SEROPREVALENCE AND IDENTIFICATION OF PATHOGENIC *LEPTOSPIRA* IN RODENTS FROM KUALA LUMPUR WET MARKETS, MALAYSIA

By

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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Pathogenic *Leptospira* are spiral-shaped bacteria that causes leptospirosis in infected victim. It was reported to be isolated from many mammal species all over the world, including human, establishing leptospirosis as the most widespread zoonosis disease. This bacterium species is widely known for having rodents as their main reservoir host, residing in the host's renal tubule and spread to the environment through their host's urine. Malaysia has declared leptospirosis as notifiable disease in 2010 and there has been increasing reported cases annually since then. Due to this, there is a need for a complete list or database of pathogenic *Leptospira* that circulate in Malaysia. Therefore, this study is performed in hope to contribute and provide information towards that goal. The objective of this study is to determine the seroprevalence and species distribution of pathogenic *Leptospira* in rodents captured from Kuala Lumpur wet markets. Four wet markets in Kuala Lumpur were chosen as the sampling sites of this study, namely Pasar Pudu, Pasar Chow Kit, Pasar Datuk Keramat and Pasar Petaling Street, as per recommendation by Kuala Lumpur City Hall (Dewan Bandaraya Kuala Lumpur, DBKL). Sampling was performed by live trapping for the duration of 12 months in 2017. Each rodent caught was euthanised, morphologically identified and processed for the extraction of blood serum and kidney samples. Microscopic Agglutination Test (MAT) were performed on serum samples (against a panel of 24 live *Leptospira* serovar culture) for serology test, while kidney samples were processed and subjected to molecular screening test for pathogenic *Leptospira* detection and cultured in Ellinghausen-McCullough-Johnson-Harris (EMJH) medium for *Leptospira* isolation. For each culture medium that succeeded in growing *Leptospira*, they were morphologically confirmed as *Leptospira* cultures under dark field microscope and underwent molecular characterisation using six loci Multilocus Sequence Typing (MLST) test for *Leptospira* species identification. Sampling yields 144 live captured rodents, all identified as house rat or *Rattus rattus*. MAT gave 50/144 (34.72%) positive samples for leptospiral antibody in rodents, with

the most prominent serovar detected being serovar Malaya, followed by IMR LEP 175. A total of 50/144 (34.7%) samples were detected as pathogenic *Leptospira* from molecular screening test. As for bacteria isolation, 23 out of 144 culture samples (15.97%) were successfully isolated, and from this, MLST analysis identified two *Leptospira* species, with 20/23 (87%) samples identified as *Leptospira borpetersenii* and the other remaining three (13%) samples were identified as ST149 *Leptospira interrogans* serogroup Bataviae serovar Bataviae strain Swart. The findings in this study present the evidence of pathogenic *Leptospira* presence which strive and actively circulating in the rodent population of Kuala Lumpur wet markets. Rodents are indeed the reservoir or maintenance host of pathogenic *Leptospira* in the urban, highly populated setting of Kuala Lumpur. Both serology and molecular screening test results indicate a high endemicity for leptospirosis if the rodent population is left unchecked, as control of *Leptospira* reservoir host is important for preventing future leptospirosis outbreaks in Kuala Lumpur.

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KELAZIMAN SERO DAN PENGENALPASTIAN *LEPTOSPIRA* PATOGENIK DI DALAM TIKUS DARI PASAR BASAH KUALA LUMPUR, MALAYSIA

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Leptospira patogenik ialah bakteria berbentuk lingkaran yang menyebabkan leptospirosis pada mangsa yang dijangkiti. *Leptospira* telah dilaporkan berjaya diasingkan daripada banyak spesies mamalia di seluruh dunia, termasuk manusia, menjadikan leptospirosis sebagai penyakit zoonosis yang paling meluas. Spesies bakteria ini terkenal dengan mempunyai tikus sebagai hos rizab utama mereka, tinggal di dalam tubul ginjal hos dan disebar ke persekitaran melalui air kencing hos mereka. Malaysia telah mengisytiharkan leptospirosis sebagai penyakit yang perlu dilaporkan pada tahun 2010 dan terdapat peningkatan kes yang dilaporkan setiap tahun sejak itu. Disebabkan ini, terdapat keperluan untuk senarai lengkap atau pangkalan data *Leptospira* patogenik yang berkitar di Malaysia. Jesteru itu, kajian ini dilakukan dengan harapan untuk menyumbang dan memberikan maklumat ke arah matlamat tersebut. Objektif kajian ini adalah untuk menentukan kelaziman sero dan taburan spesies *Leptospira* patogenik dalam tikus yang ditangkap dari pasar-pasar basah Kuala Lumpur. Empat pasar basah di Kuala Lumpur telah dipilih sebagai tapak persampelan kajian ini, iaitu Pasar Pudu, Pasar Chow Kit, Pasar Datuk Keramat dan Pasar Petaling Street, seperti yang disyorkan oleh Dewan Bandaraya Kuala Lumpur (DBKL). Persampelan dilakukan dengan cara tangkapan hidup selama 12 bulan pada tahun 2017. Setiap tikus yang ditangkap dieutanasi, dikenal pasti secara morfologi dan diproses untuk pengambilan serum darah dan sampel buah pinggang. Ujian Aglutinasi Mikroskopik (MAT) dilakukan pada sampel serum (terhadap panel 24 kultur hidup *Leptospira* serovar) untuk ujian serologi, manakala sampel ginjal diproses dan dikenakan ujian saringan molekul untuk mengesan *Leptospira* patogenik dan dikulturkan dalam Ellinghausen-McCullough-Johnson-Harris (EMJH) media untuk pengasingan *Leptospira*. Untuk setiap medium kultur yang berjaya membiakkan *Leptospira*, mereka disahkan secara morfologi sebagai kultur *Leptospira* di bawah mikroskop medan gelap dan menjalani pencirian molekul menggunakan ujian enam lokus Penjenisan Jujukan Multi-lokus (MLST) untuk mengenal pasti spesies *Leptospira*. Persampelan menghasilkan 144 tikus hidup yang berjaya ditangkap,

semuanya dikenal pasti sebagai tikus rumah atau *Rattus rattus*. MAT memberikan 50/144 (34.72%) sampel positif untuk antibodi *leptospiral* dalam tikus, dengan serovar paling menonjol dikesan ialah serovar Malaya, diikuti oleh IMR LEP 175. Sebanyak 50/144 (34.7%) sampel dikesan sebagai *Leptospira* patogenik daripada ujian saringan molekul. Bagi pengasingan bakteria, 23 daripada 144 sampel kultur (15.97%) telah berjaya diasingkan, dan daripada ini, analisis MLST berjaya mengenal pasti dua spesies *Leptospira*, dengan 20/23 (87%) sampel dikenal pasti sebagai *Leptospira borpetersenii* dan baki tiga lagi (13%) sampel dikenal pasti sebagai ST149 *Leptospira interrogans* serogroup Bataviae serovar Bataviae strain Swart. Penemuan dalam kajian ini menunjukkan bukti kehadiran *Leptospira* patogenik yang berusaha dan berkitar aktif dalam populasi tikus di pasar-pasar basah Kuala Lumpur. Tikus sememangnya merupakan hos rizab atau perumah penyenggaraan *Leptospira* patogenik di kawasan bandar Kuala Lumpur yang padat dengan penduduk. Kedua-dua keputusan ujian saringan serologi dan molekul menunjukkan endemisiti tinggi untuk leptospirosis jika populasi tikus dibiarkan tanpa kawalan, kerana kawalan terhadap hos rizab *Leptospira* adalah penting untuk mencegah wabak leptospirosis pada masa hadapan di Kuala Lumpur.

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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 Master of Science. The members of the Supervisory Committee were as follows:

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Date: 12 August 2021

Declaration by the Graduate Student

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

°C	Celsius
BLAST	Basic Local Alignment Search Tool
bp	Base Pair
BSA	Bovine Serum Albumin
DBKL	Dewan Bandaraya Kuala Lumpur
DNA	Deoxyribonucleic Acid
ELISA	Enzyme-Linked Immunosorbent Assay
EMJH	Ellinghausen, Mccullough, Johnson, Harris Media
flaB	Flagellin B
ft^2	Square Feet
Ictero	Icterohaemorrhagiae
IMR	Institute for Medical Research
L.	*Leptospira*
M	Molar
m^2	Square Meter
MAT	Microscopic Agglutination Test
$MgCl^2$	Magnesium Chloride
min	Minute
ml	Millilitre
MLST	Multi Locus Sequence Typing
MLST1	MLST Scheme 1
MLST3	MLST Scheme 3
NCBI	National Centre of Biotechnology
NMRR	National Medical Research Register

PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
pH	Potential Of Hydrogen
R.	*Rattus*
sec	Second
ST	Sequence Type
TE	Tris-EDTA
WHO	World Health Organisation
µl	Micrometer
µm	Micrometer
µM	Micromolar

CHAPTER 1

INTRODUCTION

1.1 Introduction

Leptospirosis is a disease caused by bacterial infection of pathogenic *Leptospira* species that occur in both human and animal. *Leptospira* has been isolated from almost all mammalian species around the world which make leptospirosis recognised as the most widespread zoonosis disease. This disease is endemic in tropical regions, having high incidence during rainy season and natural disasters such as flood or tsunami.

Leptospira are grouped into two; saprophytic (non-pathogenic) and pathogenic. Saprophytic *Leptospira* are natural, free-living bacteria that live in the environment and do not cause leptospirosis. Pathogenic *Leptospira*, that causes leptospirosis are mainly found in the renal tubules of its reservoir or carrier host which they are later secreted through the host's urine into the environment. Natural reservoirs and carriers of pathogenic *Leptospira* includes domestic and wild animals. Rodents are the most common and well-known reservoir host for *Leptospira* that are responsible for disease transmission in both urban and rural settings where they are abundant in number.

Human infection of *Leptospira* occurred through direct contact with the urine of infected carriers or through contact with contaminated environmental elements such as wet soil or stagnant water. *Leptospira* exposure in human usually happened through occupational or recreational activities in places where infected or reservoir host are plenty. In human, symptoms of leptospirosis are broad which include headache, myalgia, arthralgia, brain conjunctival suffusion and meningeal irritation. These symptoms are too broad and similar with other diseases such as dengue resulting in difficulties for quick and accurate diagnose. Owing to this reason, mortality rate of leptospirosis cases is 5–15% involving multisystem disease and organ failure (Costa et al., 2015). Combined with high global annual estimated severe leptospirosis cases (around 350 000-500 000 cases) and difficulties in quick and accurate diagnose, leptospirosis is a serious and worrisome disease that may threat human lives.

Since 2010, there are gradually increasing reported cases of human leptospirosis in Malaysia as leptospirosis was declared as a notifiable disease in Malaysia. A total of 1976 cases were reported in 2010 and the cases have quadrupled to 7806 in 2014 (Wahab, 2015). Due to these high annual cases, it is important to have a data on what kind or species of *Leptospira* that is the most commonly harboured by rodents in Malaysia especially in urban area where

rodents are abundant and have frequent interaction with human population in the area.

1.2 Problem Statement

This study was performed considering the lack of multiple information on pathogenic *Leptospira* in Malaysia. Until this day, study on detection of leptospiral antibody in Malaysian small mammals especially rodents are still very limited because most previous *Leptospira* studies mainly focused of on human leptospirosis. Data on circulating *Leptospira* serovars in rodents is important as it can be used as reference when dealing with both human and animal leptospirosis. In addition, details on detection of pathogenic *Leptospira* presence in highly populated urban setting such as Kuala Lumpur is lacking. Highly populated urban areas are at great risk for *Leptospira* infection. Hence, there is a need for source tracking of the bacteria for outbreak intervention. This study was also performed in order to obtain information or list on pathogenic *Leptospira* species that are thriving and prevailing in Malaysia. Current knowledge on this subject is insufficient since there are still only few reports on isolation and identification of local *Leptospira* strains. Information on local pathogenic *Leptospira* species is crucial since it can provide genetic variation data on local strains and the information can also be used to increase microscopic agglutination test (MAT) sensitivity by addition of local strains in MAT panel for diagnosis or serosurvey purpose.

1.3 Objectives

1.3.1 General Objective

To study the seroprevalence and distribution of pathogenic *Leptospira* in rodents captured from Kuala Lumpur wet markets.

1.3.2 Specific Objectives

1. To determine the seropositivity of anti-leptospiral antibody in rodents from Kuala Lumpur wet markets.

2. To isolate, characterise and identify pathogenic *Leptospira* in captured rodents.

3. To determine the most common pathogenic *Leptospira* species in rodents of Kuala Lumpur wet markets.

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