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Research Article

Shedding and Genetic Diversity of *Leptospira* spp. From Urban Stray Dogs in Klang Valley, Malaysia

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A B S T R A C T

Leptospirosis is an endemic zoonoses of global proportions. Stray dogs have been postulated to play a role in disease transmission; however, supporting information are still limited. Roaming behavior may not only predispose the dogs to infection, but could also contribute to disease spread. In this study, the susceptibility of urban stray dogs in shedding *Leptospira* spp. was determined. Blood, urine, and tissue samples of kidney and liver were collected from 100 dogs from 2 animal control facilities. Serological testing using microscopic agglutination test (MAT) were performed on blood against 20 leptospiral serovars with a cut-off titre of ≥ 1:100. Samples were cultured onto semi-solid Ellinghausen and McCullough modified by Johnson and Harris (EMJH) media. Isolates were identified using molecular polymerase chain reaction (PCR) using 2 primers (16s rRNA and LipL32) and hyperimmune serum (HIS) MAT. The seroprevalence for the dogs positive for leptospirosis was 32% (n=32/100) with the following detected serovars: Javanica (n=13), Bataviae (n=10), Icterohaemorrhagiae (n=3), Autumnalis (n=2), Canicola (n=1), Pyrogenes (n=1), Copenhageni (n=1), and Australis (n=1). Six *Leptospira* spp. isolated were procured from urine (n=2), kidney (n=2) and liver (n=2). All 6 isolates belonged to *L. interrogans*, a pathogenic variant of *Leptospira* spp. Serotyping and phylogenetic analysis suggested serovar Bataviae (n=5) and serovar Canicola (n=1). Presence of vaccinal serovars (Icterohaemorrhagiae and Canicola) suggested potential post-vaccination antibodies but the predominance of non-vaccinal serovars (Javanica and Bataviae) indicate the possibility of current infection or post-exposure. Isolation of *Leptospira* spp. directly from urine sample not only suggested an active infection but highlighted the potential shedding capability among these stray dogs. These findings further strengthen speculations that urban stray dogs could play a role in transmission and dissemination of leptospirosis through their constant movement. The urine of infected dogs may contaminate the environment, posing a major public health threat.

Introduction

As a zoonoses of global importance, leptospirosis remains one of the most widespread reemerging zoonoses.[1,2] Leptospirosis is caused by the pathogenic spirochetes *Leptospira* spp. harboured by animal reservoirs such as rats.[3] However, other mammals such as wild, semi-domesticated, livestock and even companion animals (e.g., dogs and cats) can be potential carriers, playing important roles as infection sources.[4-6] Transmission occurs through direct contact with reservoir animals or indirectly from exposure to leptospira-laden areas.[7]

Leptospirosis epidemics are often associated with exposure to contaminated water sources, especially during floods.[8] Over the years, there has been a shift in emphasis surrounding increased risk of human leptospirosis towards association with frequent and prolonged contact with animals, especially dogs.[9-11] Rapid urbanization in Malaysia has led to a tremendous increase in both the human and dog population. Therefore, the potential role of dogs in disease transmission due to close contact with human is becoming more important.[9-11]

Previous serological studies in the region reported a detection rate of 3 to 50% among shelter, pet and working dogs with serovars such as Icterohemorrhagiae, Canicola, Pomona, Grippotyphosa, Javanica, Bataviae, Pyrogenes and Australis being detected.[9,12-15] Dogs are presumably associated with low prevalence of leptospirosis due to immunity from vaccination. However, the absence of certain serovars in commercial vaccines poses risk to pet dogs. Stray dogs are often neglected and could be potential carriers playing a role in disease dissemination due to their natural free roaming behaviour which increases contact with other dogs and animals from widespread locations.[9,12,15-17]

Previous studies have detected leptospiral antibodies in stray dogs from Malaysia, but had limited success with *Leptospira* spp. isolation and identification.[18,19] Therefore, the primary objective of this study was to determine the predominant circulating leptospiral serovars shed among local urban stray dogs using both serology and molecular diagnostic methods. Information obtained may further elucidate the role of urban stray dogs in shedding, further contaminating the environment and thus, may assist transmission and dissemination of the disease.

Materials and Methods

Sample Size and Sampling Location

Sample size was determined based on a power calculation using 7% prevalence rate[14] at 95% confidence interval (CI). Using the formula, sample size = Z^2P (1-P)/e^2, where p=the expected prevalence, e=error rate, Z=the table value corresponding to the confidence level used [Z=1.962; *P*=7 % (0.07); e=0.05]. It was determined that a total of 100 dogs would be required.

This cross-sectional study was carried out on stray dogs from two animal control facilities within Klang Valley, Malaysia. Approval from

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the Institutional Animal Care and Use Committee (UPM/IACUC/AUP-R041/2018) and the authority of the animal control facility were obtained prior to commencement. Scheduled euthanasia of unclaimed stray dogs was carried out as part of an initiative in controlling stray dogs' overpopulation by the municipal council. These dogs were housed in groups based on the date of being caught. A convenience sample of dogs were enrolled based upon a sampling date and scheduled euthanasia.

The animal control facility was routinely cleaned after each batch of stray dogs had been cleared to ensure proper sanitation. Confidentiality of the information obtained was kept and utilized only for research purposes. The two animal control facilities (A and B) were located approximately 29.9 km apart. The area of coverage of each animal control facility surveillance and dog catching operations in these urban areas was as shown in Fig 3.

Sample Collection

The general physical condition, approximate age and gender of the stray dogs were noted. The dogs were manually restrained for blood sampling via cephalic venepuncture. Blood samples were stored in plain and ethylenediaminetetraacetic acid (EDTA) blood tubes. The dogs were then humanely euthanised as scheduled using 10 mL of 20% Sodium Pentobarbital Sodium (Dolethal, Vetoquinol UK) administered intravenously. A post-mortem examination was performed. Urine was obtained through cystocentesis and wedged tissue samples of kidney and liver were obtained. Samples were stored in 4°C chiller boxes and transported to the Bacteriology Laboratory in the Faculty of Veterinary Medicine, Universiti Putra Malaysia.

Microscopic Agglutination Test (MAT)

All plain blood tubes were centrifuged at 5000 rpm for 10 minutes. The serum obtained was aliquoted into 1.5 mL Eppendorf microcentrifuge tubes and stored at -20°C for serological analysis. Samples were tested against 20 leptospiral serovars (Table 1) of canine pathogenic, environmental pathogenic and a saprophytic serovar.[12,14,20,21] The endpoint titres were determined using serial two-fold dilutions and the last well showing 50% agglutination was recorded. A positive MAT reaction was defined as cut-off titer $\geq$ 1:100[22-24] and based on the diagnostic criteria by World Organisation for Animal Health (OIE)[25] and International Standard ISO/IEC 17025:2017 from Veterinary Laboratory Services Unit (VLSU) of Faculty of Veterinary Medicine, Universiti Putra Malaysia. Antigens were obtained from Leptospirosis Reference Laboratory, Queensland Health, Queensland, Australia. Samples were considered to have leptospiral antibodies if there was $\leq$ 50% free leptospires and $>$ 50% agglutination when compared to the positive control (hyperimmune serum) and negative control (antigen only). The suspected infecting serovar was based on the serovar with the highest titre.

Table 1
Leptospiral Antigen Panel for Microscopic Agglutination Test (MAT)

SPECIES	SEROVAR	STRAIN
L. interrogans	Icterohaemorrhagiae	RGA
	Canicola	Hond Utrecht IV
	Pomona	Pomona
	Bataviae	Swart
	Australis	Ballico
	Tarassovi	Perepelitsin
	Autumnalis	Akiyami A
	Pyrogenes	Salinem
	Hebdomadis	Hebdomadis
	Lai	Lai
	Copenhageni	Fiocruz
	Djasiman	Djasiman
L. weilii	Celledoni	Celledoni
L. kirschneri	Grippotyphosa	Moskva V
	Cynopteri	3522C
L. borgpetersenii	Ballum	Mus 127
	Hardjobovis	117123
	Javanica	Veldrat Bataviae 46
L. kmetyi	Malaysia	Bejo-Iso9^T
L. biflexa	Patoc	Patoc 1

Isolation of Leptospira *spp. From Direct Sample*

EDTA-anticoagulated whole blood, urine, kidney, and liver tissue samples were used for bacterial isolation. Sample inoculation was performed within two hours post-sampling. The protocol used was as described in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals.[25] Two drops each of blood and urine samples were inoculated separately onto semi-solid Ellinghausen and McCullough modified by Johnson and Harris (EMJH media containing 5-fluorouracil (5-FU). Approximately 1 mL of homogenized tissue samples of both kidney and liver samples were also separately inoculated onto semi-solid EMJH media. Primary cultures were incubated at 30°C for a period of 12 weeks. Routine fortnight examination of the cultures by means of dark-field microscopy (DFM) was done to observe for the presence of leptospire isolates. If leptospire isolates were observed within the 12 week time-period, the positive cultures were transferred into liquid EMJH medium to enhance their growth and filtered (0.45 μm filter, Millex, Ireland) until pure isolates were obtained. Pure isolates were maintained in liquid EMJH medium for further identification.

Identification of Pure Isolates Using Serology

The isolates that grew in liquid EMJH medium were tested using hyperimmune serum (HIS) MAT with 17 reference hyperimmune sera (Table 2). Evidence of agglutination against HIS was examined using DFM. The isolates belonged to a specific leptospiral serovar when it reacted serologically to HIS with the titre equivalent to or more than 1:5120.[26] The cut-off titre was based upon the reference value (Table 2) provided by Forensic and Scientific Services, Department of Health, Leptospirosis Reference Laboratory, Queensland, Australia.

Identification of Pure Isolates Using Molecular Method

DNA extraction of the isolates was performed using DNeasy Blood & Tissue Kit (QIAGEN, Germany) followed by polymerase chain reaction (PCR) which employed two sets of primers. Two 16S rRNA primers (5′-GAACTGAGACACGGTCCAT-3′ and 5′-GCCTCAGCGTCAGTTTTAGG-3′)

Table 2
Panel of Reference Hyperimmune Sera With Respective Titre Used for Serological Identification of *Leptospira* spp. Isolates

Species	Serovar	Strain	Titre
L. borgpetersenii	Hardjobovis	117123	1:6400
L. Interrogans	Hebdomadis	Hebdomadis	1:6400
L. weilii	Celledoni	Celledoni	1:6400
L. kmetyi	Malaysia	Bejo-ISO9	1:6400
L. interrogans	Pomona	Pomona	1:12800
L. borgpetersenii	Tarassovi	Perepelitsin	1:3200
L. interrogans	Pyrogenes	Salinem	1:12800
L. kirschneri	Cynopteri	3522C	1:6400
L. interrogans	Lai	Lai	1:1600
L. interrogans	Icterohaemorrhagiae	RGA	1:800
L. interrogans	Bataviae	Swart	1:6400
L. borgpetersenii	Javanica	Veldrat Bataviae 46	1:12800
L. interrogans	Autumnalis	Akiyami A	1:6400
L. borgpetersenii	Ballum	Mus 127	1:1600
L. interrogans	Djasiman	Djasiman	1:6400
L. biflexa	Patoc	Patoc I	1:1600
L. interrogans	Canicola	Hond Utrecht IV	1:6400

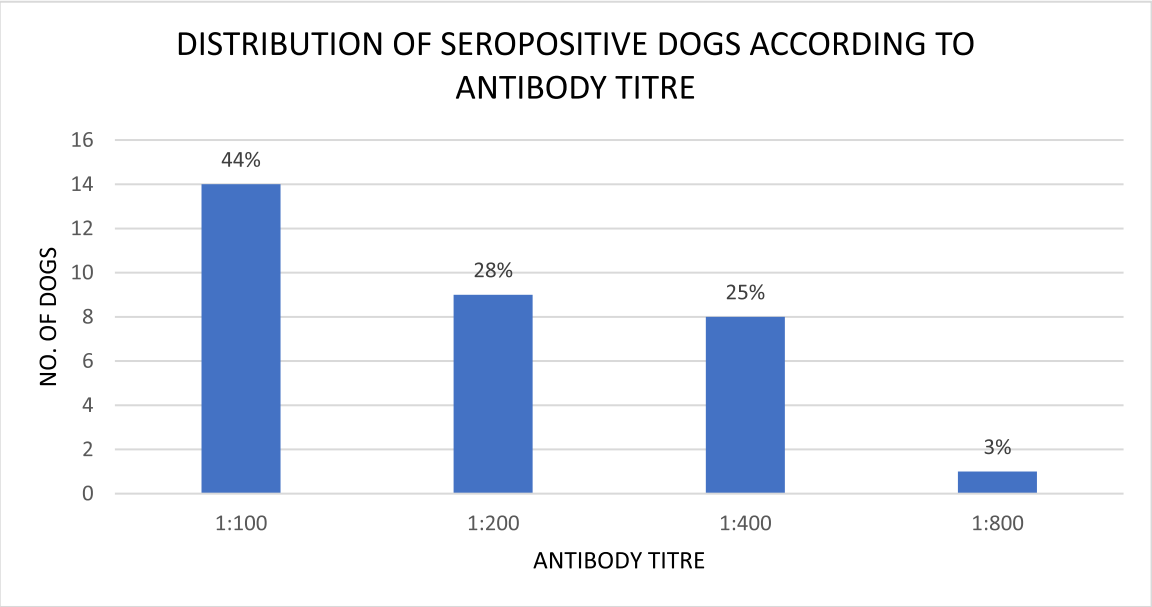

Fig 1. Distribution of the seropositive dogs based on the level of antibody titre detected by microscopic agglutination test (MAT). The values above each bar is the percentage of dogs with that antibody titre measured. Color version of figure is available online

were used to determine genus *Leptospira*. Two LipL32 primers (5′ATCTCCGTTGCACTCTTTGC3' and 5′ACCATCATCATCATCGTCCA3') were used to confirm the pathogenic nature of isolates. Both genes are present in the pathogenic *Leptospira* spp. but only 16S rRNA gene is present in the non-pathogenic variants which indicate intermediate or saprophytic *Leptospira* spp.[27] The amplicons having both genes were later subjected to partial gene sequencing (First BASE Laboratories Sdn. Bhd., Malaysia) using both the forward and reverse 16s rRNA primer sequences. The sequencing data obtained along with representative sequences from genus *Leptospira* spp. (pathogenic, intermediate and saprophytic) and *Leptonema illini* strain Habaki (as an outgroup) were compared with the GenBank database using nucleotide basic local alignment search tool (BLAST) from National Center for Biotechnology Information (NCBI) and aligned using CLUSTAL OMEGA (EMBL-EBI, UK) prior to deposition into GenBank. All the aligned sequencing data were analyzed by using phylogenetic tree analysis. Phylogenetic analysis of the sequences was computed using the Molecular Evolutionary Genetics Analysis version 7.0 (MEGA7)[28] and the Maximum Likelihood method[29] was applied.

Statistical Analysis

Data was tabulated and descriptively analysed using IBM® SPSS® Statistics Version 26 (IBM, USA). The detection rate of dogs with antibodies titres and had positive culture were presented as frequencies and percentages.

Results

One hundred stray dogs from both animal control facilities were included in this study. The range of estimated ages was 1-5 years and there were 77 (77%) intact males and 23 (23%) females. Fifty-seven (57%) dogs were included from animal control facility A and 43 (43%) from B. Most dogs were unthrifty and had a poor body condition. The vaccination history of the dogs and the neutering status of the females were not known.

Leptospiral Antibody Detection

The prevalence of positive leptospiral antibodies was 32% (32/100). Twenty-one out of the 32 stray dogs were intact males and the remaining were females with unknown neutered status. The numbers of dogs having leptospiral antibodies with their respective serovars and titres were as shown in Fig 1 and Table 3, respectively. There were slightly more seropositive dogs in animal control facility B (35%; 15/43) compared to animal control facility A (30%; 17/57) (Table 3). Antibodies to eight leptospiral serovars were detected among these urban stray dogs (Table 3) and the prominent serovars detected were Javanica (13.0%, 13/100) and Bataviae (10%, 10/100). The differences noted were that serovars Canicola and Australis were present only in dogs from animal control facility A while serovars Pyrogenes and Copenhageni were present in animal control facility B. Only 2 dogs exhibited antibodies towards multiple serovars. A single dog from animal control facility A had antibodies for Autumnalis (1:200) and Australis (1:200) while Icterohaemorrhagiae (1:400) and Javanica (1:400) was detected in a dog from animal control facility B, all of which belonged to different serogroups.

Isolation and Identification of Leptospira *spp. Isolates Using Serological and Molecular Methods*

A total of 6 isolates (Table 4) were successfully grown on EMJH medium from 4 dogs (3 males and 1 female). These 4 dogs had antibody titers ranging from 1:100 to 1:400. The isolates were successfully cultured from urine (2.0%, 2/100, 95% CI: 0.0%-4.7%) and tissue samples

Table 3
Distribution of Leptospiral Antibodies by Serovar Detected Among Urban Stray Dogs in 2 Animal Control Facilities A and B

Serovars	Animal control facility		N (%)
	A	B	
Icterohaemorrhagiae	1	2	3(3)
Canicola	1	0	1(1)
Australis	1	0	1(1)
Autumnalis	1	1	2(2)
Bataviae	7	3	10(10)
Javanica	6	7	13(13)
Pyrogenes	0	1	1(1)
Copenhageni	0	1	1(1)
Total	17	15	32(32)

Table 4
Identification of Isolates tThrough Serological (HIS MAT) and Molecular (DNA Sequencing) Methods

ID	Sample	PCR	DNA SEQUENCING	HIS MAT	ASCESION NO.
D12	Kidney	+ve	*L. interrogans*	Bataviae (1:3200)	MN218182.1
D15	Urine	+ve	*L. interrogans*	Bataviae (1:3200)	MN218183.1
	Kidney	+ve	*L. interrogans*	Bataviae (1:3200)	MN218184.1
	Liver	+ve	*L. interrogans*	Bataviae (1:3200)	MN218185.1
D16	Liver	+ve	*L. interrogans*	Bataviae (1:3200)	MN218186.1
D57	Urine	+ve	*L. interrogans*	Pomona (1:1600) Autumnalis (1:400)	MN417029.1

+ve, positive.

of kidney (2.0%, 2/100, 95% CI: 0.0%-4.7%) and liver (2.0%, 2/100, 95% CI: 0.0%-4.7%), respectively. Three dogs had a single isolate cultured from urine, kidney and liver, respectively. The fourth dog had 3 isolates procured from each from urine, kidney and liver samples. There were no *Leptospira* spp. isolates obtained from blood samples.

Through serology, 5 *Leptospira* spp. isolates agglutinated against HIS Bataviae (strain Swart) with titre of 1:6400 whereas one isolate showed reaction with HIS Pomona (1:1600) and Autumnalis (1:400). Both the 16S rRNA and LipL32 genes were amplified in all the isolates. Result indicated that the isolate obtained were of pathogenic *Leptospira* spp. DNA sequencing of 16S rRNA gene of the isolates followed by The BLAST analysis (www.ncbi.nlm.nih.gov/blast) identified isolates as *L. interrogans* with query coverage and maximum identity of $\geq$ 95%. Phylogenetic analysis placed the isolates within the pathogenic clade as shown in Fig 2. Only one isolate was closely related to serovar Canicola strain Hond Utrecht IV (GenBank accession no: FJ154561.1) while the other five isolates showed relations to serovar Bataviae (GenBank accession no: FJ154566.1) based on supporting serological results.

Discussion

Occurrence of human leptospirosis has always been associated with environmental exposure towards contaminated areas predisposed by the tropical heavy rainfall or monsoon leading to flooding favouring leptospires persistence.[30] Exposures may also occur during occupational or recreational activities.[8,9] These risk factors have been shown to have a common link, which is the direct or indirect contact with reservoir animals.[9,30,31] Dogs have been shown to be a possible source of infection; however, rats remain the primary reservoir

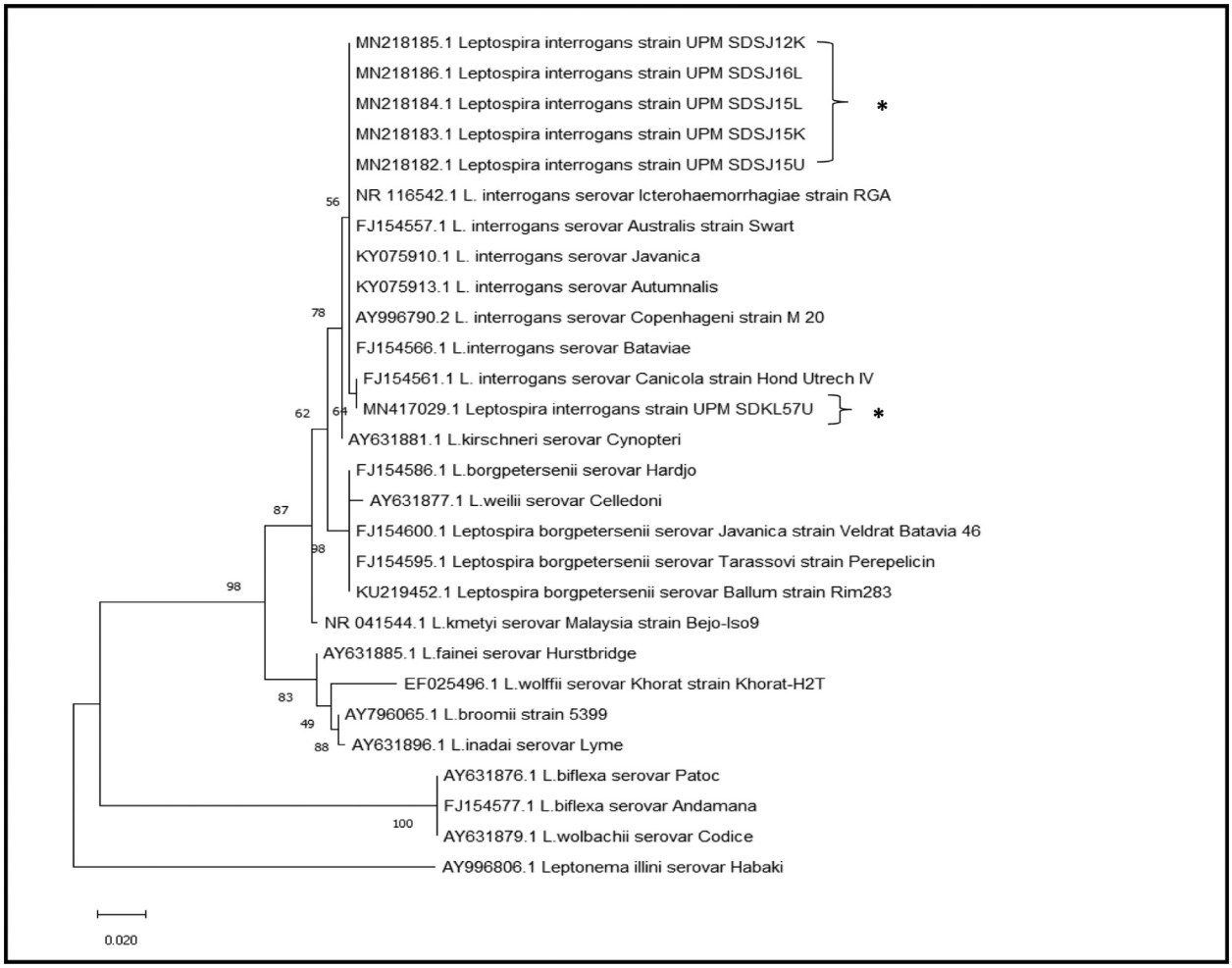

Fig 2. Phylogenetic analysis based on 16S rRNA gene for *L. interrongans* isolates retrieved from the urban stray dogs (*), representative reference *Leptospira* spp. and *Leptonema illini* serovar Habaki as an outgroup. Color version of figure is available online

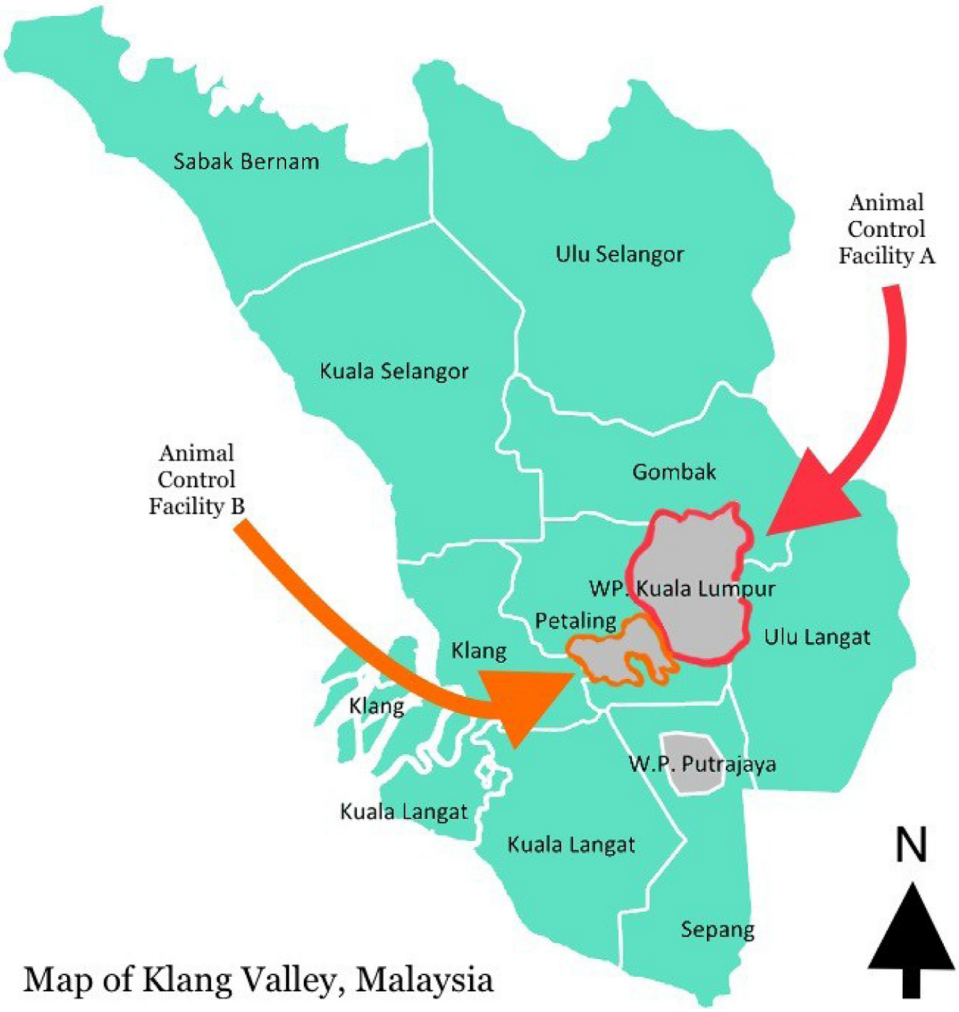

Fig 3. Location of the study area in Malaysia showing the districts in the states of Selangor and Kuala Lumpur located within the Klang Valley, Malaysia. The highlighted regions (outlined in red and orange) belonging to two different municipalities were the sampling areas for this study in which the respective animal control facility were located in. Color version of figure is available online

host.[32,33] However, since dogs are typically more exposed to environmental sources of infection than humans, they may act as sentinels of the presence of leptospires in the environment or as potential epidemiological links between the surrounding environment and humans.[32-35] Hence, it is important to unravel the dynamics of dissemination to suggest appropriate control and preventive methods, especially locally in Malaysia.

In this study, 32% of the urban stray dogs had leptospiral antibodies and falls within the wide range of 3%-50% reported from different groups such as pet dogs, shelter dogs and working dogs.[9,12,14] The differences could be related to the different localities, different exposure levels to rats/small mammals and the different control and preventive measures implemented. In this study, the two animal control facilities were situated in two different municipalities approximately 29.0 km apart (Fig 3). To our knowledge, there were no reports of flooding during the sampling period where these stray dogs were caught from their respective areas. It could be speculated that the potential causes for seroconversion were either due to indirect contact with dirty contaminated areas or directly with carrier animals (i.e. rats, small mammals or infected dogs) as these urban stray dogs live in highly dense residential and commercial areas. But, the possibility of an infected stray dog transmitting the disease to the other stray dogs within the same batch kept together for a month could not be ruled out.[9] Investigators did not have full access to the entire animal control facility and was only limited to small batches of dogs provided during each sampling weekly. Anecdotal reports from animal control officers indicated that rats were occasionally identified within the facilities. Therefore, in combination with exposure to potentially infected stray dogs not only puts the dogs at high risk of infection but the animal control facility employees as well.[10]

The predominant serovars (Javanica and Bataviae) detected through serology in this current study were of non-vaccinal serovars, indicative of past exposure or active infection.[36] The presence of serovar Javanica and Bataviae could be due to exposure to rats as these serovars have been isolated from local urban rat population.[37] Findings were similarly observed in other dog populations such as working dogs[9,14] and shelter dogs[9,12] that may suggest the widespread and local persistence of leptospirosis. The predominance of serovar Bataviae among urban stray dogs was similar to another report that included dogs housed in an animal control facility in a rural region.[12] This raises concern that presence of leptospirosis in dogs housed in animal control facilities could be due to exposure from newly introduced dogs captured from the streets. Low presence of serovars Australis, Autumnalis, Pyrogenes and Copenhageni could indicate low exposure to sources of these serovars normally harbored by wild rodents[38] speculated to be less prevalent in urban regions. Serovar Pomona was not detected among the stray dogs in urban areas due to lack of contact with pigs, known carriers for this serovar.[19] The low detection of vaccinal serovars Icterohaemorrhagiae and Canicola

could indicate limited exposure; however, limited immunization among the urban stray dogs puts these dogs at risk of infection.[39]

Majority of these stray dogs were exposed to a single detected serovar however co-infection with multiple serovars is possible.[40] The 2 urban stray dogs having multiple serovars detected serologically could be due to exposure to a single or different sources of infection possibly due to the constant roaming, especially among male dogs, which had similarly been documented in other animals such as cattle.[24] However, these findings should be interpreted with caution as significant cross-reactivity within serogroups has been known to occur.[40] Therefore, the serovar with the highest MAT antibodies titre does not necessarily reflect the infecting serovar. The ability to obtain a paired serum sample was not possible in this study.

This study successfully procured 6 isolates from four dogs, more than previous studies.[18] Another study on stray dogs and dogs housed in animal control facility in Brazil obtained a lower isolation rate of *Leptospira* spp. with only 2 of 123 dogs (1.6%) being positive. Perhaps, the 4 (blood, urine and kidney and liver tissue) different samples cultured in this study could have allowed higher chance of isolation compared to previous studies (predominantly urine and kidney samples).[16] The presence of *Leptospira* spp. in an environment may vary with endemicity level as findings could vary with sampling locations, time period and exposure to various sources of infection.[18]

Leptospira spp. was not isolated from blood in this study, which is indicative of bacteraemia during acute infection.[41] Isolation from liver samples could have had an acute active infection with potential shedding.[16] Therefore, dog (ID D16) which had positive isolates only from liver could indicate chronicity and possible hepatic colonisation of *Leptospira* spp. As for the potential dogs shedding *Leptospira* spp. via urine,[16] dogs (ID D12 and D57) were suspected to be shedders as both dogs had positive cultures from kidney and urine, respectively. Only 1 dog (ID D15) with positive cultures from urine, liver and kidney samples could have an acute active infection and potentially shedding. However, the true clinical condition of these urban stray dogs was not known as complete blood profiling was not carried out. Shedding in dogs with leptospirosis generally occurs in chronic infections or carriers[42]. Therefore, these infected urban stray dogs could either be chronically infected and/or carriers as some appeared clinically healthy while others not. Direct molecular detection of the samples could have provided a better conclusion.

The effectiveness of leptospiral isolation is dependent on many factors but the hardest to control was sample contamination. Hence, the actual burden of the disease could have easily been underestimated. This study only managed to recover the organisms either from urine or kidney samples from specific dogs. Isolates from urine, kidney and liver samples were retrieved from only 1 dog possibly due to lesser contaminants. Culturing leptospires still stands as the gold standard reference test for confirmation of leptospiral infection, albeit the low sensitivity of leptospiral isolation as compared to other detection method such as PCR.[42]

Different geographical regions with variety of reservoir animals accounts for the diversity in circulating serovar.[16] Through isolation and identification, a current local predominance of serovar Bataviae further supported the previously reported local detection data. Although these serovars were isolated from dogs, they are more commonly associated with rodents.[37,38] This finding suggests a potential link between rodents and dogs. The urine of infected roaming rodents could have contaminated the environment becoming a source of infection for the stray dogs. The inclusion of rat trapping and soil sampling from the same area where the dogs were caught would have strengthened the link. Leptospire shedding from infected urban stray dogs identified in this study indirectly supports that dogs could have a role in dissemination of the disease.[16,17] The dense urban regions not only foster interaction between animal and human but facilitate stray dogs movement across large areas easing disease transmission evident by similar serovars detected in both dog animal control facilities located in separate town municipalities.[43] Environmental variants of *Leptospira* spp. which are commonly associated with recreational areas[7] and forest parks[44] were not detected as these roaming urban stray dogs were not within the vicinity of such areas.[45]

Human-animal interaction has played a role in the occurrence of many zoonotic disease with leptospirosis being no different.[46] To date, there is limited evidence that proves stray dogs have an increased risk of *Leptospira* spp. shedding. Individuals with frequent contact with stray dogs such as dog handlers, shelter workers and animal rescuers have been shown to be at risk of infection.[9,10] Dog owners adopting from shelters or individuals who feed strays should be made aware of the potential public health threat. Proper personal protective equipment usage and frequent hand washing should be advocated among such individuals in order to mitigate risk of infection.[47] Development of suitable vaccines with specific serovars may prevent the occurrence of the disease. However, providing widespread immunization to stray dogs would be a challenging endeavur. Ongoing continuous rodent and stray dog control may provide a more sustainable means to reduce disease transmission.

Conclusion

Dogs could potentially be infected by different leptospires and may play an important role in disease dissemination, posing a public health risk. Leptospiuric dogs could be rescued from the streets and placed in animal control facilities, thus may pose a risk to animal shelter employees and prospective dog adopters. Information obtained may assist in creating awareness towards canine leptospirosis among individuals involved directly with the handling of stray dogs such dog rescuers, veterinarians, municipal council dog catchers and even dog owners. More work needs to be done to determine the roles of dogs in disease transmission and dissemination. Further investigations incorporating more localities should be undertaken to further document the epidemiology of canine leptospirosis among strays. Knowing locally predominant serovars through both serological and molecular means will allow improvements to commercial canine vaccines by incorporating these serovars may assist disease prevention. Improving public health awareness may assist sustainable zoonotic risk mitigation.

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Declaration of Competing Interest

All authors report no conflict of interest relevant to this article.

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