namely Asian elephant, should not be neglected for their role in zoonotic disease transmission. MAC infections have been sporadically reported in studies on free-ranging wild boars from Spain, Korea, Czech Republic and Portugal (Sevilla *et al*., 2015; Kim *et al*., 2013; Kopecna *et al*., 2008; Matos *et al*., 2012). PCR results from fecal and tissue samples of ruminants from zoo tested positive for MAC by culture and IS900 PCR analysis from suspected wild boar (Roller *et al*.,2020). As a result of these MAC has become an essential part of disease prevention and surveillance protocols of many zoological institutions (Roller *et al*.,2020). Data on disease surveillance in wildlife is needed to predict future disease outbreak. The country needs to prepare disease prediction models for zoonotic pathogens from the wild animals since these two species of wildlife are in abundance, and in close contact with human population competing for food. Data and

in-house ELISA develop from this study will improve our understanding on mycobacterial pathogens present in Malaysia wildlife.

1.4 Hypothesis

Null hypothesis

We hypothesized that antibodies and antigens of *Mycobacterium tuberculosis* complex (MTBC) and *M. avium* complex (MAC) cannot be detected free-ranged wild boars, free-ranged wild macaques and captive Asian elephant and modification on an in-house ELISA cannot detect antibodies against MTBC and MAC in selected wildlife species.

Alternative hypothesis

Antibodies and antigen of Mycobacterium *tuberculosis* complex (MTBC) and *M. avium* complex (MAC) can be detected in free-ranged wild boars, free-ranged wild macaques and captive Asian elephant, and modification on an in-house ELISA can detect antibodies against MTBC and MAC in selected wildlife species.

1.5 General Aim and Objectives

This study aimed to detect the current status of MTBC and MAC in Malaysian wildlife, targeting free-ranged wild boar, wild macaque, captive Asian elephant and captive non-human primates (NHPs) to produce a baseline data for future nationwide surveillance.

1.6 Specific Objectives

1. To determine the seroprevalence status and associated risk factors (sex, age, and location of farms) of *Mycobacterium tuberculosis* complex (MTBC) among free-ranged wild boars and wild macaques in selected districts in Selangor and captive Asian elephants in conservation center, Pahang.
2. To detect the antibodies and antigens of MTBC and *M. avium* complex (MAC) in free-ranged wild boar, wild macaques and captive Asian elephants through tuberculosis-like lesion (TBLL) detection, culture and molecular analysis.
3. To detect MTBC and MAC in captive non-human primates (NHPs) in zoological parks by tuberculin skin test, serology and molecular analysis.
4. To evaluate and modify the IDEXX® *M. bovis* ELISA kit in detecting IgG antibodies to MTBC in wild boars and to develop an in-house ELISA aPPD in detecting IgG antibodies to MAC in NHPs.

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