UNIVERSITI PUTRA MALAYSIA

PROTEIN PROFILE AND ANTIGENICITY OF REPRESENTATIVE LEPTOSPIRAL SEROVARS REPORTED IN MALAYSIA AND EXPERIMENTAL *LEPTOSPIRA INTERROGANS* INFECTION IN DOGS

CHENG KIM SING

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By

CHENG KIM SING

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Chairman: Professor Abdul Rani Bahaman, PhD

Faculty: Veterinary Medicine

Wide distribution of leptospires in the world has caused tremendous economic losses in agricultural sector due to decrease in animal production, quality of animal products and increased cost of treatments and also one of the public health concerns as this zoonosis has caused fatalities in human beings.

In the study, immunoprobing experiments with rabbit antisera against serovars *canicola, icterohaemorrhagiae, hardjobovis, pomona* and *australis*, band with molecular weight 137.6 kDa is unique in serovar *canicola* 35 kDa in *icterohaemorrhagiae*, 10.5 kDa and 71.4 kDa in *hardjobovis*, 48.1 kDa in *pomona* while 20.9 kDa and 25.0kDa in *australis*. These distinct bands could

have explained the selectiveness of different serovars on the target hosts, organs or perhaps tissues. In the experiment, *Leptospira interrogans* serovar *canicola* caused interstitial nephritis while serovar *icterohaemorrhagiae* caused liver damage in local stray dogs.

Two dimensional SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE) on both serovars *canicola* and *icterohaemorrhagiae* have revealed differences in the protein distributions. Three different protein spots sharing the same molecular weights at 42.1kDa, 126kDa and 136 kDa while at 60.9kDa, 65.2kDa and 89.6kDa, two proteins spots sharing the same molecular weight were detected in serovar *canicola*. Serovar *icterohaemorrhagiae* on the other hand has three protein spots at 31kDa, 36kDa and 45kDa while 5 protein spots at 32 kDa were detected.

Antibody titres peaked between 6 to 11 post inoculation day (P.I.D) with the highest titre at 1:1,600 in dogs infected with *Leptospira canicola* through intravenous route (Group 1). While dogs infected with serovar *icterohaemorrhagiae* through intravenous route had peak antibody titre between 9 to 11 (Group 2) P.I.D, with the highest titre at 1:1,600. Dogs infected with serovar *icterohaemorrhagiae* through oculonasal route (Group 3), the peak titre of antibody production was much delayed, at between 19 to 23 P.I.D but with much higher than the two previous tests (Group 1 and Group 2) at the highest titre reached was 1:3,200. The study also shows that

dogs once infected with leptospiral serovar, especially through oculonasal route, would shed the organism in its urine for a long period of time up till the end of study (nine months). This reflects the oculonasal route to be the actual route which dogs are most likely to be infected by leptospires in natural environment.

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

PROFIL PROTEIN DAN ANTIGENIK TERHADAP SEROVAR-SEROVAR *LEPTOSPIRA* CONTOH YANG DILAPORKAN DI MALAYSIA DAN JANGKITAN UJIKAJI *LEPTOSPIRA INTERROGANS* PADA ANJING

Oleh

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Kadar taburan kuman leptospira yang tinggi di dunia ini telah mengakibatkan kerugian-kerugian yang amat tinggi di dalam sektor pertanian, kerana menyebabkan pengeluaran hasil yang semakin rendah, mutu kualiti yang semakin merosot dan kos rawatan yang tinggi serta ia juga merupakan salah satu kebimbangan kesihatan umum kerana berpotensi untuk menyebabkan kematian di kalangan manusia.

Di dalam kajian ini, pengesanan immuno dengan antisera-antisera arnab terhadap serovar-serovar *canicola, icterohaemorrhagiae, hardjobovis, pomona* serta *australis* telah dikaji dan didapati jalur protein dengan berat molekul 137.6 sangat unik bagi serovar *canicola*, 35 kDa bagi *icterohaemorrhagiae*, 10.5 kDa dan 71.4 kDa dalam *hardjo –bovis*, 48.1 kDa

dalam *pomona* dan akhir sekali 20.9 kDa serta 25.0 kDa dalam *australis.* Jalur-jalur yang nyata ini mungkin boleh menjelaskan tentang cirri-ciri pemilihan terhadap sasaran pembawa-pembawa, organ-organ serta tisu-tisu tertentu oleh pelbagai serovar. Di dalam kajian ini juga, didapati serovar canicola telah menyebabkan berlakunya nefritis interstisial (interstitial nephritis), manakala serovar *icterohaemorrhagiae* pula menyebabkan berlakunya kerosakan hati pada anjing-anjing liar yang dijangkitkan dengan kuman-kuman leptospira.

Elektroforesis gel SDS Poliakrilamida (SDS-PAGE) dua dimensi yang telah dijalankan terhadap kedua-dua serovar *canicola* dan *icterohaemorrhagiae* telah menunjukkan beberapa perbezaan dari segi taburan protin-protin. Tiga tompok protin yang berbeza tetapi mempunyai berat molekul yang sama telah dikesan pada berat molekul 42.1 kDa, 126 kDa dan 136 kDa manakala pada berat molekul 60.9 kDa, 65.2 kDa dan 89.6 kDa, dua tompok protin telah dikesan pada serovar *canicola*. Serovar *icterohaemorrhagiae* pula mempunyai tiga tompok protin pada kedudukan berat molekul pada 31 kDa, 36 kDa dan 45 kDa, manakala dikedudukan berat molekul 32 kDa, lima tompok protin dikesan.

Kemuncak antibodi telah dicapai dalam lingkungan enam hingga sebelas hari pasca-inokulasi dengan titer maksima pada 1:1,600 dalam anjing-anjing yang dijangkitkan dengan kuman *Leptospira canicola* melalui injeksi

intravena (Kumpulan 1). Manakala, anjing-anjing (Kumpulan 2) yang dijangkitkan dengan kuman *Leptospira icterohaemorrhagiae* melalui suntikan intravena, mencapai puncak titer antibodi pada hari sembilan hingga sebelas hari pasca-inokulasi dengan titer maksima pada 1:1,600. Anjing-anjing dalam Kumpulan 3 juga dijangkitkan dengan kuman *Leptospira icterohaemorrhagiae* tetapi melalui laluan okulonasal, mencapai puncak pengeluaran antibodi yang lebih lewat, iaitu pada hari 19 hingga 23 pasca-inokulasi tetapi mempunyai titer yang lebih tinggi berbanding dengan kumpulan-kumpulan yang terdahulu (Kumpulan 1 dan Kumpulan 2). Titer yang tertinggi dicapai ialah 1:3,200. Kajian ini juga menunjukkan bahawa sekiranya anjing yang telah dijangkiti kuman leptospira, terutamanya melalui laluan okulonasal, akan menyebarkan kuman ini melalui air kencingnya untuk suatu masa yang lama sehingga tamat tempoh kajian (sembilan bulan). Perkara ini menunjukkan bahawa laluan okulonasal adalah besar kemungkinan merupakan laluan yang sebenarnya apabila berlaku sesuatu jangkitan kuman leptospira pada anjing-anjing di dalam alam-sekitar.

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This thesis 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 are as follow:

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DECLARATION

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

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CHENG KIM SING

Date: 18th July 2007

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LIST OF ABBREVIATIONS

%	percentage
x g	gravity
°C	Degree Celcius
2D PAGE	Two-Dimensional-Polyacrylamide Gel Electrophoreis
5-FU	5-Fluorouracil
µg	microgramme
µl	microliter
µm	micrometer
bp	base pair
BRENDA	Bacterial Restriction Endonuclease
BSA	Bovine Serum Albumin
cm	centimeter
CSF	Fluid
DBKL	Dewan Bandaraya Kuala Lumpur
ddH_2O	double distilled water
DNA	Deoxyribonucleic acid
DTT	Dithiothreitol
EDTA	ethylenediamine tetraacetic acid
ELISA	Enzyme-linked Immunosorbent Assay
H & E	haematoxylin and eosin
HCl	hydrochloric acid

HIS	hyperimmune serum
IEF	isoelectric focusing
IPG	immobilized pH gradient
JS	Johnson and Seiter
KCl	kalium chloride
kDa	kilo Dalton
L. spp.	*Leptospira* spp.
LipL	Lipoprotein
LPS	Lipopolysaccharide
M	Molar
mA	milliampere
MAT	microscopic agglutination test
min	minute
ml	milliliter
mm	millimeter
mM	millimolar
N.C	nitrocellulose
NaCl	Natrium chloride
nm	nanometer
OMP	outer membrane protein
P	pico
P.I.D	post inoculation day
PBS	phosphate buffered saline

PBS-T	phosphate buffered saline with Tween
PCR	polymerase chain reaction
PFGE	pulsed field gel electrophoresis
pH	hydrogen ion exponent
PVDF	polyvinylidene difluoride
rpm	revolution per minute
SDS-PAGE	Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoreis
spp.	species
T.B	transfer buffer
TBE	Tris-borate-EDTA electrophoresis buffer
TEMED	N,N,N',N'-tetramethylethylenediamine
TMB	3,3',5,5'-tetramethylbenzidine
Tris-HCl	Tris (hydroxymethyl) aminomethane hydrochloride
U.K.	United Kingdom
v/v	volume per volume
W.H.O.	World Health Organization
w/v	weight per volume