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DETECTION OF LEPTOSPIROSIS IN A DOG SHELTER

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DETECTION OF LEPTOSPIROSIS IN A DOG SHELTER

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It is hereby certified that we have read this project paper entitled “Detection of Leptospirosis in A Dog Shelter” by Boo Ao Lin and in our opinion it is satisfactory in terms of scope, quality and presentation as partial fulfillment of the requirement for the course VPD 4999-Final Year Project.



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DEDICATIONS

ALMIGHTY GOD

FAMILY

To my parents and siblings who give me continuous support all the time

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To my supervisor and co-supervisors for all their guidance and assistance

To all lecturers and staffs of Faculty of Veterinary Medicine, UPM, for all the
dedications and contributions in veterinary education

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To my beloved classmates of batch 2012/2017

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LIST OF ABBREVIATIONS

μl	microliter
$^{\circ}\text{C}$	degree Celsius
CDC	Centers for Disease Control and Prevention
CFR	Case Fatality Rate
CFU	Colony forming units
ELISA	Enzyme-linked immunosorbent assay
EMJH	Ellinhausen-McCullough-Johnson-Harris
IACUC	Institutional Animal Care and Use Committee
LPHS	Leptospirosis Pulmonary Haemorrhage Syndrome
LPS	lipopolysaccharide
MAT	Microscopic Agglutination Test
mL	milliliter
<i>n</i>	sample size
OIE	World Organisation for Animal Health
PBS	Phosphate Buffer Saline
PCR	Polymerase Chain Reaction
pH	Potential of Hydrogen
rpm	round per minute
sv.	serovar
UPM	Universiti Putra Malaysia

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakultin Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999- Projek Ilmiah Tahun Akhir

PENGESANAN LEPTOSPIROSIS DI DALAM SATU PUSAT

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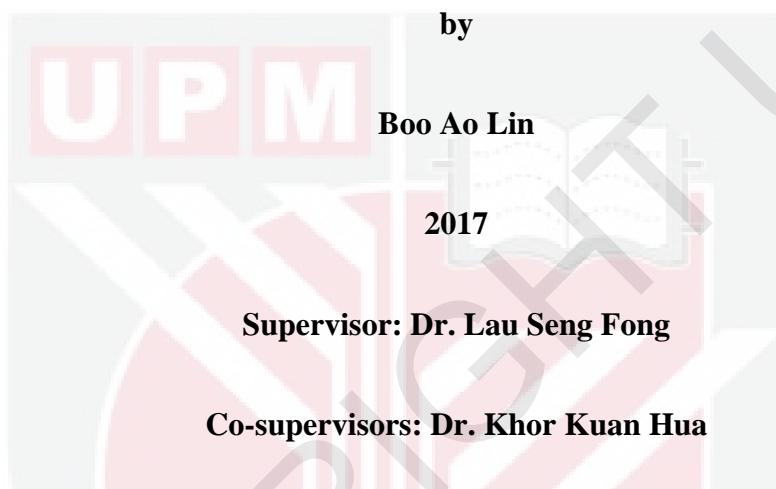
Leptospirosis (Penyakit Kencing Tikus) adalah penyakit bakteria zoonotik yang dilaporkan di seluruh dunia dengan rekod lebih daripada satu juta kes manusia setiap tahun di dunia. Kajian yang dijalankan ke atas Leptospirosis dalam kalangan anjing adalah tidak mencukupi di Malaysia walaupun mereka mungkin mempunyai risiko yang tinggi untuk menyebar penyakit ini kepada manusia. Tujuan kajian ini adalah untuk mengesan Leptospirosis dan serovars yang terlibat dalam pusat perlindungan anjing di Johor, Malaysia. Sampel darah telah dikumpul daripada 73 anjing terdiri

daripada 50 anjing yang telah menerima vaksinsi dan 23 anjing yang tidak pernah menerima vaksinasi. Ujian serologi MAT (Microscopic Agglutination Test) telah digunakan untuk mengesani antibodi anti-leptospiral dalam serum sampel. Di titer antibody penentuan iaitu 1:80, dua daripada 73 anjing (2.7%) adalah positif kepada *Leptospira borgpetersenii* serovar javanica. Dua daripada 73 anjing (2.7%) adalah positif kepada *L. interrogans* serovar Icterohaemorrhagiae dan satu daripada 73 anjing (1.4%) menunjukkan titers antibodi terhadap *L.interrogans sv. Australis*. Kelaziman keseluruhan leptospirosis adalah 6.8% ($n = 5/73$) dalam 73 anjing yang telah disampelkan. Antara kelima-lima anjing yang positif, 80% adalah betina dan 20% adalah jantan, dan kesemuanya telah menerima vaksinasi. Dapatkan kajian ini menunjukkan bahawa anjing berpotensi menyebarkan penyakit ini kepada manusia dan binatang lain. Justeru, kajian lanjutan untuk menyelidik peranan epidemiologi anjing dalam Leptospirosis adalah diperlukan.

Kata kunci: Leptospirosis, Penyakit Kencing Tikus, anjing, MAT, kelaziman, antibody

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfillment of the course VPD 4999 Final Year Project.

DETECTION OF LEPTOSPIROSIS IN A DOG SHELTER

Leptospirosis is a zoonotic bacterial disease of worldwide distribution with more than one million human cases reported annually in the world. Limited study has been conducted on canine leptospirosis in Malaysia despite they may have high risk of transmitting the disease to human. The purpose of this study was to detect the canine leptospirosis and possible serovars involved in a dog shelter in Johor, Malaysia. Blood samples were collected from 73 dogs consisted of 50 vaccinated dogs and 23 non-vaccinated dogs. Microscopic agglutination test (MAT) was used to screen the serum samples for anti-leptospiral antibodies. At the cut –off titer of 1:80, two out of 73 dogs (2.7%) were seropositive for *Leptospira borgpetersenii* serovar Javanica. Another two out of 73 dogs (2.7%) were seropositive for

L.interrogans serovar Icterohaemorrhagiae and one out of 73 dogs (1.4%) showed antibody titers against *L.interrogans* sv. Australis. The overall seroprevalence was 6.8% (n=5/73) in the 73 dogs studied. All seropositive dogs are vaccinated, consisting of 80% females and 20% males. The seropositive status of these shelter dogs showed that they could be potential disease disseminator to human and other animals warrant further investigation for their potential epidemiological role in leptospirosis.

Keywords: Leptospirosis, canine, MAT, seroprevalence, anti-leptospiral antibodies

1.0 INTRODUCTION

Leptospirosis is a zoonotic bacterial disease of worldwide distribution with more than one million human cases reported annually in the world. It is caused by a spirochete of the *Leptospira* genus which belongs to the family *Leptospiraceae*, order Spirochaetales. Leptospires comprise of both saprophytic and pathogenic species. Among the 300 serovars classified based on the expression of the surface-exposed epitopes in a mosaic of the lipopolysaccharide (LPS) antigens, 250 are pathogenic (Adler & Moctezuma, 2010; Goris, 2016). This wide spread zoonosis is depicted by Alder and Moctezuma (2010) as disease incidence have been reported in all continents and virtually all mammalian species examined. This disease has been recognized as a re-emerging disease particularly in tropical countries and is significant for public health concerns due to its zoonotic risk. Besides, leptospirosis is a main cause of disease in production and companion animals such as dogs, cattle, swine, horses, deer and probably sheep (Ellis, 2015).

Natural reservoir hosts of the disease range from rodents, companion animals such as dogs, livestock such as cattle, pigs, and wild animals. Dogs are considered maintenance hosts for serovar Canicola, incidental hosts for other serovars. They are considered a potential source of infection to human due to the close association with people and their unsanitary habits (Phumoonna *et al.*, 2009).

Leptospirosis is a systemic disease both in humans and domestic animals, predominantly dogs, cattle and swine. Clinical signs are variable in various kinds of animal species with most cases are subclinical and are related to host adapted serovars

such as Canicola in dogs and Hardjo in cattle. According to Adler & Moctezuma (2010), four syndromes have been recognized in dogs including icteric, hemorrhagic, uremic and reproductive (abortion and premature or weak pups). Typical leptospirosis in dogs may show signs such as fever, jaundice, vomiting, diarrhea, intravascular disseminated coagulation, uremia caused by renal failure, hemorrhages and death (Bolin, 1996).

Canine leptospirosis was first described in 1899. The causative agents which are most common in clinical cases of canine leptospirosis are *Leptospira* interrogans and the serovars Icterohaemorrhagiae and Canicola were described in 1960 (Carrasco, 2015). The incidence of infection seems to have reduced with the widespread use of the bivalent vaccines containing these two common serovars. However, there is increased incidence of the disease reported in the past 20 years and the most prevalent serovars nowadays are *L kirschneri* serovar Grippotyphosa, *L interrogans* serovar Pomona and *L. interrogans* serovar Bratislava (Carrasco, 2015). Species such as: *L. heidelbergis*, *L. autumnalis*, *L. australis*, *L. medanensis*, *L. bataviae* and *L. sejroe* also have been observed in studies from other countries (Robert, 1955).

To date, studies on canine leptospirosis among dog population in Malaysia is still not adequate which leads to limited knowledge on the prevalence and epidemiology of canine leptospirosis in Malaysia. The purpose of this study was to detect the canine leptospirosis and possible serovars involved in a dog shelter in Johor, Malaysia. The findings would provide information on the disease status of leptospirosis in local dog population in Malaysia and provide some insight into the epidemiology of leptospirosis in Malaysia.

8.0 REFERENCES

- Adler, B., & de la Peña Moctezuma, A. (2010). Leptospira and leptospirosis. *Veterinary Microbiology*, 140(3-4), 287–296. doi:10.1016/j.vetmic.2009.03.012
- Alexander, A.D., Benenson, A.S., Byrne, R.J., Diaz Rivera, R.S., Evans, L.B., & Gochenour, W.S., ... Yager, R.H. (1963). Leptospirosis in Puerto Rico. *Zoonoses Res*, 2, 152–27.
- Alton, G.D., Berke, O., Reid-Smith, R., Ojkic, D. & Prescott, J.F. (2008). Increase in seroprevalence of canine leptospirosis and its risk factors, Ontario 1998–2006. *Canadian Journal of Veterinary Research*, 73 (3), 167–175.
- Ambily, R., Mini, M., Joseph, S., Krishna, S.V. & Abhinay, G. (2012). Canine leptospirosis- a seroprevalence study from Kerala, India. *Veterinary World*, 6 (1), 42-44. doi:10.5455/vetworld.2013.42-44
- Benacer, D., Thong, K. L., Verasahib, K. B., Galloway, R. L., Hartskeerl, R. A., Lewis, J. W., & Mohd Zain, S. N. (2016). Human Leptospirosis in Malaysia: Reviewing the challenges after 8 decades (1925–2012). *Asia Pacific Journal of Public Health*, 28(4), 290–302. doi:10.1177/1010539516640350
- Bharti, A.R., Nally, J.E., Ricardi, J.N., Matthias, M.A., Diaz, M.M., Lovett, M.A., ... Vinetz, J.M. (2003). Leptospirosis: a zoonotic disease of global importance. *The Lancet Infectious Diseases*, 3(12), 757–771. doi: http://dx.doi.org/10.1016/S1473-3099(03)00830-2
- 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.
- Carrasco, I. R. Z. (2015). Seroprevalence of Canine Leptospirosis im Dogs in Developing Countries. In O. P. Jenkins (Ed.), *Advances in animal science & zoology*, 7, 13–22.
- Centers for Disease Control and Prevention.(2015). Prevention in pets. Retrieved from <https://www.cdc.gov/leptospirosis/pets/prevention/index.html>
- Costa, F., Hagan, J. E., Calcagno, J., Kane, M., Torgerson, P., Martinez-Silveira, M. S., ... Ko, A. I. (2015). Global morbidity and mortality of Leptospirosis: A systematic review. *PLoS Neglected Tropical Diseases*, 9(9), e0003898. doi:10.1371/journal.pntd.0003898

- Cruz-Romero, A., Romero-Salas, D., Aguirre, C.A., Aguilar-Domínguez, M. & Bautista-Piña, C. (2013). Frequency of canine leptospirosis in dog shelters in Veracruz, Mexico. *African Journal of Microbiology Research*, 7 (16), 1518-1521. doi: 10.5897/AJMR12.1053
- de Paula Dreer, M., Gonçalves, D., da Silva Caetano, I., Gerônimo, E., Menegas, P., Bergo, D., ... Martins, L. de (2013). Toxoplasmosis, leptospirosis and brucellosis in stray dogs housed at the shelter in Umuarama municipality, Paraná, Brazil. *Journal of Venomous Animals and Toxins including Tropical Diseases*, 19(1), 23. doi: 10.1186/1678-9199-19-23
- Dhliwayo, S., Matope, G., Marabini, L., Dutlow, K. & Pfukenyi, D.M. (2012). Seroprevalence of leptospirosis in dogs in urban Harare and selected rural communities in Zimbabwe. *Onderstepoort Journal of Veterinary Research*, 79 (1), 1-6. doi: <http://dx.doi.org/10.4102/>
- Ellis, W.A. (2015). Animal Leptospirosis. In B. Adler (Eds), *Leptospira and Leptospirosis* (pp. 99-137). Berlin: Springer-Verlag.
- Everard, C.O., Green, A.E., & Glosser, J.W. (1976). Leptospirosis in Trinidad and Grenada, with special reference to the mongoose. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 70(1), 57-61.
- Fletcher, W. (1928). Recent work on leptospirosis, tsutsugamushi disease and tropical typhus in the Federated Malay States. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 21 (4), 265-IN9.
- Gautam, R., Wu, 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.
- Geisen, V., Stengel, C., Brem, S., Müller, W., Greene, C. and Hartmann, K. (2007). Canine leptospirosis infections – clinical signs and outcome with different suspected *Leptospira* serogroups (42 cases). *Journal of Small Animal Practice*, 48, 324–328. doi:10.1111/j.1748-5827.2007.00324.x
- Goldstein, R.E. (2010). Canine leptospirosis. *Veterinary Clinics of North America: Small Animal Practice*, 40 (6), 1091-1101. doi: 10.1016/j.cvsm.2010.07.008
- Goris, M.G.A. (2016, April 6). Leptospirosis: epidemiology, clinical aspects and diagnosis. Erasmus University Rotterdam. Retrieved from <http://hdl.handle.net/1765/80097>.

Grooms, D. L. (2006). Reproductive losses caused by bovine viral diarrhea virus and leptospirosis. *Theriogenology*, 66(3), 624–628.
doi:10.1016/j.theriogenology.2006.04.016

Hayatrohi,A., Lak, A.G., Hashempour, A., Gholizadeh, S.S., Abdollahpour, G.R. & Zadeh, R.T. (2014). Survey on seroprevalence of *Leptospira* serotypes in household dogs using MAT method in Urmia, Iran. *Bulletin of Environment, Pharmacology and Life Sciences*, 3 (special issue V), 158-162.

Joseph, P.G. (1979). Leptospirosis in animals in West Malaysia. The *Malaysian Journal of Pathology*, 2, 15-21.

Juvet, F., Schuller, S., O'Neill, E. J., O'Neill, P. A., & Nally, J. E. (2011). Urinary shedding of spirochaetes in a dog with acute leptospirosis despite treatment. *Veterinary Record*, 168(21), 564–564. doi:10.1136/vr.d740

Khor, K.H., Tan, W.X., Lau, S.F., Roslan, M.A., Radzi, R., Bejo, S.K. & Bahaman, A.R. (2016). Seroprevalence and molecular detection of leptospirosis from a dog shelter. *Tropical Biomedicine*, 33 (2), 276-284.

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

Klaasen, H. & Adler, B. (2015). Recent advances in canine leptospirosis: focus on vaccine development. *Veterinary Medicine Research and Reports*, 2015(6), 245-260. doi: <http://dx.doi.org/10.2147/VMRR.S59521>

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. doi:10.1016/j.trstmh.2010.07.002

Lau, S.F., Low, K.N., Khor, K.H., Roslan, M.A., Bejo, S.K. & 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.

Lavinsky, M.O., Said, R.A., Strenzel, G.M.R. & Langoni, H. (2012). Seroprevalence of anti-Leptospira spp. antibodies in dogs in Bahia, Brazil. *Preventive Veterinary Medicine*, 106, 79-84. doi: <http://dx.doi.org/10.1016/j.prevetmed.2012.03.015>

- Lelu, M., Muñoz-Zanzi, C., Higgins, B. & Galloway, R. (2015). Seroepidemiology of leptospirosis in dogs from rural and slum communities of Los Rios Region, Chile. *BioMed Central Veterinary Research*, 11 (31), 1-8. doi: 10.1186/s12917-015-0341-9
- Levett, P.N. (2004). Leptospirosis: a forgotten zoonosis? *Clinical and Applied Immunology Reviews*, 4, 435-448.
- Levett, P.N. & Haake, D.A. (2010). *Leptospira species (leptospirosis). Principles and practice of infectious diseases* (pp.3059-3065). Philadelphia: Churchill Livingstone Elsevier.
- Majetić, Z. S., Habuš, J., Milas, Z., Perko, V.M., Starešina, V. & Turk, N. (2012). Serological survey of canine leptospirosis in Croatia-the changing epizootiology of the disease. *Veterinarski Arhiv*, 82 (2), 183-191.
- Major, A., Schweighauser, A. & Francey, T. (2014). Increasing incidence of canine leptospirosis in Switzerland. *International Journal of Environmental Research and Public Health*, 11 (7), 7242-7260. doi: 10.3390/ijerph110707242
- 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.
- Miller, R., Ross, S., Sullivan, N., & Perkins, N. (2007). Clinical and epidemiological features of canine leptospirosis in north Queensland. *Australian Veterinary Journal*, 85(1-2), 13–19. doi:10.1111/j.1751-0813.2006.00089.x
- Moore, G.E., Guptill, L.F., Glickman, N.W., Caldanaro, R.J., Aucoin, D. & Glickman, L.T. (2006). Canine leptospirosis, United States, 2002-2004. *Emerging Infectious Disease*, 12(3), 501-503.
- Mwachui, M.A., Crump, L., Hartskeerl, R., Zinsstag, J. & Hattendorf, J. (2015). Environmental and behavioural determinants of leptospirosis transmission: a systematic review. *Leptospirosis: epidemiology, clinical aspects and diagnosis. PLoS Neglected Tropical Diseases*, 9(9), 1-15. doi: 10.1371/journal.pntd.0003843
- Oliveira, S.T., Messick, J.B., Biondo, A.W., dos Santons, A.P., Stedile, R., Dalmolin, M.L., ... González, F.H.D. (2012). Exposure to *Leptospira* spp. in sic Dogs, Shelter Dogs and Dogs from an Endemic Area: Points to Consider. *Acta Scientiae Veterinariae*, 40 (3), 1056.

- O'Keefe, JS., Sandifer, NC., Antony, A. & Williamson, NB. (2011). A serosurvey for antibodies to *Leptospira* in dogs in the lower North Island of New Zealand.*New Zealand Veterinary Journal*, 50 (1), 23-25. doi: 10.1080/00480169.2002.36245
- Petlanchanapong, W., Yasaeng, S., Chantapetch, P. & Bhudhilukul, N. (2009). The cut-off values for single serum of leptospirosis detection. *Bulletin of the Department of Medical Sciences – วารสารกรมวิทยาศาสตร์การแพทย์*, 51(2), 91-103.
- Phua, M. , Chong, C. W., Ahmad, A. H., & Hafidzi, M. N. (2017). Understanding rat occurrences in oil palm plantation using high-resolution satellite image and GIS data. *Precision Agriculture*.1-13. doi:10.1007/s11119-016-9496-z
- Phumoonna, T., Mutualib, A.R., Bahaman, A.R. & Mohd-Azmi, M.L. (2009). Leptospiral infection in stray dogs in Malaysia. *The Malaysian Journal of Pathology*, 21 (1), 23-27.
- Picardeau, M. (2013). Diagnosis and epidemiology of leptospirosis. *Médecine et Maladies Infectieuses*, 43(1), 1–9. doi:10.1016/j.medmal.2012.11.005
- Picardeau, M., Bertherat, E., Jancloes, M., Skouloudis, A. N., Durski, K., & Hartskeerl, R. A. (2014). Rapid tests for diagnosis of leptospirosis: Current tools and emerging technologies. *Diagnostic Microbiology and Infectious Disease*, 78(1), 1–8. <http://doi.org/10.1016/j.diagmicrobio.2013.09.012>
- Robert, J. B. (1955). Canine leptospirosis and public health. *Public Health Reports*, 70(12), 1229-1236.
- Romero-Vivas, C.M.E., Cuello-Pérez, M., Agudelo-Flórez, P., Thiry, D., Levett, P.N. & Falconar, A.K.I. (2013). Cross-sectional study of *Leptospira* seroprevalence in humans, rats, mice and dogs in a main tropical sea-port city. *The American Journal of Tropical Medicine and Hygiene*, 88(1), 178-183. doi:10.4269/ajtmh.2012.12-0232
- Roqueplo, C., Marié, J., André-Fontaine, G., Kodjo, A. & Davoust, B. (2014). Serological survey of canine leptospirosis in three countries of tropical Africa: Sudan, Gabon and Ivory Coast. *Comparative Immunology, Microbiology and Infectious Diseases*, 38, 57-61. doi: <http://dx.doi.org/10.1016/j.cimid.2014.10.006>
- Schller, S., Arent, Z.J., Gilmore, C. & Nally, J. (2015). Prevalence of antileptospiral serum antibodies in dogs in Ireland. *Veterinary Record*, 177(5), 126. doi: 10.1136/vr.102916

- Shi, D., Liu, M., Guo, S., Liao, S., Sun, M., Liu, J., ... Chai, T. (2011). Serological survey of canine leptospirosis in Southern China. *Pakistan Veterinary Journal*, 32(2), 280-282.
- Shivakumar, S., & Krishnakumar, B. (2006). Diagnosis of leptospirosis-role of MAT. *The Journal of the Association of Physicians of India*, 54, 338-339.
- Smith, C.E.G., Turner, L.H., Harrison, J.L. & Broom, J.C. (1961). Animal leptospirosis in Malaya. 1. Methods, zoogeographical background and broad analysis of results. *Bulletin of the World Health Organization*, 24(1), 5-21.
- Stokes, J. E., Kaneene, J. B., Schall, W. D., Kruger, J. M., Miller, R., Kaiser, L., & Bolin, C. A. (2007). Prevalence of serum antibodies against six Leptospira serovars in healthy dogs. *Journal of the American Veterinary Medical Association*, 230(11), 1657-1664. doi:10.2460/javma.230.11.1657
- Suepaul, S.M., Carrington, C.V., Campbell, M., Borde, G., & Adesiyun, A.A. (2014). Seroepidemiology of leptospirosis in dogs and rats in Trinidad. *Tropical Biomedicine*, 31(4), 853-861.
- Sulzer, C. (1975). Leptospiral serotype distribution lists according to host and geographic area July 1966 to July 1973. Atlanta: US Department of Health, Education, and Welfare.
- Thaipadunpanit, J., Chierakul, W., Wuthiekanun, V., Limmathurotsakul, D., Amornchai, P., Boonslip, S., ... 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).doi: http://dx.doi.org/10.1371/journal.pone.0016236
- Thayaparan, S., Robertson, I.D., Fairuz, A., Suut, L. & Abdullah, M.T. (2013). Leptospirosis, an emerging zoonotic disease in Malaysia. *The Malaysian Journal of Pathology*, 35(2), 123-132.
- Tomich, P. (1979). Studies of leptospirosis in natural hostpopulations I. Small mammals of Waipio Valley, Island of Hawaii. *Pacific Science*, 33(3), 257-79.
- Victoriano, A., Smythe, L. D., Gloriani-Barzaga, N., Cavinta, L. L., Kasai, T., Limpakarnjanarat, K., ... Adler, B. (2009). Leptospirosis in the Asia Pacific region. *BioMed Central Infectious Diseases*, 9(1), 147.doi:10.1186/1471-2334-9-147

- Vojinović, D., Bogićević, N., Vasić, A., Manić, M., Radoanović, M. E., Rogozarski, D., ... Valčić, M. (2015). Seroepidemiological survey of leptospiral infection in stray dogs in Serbia. *Turkish Journal of Veterinary and Animal Sciences*, 39, 719-723. doi: 10.3906/vet-1505-18
- Wilson, S., Stirling, C., Thomas, A., King, V., Plevová, E., Chromá, L., ... Sture, G. (2013). Duration of immunity of a multivalent (DHPPI/L4R) canine vaccine against four *Leptospira* serovars. *Vaccine*, 31(31), 3126–3130. doi:10.1016/j.vaccine.2013.05.043
- Wisseman, C.L., Traub Jr.R., Gochenour,W.S., Smadel Jr. J. E. & Lancaster W.E. (1955). Leptospirosis of man and animals in urban,rural and jungle areas of Southeast Asia. *The American Journal of Tropical Medicine and Hygiene*, 4(1), 29-40.
- Wong, J.Y. (2016). *Seroprevalence and molecular detection of leptospirosis among working dogs population in Malaysia.*(Unpublished degree's thesis). University Putra Malaysia, Serdang.
- World Organization for Animal Health, (2016).Leptospirosis.In *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2016.*(Chapter 2.1.12). Retrieved from
http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.01.12_LEPTO.pdf
- 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), 1-8.
- Zwijnenberg, R., Smythe, L., Symonds, M., Dohnt, M., & Toribio, J.-A.(2008). Cross-sectional study of canine leptospirosis in animal shelter populations in mainland Australia. *Australian Veterinary Journal*, 86(8), 317–323. doi:10.1111/j.1751-0813.2008.00324.x