UNIVERSITI PUTRA MALAYSIA

SEROPREVALENCE OF LEPTOSPIROSIS AND BRUCELLOSIS IN LONG-TAILED MACAQUES (MACACA FASCICULARIS) OF PENINSULAR MALAYSIA

YONG SUIT-B, CHYNA

FPV 2017 3

SEROPREVALENCE OF LEPTOSPIROSIS AND BRUCELLOSIS IN LONG-TAILED MACAQUES *(MACACA FASCICULARIS)* OF PENINSULAR MALAYSIA

YONG SUIT-B, CHYNA

A project paper submitted to the

Faculty of veterinary Medicine, University Putra Malaysia

In partial fulfilment of the requirement for the

DEGREE OF DOCTOR OF VETERINARY MEDICINE

Universiti Putra Malaysia

Serdang, Selangor Darul Ehsan

FEBRUARY 2017

It is hereby certified that I/we* have read this project paper entitled "Seroprevalence of Leptospirosis and Brucellosis in Long-tailed macaques (*Macaca fascicularis*) of Peninsular Malaysia', by Yong Suit-B, Chyna and in my/our* opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfilment of the requirement for the course VPD 4901 – Final Year Project.

_____

ASSOC. PROF. DR. LATIFFAH HASSAN

DVM (UPM), PHD (CORNELL)

Head of Department,

Department of Veterinary Laboratory Diagnostics

Faculty of Veterinary Medicine

Universiti Putra Malaysia

(Supervisor)

ASSOC. PROF. DR. SITI KHAIRANI BEJO

DVM (UPM), MSC (UPM), PHD (UPM)

Department of Veterinary Pathology & Microbiology,

Faculty of Veterinary Medicine

Universiti Putra Malaysia

(Co-Supervisor)

DR. RUEBEN SUNIL KUMAR SHARMA

DVM (UPM), MVSC (UPM), PHD (CAMBRIDGE)

Senior Lecturer,

Department of Veterinary Laboratory Diagnostics,

Faculty of Veterinary Medicine

Universiti Putra Malaysia

(Co-Supervisor)

DEDICATION

I dedicate this dissertation to

My mother who has been a constant support and encouragement for everything I choose to pursue. Her unconditional love and dedication is irreplaceable and she is an inspiration for me to always do my best. She is precious and I am grateful to have a superwoman like her every day of my life.

My four-legged best friend, Twinkle, who has been a treasured companion for the past 15 years. No words can describe how she continues to inspire me to carry on with this veterinary course. I will always remember the silent intimate moments we share, how she makes me smile through challenging times and cherish all the time she has left in this world.

Nature and her beautiful residents.

ACKNOWLEDGEMNTS

I owe my deepest gratitude to my supervisor, Assoc. Prof. Dr. Latiffah Hassan for her patience and generosity with time and knowledge. Her support has been tremendous from the very beginning of this project and it has been an honour working with someone who has so much experience in epidemiology. Not to mention, her eloquence and grace is inspiring!

I am immensely grateful to be co-supervised by Assoc. Prof. Dr. Khairani Bejo and Dr. Rueben Sunil Kumar Sharma who are both experts in their field. I am grateful for Assoc. Prof. Dr. Khairani Bejo's motherly instinct who was always patient in repeating her explanations until she saw sparks in my eyes. It has also been enjoyable working with Dr. Rueben, who enlightened me about primate behaviour and diseases. I would like to thank him for opening up the opportunity to use the macaques serum from his laboratory.

I would also like to extend my gratitude to Prof. Dato. Dr. Abdul Rani Bahaman who shared his enthusiasm for this project. He has been uplifting and a source of motivation for me to strive.

I would like to share the credit of my project to the cheerful team ofstaffs in the Bacteriology laboratory, especially Mr. Mohd. Azri Roslan and Miss Krishnammah Kuppusamy who taught me everything I needed to know and were wonderful companions over tea breaks. Thank you to Byron Chong Yeu Liang too who was a helpful colleague.

Special thanks and gratitude goes to Dr. Mohammad Sabri Bin Abdul Rahman for his guidance and patience. My deepest appreciation and thanks to the three heroines, Ms. Nor Dilaila Mohd Saidi, Ms. Zarith Suraya Shahar, and Norhadila Zuklifli, who were on field to collect the macaque blood samples and generosity for sharing them with me.

I am thankful for my friends whom I admire most and lean on in times of need. They are Tenri Ashari, Kiven Kumar A/L Karunanithi, Amir Hakim Jasmani, and Hameedunisha Tajudeen.

Last but not least, my heartfelt gratitude goes to my family for their everlasting love and support throughout my final year project. And Twinkle, my faithful companion who always puts a smile on my face.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4901 – Projek Ilmiah Tahun Akhir.

SEROPREVALENS*LEPTOSPIROSIS* DAN *BRUCELLOSIS* PADA KERA KETAM (*MACACA FASCICULARIS*) DI SEMENANJUNG MALAYSIA

Oleh

Suit-B, Y

2017

Penyelia: Prof Madya Dr. Latiffah Hassan

Penyakit *leptospirosis* dan *brucellosis* adalah penyakit zoonotik yang penting di seluruh dunia dengan insiden yang tinggi di negara tropika yang menjejaskan biodiversiti, kesihatan manusia danhaiwan, kebajikan haiwan dan ekonomi (OIE, 2014; WHO, 2011).Populasi manusia yang semakin berkembang dan pembandaran yang pesat telah menyebabkan peningkatan interaksi hidupan liar dan manusia. Di Malaysia, penambahan konflik antara manusia dan kera (Hambali, 2012) meningkatkan risiko jangkitan penyakit. Objektif kajian ini adalah untuk menentukan seroprevalens *leptospirosis* dan *brucellosis* pada kera ketam Semenanjung Malaysia. Seratus sampel serum telah diuji untuk antibodi terhadap*leptospirosis* dan *brucellosis* dengan mengunakan Microscopic Agglutination Test (MAT) dan Rose Bengal Plate Test (RBPT) masing-masing. Empat belas peratus (14/100) didapati positif untuk *leptospirosis*.Serovar yang paling

lazimdikenalpasti adalah Cellodoni (4%) dan Pyrogenes (4%), diikuti Icterohaemorrhagiae (3%), Bataviae(2%) dan Lai (1%).Seroprevalens *leptospirosis* pada kera jantan adalah lebih tinggi dibandingan kera betina.Kera jantan adalah 4.5 kali lebih mungkin seropositif dibandingkan dengan kera betina.Ini mencadangkan bahawa perbezaan tingkah laku jantina mempengaruhi pendedahan kepada *leptospirosis*.Tiada perbezaan didapati antara seroprevalens dengan umur, habitat dan kawasan.Semua sampel adalah seronegatif terhadap *brucellosis*.Kajian ini menunjukkan bahawa kera ketam terjangkit*leptospirosis* menimbulkan risiko kesihatan awam kerana boleh berlakunya penyebaran silang spesis.

Kata Kunci: *Leptospirosis, Brucellosis, Microscopic agglutination test (MAT), Rose Bengal Plate test (RBPT), Zoonotik, Kera ketam (Macaca fascicularis), Primata, Semenanjung Malaysia*

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary medicine in partial fulfilment of the course VPD 4901– Final Year Project.

SEROPREVALENCE OF LEPTOSPIROSIS AND BRUCELLOSIS IN LONG-TAILED MACAQUES (*MACACA FASCICULARIS*) OF PENINSULAR MALAYSIA

by

Suit-B, Y

2017

Supervisor: Assoc. Prof. Dr. Latiffah Hassan

Leptospirosis and brucellosis are important zoonotic diseases worldwide with high incidence in tropical countries affecting biodiversity, human and livestock health, animal welfare and the economy (OIE, 2014; WHO, 2011). The expanding human population along with rapid urbanization have increased the likelihood of wildlife and human interaction. In Malaysia, the increased human-macaque conflicts (Hambali, 2012) have resulted in the concern about zoonotic disease transmission. This study was conducted to determine the seroprevalence of leptospirosis and brucellosis in wild long-tailed macaques of Peninsular Malaysia. A hundred serum samples were screened for antibodies against *Leptospira*and *Brucella* using microscopic agglutination test (MAT) and Rose Bengal Plate test (RBPT) respectively. Fourteen percent of macaques were seropositive for leptospirosis with serovar Cellodoni(4%), and Pyrogenes (4%) as the most common serovar identified, followed by Icterohaemorrhagiae (3%), Bataviae(2%)

and Lai(1%). The prevalence in males were significantly higher than females. Males were 4.5 times more likely to be seropositive for leptospirosis compared to females. This suggests that sex differences in behaviour influences exposure of macaques to leptospirosis. There were no significant difference in seroprevalence with age, habitat and region. All samples were seronegative for brucellosis. This study concludes that leptospirosis are prevalent in long-tailed macaques and poses a public health risk of cross-species transmission.

Keywords: *Leptospirosis, Brucellosis, Microscopic agglutination test (MAT), Rose Bengal Plate test (RBPT), Zoonosis, Long-tailed macaques (Macaca fascicularis), Non-human primate, Peninsular Malaysia*

1.0 INTRODUCTION

Zoonotic diseases are defined as diseases and infections which are naturally transmitted between vertebrate animals and man (WHO, 2017). The trend on wildlife emerging infectious diseases have been associated to the increase population density, encroachment into wildlife habitat, mismanagement of captive wildlife, change in agriculture practices, climate change, wildlife and exotic pet trade and ecotourism (Daszak et al., 2000; Guerra, 2013). Leptospirosis is an important worldwide zoonotic disease with high incidence in tropical countries, while brucellosis is a 'neglected zoonotic disease' (Thayaparan 2013; OIE 2014). Both diseases affects biodiversity, human and livestock health, animal welfare and economy (WHO 2011).

Leptospirosis is an endemic disease first reported in Malaysia in 1920 (Ministry of Health Malaysia, 2011) and has been recognized as a re-emerging public health problem in Malaysia (Arief, 2013). Factors for re-emergence are related to conditions favourable for maintenance and transmission of leptospirosis such as favourable reservoir and carrier hosts, flooding, animal-human interface and human host factors (Ministry of Health Malaysia, 2011). A seroprevalence study in Sarawak wildlife found 80% primates, 44% bats, 100% squirrels and 100% mongoose reacted positively to one or more serovars of *Leptospira sp.*(Thayaparan, 2013). Thayaparam (2013) emphasized on the importance of surveying wildlife species which lives at periphery of forests with potential to interact with humans, such as wild rats, carnivores and bats, but did not include non-human primates. Several seroprevalence studies conducted over the years revealed that non-human primates are susceptible to experimental leptospirosis and naturally acquired leptospirosis (Ibanez-Contreras et al 2010; Szonyi B 2011; Desvars

2013) and are clinically asymptomatic (Astudillo et al. 2012). Although, not much is known on leptospirosis transmission between humans and non-human primates, non-human primates should be considered as a possible asymptomatic carrier (Szonyi, 2011).

Brucellosis is the most common zoonotic infection worldwide, but is particularly neglected in Asia, leading to the emergence of this disease (Pappas et al., 2006). This disease is endemic in Malaysia (Bamaiyi et al., 2014) and high number of cases have been reported among cattle populations under the integration-plantation system (Palanisamy et al., 1999). Livestock and wildlife interaction are drivers for disease transmission. Anka et al (2014) suggested that presence of wildlife and non-cattle species on same farm are significant to bovine brucellosis in Malaysia. Prevalence studies on animal brucellosis in Malaysia has been done on goats, cattle, buffaloes and dogs (Bamaiyi et al., 2014). The epidemiology and ecology of wildlife brucellosis is still poorly understood (Godfroid et al. 2013). Multiple studies have found that non-human primates are good models to study human brucellosis (Henning, 2011; Yingst, 2010). A novel Brucella sp. was isolated in wild caught baboons (Schlabritz-Loutsevitch et al, 2009), but no studies have been performed locally on seroprevalence of brucellosis in non-human primates.

The long-tailed macaques has dominated the human-wildlifeconflict complaints received by the Department of Wildlife and National Parks in Malaysia (Saaban et al., 2016). The increase in human-macaque conflicts in Malaysia driven by loss of habitat and food sources, supported by subsequent adaptation to urbanized human environments result in higher interactions of humans and macaques (Hambali, 2012). Other contact

opportunities such as feeding in public recreational areas, capture of wild macaques for the pet trade or biomedical research colonies, consumption, or population management by wildlife authorities also increases human-macaque contact, thus increasing direct and indirect exposure to macaque body fluids (Lee et al., 2015). It is important to screen for particular species that lives at the periphery of forests and have the potential to interact with humans (Thayaparan et al., 2013), especially when wildlife serves as sinks for human pathogen (Muehlenbein, 2013).

No recent studies have been done to investigate seroprevalence of leptospirosis and brucellosis in primates of Peninsular Malaysia. The increase human-macaque conflicts and interactions in Malaysia which poses public health risk enhance the need to understanding prevalence of zoonotic diseases in the macaques.

This study was conducted to investigate the following objectives:-

1. To determine seroprevalence of leptospirosis and brucellosis in long-tailed macaques (*Macaca fascicularis*) of Peninsular Malaysia.
2. To investigate the association between seroprevalence of the two diseases to risk factors such as age, sex, habitat and region of long-tailed macaques (*Macaca fascicularis*).

The hypotheses of this study are:-

1. H_o: Long-tailed macaques of Peninsular Malaysia are seronegative for leptospirosis and/or brucellosis.
2. H_a: Long-tailed macaques of Peninsular Malaysia are seropositive for leptospirosis and/or brucellosis.

In this study, prevalence and risk factors for leptospirosis and brucellosis in long-tailed macaques are described using sera samples from human-macaque conflict cases reported in Peninsular Malaysia from 2015 to 2016. This study provides useful pilot information on the prevalence of these important zoonotic diseases in the macaques for more well designed studies in the future. The association between prevalence of disease and risk factors also gives us an insight on the possible effects of social structure and demography on disease persistence in primates.

REFERENCES

1. Anka, M.S, Hassan, L., Adzhar, A., Khairani-Bejo, S., Mohamad, R., and Zainal, M.A. (2013). Bovine brucellosis trends in Malaysia between 2000 and 2008. *PLoS ONE 9(9): e108673.*
2. Al-Mariri A, and Haj-Mahmoud, N. (2010). Detection of *Brucella abortus* in bovine milk by polymerase chain reaction. *Acta Vet. Brno.* 79:277280.
3. Adler, B., and Moctezuma, A.P. (2010). Leptospira and leptospirosis. *Veterinary Microbiology, 140,* 287-296.
4. Astudillo, V.G., Hernandez, D.W., Stadlin, J. P., Bernal, L. A., Rodriguez, D. R. L. and Hernandez, M. A. (2012). Comparative seroprevalence of *Leptospira interrogans* in Colombian mammals along a climatic gradient. *Journal of Zoo and Wildlife Medicine, 43 (4),* 768–775.
5. Al Dahouk S, Nöckler K, Scholz HC, Tomaso H, Bogumil R, et al. (2006). Immunoproteomic characterization of *Brucella abortus* 1119-3 preparations used for the serodiagnosis of *Brucella* infections. *Journal of Immunology Method, 309,* 34-47.
6. Alton, G.G., and Forsyth, J.R.L. (1996). Chapter 28, Brucella. In Baron S. Galveston (Eds.), *Medical microbiology 4th Ed*. The University of Texas Medical Branch at Galveston.
7. Angst, W. (1975). Basic Data and Concepts on the Social Organization of *Macaca fascicularis* in Primate Behaviour. In L.A. Rosenblum. (Eds.), *Developments in Field and Laboratory Research, Vol 4.* (pp. 325-388). Academic Press New York.
8. Australian Bureau of Statistics. (2013). Statistical language, census and sample. Retrieved from www.abs.gov.au
9. Arief, M. T. (2013). Changing nature of health crisis from infectious diseases and responses over three decades in Malaysia, Borneo. *Journal of Resource Science and Technology 2,* 1-11.
10. Bahaman, A.R. and Ibrahim, A.L. (1988). A review of Leptospirosis in Malaysia. *Veterinary Research Communications, 12,* 179-189.
11. Bahaman, A. R. (1991). Leptospiral infection in domestic animals in Malaysia: Its importance, epidemiology and control. *Proceedings of the 6th International Symposium on Veterinary Epidemiology and Economics, 1991,* 227-229.
12. Bahaman, A.R., Joseph, P.G., and Siti-Khairani, B. (2007). A review of the epidemiology and control of brucellosis in Malaysia. *1. Vet. Malaysia, 19 (1),* 1 – 6.
13. Bamaiyi, P.H. (2014). Updates on brucellosis in Malaysia and Southeast Asia. *Malaysian Journal of Veterinary Research, 5 (1),* 71-82.
14. Bamaiyi, P.H. (2016). Prevalence and risk factors of brucellosis in man and domestic animals: A review. *International Journal of One Health, 2,* 29-34.
15. Baitchman, E.J., Calle, P.P., James, S.B., Linn, M.J., Raphael, B.L. (2006). *Leptospirosis in Wied's marmosets (Callithrix kuhlii). Journal of Zoo and Wildlife Medicine, 37,* 182–185.
16. Baulu, J., Everard, C.O.R., Everard, J.D. (1987). Leptospires in vervet monkeys (*Cercopithecus aethiops sabaeus*) on Barbados. *Journal of Wildlife Diseases, 23 (1),* 60-66.

17. Berg, H. C., D. B. Bromley, and N. W. Charon. (1978). Leptospiral motility. In Davis, R.E. (Eds.), *Symp. Soc. Gen. Microbiol, 28*, 285-294.
18. Benacer, D., Thong, K.L., Verasahib, K., Galloway, R.L., Hartskeerl, R.A., Lewis, J.W., and Md Zain, S.N. (2016). Human leptospirosis in Malaysia: Reviewing the challenges after 8 decades (1925-2012). *Asia Pacific Journal of Public Health*, 1-13.
19. Benacer, D., Thong, K.L., Verasahib, K., Galloway, R.L., Hartskeerl, R.A., Souris, M., and Md Zain, S.N. (2016). Epidemiology of human leptospirosis in Malaysia, 2004-2012. *Acta Tropica, 157*, 162-168.
20. Berger, S. (2016). Bruellosis. In S. Berger (Eds.), *Infectious Diseases of Malaysia: 2016 Edition*. (pp. 38-41). GIDEON Informatics, Inc. pp. 61-63.
21. Bharti, A.R., Nally, J.E, Ricaldi, J.N, Matthias, A.M, Diaz, M.M, Lovett, M.A, Levett, P.N, Gilman, R.H, Willig, M.R, Gotuzzo, E., and Vinetz, J.M. (2003). Leptospirosis: a zoonotic disease of global importance. *Lancet Infect Dis, 3*, 757–71.
22. Bolin, C. (2000). Chapter 9, Leptospirosis. In C. Brown and C. Bolin (Eds.), *Emerging Disease of Animals* (pp. 185-200). ASM Press, Washington, DC.
23. Burgos-Rodriguez (2011). Zoonotic diseases of primates. *Vet Clin Exot Anim 14*, 557-575.
24. Budihal, S.V and Perwez, K. (2014). Leptospirosis diagnosis: Competency of various laboratory tests. *Journal of Clinical and Diagnostic Research. 8 (1)*, 199-202.
25. Cawthon Lang KA. (2006). Primate Factsheets: Long-tailed macaque (*Macaca fascicularis*) Taxonomy, Morphology, & Ecology. *Primate Info Net, Library and Information Service, National Primate Research Center.* Retrieved from http://pin.primate.wisc.edu/factsheets/entry/long-tailed_macaque.
26. Cleaveland, S., Laurenson, M.K., Taylr, L.H. (2001). Diseases of humans and their domestic mammals: Pathogen characteristics, host range and risk of emergence. *Philos. Trans. roy. Soc. Lond., B, biol. Sci., 356 (1411)*, 991-999.
27. Colagross-Schouten, A.M., Mazet, J.A.K., Gulland, F.M.D., Miller, M.A., and Hietala, S. (2002). Diagnosis and seroprevalence of leptospirosis in California sea lions from coastal California. *Journal of Wildlife Diseases, 38 (1)*, 7–17.
28. Corbel, M.J. (2006). Brucellosis in Humans and Animals. In *WHO Library Cataloguing-in-Publication Data*. World Health Organization.
29. Daszak, P., Cunningham, A.A and Hyatt, A. D. (2000). Emerging infectious diseases of wildlife - Threats to biodiversity and human health. *Science's Compass. Science,* 297, 443-449.
30. de Jong G, de Ruiter JR, Haring R. (1994). Genetic structure of a population with social structure and migration. In Loeschcke, V., Tomiuk, J., Jain, S.K. (Eds.), *Conservation genetics*. (pp. 147-64). Basel (Switzerland): Birkhäuser Verlag.
31. Desvars, A., Michault, A., and Bourhy, P. (2013). Leptospirosis in the western Indian Ocean islands: What is known so far? *Veterinary Research 2013*, 44-80.
32. Diaz, R., Casanova, A., Ariza, J., and Moriyon, I. (2011). The rose bengal test in human brucellosis: A neglected test for the diagnosis of a neglected disease. *PLoS Negl Trop Dis 5(4)*, e950.
33. Ellis, W.A. (2015). Animal Leptospirosis. In B. Adler (Eds.), *Leptospira and Leptospirosis, Current Topics in Microbiology and Immunology* (pp. 99- 137). Springer-Verlag Berlin Heidelberg.

34. Everard, C.O.R., Baulu, J., Carrington, D.G., Korver, H., and Terpstra, W.J. (1991). Retention of leptospiral agglutinins and long-term response to administration of monoclonal antibodies in vervet monkeys (C*ercopithecus aethiops*) on Barbados. *Eur. J. Epidemiol,* 392-299, 396-402.
35. Faine, S. (1962). The growth of *Leptospira australis B* in the kidneys of mice in the incipient experimental carrier state. *J. Hyg., Camb. 60,* 435.
36. Figueiredo, P.D., Ficht, T.A., Rice-Ficht, A., Rossetti, C.A., and Adams, L.G. (2015). Pathogenesis and immunobiology of brucellosis: Review of brucella-host interactions. *American Journal of Pathology*, 185 (6), 1505-1517.
37. Ficht, T. (2010). Brucella taxonomy and evolution. *Future Microbiology, 5 (6),* 859–866.
38. Feto, B., and Urgesa, K. (2008). Serology [Lecture notes]. *Ethiopia Public Health Training Initiative*.
39. Fooden, J. (1995). Systemic review of Southeast Asian longtail macaques, *Macaca fascicularis* (Raffles, [1821]). *Field Museum of National History, 20 (3), 431-440.*
40. Godfroid, J., Nielsen, K., and Saegerman, C. (2010). Diagnosis of Brucellosis in Livestock and Wildlife. *Croat Med J, 51,* 296-305.
41. Godfroid, J., Dahouk, S.A., Pappas, G., Roth, F., Matope, G., Muma, J., Marcotty, T., Pfeiffer, D., and Skjerve, E. (2013). A 'One Health' surveillance and control of brucellosis in developing countries: Moving away from improvisation. *Comparative Immunology, Microbiology and InfectiousDiseases*, 36, 241-248.
42. Gordon-Smith, C. E., Turner, L. H., Harrisson, J. L. and Broom, J. C., (1961). Animal leptospirosis in Malaya. 1. Methods, zoogeographical background and broad analysis of results. *Bulletin of the World Health Organisation, 24*, 5-21.
43. Goris, M.A. and Hartskeerl R.A. (2014). Leptospirosis serodiagnosis by the microscopic agglutination test. *Current protocols in microbiology 32*, 12E.5.1-12E.5.18.
44. Guerra, M.A. (2013) Leptospirosis: Public health perspectives. *Biologicals, 41,* 295-297.
45. Global Invasive Species Database (GISD) (2015). Species profile *Macaca fascicularis*. *Global Invasive Species Database*. Retrieved from http://www.iucngisd.org/gisd/species.php?sc=139
46. Hambali, K., Ismail, A., Zulkifli, S.Z., Md-Zain, B.M., and Amir, A. (2012). Human-macaque conflict and pest behaviors of long-tailed macaques (*Macaca fascicularis*) in Kuala Selangor Nature Park. *Tropical Natural History, 12(2),* 189-205.
47. Haake, D.A., and Levett, P.N. (2015). Leptospirosis in Humans. *Curr Top Microbiol Immunol, 387*, 65–97.
48. Henning, L.N., Miller, S.M., Pak, D.H., Lindsay, A., Fisher, D.A., Barnewall, R.E., Briscoe, C.M., Anderson, M.S., and Warren, R.L. (2011). Pathophysiology of the rhesus macaque model for inhalational brucellosis. *American Society for Microbiology, 80 (1),* 298-310.
49. Ibanez-Contreras A, Hernandez-Godinez B, Torres-Barranca JI, and Melendez-Velez P (2010) Antibodies findings against Leptospira spp., of the serovars Panama, Lai, Australis, Shermani, and Patoc, in a group of rhesus monkeys (Macaca mulatto) in conditions of captivity. *Archivos De Medicina Veterinaria 42*,101–104.

50. IUCN. (2005). Benefits beyond boundaries. *Proceedings of the Vth IUCN World Parks Congress*. IUCN, Gland, Switzerland and Cambridge, UK, 306.
51. Joseph, P.G. (1979). Leptospirosis in Animals in West Malaysia. *Malaysian J Pathol, 2*, 15-21.
52. Jaffe, J.E., Hartskeerl, R.A., Bolhuis, H.G.H., Ahmed, A., and Houwers, D.J. (2007). Serological survey of leptospirosis in a dutch primate colony and the local rodent population. *43rd International Symposium on Diseases of Zoo and Wild Animals 2007*, 258-258.
53. Kamarul, H., Ahmad, I., Badrul-Munir, M.Z., Syaizan, Z., and Aainaa, A. (2014). Ranging behavior of long-tailed macaques (*Macaca fascicularis*) at the entrance of Kuala Selangor Nature Park. *Malaysia Applied Biology, 43 (2),* 129-142.
54. Karimullah and Anuar, S. (2011).Determination of sexual behaviors in *Macaca fascicularis*. *International Conference on Medical, Biological and Pharmaceutical Sciences (ICMBPS'2011)*, Pattaya.
55. Kaltungo, B.Y., Saidu, S.N.A., Sackey, A.K.B., and Kazeem, H.M. (2014). A review on diagnostic techniques for brucellosis. *African Journal of Biotechnology, 13 (1*), 1-10.
56. Karuppannan, K., Saaban, S., Mustapa, A.R., Zainal Abidin, F.A., Azimat, N.A., and Keliang, C. (2014). J Primatol 2014, 3, 1.
57. Kilbourn, A.M., Karesh, W.B., Wolfe, N.D., Bosi, E.J., Cook, R.A., and Andau, M. (2003). Health evaluation of free-ranging and semi-captive orangutans (Pongo pygmaeus pymaeus) in Sabah, Malaysia. *Journal of Wildlife Diseases, 39(1),* 73–83.
58. Kumar, R.S.,Pillai, R.M., Mukhopadhyay, H.K., Antony, P.X., Thanislass, J., Srinivas, V.M.V., and Vishnupriya, S. (2013). Seroepidemiology of canine leptospirosis by iELISA and MAT. *Veterinary World, 6 (11),* 926-930.
59. Leong, K.N., Chow, T.S., Wong, P.S., Hamzah, S.H., Ahmad, N., and Ch'ng, C.C. (2015). Outbreak of human brucellosis from consumption of raw goats' milk in Penang, Malaysia. *Am J Trop Med Hyg 2, 93 (3),* 539–541.
60. Lilenbaum, W., Varges, R., Moraes, I.A., Ferreira, A.M.R, and Pissinatti, A. (2005). Leptospiral antibodies in captive lion tamarins (*Leontopithecus sp*) in Brazil. *The Veterinary Journal, 169*, 462-464.
61. Lilenbaum, W., Monterio, R.V., Ristow, P., Fraguas, S., Cardoso, V.S., and Fedullo, L.P.L. (2002). Leptospirosis antibodies in mammals from Rio de Janeiro Zoo, Brazil. *Research in Veterinary Science, 73*, 319–32.
62. Lins ZC, Lopes ML. (1984). Isolation of Leptospira from wild forest animals in Mazonian Brazil. *Transaction of Royal Society of Tropical Medicine and Hygiene 78*, 124–126.
63. Levett, P.N. (2001). Leptospirosis. *Clinical Microbiology Reviews, 14(2),* 296-326.
64. Levett, P.N. (2004). Leptospirosis: A forgotten zoonosis? *Clinical and Applied Immunology Reviews, 4*, 435–448.
65. Levett, P.N. and Haake, D.A., (2015). Leptospirosis in humans. *Curr Top Microbiol Immunol., 387*, 65-97.
66. Lee, M.H., Rostal, M.L., Hughes, T., Sitam, F., Lee, C.Y., Japning, J., Harden, M.E., Griffiths, A., Basir, M., Wolfe, N.D., Epstein, J.H., and Daszak, P. (2015). Macacine Herpesvirus 1 in Long-Tailed Macaques, Malaysia, 2009–2011. *Emerging Infectious Diseases, 21 (7),* 1107-1113.

67. MacMillan, A.P. (1997). Investigation of the performance of the Rose Bengal plate test in the diagnosis of *Brucella melitensis* infection in sheep and goats. *FAO Corporate Document Repository*.
68. Malaysian baby killed by macaque monkey. (2010, October 7). BBC News. Retrieved from www.bbc.com
69. Mense, M.G., Borschel, R.H., Wilhelmsen, C.L., Pitt, M.L., and Hoover, D.L. (2003). Pathologic changes associated with brucellosis experimentally induced by aerosol exposure in rhesus macaques (Macaca mulatta). *Am J Vet Res, 65*, 644–652.
70. Minette, H.P. (1966). Leptosspirosis in primates other than man. *American Journal of Tropical Medicine and Hygiene, 15 (2)*, 190-198.
71. Minette, H.P., and Shaffer, M.F. (1968). Experimental leptospirosis in monkeys. *The American Journal of Tropical Medicine and Hygiene*, 17 (2), 202-212.
72. Murali, R.S.N. (2017, Jan 8). Mischievous macaques in Malacca to be moved. *The STAR online*. Retrieved from http://www.thestar.com.my
73. Mohammed, H., Nozha, C., Hakim, K., Abdelaziz, F., and Rekia, B. (2011). Leptospirosis: Epidemiology and usual manifestations. *Bacteriology Journal, 1 (1)*, 1-7.
74. Molina, C.V., Catao-Dias, J.L., Neto, J.S.F., Vasconcellos, S.A., Gennari, S.M., Valle, R.D.R.D., Souza, G.O.D., Morais, Z.M.D., Vitaliano, S.N., Strefezzi, R.D>F., and Bueno, M.G. (2014). Sero-epidemiological survey for brucellosis, leptospirosis, and toxoplasmosis in free-ranging Alouatta caraya and Callithrix penicillata from S~ao Paulo State, Brazil. *J Med Primatol, 43*, 197–201.
75. Md-Zain, B.M., Ruslin, F., Idris, and W.N.R. (2014). Human-Macaque Conflict at the Main Campus of Universiti Kebangsaan Malaysia. *Pertanika J. Trop. Agric. Sci., 37 (1)*, 73 - 85
76. MyIrea. G., (2015). OIE standards relevant to neglected zoonoses [Powerpoint presentation]. *FAO-APHCA/OIE/USDA Regional Workshop on Prevention and Control of Neglected Zoonoses in Asia. Obihiro, Japan.*
77. Muehlenbein, M.P. (2013). Chapter 4, Human-Wildlife Contact and Emerging Infectious Diseases. In E.S. Brondizio and E.F. Moran (Eds.), *Human-Environment Interactions in Current and Future Directions, Human-Environment Interactions 1*. Springer Science C Business Media.
78. Ministry of Health Malaysia. (2011). *Guidelines for the Diagnosis, Management, Prevention and Control of Leptospirosis in Malaysia. Disease Control Division 1st Edition*. Disease Control Division, Department of Public Health, MOH.
79. Nielsen, K., and Yu, W.L (2010). Serological diagnosis of brucellosis. *Prilozi, 31 (1)*, 65–89.
80. Niloofa, R., Fernando, N., de Silva, N.L., Karunanayake, L., Wickramasinghe, H., and Dikmadugoda, N. (2015) Diagnosis of Leptospirosis: Comparison between microscopic agglutination test, IgM-ELISA and IgM rapid immunochromatography test. P*LoS ONE, 10 (6)*, e0129236.
81. The National Invasive Species Council (NISC) (2006). *Invasive Species Definition Clarification and Guidance White Paper*. Definitions Subcommittee of the Invasive Species Advisory Committee. The National Invasive Species Council (NISC).
82. Ong, P. and Richardson, M. (2008). *Macaca fascicularis*. *The IUCN Red List of Threatened Species.* Retrieved from http://dx.doi.org/10.2305/IUCN.UK.2008.RLTS.T12551A3355536.en

83. OIE (2014). Leptospirosis. *OIE Terrestrial Manual 2014*. World Assembly of Delegates of the OIE, May 2014.
84. OIE (2008). Leptospirosis. *OIE Terrestrial Manual 2008*. OIE.
85. OIE (2016). Brucellosis (*B. abortus, B. melitensis* and *B. suis*) (Infection with *B. abortus, B. melitensis* and *B. suis*). *World Assembly of Delegates of the OIE, May 2016.*
86. Pappas, G., Akritidis, N., Bosilkovski, M., and Tsianos, E. (2005). Brucellosis. *N Engl J Med, 352*, 2325-36.
87. Palanisamy, K., Mahendran, R., Idris, K., Brucellosis – a serious threat to cattle production in plantations. *Proceedings of the National Congress on Animal Health and Production, Malaysia, VAM*, 331-335.
88. Percy, D.H., Egwu, I.N., and Jonas, A.M. (1972). Experimental *Brucella canis* Infection in the monkey (*Macaca arctoides*). *Can. J. comp. Med, 36*, 221-225.
89. Poester, F.P., Nielsen, L.K., Samartino, L.E., and Yu, W.L. (2010). Diagnosis of brucellosis. *The Open Veterinary Science Journal, 4*, 46-60.
90. Pinna, M.H., Martins, G., Pinheiro, A.C.O., Almeida, D.S., Oria, A.O., and Lilenbaum, W. (2011). Detection of anti-leptospira antibodies in captive nonhuman primates from Salvador, Brazil. *American Journal of Primatology, 74*, 8–11
91. Pinkerton, M.E. (1972). Brucella. In Fiennes (Eds.), *Pathology of Simian Primates. Part II. Infectious and Parasitic Diseases* (pp. 289-93). S. Karger; Basel, New York.
92. Purcell, B.K., Hoover, D.L., and Friedlander, A.M. (2007). Chapter 9, Brucellosis. In Z.F. Dembek (Eds.), *Medical Aspects of Biological Warfare: Textbooks of Military Medicine.* Office of the Surgeon General, US Army Medical Department Center and School, Bodern Institute, Washington, DC.
93. Pumipuntu, N. (2015). Detection of anti-lepospira antibodies and study on hematological values of long-tailed macaque (*M. fascicularis)* at Kosumpee Forest Park, Maha Sarakham. *Journal of Wildlife in Thailand, 22 (1)*, 37-45.
94. Salman, A.M.A., and Nasri, H.A.E. (2012). Evaluation of four serological tests to detect prevalence of bovine brucellosis in Khartoum state. *Journal of Cell and Animal Biology, 6 (9)*, 140-143.
95. Saaban, S., Yazid, A. Z., Abdul, R. M., and Keliang, C. (2016). Human-Wildlife Conflict In Peninsular Malaysia - Current Status And Overview [PowerPoint Slides]. *Biodiversity Forum 2016: Human-Wildlife Conflict Mitigation and Action in Agricultural Sector.* Department of Wildlife and National Parks of Peninsular Malaysia.
96. Samsi, N.S., Zainol, A., Darus, A., Zainun, Z., Chin, S.W., Zainal, Z. and Hashim, M.A. (2013). Serodiagnosis of leptospirosis in domestic animals and humans. *Malaysian Journal of Veterinary Research, 4 (2*), 21-26.
97. Schlichting, D., Nöckler, K., Bahn, P., Luge, E., Greiner, M., Müller-Graf, C., and Mayer-Scholl, A. (2015).Estimation of the sensitivity and specificity of a *Leptospira spp*. in-house ELISA through Bayesian modelling. *International Journal of Medical Microbiology, 305 (7)*, 756-761.
98. Schlabritz-Loutsevitch, N.E., Whatmore, A.M., Quance, C.R., Koylass, M.S., Cummins, L.B., Dick Jr, E.J., Snider, C.L.s, Cappelli, D., Ebersole, J.L., Nathanielsz, P.W., and Hubbard, G.B. (2009). A novel Brucella isolate in association with two cases of stillbirth in non-human primates – first report. *J Med Primatol 38*, 70–73.
99. Sussman, R.W., and Tattersall, I. (1981). Behavior and Ecology of *Macaca fascicularis* in Mauritius: A Preliminary Study. *Primates, 22(2)*, 192-20.

100. Seethamchai, S., Putaporntip, C., Malaivijitnond, S., Cui, L., and Jongwutiwes, S. (2008). Malaria and Hepatocystis species in wild macaques, Southern Thailand. *Am. J. Trop. Med. Hyg, 78 (4),* 646-653.
101. Seleem, M.N., Boyle, S.M., and Sriranganathan, N. (2010). Brucellosis: A re-emerging zoonosis. *Veterinary Microbiology 140,* 392-398.
102. Shivakumar, S., and Krishnakumar, B. (2006). Diagnosis of Leptospirosis - Role of MAT. JAPI, 54, 338-339.
103. Szonyi. B., Agudelo-Flórezb, P., Ramírezb, M., Morenob, N., and Ko, A.I. (2011). An outbreak of severe leptospirosis in capuchin (Cebus) monkeys. *Vet J, 188 (2),* 1-6.
104. Thayaparan, S., Robertson, I., Amraan, F., Su'ut, L., and Abdullah, M.T. (2013). Serological prevalence of leptospiral infection in wildlife in Sarawak, Malaysia. *Borneo J. Resour. Sci. Tech., 2 (2),* 71-74.
105. Thayaparan, S., Robertson, and Abdullah, M.T. (2014). Leptospiral agglutinins in captive and free ranging non-human primates in Sarawak, Malaysia. *Veterinary World, 7 (6),* 428-431.
106. Thayaparan, S., Robertson, Fairuz, A., Su'ut, L., and Abdullah, M.T. (2013). Leptospirosis, an emerging zoonotic disease in Malaysia. *Malaysian J Pathol, 35 (2),* 123 – 132.
107. Thirteen-year-old bitten by monkey. (2015, June 8). *Free Malaysia Today (FMT)*. Retrieved from freemalaysiatoday.com
108. Two kids attacked by pet monkeys in separate incidents. (2016, June 2). *Free Malaysia Today (FMT)*. Retrieved from www.freemalaysiatoday.com
109. Rodman PS. (1991). Structural differentiation of microhabitats of sympatric *Macaca fascicularis* and *M. nemestrina* in East Kalimantan, Indonesia. *Int J Primatol 12 (4),* 357-75.
110. van Noordwijk MA, van Schaik CP. (1999). The effects of dominance rank and group size on female lifetime reproductive success in wild long-tailed macaques, *Macaca fascicularis*. *Primates, 40 (1),* 105-30.
111. van Noordwijk, M.A., van Schaik, C.P. (2001). Career moves: Transfer and rank challenge decisions by male long-tailed macaques. *Behaviour, 138,* 359–395.
112. Victoriano, A.F.B., Smythe LD, Gloriani-Barzaga N. (2009). Leptospirosis in the Asia Pacific region. *BMC Infect Dis, 9 (1),* 147.
113. Ward, M.P., Glickman, L.T., and Guptill, L.F. (2002). Prevalence of and risk factors for leptospirosis among dogs in the United States and Canada: 677 cases (1970–1998). *JAVMA, 220,* (1), 53-58.
114. Wareth, G., Hikal, A., Refai, M., Metzer, F., Roesler, U., and Neubauer, H. (2014). Animal brucellosis in Egypt. *J Infect Dev Ctries, 8 (11),* 1365-1373.
115. Whatmore, A.M., Davison, N., Cloeckaert, A., Dahouk, S.A., Zygmunt, M.S., Brew, S.D., Perrett, L.L., Koylass, M.S., Vergnaud, G, Quance, C., Scholz, H.C., Dick Jr, E.J., Hubbard, G., and Schlabritz-Loutsevitch, N.E. (2014*). International Journal of Systematic and Evolutionary Microbiology*, *64*, 4120–4128.
116. Wilson, D.E. (1936). The pathogenicity of tanganyika strains of *Brucella abortus* and *B. melitensis* for a local species of monkey (*Cercopithicus sp*.). *J Hyg (Lond), 36*, 125–7.
117. World Wildlife Fund (WWF) (2005). Human Wildlife Conflict Manual. *WWF-World Wide Fund for Nature*.

118. World Health Organization (WHO). (1999). Leptospirosis, India: Report of the investigation of a post-cyclone outbreak in Orissa, November 1999. *Weekly Epidemiological Report 2000, 75*, 217-223.
119. World Health Organization (2011). Report of the Second Meeting of the Leptospirosis Burden Epidemiology Reference Group. *World Health Organization.* Retrieved from http://apps.who.int/iris/bitstream/10665/44588/1/9789241501521_eng.pdf
120. World Health Organization website. (2017). Zoonoses and the human-animal-ecosystems interface. Retrieved from http://www.who.int/zoonoses/en/.
121. Xavier, M.N., Paixao, T.A., Hartigh, A.B.D., Tsolis, R.M., and Santos, R.L. (2010). Pathogenesis of Brucello spp. *The Open Veterinary Science Journal 4*, 109-118.
122. Xing,J., Wang, H., Han, K., Ray, D.A., Huang, C. H., Chemnick, G., Stewart, C.B, Disotell, T.R, Ryder, O.A., and Batzer, M.A. (2005). A mobile element based phylogeny of Old World Monkeys. *Molecular Phylogenetics and Evolution, 37 (3),* 872-880.
123. Yingst, S.L., Huzella, L.M., Chubala, L., and Wolcott, M. (2010). A rhesus macaque (*Macaca mulatta*) model of aerosol-exposure brucellosis (*Brucella suis*): Pathology and diagnostic implications. *Journal of Medical Microbiology, 59*, 724–730.
124. Yusof, S.S.M, Haron, A.W., Bejo, K.S. (2012). Disease detection of brucellosis in goat population in Negeri Sembilan, Malaysia. *7th Proceedings of the Seminar in Veterinary Sciences.*
125. Zamri-Saad, M., and Kamarudin, M.I. (2016). Control of animal brucellosis: The Malaysian experience. *Asian Pacific Journal of Tropical Medicine*, 1-5.