UNIVERSITI PUTRA MALAYSIA

OCCURRENCE AND CHARACTERISATION OF* Mycobacterium avium *COMPLEX IN CHICKEN AND CAPTIVE BIRDS IN SELECTED STATES IN PENINSULAR MALAYSIA

ABDUL SATTAR

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By

ABDUL SATTAR

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

October 2018

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DEDICATION

I specifically wish to dedicate this dissertation work to my late parents and my entire family. An endless feeling of gratitude to my siblings, wife and children whose love and prayers kept me on and saw me through this most challenging part of my life.

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

OCCURRENCE AND CHARACTERISATION OF *Mycobacterium avium* COMPLEX IN CHICKEN AND CAPTIVE BIRDS IN SELECTED STATES IN PENINSULAR MALAYSIA

By

ABDUL SATTAR

October 2018

Chairman : Associate Professor Zunita Zakaria, PhD
Faculty : Veterinary Medicine

Avian mycobacteriosis is a chronic gastrointestinal disease of the birds. It is caused mainly by *Mycobacterium avium* complex (MAC) and *Mycobacterium genavense*. Almost all species of the birds are susceptible to mycobacteriosis. *Mycobacterium avium* complex is a group of opportunistic pathogens, which are ubiquitous in the environment. It consists of two closely related species; *M. avium* and *M. intracellulare*. *Mycobacterium avium* complex has a high public health significance and its prevalence in human and animals has reportedly been increasing throughout the world. It causes disseminated diseases in immunocompromised population, pulmonary infection in elder people and facial lymphadenitis in the children. In the birds, economic losses due to mycobacteriosis include low meat and egg production, high treatment costs and loss of endangered species of birds. Infected birds excrete MAC through their feces, therefore they (chicken and captive birds) may pose a zoonotic threat to immunocompromised owners. Avian mycobacteriosis is worldwide in distribution and is frequently reported from the northern temperate zone and to a lesser extent from the tropical areas. This study was conducted due to lack of recognized research about the occurrence of mycobacteriosis in chicken and captive birds in Peninsular Malaysia. This cross sectional study detected MAC by Ziehl-Neelsen (Z-N) staining, culture and direct PCR using 300 fecal samples from village chickens (n = 100), layer chickens (n = 100) and captive birds (n = 100). Due to small quantity of feces, 4 samples were excluded from culture and 58 samples (20 due to small quantity and 30 samples due failure in yielding PCR quality DNA) were excluded from direct PCR. Total number of fecal samples were 296 and 242 for microbiology and direct PCR respectively. Successful isolation of MAC on culture media mainly depends on decontamination of the

samples. Even though several decontamination procedures are available, there is no consensus on a single procedure. Therefore, this study also aimed to evaluate six decontamination procedures for effective isolation of *M. avium* from spiked culture negative controls (village chickens *n* = 2) as well as fresh feces (chickens *n* = 35 captive birds *n* = 7). Decontamination procedures included (1) 4% NaOH, (2) 12% H_2SO_4 (3) 1% cetylperidinium chloride (CPC) (4) 4% NaOH-VNA, (5) 12% H_2SO_4 (6) CPC-VNA, (VNA referred to mixture of antibiotics containing vancomycin 100 µg/ml, nalidixic acid 100 µg/ml and amphotericin B 50 µg/ml). This study evaluated Löwenstein Jensen and calorimetric Middlebrook 7H9 culture media for rapid isolation of *M. avium* from spiked culture negative controls (layer chicken *n* = 4) and fresh feces (captive birds *n* = 45). Results of the evaluation of decontamination procedures revealed that CPC-VNA was the most favorable decontamination procedure for isolation of *M. avium* and other mycobacteria spp from feces. This method isolated mycobacteria from 2.4% fresh feces (chicken) and recovered 66.7% *M. avium* from spiked feces with 19% and 5.5% contamination of fresh fecal and spiked cultures respectively. This study also showed that CPC-VNA and L-J combination is the most favorable culture combination to isolate more mycobacteria with low contamination rate. This combination is cost effective, simple, reduces the workload on the bench and increases the recovery of *M. avium* from avian fecal samples. Results of the cross sectional study showed that all samples (296) were Z-N negative. Proportion of positive samples (by culture and PCR) was 4.0% (12/300). Proportion of Z-N positive cultures was 2.02% (6/296) and proportion of PCR positive samples was 2.5% (6/242). A total of 4% (4/100) village chickens and 2.08% (2/96) captive birds were found to be culture positive. Furthermore, PCR detected DNA of *M. avium* subspecies *avium* in 1.7% (1/58) feces from village chickens and 5.9% (5/84) feces from captive birds. No mycobacteria were isolated and detected in layer chickens. Sequence analysis confirmed three isolates (one IS*901* and two 16S rRNA) as *M. avium* subspecies *avium, M. terrae* and *M. engbaekii. Mycobacterium avium* subspecies *avium* was isolated from a White Pelican (*Pelecanus onocrotalus*). *Mycobacterium terrae* and *M. engbaekii* were isolated from village chickens (*Gallus domesticus*). Direct PCR (IS*901*) detected DNA of *M. avium* subspecies *avium* in 2.5% (6/242) feces (chicken *n* = 1 and captive birds *n* = 5) and PCR results were further verified by sequencing. *Mycobacterium avium* subspecies *avium* DNA was detected in the feces of macaw parrot (*n* = 2) namely, Green Winged macaw (*Ara chloropterus*) and Blue and gold macaw (*Ara ararauna*), Cockatoo parrot (*n* = 2) namely, Umbrella cockatoo (*Cacatus alba*) and Galah cockatoo (*Eolophus resicapilla*), Black Hornbill *Anthracoceros malayanus* (*n* = 1) and village chicken *Gallus domesticus* (*n* = 1). Phylogenetic analysis of DNA sequences of *M. avium* subspecies *avium* obtained during this study revealed close relatedness to themselves and to *M. avium* strain RCAD0278. In conclusion, this study reports the occurrence of MAC in the chickens and captive birds in Peninsular Malaysia. Furthermore, this study also revealed that culture using CPC-VNA decontamination and direct PCR can be used as referential methods for detection of MAC and other members of genus *Mycobacterium*.

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

KEHADIRAN DAN PENCIRIAN KOMPLEKS *Mycobacterium avium* PADA AYAM DAN BURUNG DI KAWASAN TENGAH SEMENANJUNG MALAYSIA

Oleh

ABDUL SATTAR

Oktober 2018

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Mikobakteriosis burung adalah penyakit gastrousus yang kronik dalam kalangan burung. Ia disebabkan oleh *Mycobacterium avium* kompleks (MAC) dan *Mycobacterium genavense*. Hampir kesemua spesis burung adalah terdedah kepada mikobakteriosis. *Mycobacterium avium* kompleks adalah kumpulan patogen oportunistik, yang berada di alam persekitaran. Ia terdiri daripada dua spesis iaitu *M. avium* dan *M. intracellulare*. *Mycobacterium avium* kompleks (MAC) memainkan peranan penting dalam kesihatan awam. Jangkitan pada manusia dan haiwan dilaporkan meningkat di seluruh dunia. Ia menyebabkan penyakit-penyakit seperti jangkitan paru-paru dalam kalangan orang tua, limfadenitis muka dalam kalangan kanak-kanak dan penyakit ini juga tersebar dalam kalangan populasi yang mempunyai sistem imun yang rendah. Kerugian ekonomi dalam kalangan pemilik atau penternak burung akibat mikobakteriosis dapat dilihat melalui penurunan pengeluaran daging dan telur, kos rawatan tinggi dan juga kehilangan spesis burung yang terancam. Burung-burung yang telah dijangkiti akan menyahtinja MAC melalui najisnya. Oleh yang demekian, burung-burung tersebut boleh menimbulkan ancaman zoonotik kepada individu yang terjejas imunnya. Mikobakteriosis burung tersebar ke seluruh dunia dan sering dilaporkan b terutamanya di zon utara yang beriklim sederhana dan juga di kawasan tropika yang tertentu. Oleh kerana kekurangan penyelidikan tentang mikobakteriosis dalam burung termasuk ayam dan juga burung belaan di Semenanjung Malaysia, kajian ini telah dijalankan. Kajian keratan rentas ini mengesan MAC menggunakan teknik pewarnaan Ziehl-Neelsen (Z-N), pengasingan bakteria dan juga reaksi polimerasi berantai (PCR) daripada 300 sampel najis ayam kampung (n = 100), ayam penelur (n = 100) dan burung eksotik belaan (n = 100). Oleh kerana kuantiti sampel yang kecil,

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empat sampel telah dikecualikan daripada proses pengasingan dan 58 sampel dikecualikan daripada PCR. Jumlah sampel najis keseluruhannya adalah 296 dan 242 masing-masing untuk mikrobiologi dan PCR. Pengasingan MAC pada kultur media adalah sangat bergantung kepada kaedah dekontaminasi sampel. Walaupun beberapa prosedur dekontaminasi telah sedia ada digunapakai, namun tiada keseragaman mengenai satu prosedur yang terbaik. Oleh itu, kajian ini juga bertujuan untuk menilai enam prosedur dekontaminasi untuk mengasingkan *M. avium* daripada sampel kawalan negatif yang ditambah dgn bakteria (ayam kampung n = 2) dan juga najis segar (ayam n = 35 ; burung belaan n = 7). Prosedur dekontaminasi termasuk (1) 4% NaOH, (2) 12% H_2SO_4, (3) 1% cetylperidinium klorida (CPC), (4) 4% NaOH-VNA, (5) 12% H_2SO_4, (6) CPC-VNA, (VNA dirujuk kepada campuran antibiotik yang mengandungi vankomisin 100 µg / ml, asid nalidisik 100 µg / ml dan amphoterisin B 50 µg / ml). Dalam kajian ini juga, keberkesanan media Löwenstein Jensen (L-J) dan Middlebrook 7H9 dinilai dalam mengasingkan *M. avium* secara daripada kawalan negatif kultur yang ditambah dengan bakteria (ayam penelur n = 4) dan najis segar (burung belaan n = 45). Didapati CPC-VNA adalah prosedur dekontaminasi yang terbaik untuk mengasingkan *M. avium* dan *Mycobacterium* sp. dari najis. Kaedah ini berjaya mengasingkan *Mycobacterium* sp. dari 2.4% najis segar (ayam) dan 66.7% *M. avium* dari najis yang ditambah dengan bakteria dengan masing-masing pada 19% dan 5.5% pencemaran kultur najis segar dan kultur yang ditambah dengan bakteria. Kajian ini menunjukkan bahawa kombinasi CPC-VNA dan L-J adalah gabungan media kultur yang paling baik untuk mengasingkan lebih banyak *Mycobacterium* sp. dengan kadar kontaminasi yang rendah. Kombinasi ini adalah kos efektif, mudah, mengurangkan beban kerja dan meningkatkan kebarangkalian pengasingan *M. avium* dari sampel najis burung. Hasil kajian keratan rentas menunjukkan bahawa semua sampel (296) adalah negatif melalui pewarnaan Z-N. Sebilangan sampel positif (oleh pengasingan dan PCR) adalah 4.0% (12/300). Kadar pengasingan positif pewarnaan Z-N adalah 2.02% (6/296) dan kadar positif pula PCR ialah 2.5% (6/242). Sejumlah 4% (4/100) ayam kampung dan 2.08% (2/96) burung belaan eksotik didapati positif kultur *Mycobacterium* sp. PCR dapat mengesan DNA *M. avium* subspecies *avium* dalam 1.7% (1/58) najis daripada ayam kampung dan 5.9% (5/84) najis daripada burung belaan eksotik. Tiada *Mycobacterium* sp. yang diasingkan dan dikesan dalam ayam penelur. Analisis penjujukan DNA mengesahkan tiga isolat (satu IS901 dan dua 16S rRNA) sebagai *M. avium* subspecies *avium*, *M. terrae* dan *M. engbaekii*. *Mycobacterium avium* subspesies *avium* diasingkan dari Pelican putih (*Pelecanus onocrotalus*). *Mycobacterium terrae* dan *M. engbaekii* diasingkan daripada ayam kampung (*Gallus domesticus*). DNA *Mycobacterium avium* subspecies *avium* dikesan di dalam najis burung nuri macaw (n = 2) iaitu Green Winged Macaw (*Ara chloropterus*) dan Macaw emas dan biru (*Ara ararauna*), burung nuri Cockatoo (n = 2) iaitu Umbrella Cockatoo (*Cacatus alba*) dan Galah Cockatoo (*Eolophus resicapilla*), Black Hornbill (*Anthracoceros malayanus*) (n = 1) dan ayam kampung (*Gallus domesticus*) (n = 1). Analisis filogenetik bagi penjujukan DNA *M. avium*

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subspesies *avium* yang diperolehi semasa kajian ini menunjukkan hubungan yang rapat dengan strain *M. avium* RCAD0278. Kesimpulannya, kajian ini melaporkan kejadian MAC di ayam dan burung belaan eksotik di semenanjung Malaysia. Tambahan pula, kajian ini juga mendedahkan bahawa yang menggunakan dekontaminasi CPC-VNA dan PCR secara langsung boleh digunakan sebagai kaedah rujukan untuk pengesanan MAC dan juga genus *Mycobacterium* yang lain.

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

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LIST OF ABBREVIATIONS

AFB	Acid fast bacilli
AIDS	Acquired immunodeficiency syndrome
AMB	Amphotericin B
ATCC	American Type Culture Collection
BCG	Bacillus Calmette-Guerin
BLAST	Basic Local Alignment Search Tool
CDC	Centre for Disease Control and Prevention
Cm	Centimeter
CFU	Colony forming unit
CI	Confidence interval
CO_2	Carbon dioxide
CPC	Cetylperidinium chloride
DGGE	Denaturing gradient gel electrophoresis
DNA	Deoxyribonucleic acid
DVS	Department of Veterinary Services
DST	Drug susceptibility test
E value	Expected Value
EDTA	Ethylene diaminetetraacetate
ELISA	Enzyme linked immunosorbent assay
G	Gram
G	Gravity
GC	Guanine and cytosine
GPL	Glycopeptidolipid
H_2SO_4	Sulfuric acid
HCl	Hydrochloric acid
HEYM	Herald's egg yolk medium
HCP	hexacetylperidinium chloride
HIV	Human immunodeficient Virus
HPLC	High performance Liquid Chromatography
Hsp	Heat Shock Protein

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ID	Identity
IS	Insertion sequence
INH	Isoniazid
IUCN	International Union for the Conservation of Nature
L-J	Löwenstein Jensen
MA	*Mycobacterium avium*
Maa	*Mycobacterium avium* spp *avium*
MAC	Mycobacterium avium complex
Mah	*Mycobacterium avium* spp *hominissuis*
Map	*Mycobacterium avium* spp *paratuberculosis*
MB7H9	Middlebrook 7H9
MGIT	Mycobacterial growth indicator tubes
Ml	Milliliter
MLST	Multilocus sequence typing
mM	Mili molar
Mm	Millimeter
NAL	Nalidixic acid
NaOH	Sodium hydroxide
NCBI	National Center for Biotechnology and Information
NCCLS	National Committee for Clinical laboratory standards
NTM	Nontuberculous mycobacteria
OADC	Oleic acid albumin dextrose catalase
OIE	Office International Epizootica
OR	Odds Ratio
P	Probability value
PANTA	Polymyxin B, Amphotericin B, Nalidixic acid, Trimethoprim and Azolocillin
PBS	Phosphate buffer solution
PCR	Polymerase chain reaction
PPD	Purified Protein derivative
PRA	PCR restriction assay
QAC	Quaternary ammonium compound

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RCF Relative centrifugation force

RFLP Restricted fragment length polymorphism

rpoB Ribonucleic acid, polymerase Beta subunit

Rpm Resolutions per minute

rRNA Ribosomal ribonucleic acid

SPSS Statistical Package for Social Sciences

STC 2,3-diphenyl-5- thienyl-(2)- tetrazolium chloride

TB Tuberculosis

TB Tuberculosis

TBE Tris-Borate EDTA

TE Tris-EDTA

TLR2 Toll Like receptor 2

UK United Kingdom

USA United States of America

USA United States of America

UV Ultra violet

VAN Vancomycin

VNA Vancomycin, nalidixic acid and amphotericin B

VNTR Variable number tandem repeats

WHO World Health Organization

% Percent

˚C Degree Celsius

< Less than

= Equal to

> Greater than

µg Microgram

µl Microliter

µm Micrometer

n Nano

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CHAPTER 1

INTRODUCTION

Avian mycobacteriosis or tuberculosis is a chronic gastrointestinal infection of the birds (Kriz et al., 2013; Dhama et al., 2011). It is mainly caused by *M. avium* complex (MAC) and *M. genavense* (Tell et al., 2001; Hoop et al., 1996). However, other pathogenic mycobacteria may also cause disease in the birds (Ikonomopoulos et al., 2009; Tell et al., 2001). *Mycobacterium avium* complex (MAC) with a high public health significance, is a group of opportunistic pathogens (Hamilton et al., 2017). It causes disseminated disease in immunocompromised population (Koirala, 2017), pulmonary infections in elderly people (Adjemian et al., 2012) and facial lymphadenitis in young children (Thegerström et al., 2008). It consists of two species of slow growing nontuberculous mycobacteria; *M. avium* and *M. intracellulare* (Kwaghe et al., 2015). The two species are similar phenotypically but genotypically they are significantly different from one another (Zhao et al., 2014). *Mycobacterium avium* complex (MAC) has been further classified into 28 serotypes (serovars) based on seroagglutination test. Serotypes are given numbers from 1 to 28 rather than name (Wolinsky & Schaefer, 1973). Presently, *M. avium* has four subspecies with wide host range. The subspecies of *M. avium* are; *M. avium* subspecies *avium*, pathogenic to birds (Pavlik et al., 2000), *M. avium* subspecies *hominissuis*, pathogenic to human and swine, *M. avium* subspecies *silvaticum*, pathogenic to birds and *M. avium* subspecies *paratuberculosis*, pathogenic to cattle (Álvarez et al., 2008; Pavlik et al., 2000). *M. avium* subspecies *paratuberculosis* is proposed as the etiology of Crohn's disease in human (Reddacliff et al., 2003).

Avian mycobacteriosis is worldwide in distribution (Millán et al., 2010). It is reported mainly in the northern temperate zone (Kriz et al., 2015 & 2013; Shitaye et al., 2008 b; Soler et al., 2009; Dvorska et al., 2007) and to a lesser extent in the tropical area (Kindu & Getaneh, 2016; Soler et al., 2009). Almost all avian species are susceptible to mycobacteriosis (Tell et al., 2001). Avian orders; *Galliformes* (domestic fowl, turkeys and pheasant), *Anseriformes* (ducks, geese, swans and screamers), *Gruiformes* (cranes, rails, trumpeters), *Columbiformes* (pigeon, doves), *psittaciformes* (parrots) and *passeriformes* (exotic birds) have been reported to be more susceptible to MAC (Kriz et al., 2013 ; Palmieri et al., 2013; Tell et al., 2001). Presently, it is more frequent in the chickens kept in small flocks for long time and in the pet birds (Shitaye, et al., 2008 b; Hoop et al., 1996). Increase in age of the birds increases the accumulative risk of exposure to this environmental mycobacteria (Fulton & Thoen, 2008). Avian mycobacteriosis is not a major problem in the commercial poultry (Tell et al., 2001) because poultry husbandry practices applied in the commercial poultry has reduced its prevalence (Dahlhausen et al., 2012). Furthermore, young age of broilers

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has reduced their exposure to the environment and MAC requires a long generation time to establish an infection (Dahlhausen et al., 2012; Tell et al., 2001). However, sporadic outbreaks have been reported in commercial chicken and duck flocks (Zhu et al., 2016; González et al., 2002).

Poultry industry has become a major contributor to the economy in Malaysia (Idris et al., 2013). Commercial poultry, the major component of the poultry industry, has developed at a rapid rate, principally due to the introduction of hybrid birds, better management system and disease control programs (Aini, 1990). However, a significant proportions of rural population is involved in keeping small flocks of indigenous or village chicken. Rural poultry is an important sources of income as well as cheap source of animal protein for villagers (Aini, 1999; Supramaniam, 1987). Rural poultry plays a significant role in improving the nutritional status, income, food security and livelihood of many small holders (Scanes, 2007). However, infectious diseases are among the major constraints in the expansion of rural poultry (Fulton & Thoen, 2008). Avian mycobacteriosis is an infectious disease affecting all species of birds (Zhu et al., 2016; Shitaye et al., 2008 b; Witte et al., 2008). It is a chronic wasting disease (VanDerHeyden, 1997) primarily effecting gastrointestinal tract, intestines, liver and spleen (Shivaprasad & Palmieri, 2012). Most of the infected birds show no clinical signs and suddenly die (Mutalib & Riddell, 1988). Rupture of liver with clotted blood in coelom is commonly observed in suddenly dying chickens (Mutalib & Riddell, 1988). Post mortem signs of mycobacteriosis are specific in the birds (Tell et al., 2001) which include pale, yellow, white and dark nodules of various size on liver, spleen, intestines, bone marrow, lung, air sacs and mesentery (Shivaprasad & Palmieri, 2012). Avian mycobacteriosis has been reported from different parts of the world (Kindu & Getaneh, 2016; Zhu et al., 2016; Kriz et al., 2013). However, due to lack of recognized research, there is no published data about the occurrence of avian mycobacteriosis in the chickens and captive birds in Peninsular Malaysia.

Diagnosis of avian mycobacteriosis in the live birds is challenging as mostly birds die without showing any clinical signs of the disease (Soler et al., 2009; Tell et al., 2001). Normally, diagnosis is performed by histopathology and culture at postmortem. Hepatomegaly (enlargement of liver), splenomegaly (enlargement of spleen), thickening of the intestinal wall and nodules (1-2 cm in diameter) on liver and spleen are helpful in postmortem diagnosis (Shivaprasad & Palmieri, 2012). Ante mortem in the live birds is a need of time. Feces as non-invasive source can be used for diagnosis of mycobacteriosis in live birds for detection of bacilli by microscopy, culture and polymerase chain reaction (PCR) (Kriz et al., 2013; Tell et al., 2003a). Microscopy is the simplest, cheap and frequently used technique for detection of bacilli (Aziz et al., 2007). However, previous studies have shown that microscopy of feces is not as sensitive as for other samples like sputum and tissue samples (Saggese et al., 2010) because shedding of bacilli

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through feces is intermittent during subclinical infection (Tell et al., 2003a). Culture is considered the definitive test for detection of bacilli as isolates are required for downstream analysis such as identification and antimicrobial susceptibility studies (Aziz et al., 2007). Fecal culture has been used as gold standard for screening of paratuberculosis in cattle (Whittington, 2009). Sensitivity of culture mainly depends on the decontamination procedure and culture media (Kantor et al., 1998). Several decontamination procedures have been developed for elimination of contaminating bacteria during primary isolation of mycobacteria (Oliveira et al., 2007). However, there is lack of consensus on a single procedure (Corner et al., 2012). Furthermore, the decontamination procedures have been evaluated for sputum, tissues, feces from human and cattle (Chatterjee et al., 2013; Corner et al., 2012). Comprehensive literature review showed that evaluation of decontamination procedures using feces (chicken and captive birds) has not been performed.

Conventional egg based solid media like Löwenstein Jensen is frequently used for culture of mycobacteria (Aziz et al., 2007). Löwenstein Jensen medium supports confluent growth of bacilli, is less prone to contamination and phenotypic identification of mycobacteria is easy (Tell et al., 2003a). However, prolonged incubation period required for the growth of mycobacteria is main disadvantage of conventional media (Aziz et al., 2007). Liquid or broth based media are preferred over conventional solid media for rapid detection of mycobacteria (Siddiqi & Gerdes, 2006). According to CDC guidelines, both liquid and solid media should be used for cultivation of mycobacteria (Siddiqi & Gerdes, 2006). Automated liquid media like BACTEC 460 and MGIT 960 have significantly reduced time to detect the growth of slow growing mycobacteria (Siddiqi et al., 2012). However, high prices of the automated liquid media and use of sophisticated machines to detect the growth of mycobacteria are the major hurdles in their routine use (Siddiqi & Gerdes, 2006). Colorimetric broth media using a reduction oxidation (redox) compounds have the potential to replace automated liquid media for rapid detection of mycobacteria and antimicrobial susceptibility test (Rojas-Ponce et al., 2013; Lee et al., 2007). Colorimetric broth media use redox compounds which make colorless solution in water. Growth of microorganism is detected by color change to red when redox come in contact with the growing microorganism (Lee et al., 2007). In the current study, calorimetric liquid media supplemented with 2, 3- Diphenyl-5-thienyl-(2) tetrazolium (STC) was compared to conventional L-J media for rapid detection of *M. avium* from the spiked cultures.

Rapid detection and identification of mycobacteria to species and subspecies level have been an important subject (Álvarez et al., 2008). Routine biochemical tests cannot differentiate closely related species of mycobacteria like *M. avium* and *M. intracellulare* (Rindi & Garzelli, 2014). Detection and characterization of mycobacteria have become easier with the progress in molecular techniques (Pavlik et al., 2000). Polymerase chain

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reaction (PCR) has been used as an alternative method for detection of mycobacteria (Kriz et al., 2013) because it rapidly detects and identifies different species of genus *Mycobacterium* (Kaevska et al., 2010; Khare et al., 2004). Polymerase chain reaction (PCR) has reduced detection time to days and it detects trace amount of DNA in the clinical samples (Ikonomopoulos et al., 2009). It (PCR) also detects those species of mycobacteria which are difficult to culture on media like *M. genavense* (Hoop et al., 1996). Subspecies of *Mycobacterium avium* possess insertion sequences which include IS*901*, IS*1245* and IS*900* which are specific to them (Rindi & Garzelli, 2014; Álvarez et al., 2008). Insertion sequence IS*901* is specific to *M. avium* subspecies *avium* (Maa) (Pavlik et al., 2000). *Mycobacterium avium* subspecies *avium* and *M. avium* subspecies *hominissuis* possess IS*1245* in their genome. Insertion sequence IS*900* is specific to *M. avium* subspecies *paratuberculosis* (Rindi & Garzelli, 2014). Amplification of IS*901* is an easy tool to detect virulent Maa in the birds (Kriz et al., 2013; Pavlik et al., 2000).

The current study was planned to determine the occurrence of *M. avium* complex in chicken and captive birds as well as to explore the most suitable decontamination procedure and culture media for successful isolation of *M. avium* complex and other mycobacteria with the hypothesis and objectives as under;

1.1 Hypothesis

It was hypothesized that:

1. All decontaminating agents have similar effects to eliminate contaminating bacteria.
2. Liquid and solid media are comparable to isolate *M. avium* and other mycobacteria.
3. The occurrence of *M. avium* complex (MAC) in chicken and captive birds in selected states of Peninsular Malaysia is 50% (Kindu & Getaneh, 2016).
4. Microscopy, culture and PCR are comparable to detect *M. avium* complex in the feces of chicken and captive birds.

1.2 Objectives

1. To evaluate six decontamination procedures to explore the most suitable procedure for isolation of *M. avium* from feces of chicken and captive birds.
2. To evaluate calorimetric Middlebrook 7H9 and Löwenstein Jensen media for rapid isolation of *M. avium* from feces of chicken and captive birds.

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3. To determine the occurrence of *M. avium* complex (MAC) in chicken and captive birds in selected states of Peninsular Malaysia and to characterize isolates of MAC.
4. To analyze the risk factors responsible for MAC infestation of the chicken and captive birds.

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