SEROLOGICAL DETECTION OF LEPTOSPIROSIS AMONG DOGS IN KELANTAN

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Serological Detection of Leptospirosis Among Dogs in Kelantan

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SEROLOGICAL DETECTION OF LEPTOSPIROSIS AMONG DOGS IN KELANTAN

ABSTRACT

Leptospirosis is a zoonotic bacterial disease affecting both humans and animals. The aim of this study is to determine the seroprevalence of leptospirosis among dogs in Kelantan and to identify the most prevalent *Leptospira* serovars in dogs in Kelantan. A total of 30 serum samples from dogs were collected from private clinics in Kelantan and Hospital Pengajaran Veterinar, Universiti Malaysia Kelantan. Thirteen male dogs and 17 female dogs' blood were collected using cephalic venepuncture. Samples were tested using Enzyme-Linked Immunosorbent Assay (ELISA) and Microscopic Agglutination Test (MAT). Among the dogs, 10% classified as young, 73% were adult and 17% were senior dogs. Based on the critical value of 0.1625 for ELISA, all of the dogs were tested negative for Canine *Leptospira* IgG. The cut-off antibody titre for MAT was ≥ 1:100, and all the samples were negative for 06 serovars tested for the MAT panel. The absence of *Leptospira* specific antibodies among study cohort carries substantial implications for public and animal health. The negative findings regarding *Leptospira* play a crucial role in contributing essential information to the local disease epidemiology, indicating a potential low risk of canine leptospirosis. These findings able to provide basis for the formulation of preventive strategies against canine leptospirosis in Kelantan, Malaysia. In conclusion, there were 0% seroprevalence of leptospirosis among dogs that enrolled for the study in Kelantan.

Keywords: Leptospirosis, Dog, Serology, ELISA, MAT

PENDETEKSIAN SEROLOGI LEPTOSPIROSIS DI KALANGAN ANJING DI KELANTAN

ABSTRAK

Leptospirosis adalah penyakit bakteria zoonotik yang menjejaskan manusia dan haiwan. Tujuan kajian ini adalah untuk menentukan seroprevalens leptospirosis di kalangan anjing di Kelantan dan mengenal pasti serovar Leptospira yang paling meluas di kalangan anjing di Kelantan. Sejumlah 30 sampel serum dari anjing telah dikumpul dari klinik swasta di Kelantan dan Hospital Pengajaran Veterinar, Universiti Malaysia Kelantan. Daripada jumlah tersebut, darah anjing sebanyak tiga belas jantan dan tujuh belas betina telah dikumpulkan menggunakan teknik vnipunktur. Sampel diuji menggunakan Enzyme-Linked Immunosorbent Assay (ELISA) dan Microscopic Agglutination Test (MAT). Di antara anjing-anjing tersebut, 10% diklasifikasikan sebagai anjing muda, 73% adalah anjing dewasa dan 17% adalah anjing tua. Berdasarkan nilai kritikal 0.1625 untuk ELISA, semua anjing diuji negatif untuk Canine Leptospira IgG. Titer antibodi yang dipotong untuk MAT adalah ≥ 1:100, dan semua sampel adalah negatif untuk enam serovar yang diuji dalam panel MAT. Ketidakwujudan antibodi khusus Leptospira di kalangan kohort kajian ini membawa implikasi besar terhadap kesihatan awam dan haiwan. Penemuan negatif mengenai Leptospira memainkan peranan penting dalam menyumbangkan maklumat penting kepada epidemiologi penyakit tempatan, menunjukkan risiko leptospirosis pada anjing mungkin rendah. Penemuan ini dapat memberikan asas untuk formulasi strategi pencegahan terhadap leptospirosis anjing di Kelantan, Malaysia. Secara keseluruhan, tiada seroprevalens leptospirosis di kalangan anjing yang disertai dalam kajian di Kelantan.

Kata kunci: Leptospirosis, anjing, Serologi, ELISA, MAT

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LIST OF ABBREVIATIONS

ELISA	Enzyme-Linked Immunosorbent Assay
IgM	Immunoglobulin M
IgG	Immunoglobulin G
LPS	Lipopolysaccharides
MAT	Microscopic Agglutination Test
OD	Optical Density
PCR	Polymerase Chain Reaction

CHAPTER 1

INTRODUCTION

Leptospirosis is a zoonotic disease that affects many species of animals worldwide. Among companion animal, dogs are commonly affected and act as carriers. It is caused by spirochete bacterium of genus *Leptospira*. *Leptospira* is a Gram negative, aerobic bacteria characterised by its thin, spiral shape with hooked ends. There are many recognised *Leptospira* spp., however, the causative agents of disease in dogs are primarily of the species *Leptospira interrogans* and *Leptospira kirschneri* (Iverson *et al*., 2021). These species are further classified into serovars based on the expression of surface-exposed lipopolysaccharides (LPS) (Patra *et al*., 2015).

The most incriminated serovars in dogs are Canicola, Ictrohaemorrhagiae, Pomona, Grippothyphosa, Ballum and Bataviae. *Leptospira* spp. able to penetrate intact mucosal surfaces or through abraded skin, leading to bacteraemia or leptospiraemia which lasts for up to 10 days (Reagan & Sykes, 2019). It will then invade the vital organs such as the kidney and liver. *Leptospira* may persist in the proximal tubular lumen of kidneys in a chronic state and thereby shed in the urine.

According to research conducted in 2021 in Klang Valley, Malaysia, a total of one hundred stray dogs were screened for leptospirosis. The prevalence of positive leptospiral antibodies was 32% with serovars detected was Javanica, Bataviae, Icterohaemorrhagiae, Autumnalis, Canicola, Pyrogenes, Copenhageni and Australis. Among enrolled dogs, there is a range of 3% to 50% reported from diverse groups such as pet dogs, shelter dogs and working dogs. These differences may relate to different localities, exposure levels to *Leptospira* vectors and control and preventive measures implemented (Goh *et al*., 2021). This research signifies that these serovars are locally circulating serovars that can be detected among dogs, which can be included for diagnostic test.

Several diagnostic tests are available for the identification of canine leptospirosis which include molecular detection by Polymerase Chain Reaction (PCR) where it allows detection of the bacteria directly and serological detection by Microscopic Agglutination Test (MAT) and Enzyme-linked Immunosorbent Assay (ELISA) that detect antibodies against *Leptospira*.

1.1 Research Problem

Leptospirosis is a zoonotic disease caused by pathogenic *Leptospira* that can affect both humans and animals. Despite *Leptosira* vaccination, dogs are known to be a significant reservoir of the bacteria and are potential sources of human infection. However, there is limited information about the prevalence and serovars of leptospirosis among dogs in Kelantan, Malaysia. Hence, this study aims to determine the seroprevalence and infected *Leptospira* serovars among dogs in Kelantan.

1.2 Research Question

- What is the seroprevalence of leptospirosis among dogs in Kelantan?
- What are the most prevalent *Leptospira* serovars in dogs in Kelantan?

1.3 Research Hypothesis

- There is high seroprevalence of leptospirosis among dogs in Kelantan, Malaysia.
- The most prevalent serovars of *Leptospira* that can be detected among dogs in Kelantan are Canicola, Ictrohaemorrhagiae, Pomona, Grippothyphosa, Ballum and Bataviae.

1.4 Research Objectives

- To determine the seroprevalence of leptospirosis among dogs in Kelantan.
- To identify most prevalent *Leptospira* serovars in dogs in Kelantan.

CHAPTER 2

LITERATURE REVIEW

2.1 Aetiology of Leptospirosis

Leptospirosis is caused by a Gram negative, motile spirochete aerobic bacterium of the genus *Leptospira*. This bacterium belongs to the family Leptospiraceae, order Spirochaetales (Adler and De la Peña Moctezuma, 2010). The bacteria are thin, flexible, motile and filamentous with distinctive hooked ends. They have a typical double membrane structure which the cytoplasmic membrane and peptidoglycan cell wall are closely associated and overlain by an outer membrane. Within outer membrane, LPS constitutes the main antigen for *Leptospira* (Cullen *et al*., 2004). The leptospiral LPS has a composition similar to that of other gram-negative bacteria but has lower endotoxic activity (Levett, 2001).

Leptospira are classified into three categories which is saprophytic, intermediate, and pathogenic *Leptospira* based on their capabilities to cause disease. Saprophytic *Leptospira* able to survive at low temperatures ranging from 5 – 35˚C and found naturally in soil and water. However, they did not have the capability to cause any infections. Members of this group include *Leptospira biflexa, L. meyeri, L. wolbachii, L yanagawae, L. vanthielii* and *L. terpstrae*.

Intermediate *Leptospira* lives between 1 – 37˚C and acts as the biochemical intermediate of saprophytic and pathogenic *Leptospira*. *Leptospira parvais* is capable of co-existing with various saprophytic and pathogenic *Leptospira*. Other significant intermediate *Leptospira* include *Leptospira broomi, L. inadai, L. licerasiae, L. wolffii* and *L. fainei*.

Favourable temperature for pathogenic *Leptospira* ranging from 20 – 35˚C and are usually obtained in rodents. Pathogenic *Leptospira* are significant to healthcare as they are capable of inflicting leptospirosis, subsequently influencing morbidity and mortality

rates. *Leptospira* species include *Leptospira interrogans*, *L. weilii*, *L. noguchii*, *L. borgpetersenii*, *L. kirschneri* and *L. santarosai* (Samrot *et al*., 2021).

2.2 Pathogenesis of Leptospirosis

Leptospira may enters through abrasions or cuts in the skin or via mucous membranes such as conjunctiva. However, the bacteria can enter via intact skin after prolonged immersion in water (Levett, 2001). After entering the host, the bacteria invade bloodstream leading to leptospiremic phase, establishing systemic infection via hematogenous route (Schuller *et al*., 2015). Leptospiremia contributes to severe vasculitis with endothelial damage leading to localised ischemia and alterations in different organs (Azocar-Aedo, 2014). *Leptospira* invade multiple organs, including liver, spleen, kidneys, eyes, central nervous system, and urogenital tract (Greene, 2012).

Leptospira replicates and persists in the renal tubular epithelium and eventually destroy the epithelial cells and tubular damage leading to renal insufficiency and renal failure. Colonisation of *Leptospira* in the kidney leads to acute interstitial nephritis and parenchymal swelling, reduce renal perfusion and the glomerular filtration rate leading to acute impairment of renal function. In the liver, *Leptospira* induces subcellular damage thus leading to hepatic dysfunction. Degree of icterus corresponds to the severity of hepatic necrosis. Prolonged infection results in extensive hepatic fibrosis and hepatic failure (Greene, 2012).

2.3 Predisposing Factors of Leptospirosis

Factors contributes to leptospirosis include exposure to contaminated water, male gender and herding or working dogs (Reagan, 2019). Leptospirosis usually occurs in dogs that are exposed to or drink from rivers, lakes, or streams. In developing countries, access to sewage increases the risk of leptospirosis. Apart from that, male dogs have a higher risk of infection compared to female dogs due to their natural straying behaviour (Azocar-

Aedo, 2014). Herding dogs, working dogs and hounds are at higher risk compared to companion dogs, presumably because of increased outdoor exposure to contaminated water and thus higher concentrations to infective leptospiral organisms (Greene, 2012).

Environmental factors associated with leptospirosis includes high rainfall, flooding, natural disasters, and high temperature. Rainfall, flooding, and natural disaster increase the risk of *Leptospira* infection as it harbours bacteria and bringing the animal hosts into closer contact with other animals and humans. Moreover, *Leptospira* able to survive in higher temperatures and humid environments. Higher temperature reduces surface water availability by evaporation and thus encourage water-based activities for both humans and animals, thereby promoting contact between humans and animals (Lau *et al.*, 2010).

2.4 Diagnosis of Leptospirosis

Diagnosis of leptospirosis can be performed by detecting either antigen (PCR and/or culture isolation) or antibodies (MAT and/or ELISA).

PCR can be used to diagnose the disease during acute phase and considered to be as highly sensitive (Greene, 2012). The limitation of PCR is that it requires large quantity of DNA and unable to identify infecting serovar (Budihal, 2014). The isolation of infectious *Leptospira* strains is important from an epidemiological perspective and able to provide information on the mammalian maintenance hosts involved in transmission.

MAT, a serogroup-specific assay is the gold standard in leptospirosis diagnosis and requires dark-field microscopy and live organisms are grown in liquid media. This method is serovar specific that involves reacting live antigens from different serogroups with serum samples to identify the infecting serovar. Once infected, individual stays MAT positive for several years, making it valuable for epidemiological purpose. However, this procedure is complex and time consuming. MAT unable to distinguish between IgM antibodies, indicative of current infection within the first seven days, and IgG antibodies,

indicative of past infection appearing after one to three weeks (Vijayachari et al., 2007, Abdoel *et al.*, 2011). For the diagnosis of acute disease by MAT, it needs to collect two consecutive samples that shows four-fold antibody titre (Musso & La Scola, 2013).

Furthermore, ELISA is used to detect the IgG, IgM or both antibodies against leptospires. IgG is produced in large quantities approximately 7 to 21 days post-infection while assays that detect IgM identifies antibody response during the first week of infection (Abdoel *et al*., 2011). The advantage of ELISA is that it able to detect IgM antibodies earlier than MAT and it allows rapid processing of large amounts of samples. However, ELISA unable to assess infecting serovar and it is comparatively less specific (Vijayachari *et al.*, 2007).

2.5 Leptospirosis in Dogs

Canine leptospirosis is an infection of *Leptospira* among dogs which spreads through bloodstream. Dogs obtain leptospirosis from puddles or water bodies that contain urine of carrier animals. Common serovars of leptospires isolated from dogs are Canicola, Icterohaemorrhagiae, Canicola and Grippotyphosa. Dogs can harbour *Leptospira* with or without any clinical symptoms. In disease conditions, clinical signs shown depends on age and immunity of the host, environmental factors, and virulence of infecting serovar. Pyrexia, shivering and generalised muscle tenderness are the initial clinical signs in acute leptospirosis followed by vomiting, dehydration, and later peripheral vascular collapse (Greene, 2012).

Furthermore, leptospirosis can result in liver and renal disease resulting in jaundice and renal failure (Garba *et al*, 2021). In healthy dogs, *Leptospira* spp. can inhabit the renal proximal tubules and chronically colonize the proximal tubules of kidney tissue without any clinical symptoms. Live organisms are shed intermittently in the urine, contaminating the surrounding environment, thus they can persist in a favourable warm humid

atmosphere for several weeks and months, until it encounters a new host (Yamaguchi *et al*., 2018).

Based on recent study of canine leptospirosis in Klang Valley, Malaysia in 2021, prevalence of positive leptospiral antibodies was 32% with seven serovars detected include Javanica, Bataviae, Icterohaemorrhagiae, Canicola, Australis, Pyrogenes and Copenhageni (Goh *et al*., 2021).

2.6 Zoonotic Impact

Leptospira is recognised as a widespread zoonosis and is an emerging infectious disease in humans and in dogs (Sykes *et al.,* 2010). Leptospirosis in humans majorly might be acquired from an animal. Human are infected through contact with the urine of an infected animal or a contaminated environment. Exposure during daily activities occurs in people with contact with pet dogs and in areas with rodent infestation.

Leptospirosis may present as per acute, acute, subacute, or chronic disease. However, signs of leptospirosis in dogs vary. Some dogs may not manifest any signs of disease and can become asymptomatic carriers of *Leptospira* in the urine, producing a risk in the context of zoonotic leptospirosis (Sant'Anna da Costa *et al*., 2021).

Leptospirosis can occur in vaccinated dogs. This incidence is possible as the vaccine might not cover all *Leptospira* serovar that is infecting the dog in that geographical area. According to a study conducted in Malaysia in 2022, current local vaccination protocol comprises of four serovars which is Icterohaemorrhagiae, Canicola, Grippothyposa and Pomona. However, seroprevalence of canine leptospirosis ranges from 3 – 50% and other serovars have been insinuated to cause disease in dogs despite good vaccination practice locally (Goh *et al.*, 2022).

CHAPTER 3

REASEARCH METHODOLOGY

3.1 Ethical Approval

This study obtained ethical approval from the Institutional Animal Care and Use Committee (IACUC), Faculty of Veterinary Medicine, Universiti Malaysia Kelantan (Appendix A) under approval code (UMK/FPV/ACUE/RES/001/2023).

3.1 Acquiring Consent and Subject Medical History

Owners and dogs were approached at selected private clinics within Kota Bharu and Veterinary Teaching Hospital, Universiti Malaysia Kelantan (HPVUMK). Prior to blood sample collection, a brief explanation of the study and the procedure was explained to the owners. Once owner agreed, consent form and contact information were signed and collected (Appendix B). Information regarding the patient's signalment, management and medical history was obtained using the study tool (Appendix C). Collected data were tabulated for further analysis.

3.2 Sample Collection

A total of thirty (30) blood samples were collected from dogs within Kelantan, Malaysia in October 2023. About 3 ml of blood were obtained by a licensed veterinarian following universal precautions. The samples were collected into a plain tube, labelled appropriately and dispatched to the laboratory in an ice box.

3.3 Serum Preparation

Blood samples collected in plain tube were allowed to clot by keeping for 30 minutes at room temperature and then centrifuged at 3,500 rpm for 15 minutes for serum separation. The serum was collected using a sterile Pasteur pipette and aliquoted into sterile microcentrifuge tubes and stored at -20 ^{o}C to perform MAT and ELISA.

3.4 ELISA

Serological detection of *Leptospira* spp. was performed using Canine *Leptospira* antibody IgG (LEP-IgG) ELISA Kit (Sunlong Biotech Co., Ltd, China). The micro-ELISA plate provided in this kit has been pre-coated with an antigen specific to canine IgG. Assay was performed according to the manufacturer's instructions.

On the day of ELISA experiment, serum specimens were taken out from the -20 ^{o}C freezer and thawed to room temperature. The ELISA kit was included with positive and negative control. Positive control, negative control, specimens and blank were carried out in duplicate. For positive and negative control wells, 50µl were added into corresponding wells. For the sample wells, 40µl sample dilution buffer was added with 10µl serum sample and mixed well with a gentle shaking. The wells for the blank control were left empty. The microplate was sealed and incubated at 37˚C for 30 minutes. Subsequently the solution was decanted from each well, 350µl of wash buffer was added to each well and soaked for 1-2 minutes. The solution was then decanted from each well and put to dry against a clean absorbent paper. This washing step was repeated 5 times. Fifty microliters of HRP conjugate reagent were added to each well and covered with the plate sealer. It was then incubated for 30 minutes at 37 ^{o}C. The solution was then decanted from each well and washing process repeated five times with the wash buffer as mentioned above. Then, colouring process was performed by adding 50µl Chromogen Solution A and 50µl Solution B to each well, mixed with gentle shaking and incubated for 15 minutes at 37 ˚C. Then 50µl of stop solution was added to each well and the absorbance at 450 nm was measured using ELISA reader. The critical value (cut off) was calculated by adding 0.15 to the average value of negative control. Judgement of positive and negative sample was made by comparing the optical density (OD) value with cut off. Negative judgement was made when the OD value was less than cut off value and positive judgement was made when the OD value was more than or equal to cut off value.

3.5 MAT

Serum samples were sent to Bacteriology Laboratory, Veterinary Laboratory Services Unit, Department of Veterinary Laboratory Diagnostics, Faculty of Veterinary Medicine, Universiti Putra Malaysia to obtain single MAT antibody titers. MAT titers were obtained using a panel of 06 *Leptospira* serogroup strains namely Canicola, Grippothyposa, Pomona, Icterohaemorrhagiae, Ballum and Batavaie. MAT titer of ≥1:100, the cut off value recommended by World Organisation for Animal Health for the diagnosis of leptospirosis in animals in Malaysia was considered as positive (Alashraf *et al.*, 2020).

CHAPTER 4

RESULT

In this study, a total of thirty dogs were sampled from different clinics in Kelantan, Malaysia. Thirty blood samples were subjected for both ELISA and MAT to detect the presence of *Leptospira* antibodies.

4.1 Demographic Data

Table 1 shows the demographic data on tested animals in the study. Of thirty dogs sampled, 57% were female and 43% were male. Their mean age was 3 years; 10% were young which aged less than 1 year old, 73% were adult, aged ranging from 1 to 6 years old and 17% were a senior dogs aged more than 6 years old. Furthermore, 47% of the dogs were managed indoor and the remaining 53% dogs were outdoors. Among thirty dogs, fourteen were vaccinated and 16 dogs were unvaccinated.

Table 1 Demographic Data on Animals Tested in the Study (n = 30)

	No. of animals	Percentage(%)
Gender		
Male	13	43%
Female	17	57%
Age group		
Young (≤ 1 y)	3	10%
Adult (1 to <6 y)	22	73%
Senior (≥ 6 y)	5	17%
Management		
Indoor	14	47%
Outdoor	16	53%
Vaccination status		
Vaccinated	14	47%
Unvaccinated	16	53%

4.2 Canine IgG ELISA

Corrected OD value for positive and negative controls were 1.2940 and 0.0125 respectively. The critical value of 0.1625 was calculated based on cut off value calculation. Result above cut-off value considered as positive and result below cut off value considered as negative. The OD value was ranged from 0.004 to 0.069 with a median value of 0.0239 for tested 30 serum samples (Appendix D). Among thirty canine serum samples, none of the sample were found leptospiral canine IgG positive according to ELISA.

4.3 MAT

Of the thirty samples, all samples showed negative results towards all 6 *Leptospira* serovars which is Canicola, Icterohaemorrhagiae, Grippotyphosa, Pomona, Ballum and Bataviae. The titre ≥ 1:100 was used as cut-off value.

CHAPTER 5

DISCUSSION

Canine leptospirosis, a globally significant zoonotic disease, is induced by diverse serovars of *Leptospira*. It poses substantial public health concerns due to the potential for human infection through close interaction with dogs, as affirmed by a study conducted in Klang Valley that supports the role of dogs in the transmission of *Leptospira* to humans from animal (Lau *et al.,* 2010). The susceptibility of dogs to leptospirosis is likely associated with their innate roaming behaviour. Additionally, dogs that roam in open fields or have access to water bodies, engaging in activities such as swimming, have a higher risk of exposure to the bacteria (Azócar-Aedo, Smits and Monti, 2014).

This study aims to ascertain the seroprevalence of leptospirosis among dogs in Kelantan and to identify the predominant *Leptospira* serovars affecting these dogs. Serological detection through ELISA provides high specificity and sensitivity resulting from the antigen-antibody reaction (Sakamoto *et al.,* 2017). Additionally, MAT is the most widely recommended diagnostic test for leptospirosis diagnosis and considered to be as the gold standard. It is predicated on the assessment of serum dilutions' capacity to agglutinate live leptospiral serovars in vitro. Reactivity to a specific serovar suggests exposure to a serovar belonging to the corresponding serogroup (Schuller *et al.,* 2015).

During investigation, ELISA kit was used for the identification of canine IgG antibodies against *Leptospira*. Additionally, the MAT was used to obtain estimate antibody titre against leptospires present in the serum. While these assays yield rapid results, they share limitations associated with potential antibody absence during early infection or presence due to recent vaccination (Schuller *et al*., 2015).

The outcome of this study indicates the absence of detectable canine IgG in all thirty samples, suggesting the absence of infections with canine leptospirosis in the sampled dogs. This result is likely attributable to limited exposure to predisposing factors of leptospirosis, including contact with contaminated water, intense rainfall, and flooding.

Moreover, irrespective of their gender, age, management and vaccination status, all dogs exhibit negative results in the MAT. The negative results observed may be attributed to a limited sample size or the absence of locally circulating serovars in the test panel. An incomplete panel could lead to false negative outcomes. Sensitivity of the test can be improved by incorporating local isolates, considering that the dogs may be infected with serovars that is not included in the panel. Based on a study conducted in 2021, the primary *Leptospira* serovars observed in Selangor, Malaysia are predominantly Bataviae, Javanica, Icterohaemorrhagiae, Australis, Ballum, Hardjobovis, Malaysia and Pomona. (Rahman *et al.*, 2021). Therefore, it is recommended to incorporate all prevalent local serovars in the MAT panel, considering the shared geographical location of Kelantan and Selangor within Malaysia. This approach could potentially provide indications as to whether the *Leptospira* serovars affecting dogs in Selangor are similarly prevalent in dogs in Kelantan.

While MAT is widely acknowledged as the gold standard for detecting leptospirosis, its efficacy is particularly notable in diagnosing acute infections, revealing limitations when applied to chronic infections. Additionally, in cases of natural infections, IgG titres may not manifest until two- or three-weeks post-infection and peak at one month after initial infection (Azocar-Aedo, 2013).

This study was not able to establish the prevalence of leptospirosis among dogs in Kelantan as both ELISA and MAT used for *Leptospira* detection, yielded negative results.

CHAPTER 6

CONCLUSION

In conclusion, this study concludes that the thirty sampled dogs exhibit no evidence of leptospirosis from both test which is ELISA and MAT. Nevertheless, it is imperative to acknowledge that a negative result does not eliminate the possibility of leptospirosis infections among other dogs in the same region.

Further investigations are required to ascertain the prevalence of leptospirosis among dogs in Kelantan, necessitating an expanded sample size that includes specimens from stray dogs or post-floodings period, given the recognised predisposition of flooding to canine leptospirosis. Additionally, efforts to enhance awareness of leptospirosis are essential as it possess significant implications for public health.

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APPENDICES

APPENDIX A

UNIVERSITI MALAYSIA KELANTAN

Fakulti Perubatan Veterinar

RUJ. KAMI *(Our Ref.)* : UMK/FPV/ACUE/RES/001/2023
TARIKH *(Date)* : 20 MARCH 2023

DR. GIGURUWA GAMAGE THILINI NISANSALA
Principal Investigator
Faculty of Veterinary Medicine
Universiti Malaysia Kelantan

Dear Dr,

APPROVAL OF INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) TO CONDUCT RESEARCH INVOLVING ANIMALS

We are pleased to inform you that your application for approval to conduct research from Institutional Animal Care and Use Committee (IACUC), Faculty of Veterinary Medicine, Universiti Malaysia Kelantan has been approved. Please refer the table below for approval code:

APPROVAL CODE	*UMK/FPV/ACUE/RES/001/2023*
TITLE	*ELUCIDATION OF LEPTOSPIRA OCCURRENCE IN COMPANION DOGS IN KELANTAN*

3. You are advised to always follow "3R" (REDUCE, REFINE, & REPLACE) and all animal ethics and animal welfare principles to reduce suffering in animal.

Thank you.

"ALLAH DITAATI, RAJA DISANJUNGI, RAKYAT DIKASIHI"
"MALAYSIA MADANI"
"BERKHIDMAT UNTUK NEGARA"

Yours sincerely,

DR. NOR FADHILAH BINTI KAMARUZZAMAN
Chairman
Institutional Animal Care and Use Committee
Faculty of Veterinary Medicine
Universiti Malaysia Kelantan

UNIVERSITI MALAYSIA KELANTAN اونيۏرسيتي مليسيا كلنتن | Karung Berkunci *(Locked Bag)* 36, Pengkalan Chepa, 16100 Kota Bharu, KELANTAN, MALAYSIA | Tel : 609 771 7277 Fax : 609 771 7282

FACULTY OF VETERINARY MEDICINE
UNIVERSITI MALAYSIA KELANTAN

Borang Persetujuan Pelanggan

Tajuk Penyelidikan: Penjelasan mengenai jangkitan Leptospira dikalangan anjing peliharaan di Kelanta.

Kami ingin menjemput anda untuk mengambil bahagian dalam kajian untuk mengesan kehadiran Leptospira pada anjing.

Untuk menjalankan kajian ini, kami ingin mendapatkan persetujuan anda untuk mengumpul sampel darah dan air kencing daripada anjing untuk digunakan bagi kultur Leptospira, serologi dan ujian molekul. Pengumpulan sampel akan dikendalikan oleh doktor haiwan yang berdaftar.

Setiap langkah akan diambil untuk memastikan proses pengumpulan sampel dilakukan dengan cermat dengan risiko yang minimum atau sifa kepada anjing peliharaan.
Jika anda memutuskan untuk mengambil bahagian dalam projek ini, kami menawarkan ujian diagnostik Leptospirosis percuma anggaran RM200 untuk anjing anda.
Kami akan memastikan bahawa semua maklumat peribadi yanh diperoleh akan dirahsiakan. Kami akan memaklumkan kepada anda mengenai keputusan ujian diagnostik yang dijalankan kerana Leptospirosis merupakan penyakit zoonotik.
Terima kasih atas penyertaan anda dan telah membantu kami dalam kajian ini.

Saya secara sukarela bersetuju untuk mengambil bahagian daam projek ini. Saya faham bahawa saya boleh menarik balik persetujuan saya pada bila-bila masa. Saya dengan ini memberi persetujuan untuk penyertaan dalam projek ini.

Nama :_____Nama Haiwan:_____No. Case: _____

Tandatangan: _____

Email : _____

Tarikh: _____

Individu untuk dihubungi sekiranya timbul sebarang masalah: Dr. Thilini Nisansala (019-8909753) / Dr. Intan Noor Aina Binti Kamaruzaman (013-2243453) Dr. Mohammad Sabri Bin Abdul Rahman (013-6339874) Email address: thilini@umk.edu.my / intanaina@umk.edu.my / sabri.ar@umk.edu.my

Questionnaire (version 1)

Elucidation of *Leptospira* occurrence in companion dogs in Kelantan

Case number: ..... Date: .....

Hospital: ..... Specimen number: .....

Pet's Information

1. Dog's Name: .....
2. Dog's Age: .....
3. Dog's Breed: .....
4. Sex of the Dog: Male ☐ Female ☐ Entire ☐ Sterilized ☐
5. Place of residence: Urban area ☐ Rural area ☐
6. Housing of your Dog: Free roaming/outdoor ☐ Indoor ☐ Caged ☐
7. Are there any other pets in the home? Yes ☐ No ☐

   If yes, please check all that apply and indicate the number of additional pets.

   Dogs .....

   Cats .....

   Other (Specify) .....
8. Dog's vaccination status: Yes ☐ No ☐

   If yes, whether dog vaccinated against leptospirosis: Yes ☐ No ☐

   If yes, number of vaccines received: .....

   Date of last vaccination against leptospirosis: .....
9. Does your dog has any medical condition, Yes ☐ No ☐

   If yes, what is the condition? .....
10. Is your dog receiving any medication currently? Yes ☐ No ☐

    If yes, which medications? .....
11. Has your dog received antibiotics during the past six months? Yes ☐ No ☐
12. Does your dog come into any contact with rats? Yes ☐ No ☐

Questionnaire (version 1)

13. Does your dog drink water outside? Yes ☐ No ☐

    If yes, where? ..... (puddles, ditches, lakes, sea etc.)
14. Does your dog have any recent outdoor/recreational activity? Yes ☐ No ☐

    If yes, what is it? ..... (Outdoor walk, swimming in sea etc.)
15. Does your dog expose to flooding recently?

    If yes, month and year of exposure .....

Samples obtained.

Blood ☐

Urine ☐

Voided ☐

Manual compression ☐

Cystocentesis ☐

ELISA RESULT

READING 1				READING 2			
SAMPLE	**RAW**	**CORRECTED**	**RESULT**	**SAMPLE**	**RAW**	**CORRECTED**	**RESULT**
POSITIVE	1.539	1.486		POSITIVE	1.156	1.102	
NEGATIVE	0.064	0.011		NEGATIVE	0.068	0.014	
1	0.065	0.011	N	1	0.062	0.008	N
2	0.069	0.015	N	2	0.072	0.019	N
3	0.065	0.011	N	3	0.075	0.022	N
4	0.063	0.01	N	4	0.064	0.011	N
5	0.085	0.032	N	5	0.08	0.026	N
6	0.08	0.026	N	6	0.073	0.02	N
7	0.071	0.017	N	7	0.066	0.013	N
8	0.06	0.006	N	8	0.068	0.014	N
9	0.065	0.011	N	9	0.067	0.014	N
10	0.07	0.016	N	10	0.074	0.021	N
11	0.07	0.017	N	11	0.069	0.015	N
12	0.081	0.028	N	12	0.061	0.007	N
13	0.067	0.013	N	13	0.06	0.006	N
14	0.061	0.007	N	14	0.057	0.004	N
15	0.063	0.009	N	15	0.06	0.006	N
16	0.066	0.013	N	16	0.123	0.069	N
17	0.081	0.027	N	17	0.072	0.018	N
18	0.066	0.012	N	18	0.063	0.009	N
19	0.066	0.013	N	19	0.06	0.006	N
20	0.064	0.01	N	20	0.065	0.012	N
21	0.07	0.017	N	21	0.073	0.019	N

22	0.079	0.025	N	22	0.066	0.013	N
23	0.111	0.062	N	23	0.099	0.049	N
24	0.093	0.044	N	24	0.084	0.035	N
25	0.077	0.028	N	25	0.105	0.056	N
26	0.115	0.066	N	26	0.112	0.063	N
27	0.089	0.04	N	27	0.088	0.038	N
28	0.104	0.055	N	28	0.108	0.059	N
29	0.087	0.038	N	29	0.086	0.036	N
30	0.09	0.041	N	30	0.08	0.03	N

AVERAGE	
1.294	**Positive**
0.0125	**Negative**

CRITICAL VALUE
0.1625

JUDGEMENT	
OD Value < 0.1625	N
OD VALUE ≥ 0.1625	P