



UNIVERSITI PUTRA MALAYSIA

***PREVALENCE AND TRANSMISSION OF LEPTOSPIRAL INFECTION IN
RATS IN MALAYSIA***

SITI NABILA BINTI MOHAMED HASSAN

FPV 2012 6

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By

SITI NABILA BINTI MOHAMED HASSAN

**This Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia in
Fulfilment of the Requirements for the Master of Science**

February 2012

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

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Chairman: Prof. Dato' Abdul Rani Bahaman, PhD

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Rats are considered as one of the most important sources of leptospirosis as they are present in abundance in many environments. They caused significant economic losses and served as reservoirs for many zoonotic diseases. One of the diseases is leptospirosis which is considered a re-emerging disease in Malaysia. However, knowledge of the epizootic of leptospirosis and leptospiral serovars associated with rats is lacking. The objectives of the study therefore, were to determine the distribution of rats species and their carrier status. In addition to the determination of the seroprevalence of leptospiral infection in rats, the transmission of leptospiral infection from leptospire-contaminated

water to rats was also determined. In Malaysia, *Rattus tiomanicus* was found to be the dominant species found in Malaysian environments and it constitutes 86.0% (420 out of 488 rats) of the rats caught in different types of localities; National Service Training Camps (Kelantan, Terengganu, Malacca and Selangor), oil palm estates (Terengganu and Malacca), Royal Belum rainforest (Perak), suburban areas (Kelantan and Perak) and PULAPOL (Negeri Sembilan). Sixty leptospiral isolates (12.3%) were successfully cultured from the kidneys of the rats caught. Polymerase chain reaction (PCR) assay revealed forty-two (8.6%) of the isolates were pathogenic as disclosed by the 16S primers. Majority of the pathogenic leptospire were isolated from rats caught in the National Service Training Camps (NSTC). The high rate in NSTC posed a major threat to the trainees as they were frequently involved in outdoor activities and exposed to infected environment.

Microscopic agglutination test (MAT) and enzyme-linked immunosorbent assay (ELISA) were applied in the detection of leptospiral antibodies in the rat sera. Screening of the rat sera by ELISA disclosed a high seroprevalence (78.1%) of leptospiral infection among the captured rats. Higher prevalence of leptospiral IgG antibodies were observed in the NSTC than other selected places. In Malaysia, little information is available regarding the prevalence of leptospiral serovars in the rat population. Results from the MAT showed leptospiral infection due to eight serovars; *icterohaemorrhagiae*, *canicola*, *djasiman*, *bataviae*, *ballum*, *australis*, *pyrogenes* and *hebdomadis*. *Icterohaemorrhagiae* was seen as the dominant serovar detected in the rat sera. The titers ranged from 1:40 to

1:2560. However, the titers were frequently recorded between 1:40 to 1:80. The highest titer (1:2560) observed belonged to serovar *australis*.

Water contaminated with leptospires was an important source for leptospiral infection in rats. Rats that have been exposed for 10 days with leptospire-contaminated water, appeared healthy and having no symptoms related to leptospiral infection. However, antibody to serovar *icterohaemorrhagiae* was detected with titer ranged from 1:80 to 1:320. As demonstrated by silver staining method, leptospires were observed from Day 10 to Day 28 in the liver, spleen and kidneys. Inflammation of the hepatocytes and spleen cells were marked by the presence of neutrophils, macrophages and lymphocytes as shown by Hematoxylin and Eosin staining. Interstitial nephritis was observed in the infected kidneys throughout the experiment. Inflammation of the tubules and glomeruli in the kidneys indicated chronic infection of leptospirosis in the infected rats.

The findings of the present study provided information about the seroprevalence and carrier status of rats for pathogenic leptospires in Peninsular Malaysia and their roles as reservoirs for the organisms. Leptospiral transmission within rat population occurs mainly through contact with leptospires-contaminated environments. Infected rats may become carriers and maintained infection for a long period, thus ensure the continuous circulation of leptospires in nature. Therefore, control of rat population is crucial in minimizing the risk of transmitting leptospirosis to human.

Abstrak tesis yang dikemukakan kepada senat Universiti Putra Malaysia adalah sebagai memenuhi keperluan untuk ijazah Master Sains

**PREVALEN DAN PENYEBARAN JANGKITAN LEPTOSPIRAL PADA TIKUS
DI MALAYSIA**

Oleh

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February 2012

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Tikus dianggap sebagai salah satu sumber yang paling penting kepada penyakit leptospirosis kerana mereka hadir dengan banyaknya di kawasan persekitaran. Tikus menyebabkan kerugian ekonomi yang signifikan dan bertindak sebagai takungan untuk banyak penyakit zoonotik. Salah satu daripadanya ialah leptospirosis yang dianggap sebagai penyakit yang muncul semula di Malaysia. Walau bagaimanapun pengetahuan mengenai epizootik leptospirosis dan serovar *Leptospira* yang berkaitan dengan tikus di Malaysia adalah kurang. Oleh itu, objektif kajian ini adalah untuk menentukan taburan spesies tikus dan status pembawa mereka. Selain daripada penentuan seroprevalen jangkitan *Leptospira* pada tikus, kajian mengenai penyebaran jangkitan leptospirosis

daripada air yang tercemar dengan bakteria *Leptospira* juga telah ditentukan. Di Malaysia, *Rattus tiomanicus* merupakan spesies yang dominan terdapat dalam persekitaran Malaysia dan ia menyumbang sebanyak 86.0 % (420 daripada 488 tikus) tikus yang berjaya ditangkap dalam pelbagai jenis lokaliti; Kem Latihan Khidmat Negara (Kelantan, Terengganu, Melaka dan Selangor), ladang kelapa sawit (Terengganu dan Melaka), hutan hujan Royal Belum (Perak), kawasan pinggir bandar (Kelantan dan Perak) dan PULAPOL (Negeri Sembilan). Enam puluh isolat leptospira (12.3%) telah berjaya diasingkan dari buah pinggang tikus yang ditangkap. Ujian tindakbalas berantai polimer (PCR) mendedahkan 42 (8.6%) daripada isolat yang diasingkan adalah patogen seperti yang dibuktikan oleh pasangan primer 16S. Majoriti patogen *Leptospira* telah diasingkan daripada tikus yang ditangkap di dalam Kem Khidmat Latihan Negara (PLKN). Oleh hal yang demikian, ia mewujudkan ancaman kesihatan kepada pelatih kerana mereka sering terlibat dalam aktiviti luar dan terdedah kepada persekitaran yang tercemar.

Ujian agglutinasia mikroskopik (MAT) dan enzim-immunosorbent (ELISA) telah digunakan untuk mengesan antibodi leptospira dalam darah tikus. Saringan darah tikus oleh ujian ELISA menunjukkan kadar seroprevalen yang tinggi (78.1%) di kalangan tikus-tikus yang ditangkap. Kadar prevalen antibodi IgG leptospiral diperhatikan lebih tinggi di Kem Khidmat Latihan Negara berbanding di tempat-tempat kajian yang lain. Di Malaysia, maklumat mengenai prevalen pelbagai serovar leptospiral dalam populasi tikus adalah terhad. Keputusan daripada ujian MAT menunjukkan jangkitan leptospiral adalah disebabkan oleh lapan serovar; *icterohaemorrhagiae*, *canicola*, *djasiman*, *bataviae*, *ballum*, *australis*, *pyrogenes* dan *hebdomadis*. *Icterohaemorrhagiae*

merupakan serovar dominan yang dikesan di dalam darah tikus dengan titers antibodi dari 1:40 ke 1:2560. Walau bagaimanapun, titer yang kerap direkod adalah di antara 1:40 ke 1:80. Titer tertinggi (1:2560) yang dikesan adalah daripada serovar *australis*.

Air yang tercemar dengan bakteria *Leptospira* merupakan medium terpenting bagi jangkitan leptospiral pada tikus. Tikus yang terdedah dengan air yang disuntik dengan bakteria *Leptospira* selama 10 hari tidak menunjukkan gejala-gejala yang berkaitan dengan jangkitan leptospirosis. Walau bagaimanapun, antibodi untuk serovar *icterohaemorrhagiae* dikesan dengan titer antibodi di antara 1:80 ke 1:320. Seperti yang ditunjukkan oleh kaedah pewarnaan silver, bakteria *Leptospira* dapat diperhatikan dari hari ke-10 hingga hari ke-28 dalam hati, limpa dan buah pinggang. Keradangan hepatosit dan sel-sel limpa yang telah ditandai oleh kehadiran neutrofil, makrofaj dan limfosit seperti yang ditunjukkan oleh pewarnaan Hematoxylin dan Eosin. Keradangan nefron interstitial dapat diperhatikan di dalam buah pinggang yang dijangkiti selama tempoh eksperimen. Keradangan tubul dan glomeruli dalam buah pinggang menunjukkan jangkitan kronik leptospirosis di dalam tikus yang dijangkiti.

Hasil kajian ini dapat menyediakan maklumat mengenai seroprevalen dan status pembawa tikus untuk *Leptospira* jenis patogen di Semenanjung Malaysia dan peranan mereka sebagai takungan untuk bakteria tersebut. Penyebaran leptospirosis di dalam populasi tikus berlaku terutamanya melalui sentuhan dengan persekitaran yang tercemar dengan bakteria *Leptospira*. Tikus yang dijangkiti boleh menjadi pembawa dan jangkitan berlarutan untuk tempoh yang lama, sekali gus memastikan kitaran yang

berterusan bakteria *Leptospira* pada alam semulajadi. Oleh itu, kawalan populasi tikus adalah penting dalam mengurangkan risiko penyebaran leptospirosis kepada manusia.



ACKNOWLEDGEMENTS

First of all, I would like to express my sincere gratitude to Professor Dato' Dr. Abdul Rani Bahaman, who has been my supervisor since the beginning of my study. He provided me with many helpful suggestions, important advice and constant encouragement during the course of this work.

I also wish to express my appreciation to the members of my supervisory committee, Associate Professor Abdul Rahim Mutalib and Associate Professor Dr. Siti Khairani Bejo for their valuable suggestions and constructive advice.

My keen appreciation goes to Fairuz Ridzlan, Encik Rahmat and Mr Supra for their valuable assistance in the field. Without their help, the field work would not have been accomplished in time.

I would like to express special thanks to my family members for supporting and encouraging me to pursue this study. Without their encouragement, this study would not have been completed.

APPROVAL SHEET

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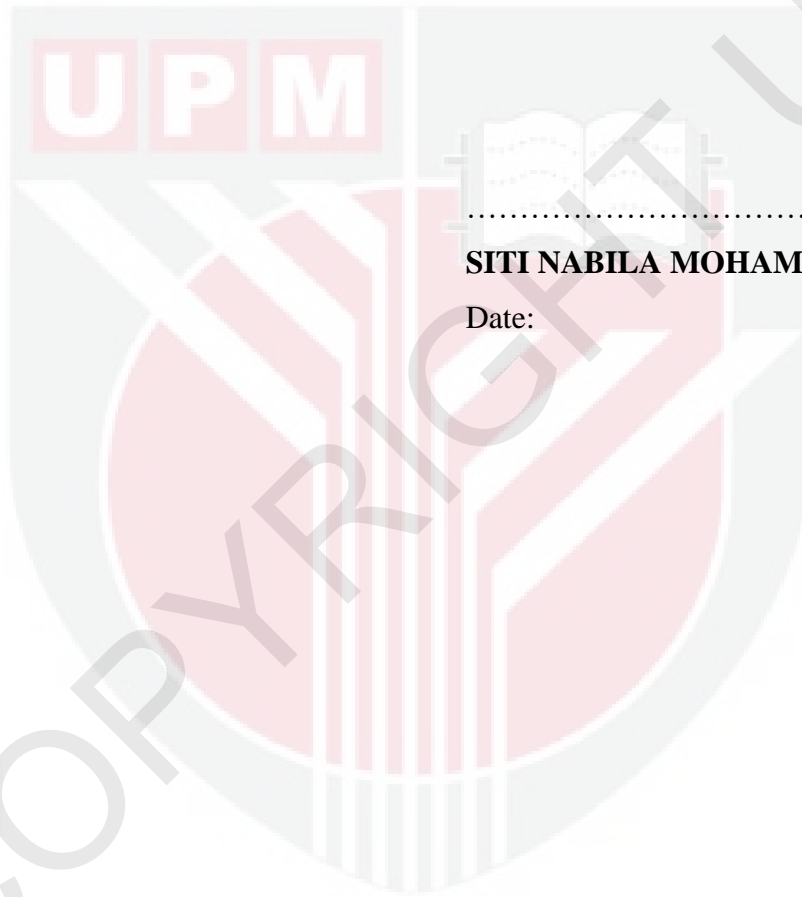
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I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently submitted for any other degree at Universiti Putra Malaysia or at any other institutions.



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LIST OF ABBREVIATIONS

| | |
|-------------------------------|--|
| A.M. | Ante Meridiem |
| bd | twice a day |
| bp | base pair |
| °C | degree Centigrade |
| dH ₂ O | distilled water |
| DNA | deoxyribonucleic acid |
| dNTP | Deoxynucleotide triphosphate |
| ABTS | 2,2-Azino-bis(3-Ethylbenzthiazoline-6-Sulfonic-Acid) Diammonium Salt |
| H ₂ O ₂ | Hydrogen Peroxide |
| H&E | Hematoxylin and Eosin staining |
| IgG | Immunoglobulin G |
| IgM | Immunoglobulin M |
| PBS | Phosphate Buffer Saline |
| PBST | Phosphate Buffer Saline-Tween 20 |
| pH | Puissance hydrogen (Hydrogen-ion concentration) |
| P.M. | Post Meridiem |
| OD | Optical Density |
| EDTA | Ethylenediamine tetraacetic acid |
| EtBr | Ethidium bromide |
| g | Gram |
| H ₂ O | Water |
| HCl | Hydrochloric acid |
| kb | Kilobase pair (number of bases in thousands) |
| M | Molar, or molarity, moles of solute per liter of solution |
| <i>M.</i> | <i>Maxomys</i> |
| MgCl ₂ | Magnesium chloride |
| ml | Mililiter |
| mm | Milimeter |
| mM | Milimolar |

| | |
|---------------|---|
| μg | Microgram |
| μl | Microliter |
| Mol | Mole |
| NaCl | Sodium chloride |
| NaOH | Sodium hydroxide |
| % | Percent |
| <i>R.</i> | <i>Rattus</i> |
| RNA | Ribonucleic acid |
| RNase | Ribonuclease |
| Rpm | revolution per minute |
| SS | Silver Staining |
| Taq | Thermus aquaticus DNA (polymerase) |
| TBE | Tris borate EDTA electrophoresis buffer |
| UV | Ultraviolet |
| V | Volts |



CHAPTER 1

INTRODUCTION

Nearly four millions of rats are born every day in developing nations (Hinds *et al.*, 2003), the increase in rat population poses a health risk to humans and domestic animals as they tend to live in proximity with them. Rats present in abundance in nature and they make up to 42% of the known mammalian species (Singleton *et al.*, 2003). Their role as pest in the developed and developing countries caused significant losses primarily in agricultural and urban environments. Control of rat population with rodenticides and mechanical control devices may help in reducing its population, with benefits not only for economic importance but improvement in public health as well. Rodents from the *Muridae* family have been extensively studied because of their role as reservoirs for many zoonotic diseases. One of the diseases is leptospirosis which is considered an emerging disease in some countries. In Malaysia, leptospirosis is a re-emerging disease as a number of outbreaks were reported recently, however, little is known about the epizootic of leptospirosis and leptospiral serovars associated with them.

Leptospirosis often occurs in tropical regions as the regions provided conditions favorable for the survival of leptospire outside their hosts. Risk of contracting leptospire is higher for people living in proximity with carrier animals especially rats. In Malaysia, the concern about leptospirosis has increased due to several outbreaks

reported recently which were related with exposure to urine-contaminated water. Potential of rats as the main contributor for the human leptospiral cases in Malaysia should be investigated since the country has many species of rats. A study on the diversity of rats in four habitats (forest, urban, rice-field and coastal habitat) in the states of Selangor and Negeri Sembilan has been conducted recently. The outcomes of the study showed that three of the habitats (urban, coastal and rice-field) were dominated with *R. rattus diardi*. However, various species of rats were found to dominate the forest habitats (Paramasvaran *et al.*, 2009). Rats are natural carrier for leptospires, their presence increase chances of getting infection from the environment. Different species of rats can be found in different geographical backgrounds, and they are carrier for many serovars of pathogenic *Leptospira*.

In the old classification, there were two species for genus *Leptospira*: *Leptospira interrogans* and *Leptospira biflexa*. The concern is more toward *Leptospira interrogans* as they comprise all pathogenic strains, and consists of at least 250 antigenically distinct variants known as serovars belonging to 23 serogroups. Identification and classification of leptospiral serovars are crucial because of different host speciation (Natarajaseenivasan *et al.*, 2002). Various leptospiral serovars have been reported to be associated with different species of rats as demonstrated serologically (Priya *et al.*, 2007). Prevalence of leptospirosis in rats poses a health risk to humans as humans are known to be susceptible to infection and may develop symptoms ranging from mild flu-like illness to severe infection with renal and hepatic failure. The epidemiology of leptospirosis of any region is reflected by the serovars carried by the rats in that region,

therefore, a study is needed to determine the seroprevalence of leptospirosis in rats in Malaysia.

Rats are known to be sources for many zoonotic diseases as they are able to transmit infections to humans in many ways. The diseases can be transmitted directly to humans through bite wounds, contact with urine-contaminated environments and can also be spread indirectly by ticks, mites and fleas. Leptospirosis can be acquired by contact with urine-contaminated water. Several outbreaks related with contaminated water have been recorded in human cases (Sejvar *et al.*, 2003; Badrul-Hisham *et al.*, 2009). Rats play an important role in leptospirosis as they serve as reservoirs of the causative agent in nature. Transmission of infection within rat population could be by horizontal and/or vertical transmission (Faine, 1982). Lake, ponds or puddle contaminated with pathogenic leptospires may serve as the source of infection for humans and rats as well if they drink or immerse in that water. Besides human, rats are also susceptible to infection, however, the symptom is mild or inapparent (Easterbrook *et al.*, 2007). Infected rats may develop carrier state as the leptospires reside in the kidneys for a long period and infection to a new host continues as they contaminate the environment with leptospires in their urine. A study on transmission and maintenance of leptospiral infection within rat population is crucial in places normally infested with rats.

Six states in Peninsular Malaysia were selected for their unique ecological settings and different human activities. These six states cover National Service Training Centre

(NSTC), PULAPOL, oil palm estates and forest habitat. The National Service Training Centre (NSTC) or locally known as PLKN training camps are normally situated in rural areas. Human cases in NSTC related with urine-contaminated water have drawn public attention about the safety of the trainees as they might be exposed to the urine-contaminated water through outdoor activities in the training camps. Leptospirosis is also known as occupational-related disease. Two oil palm estates situated in Terengganu and Malacca were selected in this study as various species of rats can be found such as *R. tiomanicus* (Miller), *R. argentiventer* and *R. rattus diardi* (Puan *et al.*, 2011). Apart from causing damage to the plantation through their destructive behavior, the presence of rats poses a health risk to workers. The Royal Belum Rainforest was chosen because it has been subjected to various human activities; military training, hunting, ecotourism and home to the aboriginal people.

The objectives of this present study were:

1. to determine the distribution of rats species in the selected environments and their carrier status for leptospirosis,
2. to determine the seroprevalence of leptospiral infection in rats in the selected environments,
3. to study the transmission of leptospiral infection from leptospire-contaminated water to animals.

Based on the current information, the following hypotheses have been identified:

1. Rats are natural carrier for pathogenic leptospires.
2. Various species of rats are associated with different leptospiral serovars.
3. Rats are susceptible to leptospiral infection and can be infected through contact with leptospires-contaminated water.



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