



UNIVERSITI PUTRA MALAYSIA

**EPIDEMIOLOGY AND DIAGNOSIS OF HUMAN LEPTOSPIROSIS IN
MALAYSIA**

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IB 2000 2

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MALAYSIA**

By

ISAM MOHAMED ALI MOHAMED EL-JALII

**Thesis Submitted in Fulfilment of the Requirements for the Degree of
Doctor of Philosophy in the Institute of Bioscience
Universiti Putra Malaysia**

September 2000



DEDICATION

**TO THE MEMORY OF MY MOTHER, FATHER AND NEPHEW
KHATAB**

TO MY WIFE EMTENAN AND DAUGHTER RAYAN

TO MY BROTHERS AND SISTERS

TO ALL OF THEM WITH LOVE AND GRATITUDE

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

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Chairman: Prof. Dr. Abdul Rani Bahaman

Institute of Bioscience

Retrospective study of human leptospirosis in Malaysia based on microscopic agglutination test (MAT) showed 13% overall prevalence of infection for the period 1983-1998. Results indicated that the prevalence was decreasing in the last five years (1994-1998). The prevalence was highest among Indians (16.67%) followed by Malays (11.48%) and the least among Chinese (5.88%). The 20-29 year-old group showed the highest prevalence of infection (17.13%). Less than 10 year-old group showed the least prevalence of infection (5.66%). Generally, many of the cases occurred between the ages of 20-50 years. Serological survey based on enzyme-linked immunosorbent assay (ELISA) showed a high overall prevalence (12.56%) of leptospiral infection. Kuala Lumpur showed the highest prevalence (19%) whilst Penang recorded the lowest prevalence (6.67%). No significant differences in the prevalence between the other states was noted.

A comparative study of three serological tests, namely enzyme-linked immunosorbent assay (ELISA), microscopic agglutination test (MAT) and indirect hemagglutination (IHA), test was carried out to evaluate these tests in the diagnosis of human leptospirosis. A total of 3000 serum samples from three groups of people were examined. In Group I, IgM and IgG-ELISA were able to detect a number of cases in the first sampling before MAT titres were detectable. In the second sampling, all samples positive for MAT were also positive for IgM-ELISA. IHA test gave positive reactions with only 38% of the samples while all samples were positive for ELISA. In Group II, ELISA detected IgM and IgG to leptospire in the samples which were negative to MAT. These were samples from patients with clinical signs of leptospirosis.

The polymerase chain reaction (PCR) was evaluated as a tool for diagnosis of leptospirosis and differentiation of leptospiral strains. Urine samples with as little as 10 serovar *hardjo* cells per ml of urine were positive on PCR indicating high sensitivity of the test. Detection of small number of leptospiral cells in urine by PCR was an advantage over culture. Random amplification polymorphic DNA (RAPD) fingerprinting was applied to differentiate leptospiral strains. Two primers were tested for their abilities to generate individual RAPD fingerprints. The DNA profiles obtained with each primer were distinct and reproducible. The fingerprint obtained could be useful for distinguishing the serovars up to the strain level. Profiles obtained revealed genetic heterogeneity between serovars belong to one serogroup.

Analysis of leptospiral DNA with restriction enzymes, *Hind* III, *Bam*H I and *Eco*R I revealed a high heterogeneity between the serovars examined. This high heterogeneity may be due to the large genome of the genus *Leptospira*. No relationship was found between the restriction patterns and the species from which the isolate was isolated. Similarities were observed among isolates of the same serovar. The 10 leptospiral field isolates were assayed for presence of plasmid DNA. Only two isolates were found to harbour plasmid DNA. The plasmid profiling obtained is of limited epidemiological value for differentiation of leptospiral isolates.

It appears that human leptospirosis is an endemic infection in Malaysia. The findings showed that ELISA was a suitable serological test for diagnosis of leptospirosis compared to MAT and IHA tests. On the other hand, the application of PCR and REA in the diagnosis of leptospirosis would be useful.

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

EPIDEMIOLOGI DAN DIAGNOSIS LEPTOSPIROSIS DI MALAYSIA

Oleh

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Kajian retrospektif terhadap leptospirosis manusia di Malaysia berasaskan ujian pengaglutinatan mikroskopi (MAT) menunjukkan prevalens jangkitan keseluruhan 13% untuk tempoh 1983 - 1998. Hasil kajian menunjukkan prevalens ini semakin kurang dalam tempoh lima tahun terakhir (1994-1998). Prevalens ini paling tinggi di kalangan kaum India (16.67%) diikuti kaum Melayu (11.48%) dan paling sedikit di kalangan kaum Cina (5.88%). Kumpulan umur 20-29 menunjukkan prevalens jangkitan paling tinggi (17.13%). Kumpulan umur kurang daripada 10 tahun menunjuk prevalens paling rendah (5.66%). Umumnya, banyak daripada kes ini berlaku pada kumpulan umur 20 - 25 tahun. Tinjauan serologi berasaskan asai imunoserap terangkai enzim (ELISA) menunjukkan prevalens jangkitan leptospira keseluruhan tinggi (12.56%). Kuala Lumpur menunjukkan prevalens paling tinggi (19%), manakala Pulau Pinang merekodkan prevalens paling rendah (6.67%). Tiada kelainan tererti wujud di antara negeri lain.

Suatu kajian perbandingan terhadap tiga ujian serologi, iaitu asai immunoerap terangkai enzim (ELISA), ujian pengaglutinatan mikroskopi (MAT), dan ujian penghemaglutinatan tak langsung (IHA) telah dijalankan untuk menilai ujian ini dalam diagnosis leptospirosis manusia. Sejumlah 3000 sampel serum manusia telah diperiksa. Dalam kumpulan I, IgM- dan IgG-ELISA dapat mengesan beberapa kes dalam pensampelan pertama, sebelum titer MAT dapat dikesan. Dalam pensampelan kedua, kesemua sampel yang positif dengan MAT juga positif dengan IgM-ELISA. Ujian IHA memberikan tindak balas positif untuk 38% daripada sampel sahaja, sambil kesemua sampel positif dengan ELISA. Dalam kumpulan II, ELISA mengesan IgM dan IgG terhadap leptospira dalam sampel yang negatif dengan MAT. Ini ialah daripada pesakit yang menunjukkan petanda klinikal untuk leptospira.

Tindak balas berangkai polimerase (PCR) telah dinilai sebagai alat untuk diagnosis leptospirosis dan untuk pembezaan strain leptospira. Sampel urin yang mengandungi hanya 10 *hardjo* sel per ml urin masih positif dalam ujian PCR, menunjukkan tingginya kepekaan ujian tersebut. Pengesanan sejumlah sel yang begitu kecil dalam urin melalui PCR memberi ujian ini kelebihan berbanding pengkulturan. Penyidikjarian DNA polimorfus penguatan rawak (RAPD) telah diguna untuk membezakan strain leptospira. Dua primer telah diuji untuk keupayaannya menjanakan sidikjari RAPD individu. Profil DNA yang diperolehi daripada setiap primer ini adalah nyata dan boleh dihasil semula. Sidikjari yang diperolehi mungkin

berguna untuk membeza serovar sehingga pada aras strain. Profil diperolehi menunjukkan keheterogenan genetik di antara serovar daripada satu serokumpulan.

Analisis DNA leptospira dengan enzim pengehadan, *Hind* III, *BamH* I dan *EcorR* I menunjukkan keheterogenan tinggi di antara serovar yang diperiksa. Heterogenan tinggi ini mungkin disebabkan oleh genom besar pada genus *Leptospira*. Tiada perkaitan yang ditemui di antara pola pengehadan dan spesies daripada mana pencilan itu diperolehi. Persamaan telah dicerap di kalangan pencilan yang serovarnya sama. Pencilan 10 leptospira liar telah diasakan untuk kewujudan DNA plasmid. Hanya dua pencilan telah ditemui mengandungi DNA plasmid. Pemprofilan plasmid yang diperolehi itu mempunyai nilai epidemiologi terhadap dalam pembezaan pencilan leptospira.

Nampaknya leptospirosis manusia itu adalah suatu jangkitan endemik di Malaysia. Penemuan ini menunjukkan ELISA itu merupakan ujian serologi yang sesuai untuk diagnosis leptospirosis berbanding ujian MAT dan IHA. Sebaliknya, penggunaan PCR dan REA dalam diagnosis leptospirosis adalah memuaskan.

ACKNOWLEDGEMENTS

First, my praise to Almighty Allah for giving me the power and will to complete this study.

My deepest gratitude and appreciation to my supervisor, Professor Dr. Abdul Rani Bahaman, Chairman of my Supervisory Committee, for his kind help, guidance, encouragement and invaluable assistance during the course of this study.

I would like to thank and express my deepest appreciation to Dr. Mohd. Azmi Mohd. Lila and Dr. Abdul Rahim Mutalib, members of my Supervisory Committee, for their invaluable advice and comments.

I wish to express my sincere gratitude to the Director of the Institute of Bioscience, former and present Deans of the Faculty of Veterinary Medicine, Universiti Putra Malaysia for allowing me to use the facilities of their laboratories during the course of this study.

I would like to thank Dr. Mangalam Sinniah from Institute for Medical Research (IMR), Kuala Lumpur for providing some of the serum samples. I am also grateful to Dr. Nadzri Salim from Faculty of Veterinary Medicine, Universiti Putra Malaysia for his help and advice.



I am most grateful to L. D. Smythe from WHO/FAO Collaboration Centre of Reference and Research on Leptospirosis, Queensland, Australia and Dr. C. A. Bolin from U. S. Department of Agriculture, USA for providing me with field and reference leptospiral strains used in this study.

I would also like to thank the staff members of Bacteriology Laboratory, Mr. Hajariah Salamah and Mr. Hamzah Adam who gave invaluable technical assistance. At the same time, I am very grateful to Mr. Zaid Othman, M. Munisamy and Omar Mohaideen for providing transportation during sample collection.

I would never forget those good friends and colleagues from whom I received kind assistance and help. I would like to thank Dr. Khairani Bejo, Dr. Abdul Wahid Saeed, Dr. Muthafar Elhaddawi, Dr. Izzeldin Babiker, Dr. Omer Arabi, Puan Zunita Zakaria, Dr. Awad El-Karim Mohd Bakhiet, Waleed Awad, Tongted Phumoonna, Dr. Md. Firoz Main. Dr. Karim EL-Jashamy and those who contributed in the completion of this study.

I am most grateful to the University of Khartoum (Sudan) for financial support. The research was wholly supported by the IRPA Grant (No. 06-02-04-055) obtained from the Ministry of Science, Technology and Environment (Malaysia).



Finally, I wish to express my deepest appreciation to my wife Emtenan, daughter Rayan and brothers Eltaib Saeed and Galal El-Jalii for their moral support and encouragement.

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LIST OF ABBREVIATIONS

Ab	Antibody
ABTS	2,2-azino-bis (3-ethylbenzthiazoline-6-Sulfonic acid)
AP-PCR	Arbitrary primed polymerase chain reaction
Bp	Base pair
BSA	Bovine serum albumin
°C	Degree Celcius
CAAT	Cross agglutination absorption test
CFT	Complement fixation test
CHEF	Contour-clamped homogenous electric field
CSF	Cerebrospinal fluid
D.D	De-ionized distilled
dNTP	Deoxy-nucleotide triphosphate
DFM	Dark-field microscopy
DNA	Deoxyribonucleic acid
Dr.	Doctor
EDTA	Ethylene diamine tetra-acetone
ELISA	Enzyme-linked Immunosorbent assay
FAO	Food and Agriculture Organization
FAT	Fluorescent antibody test
g	Gram
g/l	Gram/litre
G+C	Guanine+Cytisine
H ₂ O ₂	Hydrogen peroxide
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IHA	Indirect haemagglutination
IMR	Institute for Medical Research
JS	Johnson and Seiter
Kbp	Kilobase pairs
K L	Kuala Lumpur
L	Leptospira
LPS	Lipopolysaccharide
M	Molar
MAT	Microscopic agglutination test
Mda	Megadalton
mM	Milimole
M.S	Molecular size
M.W	Molecular weight
N.S	Negri Sembilan
O.D	Optical density
PBS	Phosphate buffer saline
PCR	Polymerase chain reaction

pH	Hydrogen-ion concentration
PLGE	Pulse field gel electrophoresis
PUO	Pyrexia of unknown origin
RAPD	Random amplified polymorphic DNA
REA	Restriction endonuclease analysis
RNA	Ribonucleic acid
rpm	Round per minute
SAT	Slide agglutination test
SDS	Sodium dodecyl sulphate
U	Unit
UK	United Kingdom
UM	Universiti Malaya
UPM	Universiti Putra Malaysia
US	United States
USA	United States of America
UV	Ultraviolet
µg	Microgram
µl	Microlitre
µM	Micro mole
V	Volt
VRI	Veterinary Research Institute
V/V	Volume/volume
WHO	World Health Organization
%	Percent

CHAPTER I

INTRODUCTION

Leptospirosis, also known as “march fever” and “mud fever,” is an important zoonotic disease, with important veterinary and public health impact (Dikken and Kmety, 1978; Gussenhoven *et al.*, 1997; Marcos *et al.*, 1997). Leptospirosis is caused by *Leptospira interrogans*. Based on immunological tests, more than 200 leptospiral serovars have been identified. The serovars could be placed into 23 serogroups (Soltys, 1979; Woodward *et al.*, 1997; Chu *et al.*, 1998). In Malaysia, 38 serovars from 13 serogroups have been known to occur (Bahaman, 1988).

In livestock, the disease causes important economic losses. Although this disease is usually mild and often subclinical, it can lead to great losses due to abortions, stillbirths, infertility, mastitis, weak progeny, decreased milk production, and with certain leptospiral serovars, death (Songer *et al.*, 1983; Thiermann, 1984; Bey and Johnson, 1986).

Leptospiral serovars can infect mammals including man. However, the pathogenicity and clinical manifestations of the disease depend on the animal