Universiti Malaysia Terengganu Journal of Undergraduate Research *eISSN: 2637-1138*
Volume 5 Number 4, October 2023: 25-33

PREVALENCE OF PATHOGENIC *Leptospira* spp. IN SMALL MAMMAL HOSTS FROM DUNGUN, TERENGGANU

NUR JULIANI SHAFIE*, MOHAMMAD IZUAN MOHD ZAMRI, WAN SITI MARIAM WAN SA'IDI, MUHAMMAD SYIMIR NUR AZIM AZHAR AND MOHAMMAD RIDHUAN MOHD ALI

Faculty of Science and Marine Environment, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia.

**Corresponding author: nur.shafie@umt.edu.my* *https://doi.org/10.46754/umtjur.v5i4.410*

Abstract: Leptospirosis is a zoonotic disease caused by *Leptospira*, which is transmitted through infected mammalian species, including small mammals. However, the prevalence of pathogenic *Leptospira* spp. in small mammals, particularly between different site categories in the eastern part of Peninsular Malaysia, is still underexplored. Therefore, in this study, we aim to determine the species composition of small mammals and the prevalence of pathogenic *Leptospira* spp. between different site categories from Dungun, Terengganu. We selected five case sites (leptospirosis cases were recorded) and five control sites (no leptospirosis cases were recorded) in Dungun, Terengganu, based on previous reports by the Ministry of Health (MOH) Malaysia (2016-2020). For three consecutive nights, 100 cage traps were used for each trapping session between September 2021 and January 2022. In addition, DNA from kidney samples of captured individuals was extracted for the Polymerase Chain Reaction (PCR) detection of pathogenic *Leptospira* spp. Overall, 89 individuals from five species were captured (6.43% trapping success), consisting of *Rattus norvegicus* (n = 39), *Rattus rattus* (n = 27), *Suncus murinus* (n = 11), *Rattus exulans* (n = 10) and *Tupaia glis* (n = 2). Out of 89 individuals captured, 14 tested positive with a 15.7% (n = 14/89) prevalence rate. *R. norvegicus* was the most dominant captured species and had the highest number of infected individuals with pathogenic *Leptospira* (20.5%, n = 8/39). The statistical analysis revealed a significant difference in pathogenic *Leptospira* spp. between case and control sites ($P < 0.05$), with control sites recording higher positive individuals (78.6%, n = 11/14) compared to case sites (21.4%, n = 3/14). However, no significant differences were observed between species and sex ($P > 0.05$). The results of the study show that the monitoring of leptospirosis cases should not only focus on case sites but also on the control sites, even if there were no reports of leptospirosis cases in the area, in order to prevent outbreaks of this disease in the future.

Keywords: *Leptospira*, pathogenic, prevalence, small mammals, Dungun.

Introduction

Leptospira is a genus of bacteria that causes a disease called leptospirosis from the family Leptospiraceae and order Spirochaetales, which includes both saprophytic and pathogenic species (Faine *et al.,* 1999). *Leptospira* comprises over 300 serovars, which are further subdivided into over 24 serogroups (Picardeau, 2013). The first case of human leptospirosis was reported in 1925 (El Jalii & Bahaman, 2004). Since then, recorded cases have risen rapidly amongst vulnerable animal species as well as humans (Benacer *et al.,* 2016a; Tan *et al.,* 2016), making it the preeminent zoonosis that causes morbidity and fatality in Malaysia (Garba *et al.,* 2017). The World Health Organization (WHO) reported that it is considered the most widely spread zoonotic disease that has emerged (Wong *et al.,* 2012). The disease is commonly known in countries with humid tropical and subtropical climates (Villanueva *et al.,* 2010). Transmission of leptospirosis is aided through interactions between the maintenance host, infected atmosphere, and vulnerable animals. The disease can spread either through direct contact with skin lesions coming in direct contact with the urine of an infected carrier or through indirect contact such as contaminated water (Oblegala *et al.,* 2016).

Small mammals are the most significant maintenance reservoirs for leptospirosis and a key vector for various pathogenic *Leptospira* spp. (Azhari *et al*., 2018; Yusof *et al*., 2019; Mohd-Taib *et al*., 2020). Due to the favourable atmospheric and climatic circumstances that support the growth and development of the bacteria, Malaysia is considered an epicentre of leptospirosis (Trueba *et al*., 2004). The vast number of rodent reservoirs and wildlife habitats serves to aggravate the situation (Garba *et al.,* 2018). Two major vectors of *Leptospira* infection are *Rattus norvegicus* (Norway/Brown rat) and *Rattus rattus* (Black rat), which are abundant in peridomestic settings and urban areas (Boey *et al.,* 2019).

Based on previous studies, leptospirosis is a reemerging infectious disease affecting more than one million people globally yearly (Cosson *et al.,* 2014). Since 2010, when the Ministry of Health Malaysia (MOH) declared leptospirosis as a notifiable disease (Ministry of Health, 2015), the number of cases has risen rapidly, beginning with 284 cases in 2004 and becoming worse in 2012, with 3604 cases (Benacer *et al.,* 2016b). As a result, there were 461 confirmed cases between 2004 and 2012, with an incidence rate of 8.00 per 100,000 people (Benacer *et al.,* 2016a). In Malaysia, Terengganu is one of the five states with the highest incidence of leptospirosis cases (Ministry of Health, 2015). However, the prevalence in pathogenic *Leptospira* spp. in areas with and without reported cases of leptospirosis in suburban areas is still underexplored. Hence, the objective of this study is to determine whether there was a significant difference of pathogenic *Leptospira* spp. between control and case sites, species captured and sex in suburban areas of Dungun, Terengganu.

Materials and Methods

Study Area

This study was conducted in suburban areas in Dungun district, Terengganu (04° 46’ N, 103° 25’ E). Dungun is a coastal town in Terengganu with a population of more than 135,000 and Kuala Dungun is the capital of Dungun district. The study area was categorized into suburban based on the criteria by the National Land Use Information (Government of Malaysia, 2008). The suburban area is characterized by the presence of residential houses scattered in a village, small towns and plantation areas close to forested areas (Hassan *et al*., 2013). Ten sampling points in Dungun were selected and divided into five case and control sites, respectively. The five case sites are Kampung Limbong, Felda Kerteh 2, Felda Kerteh 3, Kampung Sura Hujung and Kampung Balai Besar, the other five control sites, include Kampung Baharu, Taman Paka Jaya 3, Taman Paka Indah 3, Taman Seri Dungun and Taman Raya (Figure 1). Case sites are the sites that have reported cases of leptospirosis based on data provided by MOH Malaysia from 2016-2020 (Ministry of Health, 2021). In contrast, control sites are the sites without reported cases of leptospirosis and situated within a radius of ≥ 110 m from the nearest case sites (Harper & Bunbury, 2015).

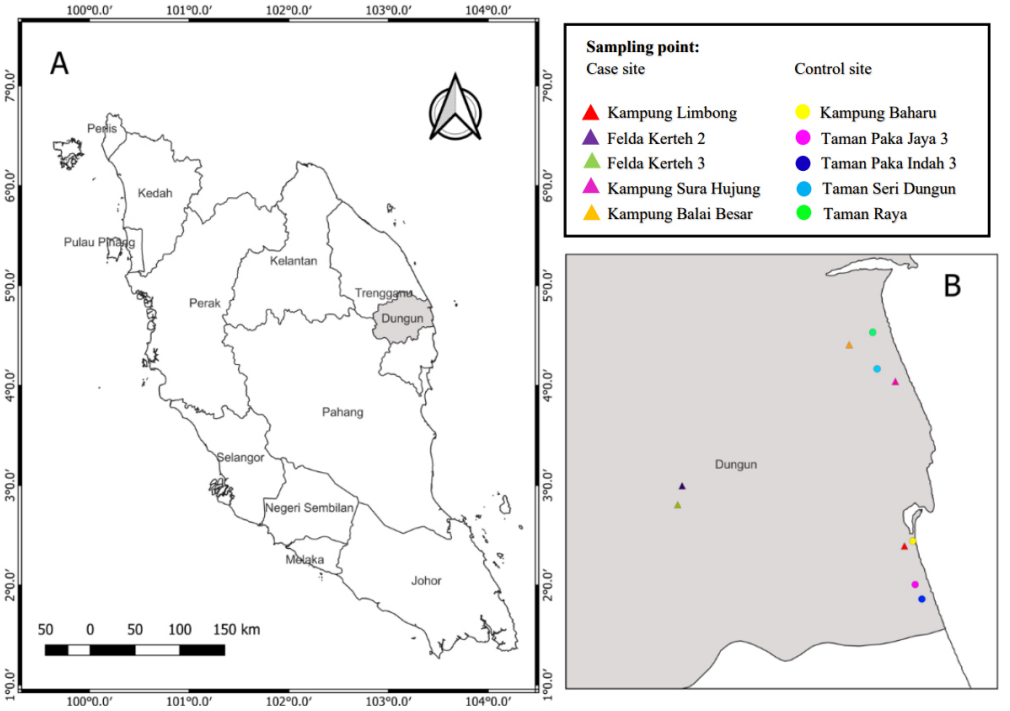

Figure 1: Map of Terengganu, Peninsular Malaysia. (A) Location of the study area in Dungun district, Terengganu; (B) location of the sampling points.

Small Mammals Trapping and Data Collection

Small mammal trapping was conducted using a total of 100 cage traps (12 cm height x 15 cm width x 25 cm length) that were positioned at 10 m between traps. The trapping was conducted for three consecutive nights during 10 trapping sessions, with a total of 3,000 trapping efforts between September 2021 and January 2022. The descriptive analysis was used to estimate the trapping success (total number of trapped individuals multiplied by 100 and divided by the total number of trapping efforts while accounting for lost and damaged traps). Sausages were placed in each cage trap as bait to attract small mammals. The sampling date, sampling sites, and the number of individuals were recorded during trapping sessions. All the captured small mammals were returned to the laboratory for further identification. Data on sex, body weight, and body length measurements were recorded and species were identified by referring to Francis (2008). This procedure has been approved by the National Medical Research Register (NMRR) with reference number: NMRR-21-743-59533 (IIR) and animal ethics from Universiti Malaysia Terengganu (UMT) with reference number: UMT/JKEPHMK/2021/53.

DNA Extraction and PCR Detection of Pathogenic Leptospira spp.

Deoxyribonucleic acid (DNA) extraction was performed from the kidneys of trapped animals using a Macherey-Nagel Nucleospin tissue kit. Briefly, 25 mg of tissue was spliced, minced, and lysed in T1 lysis buffer and 20 µL Proteinase K overnight at 56°C. A clear, lysed sample was then mixed with 200 µL Buffer B3, vortex vigorously and incubated at 70°C for 10 min. Then, 210 µL of absolute ethanol was added to the mixture, vortex vigorously, transferred to the collection tube and centrifuged at 11,000 x

g for 1 min. Next, washing was conducted using 500 µL Buffer BW and 600 µL Buffer B5 with centrifugation at 11,000 x g for 1 min. Finally, DNA was eluted in 50 µL TE buffer and kept at -20°C until further use.

The DNA of *Leptospira* was detected by the amplification of 16S rRNA and the *lipL32* gene. The LipL32 oligonucleotides used for detecting the presence of the *lipL32* gene comprised the following: forward primer 5'-AAG CAT TAC CGC TTG TGG TG-3', the reverse primer 5'-GAA CTC CCA TTT CAG CGA TT-3' and probe 5'-AA AGC CAG GAC AAG CGC CG-3' (Wunder *et al.,* 2016). The positive and negative controls were included in each PCR run. DNA of pathogenic *Leptospira interrogans* serovar Copenhageni strain M20 (20 ng) was used as the positive control, while distilled water was used as the negative control. Amplification of the DNA was conducted in a total volume of 20 µL consisting of 10 µL ready-to-use Promega GoTaq® Probe qPCR Mastermix, 400 nM forward, 400 nM reverse primer, 150 nM probes, PCR grade water (adjusted to 20 µL) and 8 µL DNA templates. These protocols of amplification were enhanced according to the BioRad CFX96 real-time PCR system and consisted of 8-min denaturation at 95℃ followed by 45 cycles of amplification at 95°C for 30 seconds, 55℃ for 15 *s*econds. Any sample with a Cq value of ≤ 40 was considered positive.

Statistical Data Analysis

The species composition of non-volant small mammals captured at the selected study areas was recorded. The significant associations between *Leptospira*-positive individuals, different sites (case and control sites), species and sex were determined using Fisher's exact test for a small sample size. All analyses were performed using Statistical Package for Social Sciences (SPSS) Version 22.0.

Results and Discussion

A total of 89 individuals of small mammals from five species, three orders and three families were captured during the 1385 trapping efforts (6.43% trapping success) in this study (Table 1). Most of the captured animals belong to the family Muridae (rats), with 76 individuals, and the family Tupaiidae (tree shrews) was the lowest, with only two individuals. *Rattus norvegicus* was the most abundant species (n = 39), followed by *Rattus rattus* (n = 27) as well as *Tupaia glis* (n = 2) was the least abundant species. Family Muridae has a higher species composition with three species, and they are the largest in the entire mammalian class, which consists of two-thirds of all rodent species, with the majority of them inhabiting Asia (Aplin *et al.,* 2003). About 18 species of rats from the family Muridae are regarded as pests in human residences, agriculture, as well as in rural and suburban areas (Parshad, 1999). The high number of rats in this study indicates high rat propagation in the area that will increase the transmission risk of rat-borne diseases including leptospirosis (Strand & Lundkvist, 2019).

All five species (n = 48) of small mammals were recorded in the case sites, whereas only four species (n = 41) were recorded in control sites, except for *Rattus exulans*. *R. rattus* was the most abundant species in case sites (n = 17), followed by *R. norvegicus* (n = 15). In control sites, *R. norvegicus* (n = 24) was the most abundant species, followed by *R. rattus* (n = 10). *T. glis* was the least abundant species in both case and control sites, with only one individual, respectively. *R. rattus* and also *R. norvegicus* are classified as commensal rodents that live in close proximity to humans and are often dependent upon human habitat for essential elements such as food, water, shelter and space (Puckett *et al*., 2020). Besides, these two species are important pests that are abundant in suburban areas (Liat, 2015) and serve as important reservoirs for the transmission of infectious diseases (Nkogwe *et al.,* 2011).

Table 1: Small mammal species recorded in control and case sites from Dungun, Terengganu

Species	Common Name	Case Site (%)	Control Site (%)	Total
Insectivora				
Soricidae				
Suncus murinus	House shrew	5 (10.4)	6 (14.6)	11 (12.4)
Scandentia				
Tupaiidae				
Tupaia glis	Common treeshrew	1 (2.1)	1 (2.4)	2 (2.2)
Rodentia				
Muridae				
Rattus norvegicus	Norway/Brown rat	15 (31.3)	24 (58.3)	39 (43.8)
Rattus rattus	Black rat	17 (35.4)	10 (24.4)	27 (30.3)
Rattus exulans	Polynesian rat	10 (20.8)	0	10 (11.2)
Total no. of individuals		48	41	89
No. of species		5	4	5
No. of family		3	3	3

Out of 89 individuals captured and screened, 14 individuals evaluated positive for pathogenic *Leptospira* spp. with a 15.7% prevalence (Table 2). The highest number of positive species was *R. norvegicus* (n = 8/89, 9.0%), followed by *R. rattus* (n = 4/89, 4.5%), *Suncus murinus* (n = 1/89, 1.1%) and *T. glis* (n = 1/89, 1.1%). Among all five small mammals captured in case sites, two species, *R. norvegicus* (n = 1, 6.7%) and *R. rattus* (n = 2, 11.8%), were detected positive for pathogenic *Leptospira* spp. While the other three species were evaluated as negative. Meanwhile, in control sites, all four small mammals captured were positive for pathogenic *Leptospira* spp. *R. norvegicus* had the highest number of positive individuals, with seven individuals assessed positive (29.2%). Previous studies stated that *R. norvegicus* is a key vector for pathogenic *Leptospira* spp. and is strongly associated with human activities (Twigg, 1973; Blasdell *et al.,* 2019). Besides *R. norvegicus*, the pathogenic leptospires were frequently isolated from other commensal rodents, such as *R. rattus* and *R. exulans,* in urban and suburban residential areas (Byers *et al.*, 2019). These two species are commonly found in residential areas while largely confined to human settlements, thus, contributing to the possible human-rat interactions and spread of zoonotic diseases (Suut *et al*., 2018; Ikbal *et al*., 2019). This study reported that *T. glis* and *S. murinus* also tested positive. These species are commonly seen in residential areas and also are classified as zoonotic carriers (Azhari *et al.,* 2018). Our results are in accordance with previous studies in Selangor, Malaysia, which also isolated pathogenic *Leptospira* spp. in *T. glis* and *S. murinus* from urban and suburban areas (Azhari *et al.,* 2018; Yusof *et al.,* 2019).

Table 2: Number of pathogenic *Leptospira* positive individuals in control and case sites from Dungun, Terengganu

Species	Case Site (%)	Control Site (%)	*Leptospira* positive individuals (%)
Suncus murinus	0/5	1/6 (16.7)	1/89 (1.1)
Tupaia glis	0/1	1/1 (100)	1/89 (1.1)
Rattus norvegicus	1/15 (6.7)	7/24 (29.2)	8/89 (9.0)
Rattus rattus	2/17 (11.8)	2/10 (20)	4/89 (4.5)
Rattus exulans	0/10	0/0	0/89
Positive *Leptospira* spp.	3	11	14
Total individual screen	48	41	89
Prevalence (%)	6.3	26.8	15.7

The prevalence of pathogenic *Leptospira* spp. was higher in control sites (26.8%) compared to case sites (6.3%). The Fisher's exact test showed that there is a significant difference ($p < 0.05$) of pathogenic *Leptospira* spp. between case and control sites. This may be due to poor waste management and the presence of open sewers near the residential areas in control sites, which provided food, water, and harborage sources for rodents, thus, making this area a potential infestation ground for leptospirosis. These results are similar to previous studies that showed open sewers with inadequate sanitation may increase *Leptospira* circulation and the risk of zoonotic transmission (Tassinari *et al.,* 2008; Shafei *et al.,* 2012). Irregular waste management and garbage disposal could increase the rodent population, thus increasing the risk of leptospirosis infection (Garba *et al.,* 2017). However, this study showed that there are no significant differences in pathogenic *Leptospira* spp. between species captured and sex ($p > 0.05$). Similarly, a previous study also found that there are no significant differences in pathogenic *Leptospira* spp. between small mammal species and sex (Agudelo-Flórez *et al.*, 2009). In contrast, previous studies conducted in Malaysia reported that a higher number of carriers were detected in males compared to females (Benacer *et al*., 2013). Adult male rats tend to have more aggressive movements and behaviours for survival, which increases the transmission risk (Mohamed-Hassan *et al.,* 2012).

Conclusion

In conclusion, this study provides useful information about the prevalence of pathogenic *Leptospira* spp. among small mammals in control and case sites from Dungun, Terengganu. These four species, *R. norvegicus*, *R. rattus*, *S. murinus* and *T. glis,* could maintain pathogenic *Leptospira* in the study areas. In addition, our results showed that the control sites recorded a higher prevalence of *Leptospira* spp. compared to case sites. Poor waste management and the presence of open sewers near the residential areas in control sites are some of the key factors that promote and attract rodents in the area. The leptospirosis cases in Dungun, Terengganu, will continue to increase if there are no efficient measures to mitigate this situation. The waste management and infrastructure intervention in this area should be improved in the future in order to prevent an outbreak of this disease. Public health agencies should not only focus on case areas in monitoring leptospirosis cases but should also pay attention to control areas, as the control areas also have the potential to become infestation grounds for leptospirosis.

Acknowledgements

We thank Universiti Malaysia Terengganu for providing funding support for this project (UMT/TAPE-RG/2020/55279). We would like to thank MOH Malaysia for approval to conduct this study and also to the residents who were willing to help during the data collection.

References

Agudelo-Flórez, P., Londoño, A. F., Quiroz, V. H., Ángel, J. C., Moreno, N., Loaiza, E. T., & 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.

Aplin, K. P., Brown, P. R., Jacob, J., Krebs, C. J., & Singleton, G. R. (2003). *Field methods for Rodent studies in Asia and the Indo-Pacific.* Australia: Australian Centre for International Agricultural Research.

Azhari, N. N., Ramli, S. N. A., Joseph, N., Philip, N., Mustapha, N. F., Ishak, S. N., Mohd-Taib, F. S., Md Nor, S., Yusof, M. A., Sah, S. A. M., Desa, M. N. Bin M., Bashiru, G., Zeppelini, C. G., Costa, F., Sekawi, Z., & Neela, V. K. (2018). Molecular characterization of pathogenic *Leptospira* sp. in small mammals captured from the human leptospirosis suspected areas of Selangor state, Malaysia. *Acta Tropica*, *188*, 68-77.

Benacer, D., Thong, K. L., Min, N. C., Verasahib, K. B., Galloway, R. L., Hartskeerl, R. A., Souris, M., & Zain, S. N. M. (2016a). Epidemiology of human leptospirosis in Malaysia, 2004-2005. *Acta Tropica*, *157*, 162-168.

Benacer, D., Thong, K. L., Verasahib, K. B., Galloway, R. L., Hartskeerl, R. A., Lewis, J. W., & Zain, S. N. M. (2016b). Human leptospirosis in Malaysia: Reviewing the challenges after 8 Decades (1925-2012). *Asia Pacific Journal of Public Health*, *28*, 290-302.

Benacer, D., Zain, S. N. M., Amran, F., Galloway, R. L., & Thong, K. L. (2013). Isolation and molecular characterization of *Leptospira interrogans* and *Leptospira borgpetersenii* isolates from the urban rat populations of Kuala Lumpur, Malaysia. *American Journal of Tropical Medicine and Hygiene*, *88*(4), 704-709.

Blasdell, K. R., Morand, S., Perera, D., & Firth, C. (2019). Association of rodent-borne *Leptospira* spp. with urban environments in Malaysia Borneo. *PLOS Neglected Tropical Disease*, *13*(2), 1-17.

Boey, K., Shiokawa, K., & Rajeev, S. (2019). *Leptospira* infection in rats: A literature review of global prevalence and distribution. *PLOS Neglected Tropical Diseases*, *13*(8), 1-24.

Byers, K. A., Lee, M. J., Patrick, D. M., & Himsworth, C. G. (2019). Rats about town: A systematic review of rat movement in urban ecosystems. *Frontier in Ecology and Evolution*, *7*(13), 1-12.

Cosson, J. F., Picardeau, M., Mielcarek, M., Tatard, C., Chaval, Y., Suputtamongkol, Y., Buchy, P., Jittapalapong, S., Herbreteau, V., & Morand, S. (2014). Epidemiology of *Leptospira* transmitted by rodents in Southeast Asia. *PLOS Neglected Tropical Diseases*, *8*(6), 1-10.

El Jalii, I. M., & Bahaman, A. R. (2004). A review of human leptospirosis in Malaysia. *Tropical Biomedicine, 21*, 113-119.

Faine, S., Adler, B., Bolin, C., & Perolat, P. (1999). *"Leptospira" and Leptospirosis*. Melbourne: MediSci. 295 pp.

Francis, C. M. (2008). *A field guide to the mammals of Thailand and South-East Asia* (pp. 392). Thailand: Asia Books.

Garba, B., Bahaman, A. R., Bejo, S. K., Zakaria, Z., & Mutalib, A. R. (2017). Retrospective study of leptospirosis in Malaysia. *EcoHealth*, *14*, 389-398.

Garba, B., Bahaman, A. R., Bejo, S. K., Zakaria, Z., Mutalib, A. R., & Bande, F. (2018). Major epidemiological factors associated with leptospirosis in Malaysia. *Acta Tropica*, *178*, 242-247.

Government of Malaysia. (2008). The Land Use Information Division. Kuala Lumpur: Federal Department of Town and Country Planning Peninsular Malaysia. *PlanMalaysia Official Portal.* Retrieved on 13 July, 2022, from https://mytownnet.planmalaysia.gov.my/index.php/townplan-journals/

Harper, G. A., & Bunbury, N. (2015). Invasive rats on tropical islands: Their population biology and impacts on native species. *Global Ecology and Conservation, 3*, 607-627.

Hassan, N., Mohd Jaafar, N. I., Raja Ariffin, R. N., Abu Samah, A., & Jaafar, M. N. (2013). Perceptions on quality of life in Malaysia: The urban-rural divide. *Journal of the Malaysian Institute of Planners*, *11*, 21-40.

Ikbal, N. H. M., Bhassu, S., Simarani, K., Uni, S., Chan, C. C., & Omar, H. (2019). Prevalence and genetic divergence of *Leptospira interrogans* and *Leptospira borgpetersenii* in house rats (*Rattus rattus*) from Peninsular Malaysia. *Asian Pacific Journal of Tropical Medicine, 12*(10), 463.

Liat, L. B. (2015). The house rodents and house shrew in Malaysia and Southeast Asia. *UTAR Agriculture Science Journal, 2*(1), 43-50.

Ministry of Health. (2015). *Epidemiology and current situation of leptospirosis in Malaysia*. Retrieved on January 2nd, 2022 from http://moh.gov.my

Ministry of Health. (2021). *Distribution of leptospirosis cases in Malaysia and Terengganu* (Unpublished data).

Mohamed-Hassan, S. N., Bahaman, A. R., Mutalib, A. R., & Khairani-Bejo, S. (2012). Prevalence of pathogenic leptospires in rats from selected locations in Peninsular Malaysia. *Research Journal of Animal Sciences, 6*(1), 12-25.

Mohd-Taib, F. S., Ishak, S. N., Yusof, M. A., Azhari, N. N., Md-Lasim, A., Md. Nor, S., Mohd-Sah, S. A., & Neela, V. K. (2020). Leptospirosis: An insight into community structure of small mammal's host in urban environment. *Tropical Biomedicine*, *37*(1), 142-154.

Nkogwe, C., Raletobana, J., Stewart-Johnson, A., Suepaul, S., & Adesiyun, A. (2011). Frequency of detection of *Escherichia coli*, *Salmonella* spp., and *Campylobacter* spp. in the faeces of wild rats (*Rattus* spp.) in Trinidad and Tobago. *Veterinary Medicine International, 20*, 1-7.

Oblegala, A., Woll, D., Karnath, C., Silaghi, C., Schex, S., Eßbauer, S., & Pfeffer, M. (2016). Pathogenic *Leptospira* species in small mammals from various habitat types in Germany. *PLOS Neglected Tropical Disease, 10*(3), 1-12.

Parshad, V. R. (1999). Rodent control in India. *Integrated Pest Management Review, 4*, 97-126.

Picardeau, M. (2013). Diagnosis and epidemiology of leptospirosis. *Médecine et Maladies Infectieuses, 43*(1), 1-9.

Puckett, E. E., Orton, D., & Munshi-South, J. (2020). Commensal rats and humans: Integrating rodent phylogeography and zooarchaeology to highlight connections between human societies. *Bioessays*, *42*(5), 1-10.

Shafei, M. N., Sulong, M. R., Yaacob, N. A., Hassan, H., Mohamad, W. M. Z. W., Daud, A., Ismail, Z., & Abdullah, M. R. (2012). Seroprevalence of leptospirosis among

town service workers in Northeastern state of Malaysia. *International Journal of Collaborative Research on Internal Medicine and Public Health, 4*, 395-403.

Strand, T. M., & Lundkvist, Å. (2019). Rat-borne diseases at the horizon. A systematic review on infectious agents carried by rats in Europe 1995-2016. *Infection Ecology & Epidemiology*, *9*(1), 1-9.

Suut, L., Mazlan, A., Arif, M. T., Katip, T., Nor Aliza, A. R., & Haironi, Y. (2018). Serovar diversity of *Leptospira* spp. infecting wild rodents in Sarawak, Malaysia. *Tropical Biomedicine*, *35*(1), 252-258.

Tan, W. L., Soelar, S. A., Mohd Suan, M. A., Hussin, N., Cheah, W. K., Verasahib, K., & Goh, P. P. (2016). Leptospirosis incidence and mortality in Malaysia. *The South-east Asian Journal of Tropical Medicine and Public Health, 47*, 434-440.

Tassinari, W. S., Pellegrini, D. C. P., Sá, C. B. P., Reis, R. B., Ko, A. I., & Carvalho, M. S. (2008). Detection and modelling of case clusters for urban leptospirosis. *Tropical Medicine & International Health, 13*(4), 503-512.

Trueba, G., Zapata, S., Madrid, K., Cullen, P., & Haake, D. (2004). Cell aggregation: A mechanism of pathogenic *Leptospira* to survive in freshwater. *International Microbiology, 7*, 35-40.

Twigg, G. I. (1973). Rat-borne leptospirosis in wildlife and on farm. *Mammal Review, 3*, 37-42.

Villanueva, S. Y. A. M., Ezoe, H., Baterna, R. A., Yanagihara, Y., Muto, M., Koizumi, N., Fukui, T., Okamoto, Y., Masuzawa, T., Cavinta., L. L., Gloriani, N., G., & Yoshida, S. (2010). Serologic and molecular studies of *Leptospira* and leptospirosis among rats in the Philippines. *The American Society of Tropical Medicine and Hygiene, 82*(5), 889-898.

Wong, M., Katz, A. R., Li, D., & Wilcox, B. A. (2012). *Leptospira* infection prevalence in small mammal host populations on three Hawaiian Islands. *The American Society of Tropical Medicine and Hygiene, 87*(2), 337-341.

Wunder, E. A., Figueira, C. P., Santos, G. R., Lourdault, K., Matthias, M. A., Vinetz, J. M., Ramos, E., Haake, D. A., Picardue, M., Reis, M. G. D., & 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.

Yusof, M. A., Mohd-Taib, F. S., Ishak, S. N., Md-Nor, S., Md-Sah, S. A., Mohamed, N. Z., Azhari, N. N., Neela, V., & Sekawi, Z. (2019). Microhabitat factors influenced the prevalence of pathogenic *Leptospira* spp. in small mammal host. *Eco Health, 16*, 260-274.