CHARACTERISATION OF *Leptospira* spp. AND RISK FACTORS OF LEPTOSPIROSIS IN DOGS DIAGNOSED WITH KIDNEY AND/OR LIVER DISEASE

By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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DEDICATION

I would like to dedicate this thesis to my mother, Sa'adiah Samad and my father, Abdul Rahman Autan. Without their kindness, generosity, and encouragement I would have been ploughing land in a remote village in Malaysia.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement of the degree of Doctor of Philosophy

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Leptospirosis is a serious bacterial disease that affects both humans and animals. A wide range of symptoms have been reported in humans; the disease in dogs is commonly associated with kidney and/or liver disease. In Malaysia, information about the common serovars infecting dogs is limited. Therefore, this study investigated the occurrences of leptospirosis in 124 pet dogs diagnosed with kidney and/or liver disease and documented information such as signalment, medical history, management, and clinical findings. Altogether, 124 whole blood, 124 sera, 113 urine, four abdominal effusions, 23 kidneys and 23 livers were collected from the dogs. The samples were analysed using microscopic agglutination test (MAT), polymerase chain reaction (PCR) and histopathological methods [Hematoxylin and Eosin (H&E); Warthin Starry (WS) staining]. Statistical analyses were descriptively analysed, and risk analyses were performed using Pearson chi-square tests and logistic regression. This study evaluated TaqMan® real-time PCR (qPCR)

assays, namely *LipL32(1)* and *LipL32(2)*, used in the diagnosis of leptospirosis and investigated its applicability in clinical samples from dogs diagnosed with kidney and/or liver disease, in comparison with conventional PCR used as a reference.

Based on MAT, 53 of 124 (42.7%) dogs were seropositive for leptospiral exposure. Sera were frequently positive for serovars Bataviae (n = 12), Javanica (n = 10), and Icterohaemorrhagiae (n = 10). Direct PCR detection showed that 42 of 124 (33.9%) of the whole blood and 36 of 113 (31.9%) urine samples were positive for pathogenic *Leptospira* spp. By PCR, 2 of 23 (9.1%) kidneys and 2 of 23 (9.1%) livers from four different dogs were positive for pathogenic *Leptospira* spp. Abdominal effusion from four dogs was PCR-positive for pathogenic *Leptospira* spp. The species detected were *L. interrogans*, *L. borgpetersenii*, *L. kirschneri*, and *L. kmetyi* by partial 16S rRNA sequencing. Eleven leptospiral isolates were successfully recovered from 8 dogs and were further identified and characterised as serovars Bataviae, Javanica, and Australis. In addition, the mortality rate of the *Leptospira*-infected dogs was high (18 of 53; 34%).

Medium and large dog breeds with a history of exposure to rats and managed outdoors had a greater risk for leptospirosis (p = 0.045). The significant predictors for the dogs' positivity were the presence of rats (p = 0.003) and acute clinical illness (p = 0.047). Administration of antibiotics (p = 0.036) and the detection of clinical illness at an early stage of the disease (p = 0.04) improved the survivability of the dogs. Identifying the profile of dogs at risk of

leptospirosis could be helpful in the design of diagnostic and treatment strategies, as well as increasing awareness for the prevention of the disease.

Results showed that the *LipL32(1)* qPCR assay was shown to have more diagnostic value than the *LipL32(2)* qPCR assay. Hence, the *LipL32(1)* qPCR assay was further analysed and revealed that the assay was able to detect DNA copies as low as 5 per reaction with high specificity against the tested leptospiral strains. No cross-amplification was observed with the other organisms. During the investigation using the clinical samples, the *LipL32(1)* qPCR assay had 100.0% sensitivity and more than 75.0% specificity. This suggested that the *LipL32(1)* qPCR assay is sensitive, specific and has the potential to be applied in a more extensive subsequent study.

The canine kidneys and livers of the *Leptospira*-infected dogs (n = 18) showed relevant histopathological lesions of canine leptospirosis, consisting of interstitial nephritis, glomerular atrophy and tubular necrosis and varying degrees of vascular and degenerative changes with scattered areas of mononuclear cell infiltration in the liver. Using WS staining, spirochete organisms were also found in the kidneys (n = 3) and livers (n = 2). Histopathology assessment of tissue samples is essential for a definitive diagnosis of the lesions and antigen demonstration that could support the evidence of canine leptospirosis.

This study has shown that at least 1 in 3 dogs with kidney and/or liver disease in Malaysia can potentially have leptospirosis. Veterinarians examining dogs

with these conditions should consider leptospirosis as a differential diagnosis. Furthermore, the isolation of serovars Bataviae, Javanica, and Australis is essential, given their absence in commercial vaccines. Therefore, proper characterisation of leptospiral isolates is critical for providing evidence-based knowledge to support developing and commercialising multivalent vaccines containing serovars circulating among local populations.

Keywords: diagnosis, dogs, kidney disease, leptospirosis, liver disease

SDG: GOAL 3: Good health and well-being

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

CIRI-CIRI *Leptospira* spp. DAN FAKTOR-FAKTOR RISIKO LEPTOSPIROSIS DALAM ANJING YANG DIDIAGNOSIS DENGAN PENYAKIT BUAH PINGGANG DAN/ATAU HATI

Oleh

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Leptospirosis adalah penyakit bakteria serius yang memberi kesan kepada manusia dan haiwan. Pelbagai gejala telah dilaporkan pada manusia; penyakit pada anjing biasanya dikaitkan dengan penyakit buah pinggang dan/atau hati. Di Malaysia, maklumat tentang serovar yang biasa menjangkiti anjing adalah terhad. Oleh itu, kajian ini menyiasat kejadian leptospirosis dalam 124 anjing peliharaan yang didiagnosis dengan penyakit buah pinggang dan/atau hati dan maklumat yang didokumenkan seperti pengenalan anjing, sejarah perubatan, pengurusan dan penemuan klinikal. Secara keseluruhan, 124 darah, 124 sera, 113 air kencing, 4 efusi perut, 23 buah pinggang dan 23 hati telah dikumpulkan daripada anjing. Sampel dianalisis menggunakan ujian aglutinasi mikroskopik (MAT), tindak balas rantai polimerase (PCR) dan kaedah histopatologi [Hematoxylin dan Eosin (H&E); Pewarnaan Warthin Starry (WS). Analisis statistik dianalisis secara deskriptif, dan analisis risiko dilakukan menggunakan ujian Pearson chi-square dan regresi logistik. Kajian

ini menilai ujian TaqMan® masa nyata PCR (qPCR), iaitu *LipL32(1)* dan *LipL32(2)*, yang digunakan dalam diagnosis leptospirosis dan menyiasat kebolehgunaannya dalam sampel klinikal daripada anjing yang didiagnosis dengan penyakit buah pinggang dan/atau hati, dalam perbandingan dengan PCR konvensional yang digunakan sebagai rujukan.

Berdasarkan MAT, 53 daripada 124 (42.7%) anjing adalah seropositif untuk pendedahan jangkitan leptospiral. Sera positif untuk serovar Bataviae (n = 12), Javanica (n = 10), dan Icterohaemorrhagiae (n = 10). Pengesanan terus menggunakan PCR menunjukkan bahawa 42 daripada 124 (33.9%) sampel darah dan 36 daripada 113 (31.9%) sampel air kencing adalah positif untuk patogenik *Leptospira* spp. Dengan PCR, 2 daripada 23 (9.1%) buah pinggang dan 2 daripada 23 (9.1%) hati dari empat anjing yang berbeza adalah positif untuk patogenik *Leptospira* spp. Efusi perut daripada 4 ekor anjing adalah PCR-positif untuk patogenik *Leptospira* spp. Spesies yang dikesan ialah *L. interrogans*, *L. borgpetersenii*, *L. kirschneri*, dan *L. kmetyi* oleh penjujukan separa 16S rRNA. Sebelas pencilan leptospiral berjaya ditemui daripada 8 ekor anjing dan seterusnya dikenal pasti dan dicirikan sebagai serovar Bataviae, Javanica dan Australis. Di samping itu, kadar kematian anjing yang dijangkiti *Leptospira* adalah tinggi (18 daripada 53; 34%).

Baka anjing sederhana dan besar dengan sejarah terdedah kepada tikus dan dijaga di luar rumah mempunyai risiko yang lebih besar untuk leptospirosis (p = 0.05). Ramalan penting untuk kepositifan anjing adalah kehadiran tikus (p = 0.003) dan penyakit klinikal akut (p = 0.047). Pemberian antibiotik (p = 0.036)

dan pengesanan penyakit klinikal pada peringkat awal penyakit (p = 0.04) meningkatkan kemandirian anjing. Mengenal pasti profil anjing berisiko leptospirosis boleh membantu dalam reka bentuk strategi diagnostik dan rawatan, serta meningkatkan kesedaran untuk pencegahan penyakit tersebut.

Keputusan menunjukkan bahawa ujian qPCR *LipL32(1)* mempunyai lebih banyak nilai diagnostik berbanding ujian qPCR *LipL32(2)*. Oleh itu, ujian qPCR *LipL32(1)* dianalisis selanjutnya dan mendedahkan bahawa ujian itu dapat mengesan salinan DNA serendah 5 setiap tindak balas dengan kekhususan tinggi terhadap strain leptospiral yang diuji. Tiada amplifikasi silang diperhatikan dengan organisma lain. Semasa penyiasatan menggunakan sampel klinikal, ujian qPCR *LipL32(1)* mempunyai sensitiviti 100.0% dan lebih daripada 75.0% kekhususan. Ini mencadangkan bahawa ujian qPCR *LipL32(1)* adalah sensitif, khusus dan berpotensi untuk digunakan dalam kajian seterusnya yang lebih meluas.

Buah pinggang dan hati anjing yang dijangkiti *Leptospira* (n = 18) menunjukkan lesi histopatologi yang berkaitan dengan leptospirosis anjing, yang terdiri daripada nefritis interstisial, atrofi glomerular dan nekrosis tiub dan pelbagai peringkat perubahan vaskular dan degeneratif dengan kawasan penyusupan sel mononuklear yang bertaburan dalam hati. Melalui penggunaan pewarnaan WS, organisma spirochete juga ditemui dalam buah pinggang (n = 3) dan hati (n = 2). Penilaian histopatologi sampel tisu adalah penting untuk mencapai diagnosis definitif lesi dan demonstrasi antigen yang boleh menyokong bukti leptospirosis anjing.

Kajian ini telah menunjukkan bahawa sekurang-kurangnya 1 dalam 3 anjing dengan penyakit buah pinggang dan/atau hati di Malaysia berpotensi menghidap leptospirosis. Doktor haiwan yang memeriksa anjing dengan keadaan ini harus mempertimbangkan leptospirosis sebagai salah satu diagnosis pembezaan. Tambahan pula, pengasingan serovar Bataviae, Javanica, dan Australis adalah penting, memandangkan ketiadaannya dalam vaksin komersial. Oleh itu, pencirian yang betul bagi isolat leptospiral adalah penting untuk menyediakan pengetahuan berasaskan bukti untuk menyokong pembangunan dan pengkomersilan vaksin multivalen yang mengandungi serovar yang beredar di kalangan anjing tempatan.

Kata Kunci: anjing, diagnosis, leptospirosis, penyakit buah pinggang, penyakit hati

SDG: MATLAMAT 3: Kesihatan Baik dan Kesejahteraan

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

%	percentage
µl	microliter
µm	micrometer
µmol	micromoles
5-FU	5-fluorouracil
AKI	acute kidney injury
ALP	alkaline phosphatase
ALT	alanine transaminase
APPT	activated partial thromboplastin time
BUN	blood urea nitrogen
CI	confidence interval
Ct	cycle threshold
DIC	disseminated intravascular coagulation
DNA	deoxyribonucleic acid (s)
EDTA	ethylene-diamine-tetraacetic-acid
ELISA	enzyme-linked immunosorbent assay
EMJH	Ellinghausen and McCullough modified by Johnson and Harris
GFR	glomerular filtration rate
GspD	general secretory protein D
H&E	Hematoxylin and Eosin
IF	immunofluorescence
IgG	immunoglobulin G
IgM	immunoglobulin M
IP	immunoperoxidase
IP	immunoperoxidase

L	liter
LOD	limit of detection
LPS	lipopolysacharide
MAT	microscopic agglutination test
ml	mililiter
MLST	multilocus sequence typing
mmol	millimoles
mPCR	multiplex polymerase chain reaction
NaCl	sodium chloride
NCBI	National Centre for Biotechnology Information
NPV	negative predictive value
OmpL1	outer membrane protein leptospiral 1
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PPV	positive predictive value
PT	prothrombin time
PUPD	polyuria and polydipsia
qPCR	quantitative real-time PCR
rpm	revolutions per minute
rRNA	ribosomal ribonucleic acid
TBE	tris-borate-EDTA
U/L	units per liter
WGS	whole genome sequencing
WS	Warthin Starry
WSAVA	World Small Animal Veterinary Association

CHAPTER 1

INTRODUCTION

The causative agent of canine leptospirosis consists of various strains of the *Leptospira* bacteria and can lead to a range of symptoms, from mild to severe. Dogs can contract leptospirosis through exposure to contaminated water, soil, or the urine of infected animals, especially rodents. Leptospirosis is a zoonotic disease that can be transmitted from animals to humans. People in close contact with infected dogs should take precautions to prevent transmission, including proper hygiene practices and avoiding contact with the dog's bodily fluids (Schuller *et al.,* 2015).

Clinical signs of leptospirosis in dogs can vary widely. They may include fever, muscle pain, stiffness, lethargy, loss of appetite, vomiting, diarrhoea, jaundice, increased thirst and urination, kidney and liver dysfunction, respiratory symptoms and bleeding. It is currently not known whether specific serovars are associated with specific clinical signs of leptospirosis in dogs, and there is no published evidence to guide therapy based on serovar identification (Sykes *et al.,* 2011). However, veterinarians must maintain a high index of suspicion for leptospirosis because this is a zoonotic disease with a wide range of clinical presentations in dogs. Any age, breed, or sex of the dog is susceptible to leptospirosis, and the diagnosis should not be excluded based on signalment or lifestyle. Canine leptospirosis is not restricted to large-breed dogs, male dogs, or dogs with a predominantly outdoor lifestyle (Lunn, 2022; Taylor *et al.,* 2021).

Diagnosing canine leptospirosis involves a combination of clinical evaluation, blood tests, and sometimes urine tests. Specific blood tests can detect antibodies or DNA of the bacteria in dogs. Diagnosing and treating the disease promptly is essential, as severe cases can lead to kidney and liver failure and even death (Budihal and Perwez, 2014). Prevention and management of canine leptospirosis include vaccination, hygiene, rodent control, monitoring clinical signs, isolation and environmental management. If a dog has been exposed to leptospirosis or shows clinical signs, consult a veterinarian immediately. Early diagnosis and treatment can improve the chances of a positive outcome (Reagan & Sykes, 2019).

In Malaysia, knowledge of the leptospiral infection in dogs diagnosed with kidney and/or liver disease is essential for epidemiologic studies. However, it is not well documented. Moreover, little is known about the association between leptospiral infection and the risk factors of dogs diagnosed with kidney and/or liver disease. Despite currently available diagnostic methods, there is still a limitation in diagnosing leptospiral infection in dogs earlier. Therefore, this study was conducted to:

1. Detect leptospiral infection in dogs diagnosed with kidney and/or liver disease using serological and molecular methods.
2. Isolate and identify *Leptospira* spp. in dogs diagnosed with kidney and/or liver disease.
3. Determine risk factors between leptospiral infection and dogs diagnosed with kidney and/or liver disease.
4. Quantify *Leptospira* spp. from infected dogs using quantitative real-time polymerase chain reaction (qPCR).

5. Describe the histopathology of kidney and liver form infected dogs using histological methods.

Thus, the hypotheses for this study were:

1. Leptospiral infection in dogs diagnosed with kidney and/or liver disease can be detected using serological and molecular methods.
2. *Leptospira spp.* can be isolated from dogs diagnosed with kidney and/or liver disease.
3. There is an association between leptospiral infection and dogs diagnosed with kidney and/or liver disease.
4. *Leptospira* spp. from infected dogs can be quantified using qPCR.
5. Histopathology of kidney and liver samples from infected dogs can be described using histological methods.

REFERENCES

Adamus, C., Buggin-Daubié, M., Izembart, A., Sonrier-Pierre, C., Guigand, L., Masson, M. T., André-Fontaine, G., & Wyers, M. (1997). Chronic hepatitis associated with leptospiral infection in vaccinated beagles. *Journal of comparative pathology*, 117(4), 311–328. https://doi.org/10.1016/s0021-9975(97)80079-5

Adler, B., & De La Peña Moctezuma, A. (2010). *Leptospira* and leptospirosis. *Veterinary Microbiology*, *140*(3–4), 287–296. https://doi.org/10.1016/j.vetmic.2009.03.012

Adler, B., Lo, M., Seemann, T., & Murray, G. L. (2011). Pathogenesis of leptospirosis: the influence of genomics. *Veterinary Microbiology*, 153(1-2), 73–81. https://doi.org/10.1016/j.vetmic.2011.02.055

Agudelo-Flórez, P., Londoño, A. F., Quiroz, V. H., Angel, J. C., Moreno, N., Loaiza, E. T., Muñoz, L. F., & Rodas, J. D. (2009). Prevalence of *Leptospira* spp. in urban rodents from a groceries trade center of Medellin, Colombia. *The American Journal of Tropical Medicine and Hygiene*, 81(5), 906–910. https://doi.org/10.4269/ajtmh.2009.09-0195

Ahmed, A., & Grobusch, M. P. (2012a). Molecular approaches in the detection and characterization of *Leptospira*. *Journal of Bacteriology and Parasitology*, 03(02). https://doi.org/10.4172/2155-9597.1000133

Ahmed, S. A., Sandai, D. A., Musa, S., Hoe, C. H., Riadzi, M., Lau, K. L., & Tang, T. H. (2012b). Rapid diagnosis of leptospirosis by multiplex PCR. *The Malaysian Journal of Medical Sciences*, 19(3), 9–16.

Alashraf, A. R., Lau, S. F., Khairani-Bejo, S., Khor, K. H., Ajat, M., Radzi, R., Roslan, M. A., & Abdul Rahman, M. S. (2020). First report of pathogenic *Leptospira* spp. isolated from urine and kidneys of naturally infected cats. *Plos One*, 15(3), e0230048. https://doi.org/10.1371/journal.pone.0230048

Alton, G. D., Berke, O., Reid-Smith, R., Ojkic, D., & Prescott, J. F. (2009). Increase in seroprevalence of canine leptospirosis and its risk factors, Ontario 1998-2006. *Canadian Journal of Veterinary Research*, 73(3), 167–175.

Alturkistani, H. A., Tashkandi, F. M., & Mohammedsaleh, Z. M. (2015). Histological Stains: A Literature Review and Case Study. *Global Journal of Health Science*, 8(3), 72–79. https://doi.org/10.5539/gjhs.v8n3p72

Andre-Fontaine G. (2006). Canine leptospirosis--do we have a problem?. *Veterinary Microbiology*, 117(1), 19–24. https://doi.org/10.1016/j.vetmic.2006.04.005

Awosanya, E. J., Nguku, P., Oyemakinde, A., & Omobowale, O. (2013). Factors associated with probable cluster of leptospirosis among kennel workers in Abuja, Nigeria. *The Pan African Medical Journal*, 16, 144. https://doi.org/10.11604/pamj.2013.16.144.3529

Azali, M. A., Yean Yean, C., Harun, A., Aminuddin Baki, N. N., & Ismail, N. (2016). Molecular characterization of *Leptospira* spp. in environmental samples from North-Eastern Malaysia revealed a pathogenic strain, *Leptospira alstonii*. *Journal of Tropical Medicine*, 2060241. https://doi.org/10.1155/2016/2060241

Azizi, S., Kheirandish, R., & Rahimi, E. (2014). Comparison of polymerase chain reaction and Warthin-Starry techniques to detect *Leptospira* spp. in kidneys of slaughtered cattle. *The Onderstepoort Journal of Veterinary Research*, 81(1), e1–e6. https://doi.org/10.4102/ojvr.v81i1.821

Azocar-Aedo, L., & Monti, G. (2016). Meta-Analyses of factors associated with leptospirosis in domestic dogs. *Zoonoses and Public Health*, 63(4), 328–336. https://doi.org/10.1111/zph.12236

Azócar-Aedo, L., & Monti, G. (2022). Seroprevalence of pathogenic *Leptospira* spp. in domestic dogs from southern Chile and risk factors associated with different environments. *Preventive Veterinary Medicine*, 206, 105707. https://doi.org/10.1016/j.prevetmed.2022.105707

Azocar-Aedo, L., Hl, S., & Monti, G. (2014). Leptospirosis in dogs and cats: epidemiology, clinical disease, zoonotic implications and prevention. *Archivos De Medicina Veterinaria*, 46(3), 337–348. https://doi.org/10.4067/s0301-732x2014000300002

Badrul Hisham, A.S., Nor Azian Shaharom, C.M.D., Marzukhi, M.I., Norli, R., Fatimah, O., Kee, K.F., Arbaiah, O., Yusof, M. & Adam, A.M. (2009). Spectrum of floodrelated diseases encountered during flood disaster in Johore, Malaysia. *Malaysian Journal of Community Health*, 15, 15-23.

Bal, A. E., Gravekamp, C., Hartskeerl, R. A., De Meza-Brewster, J., Korver, H., & Terpstra, W. J. (1994). Detection of leptospires in urine by PCR for early diagnosis of leptospirosis. *Journal of Clinical Microbiology*, 32(8), 1894–1898. https://doi.org/10.1128/jcm.32.8.1894-1898.1994

Bancroft, J.D. and Gamble, M. (2007). Theory and Practice of Histological Techniques, 6th Edn., Churchill Livingstone Publishers, London, UK.

Baskerville, A. (1986). Histopathological aspects of diagnosis of leptospirosis. *Current Topics in Veterinary Medicine and Animal Science*, 36, 33–43.

Bedir, O., Kilic, A., Atabek, E., Kuskucu, A. M., Turhan, V., & Basustaoglu, A. C. (2010). Simultaneous detection and differentiation of pathogenic and nonpathogenic *Leptospira* spp. by multiplex real-time PCR (TaqMan) assay. *Polish Journal of Microbiology*, 59(3), 167–173.

Benacer, D., Mohd Zain, S. N., Sim, S. Z., Mohd Khalid, M. K., Galloway, R. L., Souris, M., & Thong, K. L. (2016b). Determination of *Leptospira borgpetersenii* serovar Javanica and *Leptospira interrogans* serovar Bataviae as the persistent *Leptospira* serovars circulating in the urban rat populations in Peninsular Malaysia. *Parasites and Vectors*, 9, 117. https://doi.org/10.1186/s13071-016-1400-1

Benacer, D., Thong, K. L., Ooi, P. T., Souris, M., Lewis, J. W., Ahmed, A. A., & Mohd Zain, S. N. (2017). Serological and molecular identification of *Leptospira* spp. in swine and stray dogs from Malaysia. *Tropical Biomedicine*, 34(1), 89–97.

Benacer, D., Thong, K. L., Verasahib, K., Galloway, R. L., Hartskeerl, R. A., Lewis, J. W., & Zain, S. N. M. (2016). Human leptospirosis in Malaysia. *Asia-Pacific Journal of Public Health*, *28*(4), 290–302. https://doi.org/10.1177/1010539516640350

Benacer, D., Woh, P. Y., Mohd Zain, S. N., Amran, F., & Thong, K. L. (2013). Pathogenic and saprophytic *Leptospira* species in water and soils from selected urban sites in peninsular Malaysia. *Microbes and Environments*, 28(1), 135–140. https://doi.org/10.1264/jsme2.me12154

Bharti, A., Nally, J. E., Ricaldi, J. N., Matthias, M., Díaz, M. M., Lovett, M., Levett, P. N., Gilman, R. H., Willig, M. R., Gotuzzo, E., & Vinetz, J. M. (2003). Leptospirosis: a zoonotic disease of global importance. *Lancet Infectious Diseases*, 3(12), 757–771. https://doi.org/10.1016/s1473-3099(03)00830-2

Birnbaum, N., Barr, S. C., Center, S. A., Schermerhorn, T., Randolph, J. F., & Simpson, K. W. (1998). Naturally acquired leptospirosis in 36 dogs: serological and clinicopathological features. *The Journal of Small Animal Practice*, 39(5), 231–236. https://doi.org/10.1111/j.1748-5827.1998.tb03640.x

Blanchard, S., Cariou, C., Bouvet, J., Valfort, W., Oberli, F., Villard, S., Barret-Hilaire, F., Poulet, H., Cupillard, L., & de Saint-Vis, B. (2021). Quantitative Real-Time PCR Assays for the Detection of Pathogenic Leptospira Species in Urine and Blood Samples in Canine Vaccine Clinical Studies: a Rapid Alternative to Classical Culture Methods. Journal of clinical microbiology, 59(7), e0300620. https://doi.org/10.1128/JCM.03006-20

Bolin C. A. (1996). Diagnosis of leptospirosis: a reemerging disease of companion animals. *Seminars in Veterinary Medicine and Surgery (small animal)*, 11(3), 166–171. https://doi.org/10.1016/s1096-2867(96)80029-6

Bolin, C. A., Thiermann, A. B., Handsaker, A. L., & Foley, J. W. (1989). Effect of vaccination with a pentavalent leptospiral vaccine on *Leptospira interrogans* serovar hardjo type hardjo-bovis infection of pregnant

cattle. *PubMed*, 50(1), 161–165. https://pubmed.ncbi.nlm.nih.gov/2645816

Boonsilp, S., Thaipadungpanit, J., Amornchai, P., Wuthiekanun, V., Bailey, M. S., Holden, M. T., Zhang, C., Jiang, X., Koizumi, N., Taylor, K., Galloway, R., Hoffmaster, A. R., Craig, S., Smythe, L. D., Hartskeerl, R. A., Day, N. P., Chantratita, N., Feil, E. J., Aanensen, D. M., Spratt, B. G., Peacock, S. J. (2013). A single multilocus sequence typing (MLST) scheme for seven pathogenic *Leptospira* species. *PLoS Neglected Tropical Diseases*, 7(1), e1954. https://doi.org/10.1371/journal.pntd.0001954

Bourhy, P., Bremont, S., Zinini, F., Giry, C., & Picardeau, M. (2011). Comparison of real-time PCR assays for detection of pathogenic *Leptospira* spp. in blood and identification of variations in target sequences. *Journal of Clinical Microbiology*, 49(6), 2154–2160. https://doi.org/10.1128/JCM.02452-10

Bourhy, P., Collet, L., Brisse, S., & Picardeau, M. (2014). *Leptospira mayottensis* sp. nov., a pathogenic species of the genus *Leptospira* isolated from humans. *International Journal of Systematic and Evolutionary Microbiology*, *64*(Pt_12), 4061–4067. https://doi.org/10.1099/ijs.0.066597-0

Bourhy, P., Collet, L., Clément, S., Huerre, M., Ave, P., Giry, C., Pettinelli, F., & Picardeau, M. (2010). Isolation and characterization of new *Leptospira* genotypes from patients in Mayotte (Indian Ocean). *PLoS Neglected Tropical Diseases*, 4(6), e724. https://doi.org/10.1371/journal.pntd.0000724

Brenner, D. J., Kaufmann, A. F., Sulzer, K. R., Steigerwalt, A. G., Rogers, F., & Weyant, R. S. (1999). Further determination of DNA relatedness between serogroups and serovars in the family *Leptospiraceae* with a proposal for *Leptospira alexanderi* sp. nov. and four new *Leptospira* genomospecies. *International Journal of Systematic and Evolutionary Microbiology*, 49(2), 839–858. https://doi.org/10.1099/00207713-49-2-839

Brown, K. W., & Prescott, J. F. (2008). Leptospirosis in the family dog: a public health perspective. *Canadian Medical Association Journal*, 178(4), 399–401. https://doi.org/10.1503/cmaj.071097

Budihal, S. V., & Perwez, K. (2014). Leptospirosis diagnosis: competancy of various laboratory tests. *Journal of Clinical and Diagnostic Research*, 8(1), 199–202. https://doi.org/10.7860/JCDR/2014/6593.3950

Burth, P., Younes-Ibrahim, M., Gonçalez, F. H., Costa, E., & De Castro Faria, M. V. (1997). Purification and characterization of a Na+, K+ ATPase inhibitor found in an endotoxin of *Leptospira interrogans*. *Infection and Immunity*, 65(4), 1557–1560. https://doi.org/10.1128/iai.65.4.1557-1560.1997

Bustin, S. A., Benes, V., Garson, J. A., Hellemans, J., Huggett, J., Kubista, M., Mueller, R., Nolan, T., Pfaffl, M. W., Shipley, G. L., Vandesompele, J., & Wittwer, C. T. (2009). The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clinical Chemistry*, 55(4), 611–622. https://doi.org/10.1373/clinchem.2008.112797

Calderon, A., Rodríguez, V., Máttar, S., & Arrieta, G. (2014). Leptospirosis in pigs, dogs, rodents, humans, and water in an area of the Colombian tropics. *Tropical Animal Health and Production*, 46(2), 427–432. https://doi.org/10.1007/s11250-013-0508-y

Chadsuthi, S., Bicout, D. J., Wiratsudakul, A., Suwancharoen, D., Petkanchanapong, W., Modchang, C., Triampo, W., Ratanakorn, P., & Chalvet-Monfray, K. (2017). Investigation on predominant *Leptospira* serovars and its distribution in humans and livestock in Thailand, 2010-2015. *PLoS Neglected Tropical Diseases*, 11(2), e0005228. https://doi.org/10.1371/journal.pntd.0005228

Chandrasekaran, D., Prathaban, S., Dhanabalan, P., Balachandran, C., Manohar, B. M., Venkataraman, K. S. (2011). Pathological changes in canine leptospirosis. *Tamilnadu Journal of Veterinary and Animal Sciences*, 7(3), 180-183.

Cole, J. R., Jr, Sulzer, C. R., & Pursell, A. R. (1973). Improved microtechnique for the leptospiral microscopic agglutination test. *Applied Microbiology*, 25(6), 976–980. https://doi.org/10.1128/am.25.6.976-980.1973

Costa, F., Hagan, J. E., Calcagno, J., Kane, M., Torgerson, P., Martinez-Silveira, M. S., Stein, C., Abela-Ridder, B., & Ko, A. I. (2015). Global morbidity and mortality of leptospirosis: A Systematic Review. *PLoS Neglected Tropical Diseases*, 9(9), e0003898. https://doi.org/10.1371/journal.pntd.0003898

Cousins, D. V., Robertson, G. M., & Hustas, L. (1985). The use of the enzyme-linked immunosorbent assay (ELISA) to detect the IgM and IgG antibody response to *Leptospira interrogans* serovars hardjo, pomona and tarassovi in cattle. *Veterinary Microbiology*, 10(5), 439–450. https://doi.org/10.1016/0378-1135(85)90026-4

Cullen, P., Xu, X., Matsunaga, J., Sánchez, Y., Ko, A. I., Haake, D. A., & Adler, B. (2005). Surfaceome of *Leptospira* spp. *Infection and Immunity*, 73(8), 4853–4863. https://doi.org/10.1128/iai.73.8.4853-4863.2005

Cumberland, P., Everard, C. O., Wheeler, J. G., & Levett, P. N. (2001). Persistence of anti-leptospiral IgM, IgG and agglutinating antibodies in patients presenting with acute febrile illness in Barbados 1979-1989. *European Journal of Epidemiology*, 17(7), 601–608. https://doi.org/10.1023/a:1015509105668

Da Silva, J. D., Melo, D. H. M., De Barros Correia, É. L., Alves, J. R. A., Da Costa, D. F., Higino, S. S. D. S., De Azevêdo, S. S., & Alves, C. J. (2017). Epidemiological characterization and risk factors associated with *Leptospira* infection in dogs from rural settlements in the semi-arid region of Northeast Brazil. *Semina-ciencias Agrarias*, 38(4Supl1), 2531. https://doi.org/10.5433/1679-0359.2017v38n4suplp2531

Day, M. J., Horzinek, M. C., Schultz, R. D., Squires, R. A., & Vaccination Guidelines Group (VGG) of the World Small Animal Veterinary Association (WSAVA) (2016). WSAVA Guidelines for the vaccination of dogs and cats. *The Journal of Small Animal Practice,* 57(1), E1–E45. https://doi.org/10.1111/jsap.2_12431

Dias, J. P., Teixeira, M. G., Costa, M. C., Mendes, C. M., Guimarães, P., Reis, M. G., Ko, A., & Barreto, M. L. (2007). Factors associated with *Leptospira* sp infection in a large urban center in northeastern Brazil. *Revista da Sociedade Brasileira de Medicina Tropical*, 40(5), 499–504. https://doi.org/10.1590/s0037-86822007000500002

Diprete A. (2019). Leptospirosis in dogs. Today's Veterinary Nurse. https://todaysveterinarynurse.com/infectious-disease/leptospirosis-in-dogs/

Dupouey, J., Faucher, B., Edouard, S., Richet, H., Kodjo, A., Drancourt, M., & Davoust, B. (2014). Human leptospirosis: an emerging risk in Europe?. *Comparative Immunology, Microbiology and Infectious Diseases*, 37(2), 77–83. https://doi.org/10.1016/j.cimid.2013.12.002

Dziezyc J. (2000). Canine systemic bacterial infections. The Veterinary Clinics of North America. *Small Animal Practice*, 30(5), 1103–1117. https://doi.org/10.1016/s0195-5616(00)05009-9

Ellis, W. A., Bryson, D. G., O'Brien, J. J., & Neill, S. D. (1983). Leptospiral infection in aborted equine foetuses. *Equine Veterinary Journal*, 15(4), 321–324. https://doi.org/10.1111/j.2042-3306.1983.tb01811.x

Eribo, B. E., Mingmongkolchai, S., Tao, Y., Dubbs, P., & Nelson, K. E. (2012). Leptospire Genomic diversity revealed by Microarray-Based Comparative Genomic Hybridization. *Applied and Environmental Microbiology*, 78(9), 3045–3050. https://doi.org/10.1128/aem.07465-11

Eric Klaasen, H. L., & Adler, B. (2015). Recent advances in canine leptospirosis: focus on vaccine development. *Veterinary Medicine (Auckland, N.Z.)*, 6, 245–260. https://doi.org/10.2147/VMRR.S59521

Esteves, L. M., Bulhões, S. M., Branco, C. C., Carreira, T., Vieira, M. L., Gomes-Solecki, M., & Mota-Vieira, L. (2018). Diagnosis of human leptospirosis in a clinical setting: real-time PCR high resolution melting analysis for detection of *Leptospira* at the onset of disease. *Scientific Reports*, 8(1), 9213. https://doi.org/10.1038/s41598-018-27555-2

Evangelista, K. V., & Coburn, J. (2010). *Leptospira* as an emerging pathogen: a review of its biology, pathogenesis and host immune responses. *Future Microbiology*, 5(9), 1413–1425. https://doi.org/10.2217/fmb.10.102

Faine S. Guidelines for the control of leptospirosis. World Health Organization; Geneva: (1982).

Faine, S. (2015). Leptospira and leptospirosis. In *Current Topics in Microbiology and Immunology*. https://doi.org/10.1007/978-3-662-45059-8

Farouk, W. I., Hassan, N. H., Ismail, T. R., Daud, I. S., & Mohammed, F. (2018). Warthin-Starry Staining for the detection of *Helicobacter pylori* in gastric biopsies. *The Malaysian Journal of Medical Sciences*, 25(4), 92–99. https://doi.org/10.21315/mjms2018.25.4.9

Fink, J. M., Moore, G. E., Landau, R., & Vemulapalli, R. (2015). Evaluation of three 5' exonuclease-based real-time polymerase chain reaction assays for detection of pathogenic *Leptospira* species in canine urine. *Journal of Veterinary Diagnostic Investigation : official publication of the American Association of Veterinary Laboratory Diagnosticians, Inc,* 27(2), 159–166. https://doi.org/10.1177/1040638715571360

Fraga, T. R., Barbosa, A. S., & Isaac, L. (2011). Leptospirosis: aspects of innate immunity, immunopathogenesis and immune evasion from the complement system. *Scandinavian Journal of Immunology*, 73(5), 408–419. https://doi.org/10.1111/j.1365-3083.2010.02505.x

Fraune, C. K., Schweighauser, A., & Francey, T. (2013). Evaluation of the diagnostic value of serologic microagglutination testing and a polymerase chain reaction assay for diagnosis of acute leptospirosis in dogs in a referral center. *Journal of the American Veterinary Medical Association*, 242(10), 1373–1380. https://doi.org/10.2460/javma.242.10.1373

Galton, M. M. (1962). Methods in the laboratory diagnosis of leptospirosis. *Annals of the New York Academy of Sciences*, 98(3), 675–685. https://doi.org/10.1111/j.1749-6632.1962.tb30589.x

Garba, B., Bahaman, A. R., Khairani-Bejo, S., Zakaria, Z., & Mutalib, A. R. (2017). Retrospective study of leptospirosis in Malaysia. *EcoHealth*, *14*(2), 389–398. https://doi.org/10.1007/s10393-017-1234-0

Garibyan, L., & Avashia, N. (2013). Polymerase chain reaction. *The Journal of Investigative Dermatology*, 133(3), 1–4. https://doi.org/10.1038/jid.2013.1

Gautam, R., Wu, C. C., Guptill, L. F., Potter, A., & Moore, G. E. (2010). Detection of antibodies against *Leptospira* serovars via microscopic agglutination tests in dogs in the United States, 2000-2007. *Journal of*

the American Veterinary Medical Association, 237(3), 293–298. https://doi.org/10.2460/javma.237.3.293

Ghada, A. H., Bahaman, A. R., Khairani-Bejo, S., Zunita, Z., & Garba, B. (2017). Serological and molecular prevalence of *Leptospira* infection in rat populations in Kuala Lumpur. *Australian Journal of Basic and Applied Sciences*, 11(1): 62-72.

Ghneim, G. S., Viers, J. H., Chomel, B. B., Kass, P. H., Descollonges, D. A., & Johnson, M. L. (2007). Use of a case-control study and geographic information systems to determine environmental and demographic risk factors for canine leptospirosis. *Veterinary Research*, 38(1), 37–50. https://doi.org/10.1051/vetres:2006043

Goarant C. (2016). Leptospirosis: risk factors and management challenges in developing countries. *Research and Reports in Tropical Medicine*, 7, 49–62. https://doi.org/10.2147/RRTM.S102543

Goh, S. H., Ismail, R., Lau, S. F., Megat Abdul Rani, P. A., Mohd Mohidin, T. B., Daud, F., Bahaman, A. R., Khairani-Bejo, S., Radzi, R., & Khor, K. H. (2019). Risk factors and prediction of leptospiral seropositivity among dogs and dog handlers in Malaysia. *International Journal of Environmental Research and Public Health*, 16(9), 1499. https://doi.org/10.3390/ijerph16091499

Goh, S. H., Khor, K. H., Radzi, R., Lau, S. F., Khairani-Bejo, S., Abdul Rahman, M. S., & Roslan, M. A. (2021). Shedding and genetic diversity of *Leptospira* spp. from urban stray dogs in Klang Valley, Malaysia. *Topics in Companion Animal Medicine*, 45, 100562. https://doi.org/10.1016/j.tcam.2021.100562

Goldstein R. E. (2010). Canine leptospirosis. The Veterinary Clinics of North America. *Small Animal Practice*, 40(6), 1091–1101. https://doi.org/10.1016/j.cvsm.2010.07.008

Goldstein, R. E., Lin, R. C., Langston, C. E., Scrivani, P. V., Erb, H. N., & Barr, S. C. (2006). Influence of infecting serogroup on clinical features of leptospirosis in dogs. *Journal of Veterinary Internal Medicine*, 20(3), 489–494. https://doi.org/10.1892/0891-6640(2006)20[489:ioisoc]2.0.co;2

Gompf, S. G., Jimenez, J. M. H., Talavera, F., Bronze, M. S., Green-McKenzie, J., & Velez, A. P. (2021). Questions & answers. https://emedicine.medscape.com/article/220563-questions-and-answers

Goris, M. G. A., Leeflang, M. M. G., Boer, K. R., Goeijenbier, M., & Gorp, E. C. M. (2012). Establishment of valid laboratory case definition for human leptospirosis. Jounal of Bacteriology and Parasitology, 3, 132.

Greene, C. E., Miller, M. A Brown, C. A.(1998). Leptospirosis. In. Infectious diseases of dog and cat. Phildelphia, W.B. *Saunders & Co*, pp.273.

Greene, C. E., Sykes, J. F., Brown, C. A., & Hartmann, K. (2008). Leptospirosis. En: Greene C (ed). Enfermedades infecciosas del perro y el gato. *Intermédica, Buenos Aires, Argentina*, pp 448-463.

Greene, C. E., Sykes, J. F., Brown, C. A., Hartmann, K. (2006). Leptospirosis. In: Greene CE, ed. Infectious Diseases of the Dog and Cat. 3rd edition. *Saunders Elsevier*, Leptospirosis; pp 402–415

Greene, C. E., Sykes, J. F., Brown, C. A., Hartmann, K. (2012). Leptospirosis. In: Greene CE, ed. Infectious Diseases of the Dog and Cat. 4th edition. *Saunders Elsevier*, pp 431–447.

Greenlee, J. J., Bolin, C. A., Alt, D. P., Cheville, N. F., & Andreasen, C. B. (2004). Clinical and pathologic comparison of acute leptospirosis in dogs caused by two strains of *Leptospira kirschneri* serovar grippotyphosa. *American Journal of Veterinary Research*, 65(8), 1100–1107. https://doi.org/10.2460/ajvr.2004.65.1100

Guerra M. A. (2009). Leptospirosis. *Journal of the American Veterinary Medical Association*, 234(4), 472–430. https://doi.org/10.2460/javma.234.4.472

Haake, D. A., & Levett, P. N. (2015). *Leptospira* species (Leptospirosis). In Elsevier eBooks, pp 2714-2720.e2. https://doi.org/10.1016/b978-1-4557-4801-3.00241-1

Haake, D. A., Chao, G., Zuerner, R. L., Barnett, J. K., Barnett, D., Mazel, M., Matsunaga, J., Levett, P. N., & Bolin, C. A. (2000). The leptospiral major outer membrane protein LipL32 is a lipoprotein expressed during mammalian infection. *Infection and Immunity*, 68(4), 2276–2285. https://doi.org/10.1128/IAI.68.4.2276-2285.2000

Harkin, K. R., & Gartrell, C. L. (1996). Canine leptospirosis in New Jersey and Michigan: 17 cases (1990-1995). *Journal of the American Animal Hospital Association*, 32(6), 495–501. https://doi.org/10.5326/15473317-32-6-495

Harkin, K. R., & Hays, M. P. (2016). Variable-number tandem-repeat analysis of leptospiral DNA isolated from canine urine samples molecularly confirmed to contain pathogenic leptospires. *Journal of the American Veterinary Medical Association*, 249(4), 399–405. https://doi.org/10.2460/javma.249.4.399

Harkin, K. R., Roshto, Y. M., & Sullivan, J. T. (2003a). Clinical application of a polymerase chain reaction assay for diagnosis of leptospirosis in dogs. *Journal of the American Veterinary Medical Association*, 222(9), 1224–1229. https://doi.org/10.2460/javma.2003.222.1224

Harkin, K. R., Roshto, Y. M., Sullivan, J. T., Purvis, T. J., & Chengappa, M. M. (2003b). Comparison of polymerase chain reaction assay, bacteriologic culture, and serologic testing in assessment of prevalence of urinary shedding of leptospires in dogs. *Journal of the American Veterinary Medical Association*, 222(9), 1230–1233. https://doi.org/10.2460/javma.2003.222.1230

Harkin, K. R. (2012). Canine leptospirosis: A perpective on recent trends. *Today's Veterinary Practice,* 2(4), 27-33.

Harland, A. L., Cave, N. J., Jones, B. R., Benschop, J., Donald, J. J., Midwinter, A. C., Squires, R. A., & Collins-Emerson, J. M. (2013). A serological survey of leptospiral antibodies in dogs in New Zealand. *New Zealand Veterinary Journal*, 61(2), 98–106. https://doi.org/10.1080/00480169.2012.719212

Hartman, E., Van Den Ingh, T. S. G. A. M., & Rothuizen, J. (1986). Clinical, pathological and serological features of spontaneous canine leptospirosis. An evaluation of the IgM- and IgG-specific ELISA. *Veterinary Immunology and Immunopathology*, 13(3), 261–271. https://doi.org/10.1016/0165-2427(86)90078-4

Hartskeerl, R. A., & Smythe, L. D. (2014). The role of leptospirosis reference laboratories. In *Current Topics in Microbiology and Immunology*, pp 273–288. https://doi.org/10.1007/978-3-662-45059-8_11

Hartskeerl, R. A., Collares-Pereira, M., & Ellis, W. (2011). Emergence, control and re-emerging leptospirosis: dynamics of infection in the changing world. *Clinical Microbiology and Infection*, 17(4), 494–501. https://doi.org/10.1111/j.1469-0691.2011.03474.x

Hendrikse, J. L., Parsons, T. E., & Hallgrímsson, B. (2007). Evolvability as the proper focus of evolutionary developmental biology. *Evolution and Development*, 9(4), 393–401. https://doi.org/10.1111/j.1525-142X.2007.00176.x

Hennebelle, J. H., Sykes, J. E., Carpenter, T. E., & Foley, J. (2013). Spatial and temporal patterns of *Leptospira* infection in dogs from northern California: 67 cases (2001-2010). *Journal of the American Veterinary Medical Association*, 242(7), 941–947. https://doi.org/10.2460/javma.242.7.941

Heymann, D. L. (2004). Control of communicable diseases manual : an official report of the American Public Health Association. In *American Public Health Association eBooks*. https://ci.nii.ac.jp/ncid/BB20153209

Hoy, M. A. (2013). Insect Molecular Genetics: An Introduction to Principles and Application (3rd ed.). *Academic Press*.

Johnson, R. C., & Rogers, P. (1964). Differentiation of pathogenic and saprophytic leptospires with 8-azaguanine. *Journal of Bacteriology*, 88(6), 1618–1623. https://doi.org/10.1128/jb.88.6.1618-1623.1964

Khor, K. H., Tan, W. X., Lau, S. F., Mohd Azri, R., Rozanaliza, R., Siti, K. B., & Abdul Rani, B. (2016). Seroprevalence and molecular detection of leptospirosis from a dog shelter. *Tropical Biomedicine*, 33(2), 276–284.

Kikuti, M., Langoni, H., Nóbrega, D. B., Corrêa, A. P. F. L., & Ullmann, L. S. (2012). Occurrence and risk factors associated with canine leptospirosis. *Journal of Venomous Animals and Toxins Including Tropical Diseases*, 18(1), 124–127. https://doi.org/10.1590/s1678-91992012000100016

Klopfleisch, R., Kohn, B., Plog, S., Weingart, C., Nöckler, K., Mayer-Scholl, A., & Gruber, A. D. (2010). An emerging pulmonary haemorrhagic syndrome in dogs: similar to the human leptospiral pulmonary haemorrhagic syndrome?. *Veterinary Medicine International*, 928541. https://doi.org/10.4061/2010/928541

Ko, A. I., Goarant, C., & Picardeau, M. (2009). *Leptospira*: the dawn of the molecular genetics era for an emerging zoonotic pathogen. *Nature Reviews Microbiology*, 7(10), 736–747. https://doi.org/10.1038/nrmicro2208

Koay, T. K., Nirmal, S., Noitie, L., & Tan, E. (2004). An epidemiological investigation of an outbreak of leptospirosis associated with swimming, Beaufort, Sabah. *The Medical Journal of Malaysia*, 59(4), 455–459.

Kohn, B., Steinicke, K., Arndt, G., Gruber, A. D., Guerra, B., Jansen, A., Kaser-Hotz, B., Klopfleisch, R., Lotz, F., Luge, E., & Nöckler, K. (2010). Pulmonary abnormalities in dogs with leptospirosis. *Journal of Veterinary Internal Medicine*, 24(6), 1277–1282. https://doi.org/10.1111/j.1939-1676.2010.0585.x

Koizumi, N., Muto, M. M., Akachi, S., Okano, S., Yamamoto, S., Horikawa, K., Harada, S., Funatsumaru, S., & Ohnishi, M. (2013). Molecular and serological investigation of *Leptospira* and leptospirosis in dogs in Japan. *Journal of Medical Microbiology*, 62(4), 630–636. https://doi.org/10.1099/jmm.0.050039-0

Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Molecular Biology and Evolution*, 33(7), 1870–1874. https://doi.org/10.1093/molbev/msw054

Lane, A. B., & Dore, M. (2016). Leptospirosis: A clinical review of evidence based diagnosis, treatment and prevention. *World Journal of Clinical Infectious Diseases*, 6(4), 61. https://doi.org/10.5495/wjcid.v6.i4.61

Langston, C. E., & Heuter, K. J. (2003). Leptospirosis. A re-emerging zoonotic disease. The Veterinary Clinics of North America. *Small Animal Practice*, 33(4), 791–807. https://doi.org/10.1016/s0195-5616(03)00026-3

Latosinski, G. S., Fornazari, F., Babboni, S. D., Caffaro, K., Paes, A. C., & Langoni, H. (2018). Serological and molecular detection of *Leptospira* spp in dogs. *Revista da Sociedade Brasileira de Medicina Tropical*, 51(3), 364–367. https://doi.org/10.1590/0037-8682-0276-2017

Lau, C. L., Smythe, L. D., Craig, S. B., & Weinstein, P. (2010). Climate change, flooding, urbanisation and leptospirosis: fuelling the fire?. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 104(10), 631–638. https://doi.org/10.1016/j.trstmh.2010.07.002

Lau, S. F., Low, K. N., Khor, K. H., Roslan, M. A., Bejo, S. K., Radzi, R., & Bahaman, A. R. (2016). Prevalence of leptospirosis in healthy dogs and dogs with kidney disease in Klang Valley, Malaysia. *Tropical Biomedicine*, 33(3), 469–475.

Lau, S. F., Wong, J. Y., Khor, K. H., Roslan, M. A., Abdul Rahman, M. S., Bejo, S. K., Radzi, R., & Bahaman, A. R. (2017). Seroprevalence of Leptospirosis in Working Dogs. *Topics in Companion Animal Medicine*, 32(4), 121–125. https://doi.org/10.1053/j.tcam.2017.12.001

Lee, S. H., Kim, S., Park, S. C., & Kim, J. H. (2002). Cytotoxic activities of *Leptospira interrogans* hemolysin SphH as a pore-forming protein on mammalian cells. *Infection and Immunity*, 70(1), 315–322. https://doi.org/10.1128/iai.70.1.315-322.2002

Leon, A., Pronost, S., Tapprest, J., Foucher, N., Blanchard, B., André-Fontaine, G., Laugier, C., Fortier, G., & Leclercq, R. (2006). Identification of pathogenic *Leptospira* strains in tissues of a premature foal by use of polymerase chain reaction analysis. *Journal of Veterinary Diagnostic Investigation : official publication of the American Association of Veterinary Laboratory Diagnosticians, Inc*, 18(2), 218–221. https://doi.org/10.1177/104063870601800216

Levett, P. N. (2001). Leptospirosis. *Clinical Microbiology Reviews*, 14(2), 296–326. https://doi.org/10.1128/cmr.14.2.296-326.2001

Levett, P. N. (2003). Usefulness of serologic analysis as a predictor of the infecting serovar in patients with severe leptospirosis. *Clinical Infectious Diseases*, 36(4), 447–452. https://doi.org/10.1086/346208

Levett, P. N. (2004). Leptospirosis: A forgotten zoonosis? *Clinical and Applied Immunology Reviews*, 4(6), 435–448. https://doi.org/10.1016/j.cair.2004.08.001

Levett, P. N., Morey, R. E., Galloway, R. L., & Steigerwalt, A. G. (2006). *Leptospira broomii* sp. nov., isolated from humans with leptospirosis. *International Journal of Systematic and Evolutionary Microbiology*, 56(3), 671–673. https://doi.org/10.1099/ijs.0.63783-0

Levett, P. N., Morey, R. E., Galloway, R. L., Turner, D. E., Steigerwalt, A. G., & Mayer, L. W. (2005). Detection of pathogenic leptospires by real-time quantitative PCR. *Journal of Medical Microbiology*, 54(1), 45–49. https://doi.org/10.1099/jmm.0.45860-0

Lim V. K. (2011). Leptospirosis: a re-emerging infection. *The Malaysian Journal of Pathology*, *33*(1), 1–5.

Llewellyn, J. R., Krupka-Dyachenko, I., Rettinger, A. L., Dyachenko, V., Stamm, I., Kopp, P. A., Straubinger, R. K., & Hartmann, K. (2016). Urinary shedding of leptospires and presence of *Leptospira* antibodies in healthy dogs from Upper Bavaria. *Berliner und Munchener tierarztliche Wochenschrift*, 129(5-6), 251–257.

Lobetti, R. 2007. Leptospirosis. Proceedings of the World Small Animal Veterinary Congress, Sydney, Australia.

Logan, C., Habington, A., Lennon, G., Cronin, F., & O'Sullivan, N. (2010). Evaluation of the efficacy of real-time polymerase chain reaction for the routine early detection of *Pseudomonas aeruginosa* in cystic fibrosis sputum and throat swab specimens. *Diagnostic Microbiology and Infectious Disease*, 68(4), 358–365. https://doi.org/10.1016/j.diagmicrobio.2010.07.012

Lunn, K. F. (2023, October 5). Leptospirosis in animals - overview. MSD Veterinary Manual. https://www.msdvetmanual.com/generalized-conditions/leptospirosis/leptospirosis-in-animals-overview

Lunn, K. F. (2023, October 5). Leptospirosis in dogs. MSD Veterinary Manual. https://www.msdvetmanual.com/generalized-conditions/leptospirosis/leptospirosis-in-dogs

Magaldi, A. J., Yasuda, P. N., Kudo, L. H., Seguro, A. C., & Rocha, A. S. (1992). Renal involvement in leptospirosis: a pathophysiologic study. *Nephron*, 62(3), 332–339. https://doi.org/10.1159/000187069

Martín, P. L., Stanchi, N. O., Brihuega, B., Bonzo, E., Galli, L., & Arauz, M. S. (2019). Diagnosis of canine leptospirosis: evaluation of two PCR assays in comparison with the microagglutination test. *Pesquisa Veterinaria Brasileira*, 39(4), 255–262. https://doi.org/10.1590/1678-5150-pvb-5868

Martins, C. M., Barros, C.daC., Galindo, C. M., Kikuti, M., Ullmann, L. S., Pampuch, R.dosS., Hoffmann, J. L., Langoni, H., Ferreira, F., Molento, M. B., & Biondo, A. W. (2013). Incidence of canine leptospirosis in the metropolitan area of Curitiba, State of Paraná, Southern Brazil. *Revista*

da Sociedade Brasileira de Medicina Tropical, 46(6), 772–775. https://doi.org/10.1590/0037-8682-1665-2013

Matsui, M., Roche, L., Geroult, S., Soupé-Gilbert, M. E., Monchy, D., Huerre, M., & Goarant, C. (2016). Cytokine and chemokine expression in kidneys during chronic leptospirosis in reservoir and susceptible animal models. *PloS One*, 11(5), e0156084. https://doi.org/10.1371/journal.pone.0156084

Matthias, M., Ricaldi, J. N., Céspedes, M., Díaz, M. M., Galloway, R. L., Saito, M., Steigerwalt, A. G., Patra, K. P., Oré, C. V., Gotuzzo, E., Gilman, R. H., Levett, P. N., & Vinetz, J. M. (2008). Human Leptospirosis caused by a new, antigenically unique *Leptospira* associated with a *Rattus* species reservoir in the Peruvian Amazon. *PloS Neglected Tropical Diseases*, 2(4), e213. https://doi.org/10.1371/journal.pntd.0000213

McCallum, K. E., Constantino-Casas, F., Cullen, J. M., Warland, J. H., Swales, H., Linghley, N., Kortum, A. J., Sterritt, A. J., Cogan, T., & Watson, P. J. (2019). Hepatic leptospiral infections in dogs without obvious renal involvement. *Journal of Veterinary Internal Medicine*, 33(1), 141–150. https://doi.org/10.1111/jvim.15340

Meeyam, T., Tablerk, P., Petchanok, B., Pichpol, D., & Padungtod, P. (2006). Seroprevalence and risk factors associated with leptospirosis in dogs. *The Southeast Asian Journal of Tropical Medicine and Public Health*, 37(1), 148–153.

Meny, P., Menéndez, C., Quintero, J., Hernández, E., Ríos, C., Balassiano, I. T., Trindade, C. N. D. R., Vital-Brazil, J. M., Ramos, T. M. V., Ashfield, N., Feble, C., Avila, E., Schelotto, F., & Varela, G. (2017). Characterization of *Leptospira* isolates from humans and the environment in Uruguay. *Revista do Instituto de Medicina Tropical de Sao Paulo*, 59, e79. https://doi.org/10.1590/S1678-9946201759079

Merien, F., Amouriaux, P., Perolat, P., Baranton, G., & Saint Girons, I. (1992). Polymerase chain reaction for detection of *Leptospira* spp. in clinical samples. *Journal of Clinical Microbiology*, 30(9), 2219–2224. https://doi.org/10.1128/jcm.30.9.2219-2224.1992

Miller, R. B., Ross, S., Sullivan, N. D., & Perkins, N. R. (2007). Clinical and epidemiological features of canine leptospirosis in North Queensland. *Australian Veterinary Journal*, 85(1–2), 13–19. https://doi.org/10.1111/j.1751-0813.2006.00089.x

Miotto, B. A., Guilloux, A. G. A., Tozzi, B. F., Moreno, L. Z., da Hora, A. S., Dias, R. A., Heinemann, M. B., Moreno, A. M., Filho, A. F. S., Lilenbaum, W., & Hagiwara, M. K. (2018a). Prospective study of canine leptospirosis in shelter and stray dog populations: Identification of chronic carriers and different Leptospira species infecting dogs. *PloS One*, 13(7), e0200384. https://doi.org/10.1371/journal.pone.0200384

Miotto, B. A., Moreno, L. Z., Guilloux, A. G. A., Sousa, G. O., Loureiro, A. P., Moreno, A. M., Lilenbaum, W., Vasconcellos, S. A., Heinemann, M. B., & Hagiwara, M. K. (2016). Molecular and serological characterization of the first *Leptospira santarosai* strain isolated from a dog. *Acta Tropica*, 162, 1–4. https://doi.org/10.1016/j.actatropica.2016.06.007

Miotto, B. A., Tozzi, B. F., Penteado, M. S., Guilloux, A. G. A., Moreno, L. Z., Heinemann, M. B., Moreno, A. M., Lilenbaum, W., & Hagiwara, M. K. (2018b). Diagnosis of acute canine leptospirosis using multiple laboratory tests and characterization of the isolated strains. *BMC Veterinary Research*, 14(1), 222. https://doi.org/10.1186/s12917-018-1547-4

Mohammed, H., Nozha, C., Karib, H., & Fassouane, A. (2011). *Leptospira*: Morphology, classification and pathogenesis. *Journal of Bacteriology and Parasitology*, 2(6). https://doi.org/10.4172/2155-9597.1000120

Mohd-Ali, M. R., Lih Huey, L., Foo, P. C., Goay, Y. X., Ismail, A. S., Mustaffa, K. M. F., Aziah, I., Kia Kien, P., Harun, A., Ismail, N., & Yean Yean, C. (2019). Duplex TaqMan hydrolysis probe-based molecular assay for simultaneous detection and differentiation of *Burkholderia pseudomallei* and *Leptospira* spp. DNA. *BioMed Research International*, 2019, 9451791. https://doi.org/10.1155/2019/9451791

Mohd-Ali, M. R., Mohamad Safiee, A. W., Yusof, N. Y., Fauzi, M. H., Yean Yean, C., & Ismail, N. (2018). Isolation of *Leptospira kmetyi* from residential areas of patients with leptospirosis in Kelantan, Malaysia. *Journal of Infection and Public Health*, 11(4), 578–580. https://doi.org/10.1016/j.jiph.2017.12.008

Monahan, A. M., Miller, I. S., & Nally, J. E. (2009). Leptospirosis: risks during recreational activities. *Journal of Applied Microbiology*, 107(3), 707–716. https://doi.org/10.1111/j.1365-2672.2009.04220.x

Morey, R. E., Galloway, R. L., Bragg, S. L., Steigerwalt, A. G., Mayer, L. W., & Levett, P. N. (2006). Species-specific identification of Leptospiraceae by 16S rRNA gene sequencing. *Journal of Clinical Microbiology*, 44(10), 3510–3516. https://doi.org/10.1128/jcm.00670-06

Mullis K. B. (1990). The unusual origin of the polymerase chain reaction. *Scientific American*, 262(4), 56–65. https://doi.org/10.1038/scientificamerican0490-56

Musso, D., & La Scola, B. (2013). Laboratory diagnosis of leptospirosis: a challenge. *Journal of Microbiology, Immunology, and Infection*, 46(4), 245–252. https://doi.org/10.1016/j.jmii.2013.03.001

Nascimento, A. L., Ko, A. I., Martins, E. A., Monteiro-Vitorello, C. B., Ho, P. L., Haake, D. A., Verjovski-Almeida, S., Hartskeerl, R. A., Marques, M. V., Oliveira, M. C., Menck, C. F., Leite, L. C., Carrer, H., Coutinho, L. L., Degrave, W. M., Dellagostin, O. A., El-Dorry, H., Ferro, E. S., Ferro, M.

I., Furlan, L. R., … Van Sluys, M. A. (2004). Comparative genomics of two *Leptospira interrogans* serovars reveals novel insights into physiology and pathogenesis. *Journal of Bacteriology*, 186(7), 2164–2172. https://doi.org/10.1128/JB.186.7.2164-2172.2004

Natarajaseenivasan, K., Vijayachari, P., Sharma, S., Sugunan, A. P., Selvin, J., & Sehgal, S. C. (2008). Serodiagnosis of severe leptospirosis: evaluation of ELISA based on the recombinant OmpL1 or LipL41 antigens of *Leptospira interrogans* serovar autumnalis. *Annals of Tropical Medicine and Parasitology*, 102(8), 699–708. https://doi.org/10.1179/136485908x355229

Navarro, C. E., & Kociba, G. J. (1982). Hemostatic changes in dogs with experimental *Leptospira interrogans* serovar icterohaemorrhagiae infection. *American journal of Veterinary Research*, 43(5), 904–906.

Noubade, R., Krishnamurthy, G. V., Murag, S., Venkatesha, M. D., & Krishnappa, G. (2002). Differentiation of pathogenic and saprophytic leptospires by polymerase chain reaction. *Indian Journal of Medical Microbiology*, 20(1), 33–36.

Paiva-Cavalcanti, M., Régis-Da-Silva, C., & Gomes, Y. M. (2010). Comparison of real-time PCR and conventional PCR for detection of *Leishmania infantum* infection: a mini-review. *Journal of Venomous Animals and Toxins Including Tropical Diseases*, 16(4), 537–542. https://doi.org/10.1590/s1678-91992010000400004

Pedro, N., & Szyfres, B. (2003). Zoonoses and communicable diseases common to man and animals: Volume III: Parasitoses. *Revista Panamericana De Salud Publica-pan American Journal of Public Health*. https://iris.paho.org/handle/10665.2/711

Pessoa-E-Silva, R., Mendonça Trajano-Silva, L. A., Lopes da Silva, M. A., da Cunha Gonçalves-de-Albuquerque, S., de Goes, T. C., Silva de Morais, R. C., Lopes de Melo, F., & de Paiva-Cavalcanti, M. (2016). Evaluation of urine for *Leishmania infantum* DNA detection by real-time quantitative PCR. *Journal of Microbiological Methods*, 131, 34–41. https://doi.org/10.1016/j.mimet.2016.10.002

Pettersson, E., Lundeberg, J., & Ahmadian, A. (2009). Generations of sequencing technologies. *Genomics*, 93(2), 105–111. https://doi.org/10.1016/j.ygeno.2008.10.003

Philip, N., Garba, B., & Neela, V. K. (2018). Long-term preservation of Leptospira spp.: challenges and prospects. *Applied Microbiology and Biotechnology*, 102(13), 5427–5435. https://doi.org/10.1007/s00253-018-9047-9

Picardeau M. (2013). Diagnosis and epidemiology of leptospirosis. *Medecine et maladies infectieuses*, 43(1), 1–9. https://doi.org/10.1016/j.medmal.2012.11.005

Plank, R., & Dean, D. (2000). Overview of the epidemiology, microbiology, and pathogenesis of Leptospira spp. in humans. *Microbes and Infection*, 2(10), 1265–1276. https://doi.org/10.1016/s1286-4579(00)01280-6

Prescott, J. (2008) Canine leptospirosis in Canada: A veterinarian's perspective. *Canadian Medical Association Journal*, 178, 397-398. https://doi.org/10.1503/cmaj.071092

Prescott, J. F., McEwen, B., Taylor, J., Woods, J. P., Abrams-Ogg, A., & Wilcock, B. (2002). Resurgence of leptospirosis in dogs in Ontario: recent findings. *The Canadian Veterinary Journal*, 43(12), 955–961.

Rahman, S. A., Khor, K. H., Khairani-Bejo, S., Lau, S. F., Mazlan, M., Roslan, A., & Goh, S. H. (2021). Detection and characterization of *Leptospira* spp. in dogs diagnosed with kidney and/or liver disease in Selangor, Malaysia. *Journal of Veterinary Diagnostic Investigation : official publication of the American Association of Veterinary Laboratory Diagnosticians, Inc,* 33(5), 834–843. https://doi.org/10.1177/10406387211024575

Ramírez, C. V. H., Camacho, S. M. G., Ramírez, I. O., Enríquez-Verdugo, I., Del Campo, N. C., & López-Moreno, H. S. (2017). Prevalence and risk factors associated with serovars of *Leptospira* in dogs from Culiacan, Sinaloa. *Veterinaria México OA*, 4(2). https://doi.org/10.21753/vmoa.4.2.369

Reagan, K. L., & Sykes, J. E. (2019). Diagnosis of canine leptospirosis. *Veterinary Clinics of North America: Small Animal Practice*, 49(4), 719–731. https://doi.org/10.1016/j.cvsm.2019.02.008

Reyes, E. A., Cullen, P., Bulach, D., Adler, B., Haake, D., & de la Peña-Moctezuma, A. (2005). Expression in *Escherichia coli* of the gspd(l) gene of the type II secretion system in *Leptospira borgpetersenii* serovariety hardjo. *Revista Cubana de Medicina Tropical*, 57(1), 45–46.

Ribotta, M. J., Higgins, R., Gottschalk, M., & Lallier, R. (2000). Development of an indirect enzyme-linked immunosorbent assay for the detection of leptospiral antibodies in dogs. *Canadian Journal of Veterinary Research*, 64(1), 32–37.

Ricardo, T., Previtali, M. A., & Signorini, M. (2020). Meta-analysis of risk factors for canine leptospirosis. *Preventive Veterinary Medicine*, 181, 105037. https://doi.org/10.1016/j.prevetmed.2020.105037

Ridzlan, F. R., Bahaman, A. R., Khairani-Bejo, S., & Mutalib, A. R. (2010). Detection of pathogenic *Leptospira* from selected environment in Kelantan and Terengganu, Malaysia. *Tropical Biomedicine*, 27(3), 632–638.

Rissi, D. R., & Brown, C. A. (2014). Diagnostic features in 10 naturally occurring cases of acute fatal canine leptospirosis. *Journal of Veterinary Diagnostic Investigation : official publication of the American Association of Veterinary Laboratory Diagnosticians, Inc*, 26(6), 799–804. https://doi.org/10.1177/1040638714553293

Rodriguez, J., Blais, M. C., Lapointe, C., Arsenault, J., Carioto, L., & Harel, J. (2014). Serologic and urinary PCR survey of leptospirosis in healthy cats and in cats with kidney disease. *Journal of Veterinary Internal Medicine*, 28(2), 284–293. https://doi.org/10.1111/jvim.12287

Romero, E. C., Billerbeck, A. E., Lando, V. S., Camargo, E. D., Souza, C. C., & Yasuda, P. H. (1998). Detection of *Leptospira* DNA in patients with aseptic meningitis by PCR. *Journal of Clinical Microbiology*, 36(5), 1453–1455. https://doi.org/10.1128/JCM.36.5.1453-1455.1998

Sabri, A. R., Khairani-Bejo, S., Zunita, Z., & Hassan, L. (2019). Molecular detection of *Leptospira* sp. in cattle and goats in Kelantan, Malaysia after a massive flood using multiplex polymerase chain reaction. *Tropical Biomedicine*, 36(1), 165–171.

Samir, A., Soliman, R., El-Hariri, M., Abdel-Moein, K., & Hatem, M. E. (2015). Leptospirosis in animals and human contacts in Egypt: broad range surveillance. *Revista da Sociedade Brasileira de Medicina Tropical*, 48(3), 272–277. https://doi.org/10.1590/0037-8682-0102-2015

Samsi, N. S., Zainol, A., Darus, A., Zainun, Z., Wang, C. S., Zainal, Z., Hashim, M. A. (2013). Serodiagnosis of leptospirosis in domestic animals and humans. *Malaysian Journal of Veterinary Research*, 4(2):21-26.

Sandhu, R. S., Ismail, H. B., Ja'afar, M. H. B., & Rampal, S. (2020). The predictive factors for severe leptospirosis cases in Kedah. *Tropical Medicine and Infectious Disease*, 5(2), 79. https://doi.org/10.3390/tropicalmed5020079

Santanna, R., Vieira, A. S., Grapiglia, J., & Lilenbaum, W. (2017). High number of asymptomatic dogs as leptospiral carriers in an endemic area indicates a serious public health concern. *Epidemiology and Infection*, 145(9), 1852–1854. https://doi.org/10.1017/S0950268817000632

Sarrazin, C., Gärtner, B. C., Sizmann, D., Babiel, R., Mihm, U., Hofmann, W. P., von Wagner, M., & Zeuzem, S. (2006). Comparison of conventional PCR with real-time PCR and branched DNA-based assays for hepatitis C virus RNA quantification and clinical significance for genotypes 1 to 5. *Journal of Clinical Microbiology*, 44(3), 729–737. https://doi.org/10.1128/JCM.44.3.729-737.2006

Scanziani, E., Luini, M., Fabbi, M., & Pizzocaro, P. (1991). Comparison between specific immunoperoxidase staining and bacteriological culture in the diagnosis of renal leptospirosis of pigs. *Research in*

Veterinary Science, 50(2), 229–232. https://doi.org/10.1016/0034-5288(91)90112-2

Scanziani, E., Sironi, G., & Mandelli, G. (1989). Immunoperoxidase studies on leptospiral nephritis of swine. *Veterinary Pathology*, 26(5), 442–444. https://doi.org/10.1177/030098588902600510

Schneider, M. C., Najera, P., Pereira, M. M., Machado, G., dos Anjos, C. B., Rodrigues, R. O., Cavagni, G. M., Muñoz-Zanzi, C., Corbellini, L. G., Leone, M., Buss, D. F., Aldighieri, S., & Espinal, M. A. (2015). Leptospirosis in Rio Grande do Sul, Brazil: An ecosystem approach in the animal-human interface. *PLoS Neglected Tropical Diseases*, 9(11), e0004095. https://doi.org/10.1371/journal.pntd.0004095

Schrader, C., Schielke, A., Ellerbroek, L., & Johne, R. (2012). PCR inhibitors - occurrence, properties and removal. *Journal of Applied Microbiology*, 113(5), 1014–1026. https://doi.org/10.1111/j.1365-2672.2012.05384.x

Schuller, S., Francey, T., Hartmann, K., Hugonnard, M., Kohn, B., Nally, J. E., & Sykes, J. E. (2015). European consensus statement on leptospirosis in dogs and cats. *Journal of Small Animal Practice*, 56(3), 159–179. https://doi.org/10.1111/jsap.12328

Sembiring, E. (2018). Diagnostic approach in leptospirosis patients. *IOP Conference Series*, 125, 012089. https://doi.org/10.1088/1755-1315/125/1/012089

Sessions, J., & Greene, C. (2004). Canine leptospirosis: Treatment, prevention, and zoonosis. *ResearchGate*. https://www.researchgate.net/publication/287716084_Canine_leptospirosis_Treatment_prevention_and_zoonosis

Sethi, S., Sharma, N., Kakkar, N., Taneja, J., Chatterjee, S. S., Banga, S. S., & Sharma, M. (2010). Increasing trends of leptospirosis in northern India: a clinico-epidemiological study. *PLoS Neglected Tropical Diseases*, 4(1), e579. https://doi.org/10.1371/journal.pntd.0000579

Shang, E. S., Summers, T. A., & Haake, D. A. (1996). Molecular cloning and sequence analysis of the gene encoding LipL41, a surface-exposed lipoprotein of pathogenic *Leptospira* species. *Infection and Immunity*, 64(6), 2322–2330. https://doi.org/10.1128/iai.64.6.2322-2330.1996

Slack, A. T., Kalambaheti, T., Symonds, M. L., Dohnt, M. F., Galloway, R. L., Steigerwalt, A. G., Chaicumpa, W., Bunyaraksyotin, G., Craig, S. B., Harrower, B., & Smythe, L. D. (2008). *Leptospira wolffii* sp. nov., isolated from a human with suspected leptospirosis in Thailand. *International Journal of Systematic and Evolutionary Microbiology*, 58(10), 2305–2308. https://doi.org/10.1099/ijs.0.64947-0

Slack, A. T., Khairani-Bejo, S., Symonds, M. L., Dohnt, M. F., Galloway, R. L., Steigerwalt, A. G., Bahaman, A. R., Craig, S., Harrower, B. J., &

Smythe, L. D. (2009). *Leptospira kmetyi* sp. nov., isolated from an environmental source in Malaysia. *International Journal of Systematic and Evolutionary Microbiology*, 59(Pt 4), 705–708. https://doi.org/10.1099/ijs.0.002766-0

Smythe L. D. (2008). Leptospirosis. *Australia and New Zealand Standard Diagnostic Procedures*. https://www.agriculture.gov.au/agriculture land/animal/health/laboratories/procedures/anzsdp/leptospirosis

Smythe, L. D., Smith, I. L., Smith, G. A., Dohnt, M. F., Symonds, M. L., Barnett, L. J., & McKay, D. B. (2002). A quantitative PCR (TaqMan) assay for pathogenic *Leptospira* spp. *BMC Infectious Diseases*, 2(13). https://doi.org/10.1186/1471-2334-2-13

Sonja, O., Sonja, R., Nataša, S., Danica, B., Slobodanka, V. & Miroslav, V.(2014). Seroprevalence of cat leptospirosis in Belgrade (Serbia). *Acta Veterinaria*, 64(4), 510-518. https://doi.org/10.2478/acve-2014-0047

Stoddard, R. A., Gee, J. E., Wilkins, P. P., McCaustland, K., & Hoffmaster, A. R. (2009). Detection of pathogenic *Leptospira* spp. through TaqMan polymerase chain reaction targeting the LipL32 gene. *Diagnostic Microbiology and Infectious Disease*, 64(3), 247–255. https://doi.org/10.1016/j.diagmicrobio.2009.03.014

Stokes, J. E., & Forrester, S. D. (2004). New and unusual causes of acute renal failure in dogs and cats. *The Veterinary Clinics of North America. Small Animal Practice*, 34(4), 909–vi. https://doi.org/10.1016/j.cvsm.2004.03.006

Suepaul, S. M., Carrington, C. V., Campbell, M., Borde, G., & Adesiyun, A. A. (2010). Serovars of *Leptospira* isolated from dogs and rodents. *Epidemiology and Infection*, 138(7), 1059–1070. https://doi.org/10.1017/S0950268809990902

Sykes, J. E., Hartmann, K., Lunn, K. F., Moore, G. E., Stoddard, R. A., & Goldstein, R. E. (2011). 2010 ACVIM small animal consensus statement on leptospirosis: diagnosis, epidemiology, treatment, and prevention. *Journal of Veterinary Internal Medicine*, 25(1), 1–13. https://doi.org/10.1111/j.1939-1676.2010.0654.x

Szeredi, L., & Haake, D. A. (2006). Immunohistochemical identification and pathologic findings in natural cases of equine abortion caused by leptospiral infection. *Veterinary Pathology*, 43(5), 755–761. https://doi.org/10.1354/vp.43-5-755

Tan, X. T., Amran, F., Chee Cheong, K., & Ahmad, N. (2014). In-house ELISA screening using a locally-isolated *Leptospira* in Malaysia: determination of its cut-off points. *BMC Infectious Diseases*, 14, 563. https://doi.org/10.1186/s12879-014-0563-7

Tansuphasiri, U., Chanthadee, R., Phulsuksombati, D., & Sangjun, N. (2006). Development of a duplex-polymerase chain reaction for rapid detection of pathogenic *Leptospira*. *The Southeast Asian Journal of Tropical Medicine and Public Health*, 37(2), 297–308.

Taylor, C., Brodbelt, D., Dobson, B., Catchpole, B., O'Neill, D. G., & Stevens, K. (2021). Spatio-temporal distribution and agroecological factors associated with canine leptospirosis in Great Britain. *Preventive Veterinary Medicine*, 193, 105407. https://doi.org/10.1016/j.prevetmed.2021.105407

Thaipadungpanit, J., Chierakul, W., Wuthiekanun, V., Limmathurotsakul, D., Amornchai, P., Boonslip, S., Smythe, L. D., Limpaiboon, R., Hoffmaster, A. R., Day, N. P., & Peacock, S. J. (2011). Diagnostic accuracy of real-time PCR assays targeting 16S rRNA and lipL32 genes for human leptospirosis in Thailand: a case-control study. *PloS One*, 6(1), e16236. https://doi.org/10.1371/journal.pone.0016236

Thanh, T. T., Anh, N. T., Tham, N. T., Van, H. M., Sabanathan, S., Qui, P. T., Ngan, T. T., Van, T. T., Nguyet, L. A., Ny, N. T., Thanh, leT. M., Chai, O. K., Perera, D., Viet, doC., Khanh, T. H., Ha, doQ., Tuan, H. M., Wong, K. T., Hung, N. T., Chau, N. V., & Van Tan, L. (2015). Validation and utilization of an internally controlled multiplex real-time RT-PCR assay for simultaneous detection of enteroviruses and enterovirus A71 associated with hand foot and mouth disease. *Virology Journal*, 12(85). https://doi.org/10.1186/s12985-015-0316-2

Thiermann A. B. (1984). Leptospirosis: current developments and trends. *Journal of the American Veterinary Medical Association*, 184(6), 722–725.

Topçuoğlu, N., Paltura, C., Külekçi, M., Ustek, D., & Külekçi, G. (2013). Real-time polymerase chain reaction versus conventional PCR: A comparison between two methods for the detection of *Fusobacterium nucleatum* in saliva, nasopharyngeal secretion and middle ear effusion samples. *Biotechnology & Biotechnological Equipment*, 27(3), 3825–3828. https://doi.org/10.5504/bbeq.2013.0022

Torgerson, P. R., Hagan, J. E., Costa, F., Calcagno, J. I., Kane, M. J., Martinez-Silveira, M. S., Goris, M. G. A., Stein, C., Ko, A. I., & Abela-Ridder, B. (2015). Global burden of Leptospirosis: estimated in terms of disability adjusted life years. *PLoS Neglected Tropical Diseases*, 9(10), e0004122. https://doi.org/10.1371/journal.pntd.0004122

Valasek, M. A., & Repa, J. J. (2005). The power of real-time PCR. *Advances in Physiology Education*, 29(3), 151–159. https://doi.org/10.1152/advan.00019.2005

Valones, M. A., Guimarães, R. L., Brandão, L. A., de Souza, P. R., de Albuquerque Tavares Carvalho, A., & Crovela, S. (2009). Principles and applications of polymerase chain reaction in medical diagnostic

fields: a review. *Brazilian Journal of Microbiology : [publication of the Brazilian Society for Microbiology]*, 40(1), 1–11. https://doi.org/10.1590/S1517-83822009000100001

van de Maele, I., Claus, A., Haesebrouck, F., & Daminet, S. (2008). Leptospirosis in dogs: a review with emphasis on clinical aspects. *The Veterinary Record*, 163(14), 409–413. https://doi.org/10.1136/vr.163.14.409

VanGuilder, H. D., Vrana, K. E., & Freeman, W. M. (2008). Twenty-five years of quantitative PCR for gene expression analysis. *BioTechniques*, 44(5), 619–626. https://doi.org/10.2144/000112776

Vedhagiri, K., Natarajaseenivasan, K., Prabhakaran, S. G., Selvin, J., Narayanan, R., Shouche, Y. S., Vijayachari, P., & Ratnam, S. (2010). Characterization of *Leptospira borgpetersenii* isolates from field rats (*Rattus norvegicus*) by 16s rrna and lipl32 gene sequencing. *Brazilian Journal of Microbiology : [publication of the Brazilian Society for Microbiology]*, 41(1), 150–157. https://doi.org/10.1590/S1517-838220100001000022

Victoriano, A. F. B., Smythe, L. D., Gloriani-Barzaga, N., Cavinta, L. L., Kasai, T., Limpakarnjanarat, K., Ong, B. L., Gongal, G., Hall, J. M., Coulombe, C. A., Yanagihara, Y., Yoshida, S., & Adler, B. (2009). Leptospirosis in the Asia Pacific region. *BMC Infectious Diseases*, *9*(1). https://doi.org/10.1186/1471-2334-9-147

Villumsen, S., Pedersen, R., Borre, M. B., Ahrens, P., Jensen, J. S., & Krogfelt, K. A. (2012). Novel TaqMan® PCR for detection of *Leptospira* species in urine and blood: pit-falls of in silico validation. *Journal of Microbiological Methods*, 91(1), 184–190. https://doi.org/10.1016/j.mimet.2012.06.009

Waggoner, J. J., Balassiano, I., Abeynayake, J., Sahoo, M. K., Mohamed-Hadley, A., Liu, Y., Vital-Brazil, J. M., & Pinsky, B. A. (2014). Sensitive real-time PCR detection of pathogenic *Leptospira* spp. and a comparison of nucleic acid amplification methods for the diagnosis of leptospirosis. *PloS One*, 9(11), e112356. https://doi.org/10.1371/journal.pone.0112356

Waggoner, J. J., Soda, E. A., Seibert, R., Grant, P., & Pinsky, B. A. (2015). Molecular detection of *Leptospira* in two returned travelers: higher bacterial load in cerebrospinal fluid versus serum or plasma. *The American Journal of Tropical Medicine and Hygiene*, 93(2), 238–240. https://doi.org/10.4269/ajtmh.15-0174

Wang, N., Han, Y. H., Sung, J. Y., Lee, W. S., & Ou, T. Y. (2016). Atypical leptospirosis: an overlooked cause of aseptic meningitis. *BMC Research Notes*, 9, 154. https://doi.org/10.1186/s13104-016-1964-z

Ward, M. P., Glickman, L. T., & Guptill, L. E. (2002). Prevalence of and risk factors for leptospirosis among dogs in the United States and Canada: 677 cases (1970-1998). *Journal of the American Veterinary Medical Association*, 220(1), 53–58. https://doi.org/10.2460/javma.2002.220.53

Ward, M. P., Guptill, L. F., Prahl, A., & Wu, C. C. (2004). Serovar-specific prevalence and risk factors for leptospirosis among dogs: 90 cases (1997-2002). *Journal of The American Veterinary Medical Association*, 224(12), 1958–1963. https://doi.org/10.2460/javma.2004.224.1958

White, A. M., Zambrana-Torrelio, C., Allen, T., Rostal, M. K., Wright, A. K., Ball, E. C., Daszak, P., & Karesh, W. B. (2017). Hotspots of canine leptospirosis in the United States of America. *Veterinary Journal (London, England : 1997)*, 222, 29–35. https://doi.org/10.1016/j.tvjl.2017.02.009

Wild, C. J., Greenlee, J. J., Bolin, C. A., Barnett, J. K., Haake, D. A., & Cheville, N. E. (2002). An improved immunohistochemical diagnostic technique for canine leptospirosis using antileptospiral antibodies on renal tissue. Journal of veterinary diagnostic investigation : official publication of the American Association of Veterinary Laboratory Diagnosticians, Inc, 14(1), 20–24. https://doi.org/10.1177/104063870201400105

WHO (2003) Human Leptospirosis: Guidance for Diagnosis, Surveillance and Control. http://www.who.int/csr/don/en/WHO_CDS_CSR_EPH_2002.23.pdf

WHO (2011) Leptospirosis: an emerging public health problem. *Weekly Epidemiological Record*, 86(6), 45–52. http://www.who.int/wer/2011/wer8606/en [PubMed]

Woo, T. H., Patel, B. K., Cinco, M., Smythe, L. D., Norris, M. A., Symonds, M. L., Dohnt, M. F., & Piispanen, J. (1999). Identification of *Leptospira biflexa* by real-time homogeneous detection of rapid cycle PCR product. *Journal of Microbiological Methods*, 35(1), 23–30. https://doi.org/10.1016/s0167-7012(98)00095-5

World Organisation for Animal Health. (2021). Leptospirosis - WOAH Terrestrial Manual 2021. https://www.woah.org/fileadmin/Home/fr/Health_standards/tahm/3.01.12_LEPTO.pdf

Wunder, E. A., Jr, Figueira, C. P., Santos, G. R., Lourdault, K., Matthias, M. A., Vinetz, J. M., Ramos, E., Haake, D. A., Picardeau, M., Dos Reis, M. G., & Ko, A. I. (2016). Real-time PCR reveals rapid dissemination of *Leptospira interrogans* after intraperitoneal and conjunctival inoculation of hamsters. *Infection and Immunity*, 84(7), 2105–2115. https://doi.org/10.1128/IAI.00094-16

Wuthiekanun, V., Sirisukkarn, N., Daengsupa, P., Sakaraserane, P., Sangkakam, A., Chierakul, W., Smythe, L. D., Symonds, M. L., Dohnt,

M. F., Slack, A. T., Day, N. P., & Peacock, S. J. (2007). Clinical diagnosis and geographic distribution of leptospirosis, Thailand. *Emerging Infectious Diseases*, 13(1), 124–126. https://doi.org/10.3201/eid1301.060718

Xu, C., Loftis, A., Ahluwalia, S. K., Gao, D., Verma, A., Wang, C., & Kaltenboeck, B. (2014). Diagnosis of canine leptospirosis by a highly sensitive FRET-PCR targeting the lig genes. *PloS One*, 9(2), e89507. https://doi.org/10.1371/journal.pone.0089507

Yamaguchi, T., Higa, N., Okura, N., Matsumoto, A., Hermawan, I., Yamashiro, T., Suzuki, T., & Toma, C. (2018). Characterizing interactions of *Leptospira interrogans* with proximal renal tubule epithelial cells. *BMC Microbiology*, 18(1), 64. https://doi.org/10.1186/s12866-018-1206-8

Yang, C. W., Wu, M. S., Pan, M. J., Hsieh, W. J., Vandewalle, A., & Huang, C. C. (2002). The *Leptospira* outer membrane protein LipL32 induces tubulointerstitial nephritis-mediated gene expression in mouse proximal tubule cells. *Journal of The American Society of Nephrology*, 13(8), 2037–2045. https://doi.org/10.1097/01.asn.0000022007.91733.62

Yusof, N. Y., Muhammad Yusoff, F., Muhammad Harish, S., Ahmad, M. N., Khalid, M. F., Mohd Nor, F., Ismail, N., & Aziah, I. (2019). Complete genome sequence of *Leptospira kmetyi* LS 001/16, isolated from a soil sample associated with a leptospirosis patient in Kelantan, Malaysia. *Microbiology Resource Announcements*, 8(28), e00015-19. https://doi.org/10.1128/MRA.00015-19

Zakeri, S., Khorami, N., Ganji, Z. F., Sepahian, N., Malmasi, A. A., Gouya, M. M., & Djadid, N. D. (2010). *Leptospira wolffii*, a potential new pathogenic *Leptospira* species detected in human, sheep and dog. *Journal of Molecular Epidemiology and Evolutionary Genetics in Infectious Diseases*, 10(2), 273–277. https://doi.org/10.1016/j.meegid.2010.01.001

Zilber, A., Belli, P., Artois, M., Kodjo, A., & Djelouadji, Z. (2016). First observation of *Leptospira interrogans* in the lungs of *Rattus norvegicus*. *BioMed Research International*, 2016, 1–8. https://doi.org/10.1155/2016/9656274

Zwijnenberg, R. J., Smythe, L. D., Symonds, M. I., Dohnt, M. F., & Toribio, J. A. (2008). Cross-sectional study of canine leptospirosis in animal shelter populations in mainland Australia. *Australian Veterinary Journal*, 86(8), 317–323. https://doi.org/10.1111/j.1751-0813.2008.00324.x