



UNIVERSITI PUTRA MALAYSIA

***SEROPREVALENCE AND MOLECULAR DETECTION OF
LEPTOSPIROSIS AMONG WORKING DOGS POPULATION
IN MALAYSIA***

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FPV 2016 101

**SEROPREVALENCE AND MOLECULAR DETECTION OF
LEPTOSPIROSIS AMONG WORKING DOGS POPULATION
IN MALAYSIA**

Wong Jia Yun

**A project paper submitted to the
Faculty of Veterinary Medicine, University Putra Malaysia in
partial fulfillment of the requirement for the**

DEGREE OF DOCTOR OF VETERINARY MEDICINE

University Putra Malaysia Serdang, Selangor Darul Ehsan

MARCH 2015

II

It is hereby certified that we have read this project paper entitled “Seroprevalence and Molecular Detection of Leptospirosis among Working Dogs Population in Malaysia” by Wong Jia Yun and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfillment of the requirement for the course VPD 4999 – Final Year Project

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ACKNOWLEDGEMENTS

Sincere thanks to those who have contributed time, effort, and aid in the completion of this project paper:

Dr. Lau Seng Fong

Dr. Khor Kuan Hua

Dr. Rozanaliza Radzi

Dr. Mohammad Sabri Abdul Rahman

Mr. Mohd Azri Roslan & Bacteriology Laboratory Staff

Officer Handlers of The Canine Units

Also million thanks to those who have helped and encourage me during the completion of this project paper:

Dearest Family Members and Friends

Beloved DVM Classmates of Batch 2011/2016

CONTENTS

	PAGE
TITLE	I
CERTIFICATION	II
ACKNOWLEDGEMENTS	IV
CONTENTS	V
LIST OF TABLES	VII
LIST OF FIGURES	VIII
LIST OF ABBREVIATIONS	IX
ABSTRACT	X
ABSTRAK	XII
1.0 INTRODUCTION	1
2.0 LITERATURE REVIEW	
2.1 Epidemiology of Leptospirosis.....	6
2.2 Clinical Features of Canine Leptospirosis.....	8
2.3 Seroprevalence of Canine Leptospirosis.....	10
2.4 Risk Factors of Canine Leptospirosis.....	15
2.5 Leptospirosis among Working Dogs.....	16
2.6 Screening and Diagnosis of Canine Leptospirosis.....	17

3.0 MATERIALS AND METHODS

3.1 Sample Collection.....	22
3.2 Transportation and Storage of Samples.....	22
3.3 Microscopic Agglutination Test (MAT).....	23
3.4 DNA Extraction and Polymerase Chain Reaction (PCR) Assay..	25
3.5 Agarose Gel Preparation and Electrophoresis.....	27
3.6 Statistical Analysis.....	28

4.0 RESULTS

4.1 Microscopic Agglutination Test (MAT) Results.....	31
4.2 Polymerase Chain Reaction (PCR) Assay Results.....	33

5.0 DISCUSSION

5.1 Difference of Findings.....	34
5.2 Epidemiology of Canine Leptospirosis.....	39

6.0 CONCLUSION..... 41**7.0 RECOMMENDATIONS.....** 41**8.0 REFERENCES.....** 42**9.0 APPENDICES.....** 54

LIST OF TABLES	PAGE
Table 1: Document of Outbreaks of Leptospirosis in People Associated with Exposure to Dogs.....	3
Table 2: Seroprevalence of Canine Leptospirosis Reported in Different Countries.....	15
Table 3: <i>Leptospira</i> Antibody Titres Used in Microscopic Agglutination Test (MAT) for The Working Dogs Sera Sample.....	31

LIST OF FIGURES	PAGE
Figure 1: Illustrative Diagram of Respectives Microtitre Plate Wells Prepared in Microscopic Agglutination Test (MAT).....	24
Figure 2: DNA Purification from Blood Sample with Spin Protocol.....	26
Figure 3: The Percentage of Male and Female of Selected Working Dogs.....	29
Figure 4: The Percentage of Young, Middle-Age and Old Dogs of Selected Working Dogs.....	30
Figure 5: Seroprevalence of Leptospirosis among Working Dogs for 11 <i>Leptospira</i> Serovars using Microscopic Agglutination Test (MAT) with Cut-Off Antibody Titre of 1:80.....	32
Figure 6: Molecular Detection of Leptospirosis for Working Dogs Blood Samples using Polymerase Chain Reaction (PCR) Assay.....	33

LIST OF ABBREVIATIONS

bp	base pairs
CF	Complement Fixation Test
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked Immunosorbent Assay
FRET	Fluorescence Resonance Energy Transfer
IFA	Indirect Fluorescent Antibody
Ig	Immunoglobulin
IHA	Indirect Hemagglutination Assay
LFA	Lateral Flow Assay
MAT	Microscopic Agglutination Test
mL	milliliter
mm	millimeter
MSAT	Macroscopic Slide Agglutination Test
°C	degree Celsius
PBS	Phosphate Buffer Saline
PCR	Polymerase Chain Reaction
RSAT	Rapid Slide Agglutination Test
TBE	Tris-Borate-Ethylenediaminetetraacetic acid
UPM	University Putra Malaysia
μL	microliter

ABSTRACT

**An abstract of the project paper presented to the Faculty of Veterinary Medicine
in partial fulfillment of the Course VPD 4999- Final Year Project**

**SEROPREVALENCE AND MOLECULAR DETECTION OF LEPTOSPIROSIS
AMONG WORKING DOGS POPULATION**

IN MALAYSIA

By

Wong Jia Yun

2016

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XI

The aim of this study was to determine seroprevalence of leptospirosis among the working dogs population in Malaysia. Blood samples were obtained from five representative canine units, namely, Royal Malaysia Police, FELDA Security Police, Royal Malaysian Custom Department, Fire and Rescue Department and Prison Department in Malaysia. In total, 96 blood samples were collected. All the working dogs were clinically healthy during blood collection. Serum samples collected were examined by using Microscopic Agglutination Test (MAT) against 11 *Leptospira* serovars (icterohaemorrhagiae, canicola, Pomona, grippityphosa, australis, bataviae, javanica, tarassovi, hebdomadis, lai and pyrogene) to investigate the seroprevalence of canine leptospirosis. Whole blood samples collected were examined by using Polymerase Chain Reaction (PCR) assay. Primer targeting the 531 bp pathogenic-specific and 331 bp genus-specific *Leptospira* genes was used. Out of 96 sera samples, three sera samples (3.1%) were tested positive for *Leptospira javanica*, *L. australis* and *L. bataviae* based on MAT at the antibody titre cut-off point at 1:80. There was no detection of leptospire nucleic acid (DNA) for all samples in PCR. Hence those three dogs tested positively by using MAT might serve as carriers for leptospiral. Working environment of the working dogs was believed as a source of leptospiral infection. Despite annual vaccination was performed, the working dogs are still risk of ongoing exposure to leptospire. Further investigation of seroprevalence in other group of dogs is of crucial importance in order to investigate the most common serovars presence in the dogs in Malaysia.

Keywords: Canine Leptospirosis, Seroprevalence, Working dogs, Microscopic Agglutination Test, Polymerase Chain Reaction

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999-

Projek Ilmiah Tahun Akhir

KELAZIMAN SECARA SEROLOGI DAN PENGESANAN SECARA MOLEKULAR TERHADAP JANGKITAN LEPTOSPIROSIS DALAM KALANGAN POPULASI ANJING KERJA DI MALAYSIA

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Matlamat kajian ini ialah untuk menentukan kelaziman secara serologi dan pengesanan secara molekular terhadap jangkitan Leptospirosis dalam kalangan populasi anjing kerja ($n=96$) di Malaysia. Sembilan puluh enam sampel darah anjing telah diperolehi dari

XIII

lima unit anjing iaitu Polis Diraja Malaysia, Polis Bantuan FELDA, Jabatan Kastam Diraja Malaysia, Balai Bomba Dan Penyelamat dan Ibu Pejabat Penjara Malaysia. Semua anjing adalah sihat semasa proses pengambilan sampel darah. Sampel serum yang diperolehi digunakan untuk ujian serologi Microscopic Agglutination Test (MAT) terhadap 11 *Leptospira* serovar (icterohaemorrhagiae, canicola, pomona, grippityphosa, australis, bataviae, javanica, tarassovi, hebdomadis, lai and pyrogene). Sampel darah penuh digunakan untuk ujian Polymerase Chain Reaction (PCR) assay dengan menggunakan primer mensasarkan 531 bp patogenik-spesifik gen dan 331 bp genus-spesifik gen. Tiga daripada 96 ekor anjing kerja (3.1%) telah diuji positif terhadap *Leptospira javanica*, *L. australis* dan *L. bataviae* berdasarkan ujian serologi MAT pada titer 1:80. Semua sampel darah tidak mengesan sebarang kehadiran asid nukleik (DNA) *Leptospira* dengan kaedah PCR. Oleh itu, tiga ekor anjing kerja telah diuji positif dan dianggap sebagai pembawa penyakit *Leptospira*. Persekitaran terlibat dalam anjing kerja dikenalpasti sebagai punca jangkitan *Leptospira* kepada anjing-anjing kerja ini. Walaupun suntikan imunisasi tahunan *Leptospira* dijalankan di kalangan anjing kerja ini, tetapi anjing kerja ini masih mempunyai risiko terhadap jangkitan *Leptospira*. Oleh itu siasatan untuk kelaziman secara serologi dalam kalangan anjing populasi lain mestilah digalakkan untuk mengkaji *Leptospira* serovar dalam kalangan anjing di Malaysia.

Kata kunci: Leptospirosis, serologi, anjing kerja, Microscopic Agglutination Test, Polymerase Chain Reaction

1.0 INTRODUCTION

Leptospirosis is an emerging or re-emerging infectious disease and presumed as a most widespread zoonotic disease in the world (Roqueplo *et al.*, 2014). It is commonly occurring in tropical and subtropical regions with relating to the climatic and environmental condition (Bharti *et al.*, 2003; WHO, 2003; Rafizah *et al.*, 2013). It is caused by spirochetes of the genus *Leptospira*, which belong to the family Leptospiraceae, order Spirochaetales, which is characterized as highly motile, thin, flexible, filamentous and made up of fine spirals with hook-shaped ends (Adler *et al.*, 2009; Goldstein, 2010).

Leptospirosis is known as Weil's disease in human medicine and was first identified in Japan (Faine *et al.*, 1999; Rad *et al.*, 2004). According to Skouloudis (2015), it became a major public health problem in the tropical developing world due influencing factor environmental conditions such as natural disaster. Increased number of reported incident cases of human leptospirosis was noted with occurrences of natural disaster and flooding with notable outbreaks in places as follow, Nicaragua (1995), Peru and Ecuador (1998), Orrissa (1998), Malaysia (2000), Jakarta (2002), India (2000 and 2005), Sri Lanka (2008) and Philippines (2009) (Skouloudis *et al.*, 2015). However, the outbreak of the disease is not fully understood since it is a complex and dynamic bacterial disease with multitude of pathogenic serovars, numerous host and multiple modes of transmission.

The first case of human leptospirosis in Malaysia was discovered by Fletcher in 1925. In Malaysia, the risk factor of this infection was presumed due to the exposure to water and soil contamination with urine of rats and high incidences of leptospirosis especially after flood has been reported (Bahaman *et al.*, 1988). The most recent study revealed that the seroprevalence of human leptospirosis in Malaysia was 9.77% among 829 samples with using diagnostic test of Microscopic Agglutination Test (MAT) as screening (Samsi *et al.*, 2013).

According to Fletcher (1928), first canine leptospirosis in Malaysia was reported with serovar hebdomadis isolated in 1928. Two serovars were believed the main causes of canine leptospirosis throughout the world, which are; serovar icterohaemorrhagiae and serovar canicola (Michna, 1970; Sullivan, 1974). In recent years, there had been an increased numbers of clinical cases of canine leptospirosis associated with serovar grippityphosa, pomona and australis. (Klaasen *et al.*, 2013). In Malaysia, thirty-seven *Leptospira* serovars had been isolated from both animals and humans (Bahaman *et al.*, 1988). High seroprevalence of leptospirosis among dog population may contribute to public health issue due to the close relationship between reservoir dog and susceptible human (i.e. pet-owners and occupation such as veterinarian, animal handlers and dog trainers. A preliminary study of seroprevalence antibodies against *Leptospira* serovars in United State veterinarian conducted reported that 13 out of 511 veterinarians (2.5%) were association with unintentional needle stick injuries, animal bites and scratches (Whitney *et al.*, 2009). Levett (2001) and Greene

(2013) had shown that dogs are known as significant reservoir to infect human. Table 1 showed that dog act as a source of leptospirosis outbreak in many tropical countries.

Place and Year	No. of Human Leptospirosis Cases	Source of infection	Persumptive serogroup	Infecting serovar isolated	Reference
North Dakota, 1950	9	Infected family pet dog	Canicola	Not isolated	Haunz & Cardy, 1952
Texas, 1971	7	Infected pet dog	Canicola	Canicola	Barkin & Glosser, 1973
Portland, Oregon, 1972	9	Infected family pet dog	Autumnalis	Fortbragg	Fraser <i>et al.</i> , 1973
St. Louis, Missouri, 1972	5	Infected pet dog, previously immunised	Icterohaemorrhagiae	Icterohaemorrhagiae	Feigin <i>et al.</i> , 1973
Barbados, 1988	1	Infected guard dogs in kennels, immunized	Autumnalis	Bim	Everard <i>et al.</i> , 1987

Georgia, 1952	26	Swimming in creek; dogs suspected	Canicola	Not isolated	Zimmer, <i>et al.</i> , 2000
Japan, 1953	114	Swimming in river; dogs suspected	Canicola	Canicola	Misao <i>et al.</i> , 1956
Morán Cuba, 1986	6	Swimming in creek; dog suspected	Canicola	Not isolated	Hernandez <i>et</i> <i>al.</i> , 2000
Madras city, India, 1992	9	Infected pet dog, urine contaminated in flooding	Canicola	Canicola	Venkataraman & Nedunchelliya n, 1992
Ncaragua, 1995	100	Flooding, walking in creek; dogs suspected	Canicola	Portlandvere	Trevejo <i>et al.</i> , 1998; Zuerner <i>et al.</i> , 1997

Table 1: Document of Outbreaks of Leptospirosis in People Associated with Exposure to Dogs.

In Malaysia, the most recent study on the seroprevalence of canine leptospirosis in pet dogs from selected area of Klang Valley was 7% ($n = 57$) (Lau *et al.*, 2016). Another study in a selected dog shelter in Selangor reported a seroprevalence of 3.8% ($n = 80$) (Khor *et al.*, 2016). Seroprevalence of canine leptospirosis has been carried out in many countries, however, there are limited study conducted in Malaysia especially working dogs population.

Therefore, the result of this preliminary study can be used to suggest a general overview of the current status of canine leptospirosis in working dog population in Malaysia. Besides that it will provide veterinarian on the estimation of the spatial distribution and trend of this infectious disease among working dogs population and may raise public health concern for officer handler. The objectives of this study were:

1. To investigate the prevalence of canine leptospirosis among working dogs population in Malaysia.
2. To determine the most common *Leptospira* serovars in the working dogs population.

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