



**DETECTION, CHARACTERISATION AND RISK FACTOR OF *Leptospira* spp.  
AMONG DOGS AND DOG HANDLERS**

By

**GOH SOON HENG**

**Thesis Submitted to School of Graduate Studies, Universiti Putra  
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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

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**October 2022**

**Chairman : Khor Kuan Hua, PhD**  
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Leptospirosis is a re-emerging zoonosis commonly associated with dogs. However, serological diagnosis remains a challenge, especially among vaccinated dogs. Dogs pose a zoonotic threat potential to at-risk dog handlers, yet, there is limited knowledge regarding the potential risk factors and awareness levels among them. Therefore, this study determined; i) the post-vaccination anti-leptospiral antibody titres in healthy pet dogs and puppies, ii) the susceptibility of urban stray dogs in shedding *Leptospira* spp., iii) the seroprevalence and risk factors of leptospiral infection among dogs and dog handlers, and iv) the knowledge, attitude, and practice (KAP) towards leptospirosis among dog handlers. The median antibody titre in puppies was 1:100 (range: 1:50-1:200) with a median duration of 4 weeks (range: 2 weeks-9 months). For adult dogs the median antibody titre was 1:200 (range: 1:50-1:800) with a median duration of 6 months (range: 6 weeks-1 year). Icterohaemorrhagiae titres was the highest with the longest duration followed by Canicola, Grippotyphosa and Pomona, with no cross-reaction detected throughout the sampling period for both groups.

Among the urban stray dogs, 32.0% (n=32/100) was seropositive predominantly for serovars Javanica, Bataviae, Icterohaemorrhagiae, Autumnalis, Canicola, Pyrogenes, Copenhageni, and Australis (titres ranged: 1:100-1:800). Six *Leptospira* spp. isolates were procured from urine, kidney and liver samples of four dogs. Isolation and identification revealed the isolates were pathogenic *L. interrogans* serovar Bataviae (n=5) and Canicola (n=1). Among the working and shelter dogs, 26.3% (n=70/266) were seropositive predominantly for serovars Icterohaemorrhagiae, Ballum, Bataviae and Javanica (titres ranged: 1:100–1:800). The potential dog risk factors identified were presence of rats [OR = 4.61 (95% CI: 1.05, 20.33), p = 0.043] and shared common area within the organisation [OR = 5.12 (95% CI: 1.94, 13.46), p = 0.001]. As for the dog handlers, 34.5%

(n=67/194) were seropositive predominantly for serovars Grippityphosa, Icterohaemorrhagiae and Malaysia (titres ranged: 1:50–1:200).

Besides the known zoonotic risk from exposure to rats and a contaminated environment from, increased contact time with the dogs [OR = 3.28 (95% CI: 1.28, 8.40), p = 0.013] was identified as another important risk factor after controlling the other factors, which highlighted the potential transmission from dog to human. Awareness is important but unfortunately, most working dog handlers (58%, n=58/100) and shelter dog handlers (90%, n=34/38) lacked knowledge. Attitude towards leptospirosis was also poor among most working dog handlers (75%, n=75/100) and all shelter dog handlers. The working dog handlers (77%, n=77/100) had good practices while shelter dog handlers (47%, n=18/38), had moderate level. The poor knowledge was likely due to the lower education level. Lack of knowledge in terms of clinical sign of the disease will be a challenge for disease management. Despite the limited knowledge, they still know the importance of preventive measures such as vaccination in disease prevention.

In conclusion, MAT remains a gold standard test for serological diagnosis and surveillance, however, detailed considerations should be taken when interpreting results of clinical cases with the presence of anti-leptospiral antibodies, as paired serum for better interpretation was required. Although the absence of circulating environmental serovars among urban stray dogs, nonetheless the zoonotic potential remains as they can still be infected or carriers of other pathogenic serovars and disseminate the disease via urine shedding. The diverse occupational responsibilities may predispose both dogs and their handlers towards exposure to both *Leptospira* spp. contaminated environments and infected/reservoir animals. Frequent close contact with their potentially infected dogs adds additional occupational risk for dog handlers. Such risk could be mitigated with proper protection when dog handling during operations. Promoting awareness through knowledge enhancements could limit the risk of disease transmission among animals and humans.

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

**PENGESANAN, PERINCIAN DAN FAKTOR RISIKO *Leptospira* spp. DALAM  
KALANGAN ANJING DAN PENGENDALI DOG**

Oleh

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Leptospirosis merupakan zoonosis yang biasanya dikaitkan dengan anjing. Namun, diagnosis serologi tetap menjadi cabaran, terutamanya di kalangan anjing yang divaksinasi. Anjing merupakan ancaman zoonosis berpotensi bagi pengendali anjing yang berisiko, namun pengetahuan masih terbatas mengenai faktor risiko berpotensi dan tahap kesedaran dalam kalangan mereka. Oleh kerana itu, kajian ini menentukan; i) titer antibodi anti-leptospira pasca vaksinasi pada anjing dewasa dan anak anjing yang sihat, ii) keterentanan anjing liar urban dalam peluruhan *Leptospira* spp., iii) prevalens serologik dan faktor risiko jangkitan leptospira pada anjing dan pengendali anjing, dan iv) pengetahuan, sikap, dan amalan (PSA) terhadap leptospirosis di kalangan pengendali anjing. Titer antibodi median pada anak anjing adalah 1:100 (julat: 1:50-1:200) dengan durasi median 4 minggu (julat: 2 minggu-9 bulan). Untuk anjing dewasa, titer antibodi median adalah 1:200 (julat: 1:50-1:800) dengan durasi median 6 bulan (julat: 6 minggu-1 tahun). Titer Icterohaemorrhagiae adalah yang tertinggi dengan tempoh terpanjang diikuti oleh Canicola, Grippytyphosa, dan Pomona, tanpa adanya tindak balas silang yang dikesan sepanjang tempoh pengambilan sampel untuk kedua-dua kelompok.

Di kalangan anjing liar urban, 32.0% (n=32/100) adalah seropositif terutamanya untuk serovar Javanica, Bataviae, Icterohaemorrhagiae, Autumnalis, Canicola, Pyrogenes, Copenhageni, dan Australis (tajukannya berkisar antara: 1:100-1:800). Enam isolat *Leptospira* spp. diperoleh daripada sampel air kencing, buah pinggang, dan hati empat ekor anjing. Pemencilan dan pengenalpastian menunjukkan isolat tersebut adalah patogen *L. interrogans* serovar Bataviae (n=5) dan Canicola (n=1). Di kalangan anjing pekerja dan anjing pusat perlindungan, 26.3% (n=70/266) adalah seropositif terutamanya untuk serovar Icterohaemorrhagiae, Ballum, Bataviae, dan Javanica (julat titer: 1:100-1:800). Faktor risiko anjing yang berpotensi dikenal pasti adalah kehadiran tikus [OR =

4.61 (95% CI: 1.05, 20.33),  $p = 0.043$ ] dan perkongisan kawasan bersama dalam organisasi [OR = 5.12 (95% CI: 1.94, 13.46),  $p = 0.001$ ]. Untuk pengendali anjing, 34.5% ( $n=67/194$ ) adalah seropositif terutamanya untuk serovar Grippotyphosa, Icterohaemorrhagiae, dan Malaysia (julat titer: 1:50-1:200).

Selain risiko zoonotik yang diketahui dari pendedahan kepada tikus dan persekitaran tercemar, peningkatan masa sentuhan dengan anjing [OR = 3.28 (95% CI: 1.28, 8.40),  $p = 0.013$ ] dikenal pasti sebagai faktor risiko penting yang lain setelah mengawal untuk faktor lain, yang menekankan potensi penularan dari anjing kepada manusia. Kesedaran adalah penting namun kebanyakan pengendali anjing pekerja (58%,  $n = 58/100$ ) dan pengendali anjing pusat pelindungan (90%,  $n = 34/38$ ) mempunyai kekurangan pengetahuan. Sikap terhadap leptospirosis juga lemah di kalangan kebanyakan pengendali anjing pekerja (75%,  $n = 75/100$ ) dan semua pengendali anjing tempat pelindungan. Pengendali anjing pekerja (77%,  $n = 77/100$ ) mempunyai amalan yang baik manakala pengendali anjing tempat pelindungan (47%,  $n = 18/38$ ) mempunyai tahap sederhana. Kurangnya pengetahuan mungkin disebabkan oleh tahap pendidikan yang lebih rendah. Kurangnya pengetahuan dari serig tanda klinikal penyakit akan menjadi satu cabaran dalam pengurusan penyakit. Walaupun pengetahuan terhad, mereka masih tahu kepentingan langkah-langkah pencegahan seperti vaksinasi dalam pencegahan penyakit.

Secara kesimpulannya, MAT masih menjadi ujian piawai untuk diagnosis dan pengawasan serologi, namun, pertimbangan yang teliti perlu diambil apabila mentafsirkan keputusan kes klinikal dengan kehadiran antibodi anti-leptospira, kerana serum berpasangan diperlukan untuk pentafsiran yang lebih baik. Walaupun tidak ada serovar alam sekitar mengelilingi di kalangan anjing liar urban, potensi zoonosis masih wujud kerana mereka masih boleh dijangkiti atau menjadi pembawa serovar patogenik lain, dan menyebarkan penyakit melalui peluruhan air kencing. Pelbagai tanggungjawab pekerjaan mungkin menyebabkan anjing dan pengendalinya terdedah kepada persekitaran yang tercemar oleh *Leptospira* spp. dan haiwan terjangkit/pembawa. Sentuhan yang kerap dengan anjing yang berpotensi terjangkit menambah risiko pekerjaan untuk pengendali anjing. Risiko tersebut boleh dikurangkan dengan perlindungan yang sewajarnya ketika mengendalikan anjing semasa operasi. Mempromosikan kesedaran melalui peningkatan pengetahuan boleh mengurangkan risiko penularan penyakit di kalangan haiwan dan manusia.

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## LIST OF ABBREVIATIONS

%	Percentage
5-FU	5-fluorouracil
µg	Microgram
µM	Micromolar
µl	Microliter
BLAST	Basic local alignment search tool
CBC	Complete Blood Count
CI	Confidence interval
DNA	Deoxyribonucleic acid (s)
dNTP	Deoxynucleotide triphosphate
EDTA	ethylene-diamine-tetraacetic-acid
ELISA	Enzyme-linked immunosorbent assay
EMJH	Ellinghausen and McCullough modified by Johnson and Harris
FPV	Faculty of Veterinary Medicine
HIS	Hyperimmune Sera
IACUC	Institutional Animal Care and Use Committee
i.e.	id est
IgG	Immunoglobulin G
IgM	Immunoglobulin M
LR	Likelihood Ratio
MAT	Microscopic agglutination test
Md	Median
mg	Milligram
MgCl <sub>2</sub>	Magnesium chloride

ml	Millilitre
MOH	Ministry of Health
NaCl	Sodium chloride
NCBI	National Centre for Biotechnology Information
OIE	World Organisation for Animal Health
OMP	Outer Membrane Protein
OR	Odd Ratio
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PPE	Personal Protective Equipment
REC	Research Ethics Committee
rpm	Revolutions per minute
rRNA	Ribosomal Ribonucleic Acid (s)
SPF	Specific Pathogen Free
TBE	Tris-borate-EDTA
TMB	Tetra-methylbenzidine
Tris-Cl	Tric-chloride
UK	United Kingdom
UKM	Universiti Kebangsaan Malaysia
UPM	Universiti Putra Malaysia
USA	United States of America
VGG	Vaccine Guideline Group
WSAVA	World Small Animal Veterinary Association

# CHAPTER 1

## INTRODUCTION

### 1.1 Background of study

Leptospirosis, a bacterial zoonotic disease (Agampodi *et al.*, 2019; Haake *et al.*, 2015; Mohamed-Hassan *et al.*, 2010; Pappas *et al.*, 2008; Levett, 2004; Bharti *et al.*, 2003) endemic to tropics like Malaysia (Major *et al.*, 2014). The culprits are 250 pathogenic serovars of motile spirochaetal bacterium *Leptospira* spp. (Adler, 2015). Global reports estimated nearly 60000 human deaths among over a million reported annual leptospirosis cases (Philip *et al.*, 2020; Federico Costa *et al.*, 2015; Torgerson *et al.*, 2015). Locally, sporadic fatalities were reported with spiking incidence during monsoon seasons, frequently associated with exposure to *Leptospira* spp. laden areas (i.e. water and soil in forested areas) (Benacer *et al.*, 2016; Benacer *et al.*, 2013; Lim *et al.*, 2011). Reported clinical symptoms in humans ranged from mild flu-like symptoms to full blown Weil's Disease with manifestation of renal and hepatic disease, respiratory distress and death (Adler *et al.*, 2010). Transmission is mainly through direct/indirect contact with urine of infected animals, as well as contaminated soil and water which may result in epidemics (Garba *et al.*, 2018; Benacer *et al.*, 2016). Rats often implicated as they are natural carriers of these pathogenic leptospire but of recent, livestock and companion animals (cats and dogs) are becoming potential infection sources (Costa *et al.*, 2022; Ball *et al.*, 2014; Calderón *et al.*, 2014; Jimenez-Coello *et al.*, 2010). Therefore, it may pose a potential zoonotic threat to individuals with involving animal handling (Hartskeerl *et al.*, 2011).

Understanding the disease agents and its epidemiology allows implementation of prevention and control measures to mitigate risk of infection. Vaccination plays an important role in disease control and prevention of many known diseases and many attempts towards development of a suitable vaccine in an effort of preventing leptospirosis has been made (Bashiru *et al.*, 2018). To date, most of the vaccines developed are chemically inactivated whole culture vaccines (Barazzone *et al.*, 2022). In Malaysia, the vaccination protocol adopted was in accordance with the vaccination guidelines group (VGG) of the World Small Animal Veterinary Association (WSAVA) (Day *et al.*, 2016). The commercial vaccines were known to protect against four leptospiral serovars (Canicola, Icterohaemorrhagiae, Grippityphosa and Pomona).

Current assessments of the post-vaccination antibody titre show variation (minor and major) in antibody responses during the first 12 to 16 weeks post-vaccination (Barr *et al.*, 2005; Andre-Fontaine *et al.*, 2003; Klaasen *et al.*, 2003) with highest reported titre at 1:800 (Martin *et al.*, 2014). Globally, various research has reported immunity for  $\leq 12$  months (Minke *et al.*, 2009; Klaasen *et al.*, 2003). Changes in antibody level documented in the past were conducted on specific pathogen-free (SPF) animals using the commercially available inactivated bacterin vaccines. It was observed in these studies that high antibody

titres were generated, but were however not reflective of the actual antibody titre changes observed in non-SPF dogs (i.e. pet dogs). Findings of the antibody titres were serovar-specific, thus interpretation of serology using MAT to differentiate between vaccination and infection remains a challenge (Srivastava, 2006). As there is still paucity of information in terms of the duration and magnitude of the leptospiral antibody titre post-vaccination among pet dogs using global vaccination protocols (Day *et al.*, 2016), this is a pioneering study locally. These global recommendations were based mainly on information of studies conducted in temperate countries and the response to serovar-specific commercialised vaccine could be different in local tropical setting with different diversity of leptospiral serovars.

Leptospirosis infected animals that are asymptomatic may either be maintenance/reservoirs (rats) and/or potential carriers (wild and domesticated animals including companion animals) (Picardeau, 2013). Investigation of canine leptospirosis among dogs in Malaysia remains insufficient therefore contribute to potential under-reporting of cases and presumption of low prevalence resulting from vaccination protocol implementation. In recent years, three local studies in dogs (pet dogs, shelter dogs and working dogs) have detected seroprevalences between 3.1-7.0% mainly for *L. bataviae*, *L. icterohemorrhagiae*, *L. canicola*, *L. javanica*, and *L. australis* (Lau *et al.*, 2016, 2017; Khor *et al.*, 2016). Detection of non-vaccinal serovars (*L. bataviae*, *L. javanica*, and *L. australis*) indicated possible disease exposure from frequent close contact with animal reservoirs and other infected dogs which can potentially become a source of infection (Murphy, 2015; Awosanya *et al.*, 2013; Okewole *et al.*, 2009). Hence, investigating its presence among dogs in Malaysia has paramount importance as there are many occupations involving work with dogs (Lau *et al.*, 2017), as dogs are popular choice for pets as a form of psychological therapy (McNicholas, 2005; Allen *et al.*, 2001).

Khor (2016) reported a seroprevalence of 3.8%, n=3 (of 80) with *L. bataviae* being detected among dogs in a shelter. The source of the dogs relocated to shelters were stray dogs living in the streets (Khor *et al.*, 2016). It was estimated that the global stray dog population was between 700million – 1 billion which make up 75-85% of the total dog population, which increases each year (K.S. *et al.*, 2019; Hughes *et al.*, 2013). With the estimated local dog population of 6 million (Mordor Intelligence, 2022), we could be dealing with nearly 4.5 million stray dogs. These stray dogs could be chronically (carrier) or acutely infected becoming a likely risk to other animals. Depending on the location where these stray dogs roam, there is possible contact with other animals which include rats (reservoir), livestock (pig and cattle) and even other infected stray dogs (Costa *et al.*, 2022; Boey *et al.*, 2019; Benacer *et al.*, 2017; Lelu *et al.*, 2015). This may have potential economic implications due infection causing reduced production of livestock animals to loss of income among humans (Smith *et al.*, 2022; Ellis, 2015) which warrants further investigation to determine the role of stray dogs in disease transmission and dissemination (Chou *et al.*, 2014; Jimenez-Coello *et al.*, 2010).

Risk of infection among humans from animal contact has been reported (Hartskeerl *et al.*, 2011). Slaughterhouse workers were found seropositive from handling carcass and offal of infected animals (Dreyfus *et al.*, 2014). In Kenya, kennel workers were at risk of infection and dogs were speculated to spread this disease (Awosanya *et al.*, 2013). Locally, the oil palm plantations workers in Melaka and Johor and town service workers from North-Eastern Malaysia were exposed to *Leptospira* spp. which resulted from possible rodent and/or contaminated environment contact (Ridzuan *et al.*, 2016; Shafei *et al.*, 2012). Since dogs has been implicated in the transmission of leptospirosis, there may be a potential occupational hazard among dog handlers who are constantly exposed during their daily task of dog handling. Therefore, it is utmost important that these individuals to be made aware of the potential treat which they may face and to ensure a safe working environment for both the dog handlers and their dogs.

This study evaluated the duration and magnitude of the leptospiral antibody titre post-vaccination. The observation of these leptospiral antibody titres would allow a better understanding of the extent of antibody presence, potentially improving MAT interpretation. Ultimately, this study intended to understand to roles of stray dogs in leptospirosis transmission to humans, posing zoonotic concern. The investigation encompassed bacterial isolation followed by identification using molecular [polymerase chain reaction (PCR)] and serological [microscopic agglutination test (MAT)] methods. Findings of predominant leptospiral serovars among the stray dogs would allow future incorporation of local serovars found into the vaccine to expand its spectrum of protection and ensure its relevancy in protecting local dogs against the disease. Besides the urban stray dogs on the street, this study determined the serological presence and identified potential risk factors of leptospiral infection among dogs and their handlers in shelter and working dog populations. An evaluation on the level of knowledge regarding leptospiral infection as well as their attitude towards the preventive practices with accompanying practicing levels of current preventive measures was carried out. Results from this study could assist in the future development of a more targeted disease control and prevention program.

## **1.2 Problem statement**

Studies have shown that rats are the dominant reservoir with its ability to harbour a wide range of *Leptospira* spp. However, attention has been shifted towards other animals such as dogs in recent years. Dogs have been documented in the past as the reservoir for *L. interrogans* serovar Canicola, making them potentially important in leptospirosis transmission. The zoonotic potential leptospirosis has led to the practice of vaccination as an important preventive tool. However, there is still paucity of information on the antibody titre response among local pet dogs following vaccination in accordance to vaccination guidelines practiced locally.

As mentioned, dogs have been suspected to be carriers of *Leptospira* spp., thus making them a potential zoonotic threat. With the estimated large stray dog population, potentially infected stray dogs may contaminate the large areas and infecting not only other dogs but also other animals and humans as they roam, thus impacting the environment. Previous work done on dogs were not focused on stray dogs and have been serology orientated with limited success in isolation efforts. In the past there has been indication of leptospirosis presence among working dogs and shelter dogs which may become a potential zoonotic risk for their handlers. However, not much is known about the potential risk factors of leptospirosis among dogs and dog handlers and whether contact with dogs could be a potential risk factor for both groups. Besides that, there is also limited information regarding the level of knowledge, attitude, and practice of dog handlers towards leptospirosis.

### **1.3 Justification**

The absence of the post-vaccination antibody titre makes it a challenge to interpret serological findings in clinical cases. This information will assist researchers and clinicians working closely with canine leptospirosis in obtaining a more accurate interpretation of serological results. Documenting the predominant *Leptospira* spp. among the urban stray dogs is crucial in disease management especially with regards to potential vaccine development. The presence of these serovars would also indicate shedding potential of infected dogs causing disease dissemination.

With the presence of leptospirosis dogs housed in the working dogs' organizations and shelters, these dogs may potentially spread leptospirosis and puts risk of infection on both dog and their handlers. Such a situation is made complicated within the shelter environment with the practice of the "No Kill" policy. Risk factors identified may allow for a more targeted preventive program to be implemented. However, implementation is very much dependent on the level of knowledge, attitude, and practice of the target population. Prior knowledge on the matter is important towards the development of an effective control and preventive measures in mitigating the risk of this zoonotic disease.

### **1.4 Objective**

The objectives of this study were:

1. To evaluate the duration and magnitude of post-vaccination leptospiral antibody titre in pet dogs (puppies and adult dogs)
2. To determine the predominant circulating leptospiral serovars among local urban stray dogs and their susceptibility in shedding of *Leptospira* spp. using both serology and molecular diagnostic methods

3. To determine the seroprevalence of leptospiral infection among dogs and dog handlers.
4. To determine the risk factors of leptospiral infection among dogs and dog handlers.
5. To evaluate the level of knowledge, attitude, and practice towards leptospirosis among dog handlers.

## 1.5 Hypothesis

The hypotheses of this study were:

1. The duration and magnitude of post-vaccination leptospiral antibodies in pet dogs (puppies and adult dogs) will be for one year with a high antibody titre.
2. There is predominance of leptospiral serovars among local urban stray dogs which are susceptible to the shedding of *Leptospira* spp. as shown by serology and molecular diagnostic methods.
3. There is high level of seroprevalence of leptospiral infection among dog and dog handlers.
4. There is risk of leptospiral infection among dog and dog handlers.
5. Dog handlers have poor knowledge, attitude, and practice towards leptospirosis.

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