SCREENING OF *Rickettsia* sp. IN TICKS (ACARI: IXODIDAE) COLLECTED FROM SMALL MAMMALS IN THREE RECREATIONAL FORESTS IN SELANGOR, MALAYSIA.

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ABSTRACT

Rickettsia is a gram-negative, non-motile, and obligate intracellular bacterium that usually associated with arthropods vectors such as ticks, fleas, lice and mites. These bacteria have the ability to cause diseases in humans, however, in Malaysia, knowledge on the prevalence and distribution of these bacteria mainly focused on humans and information of these bacteria in small mammal hosts is limited. Thus, this study aims to investigate the presence of *Rickettsia* sp. in the DNA of tick's samples collected from small mammals in three different recreational forests in Selangor, Malaysia. Sampling was conducted in which 200 cage traps were set up randomly along streams and forest trails for five nights. A total of 106 fully engorged and adult ticks were collected from 23 individuals of seven small mammals host species. All samples were tested for *Rickettsia* bacteria based on polymerase chain reaction (PCR) using partial 17-kDa antigen gene. The PCR results obtained from this study showed no infestation of *Rickettsia* sp. in all tick samples. Our findings revealed that none of the tick samples from these forests' sites were infected with *Rickettsia* pathogen, however, more intense and extensive surveillance for *Rickettsia* sp. from other tick species is still necessary for greater geographical areas across Malaysia.

Keywords: Ticks, Small mammals, *Ixodes granulatus,* Tick-borne diseases

ABSTRAK

Rickettsia adalah bakteria gram-negatif, tidak bermotil, dan intrasel obligat bakteria yang biasanya dikaitkan dengan vektor artropod seperti sengkenit, pinjal, kutu, dan tungau. Bakteria ini mempunyai keupayaan yang menyebabkan penyakit pada manusia, namun, di Malaysia, pengetahuan mengenai prevalen dan taburan bakteria ini tertumpu hanya pada manusia dan maklumat bakteria ini pada perumah mamalia kecil adalah terhad. Oleh itu, kajian ini bertujuan untuk mengkaji kehadiran bakteria *Rickettsia* sp. dalam DNA sampel sengkenit yang diambil daripada mamalia kecil di tiga kawasan hutan rekreasi yang berlainan di Selangor, Malaysia. Persampelan dilakukan dengan menggunakan sejumlah 200 perangkap sangkar yang

diletakkan secara rawak di sepanjang sungai dan laluan hutan selama 5 malam. Sejumlah 106 individu sengkenit dewasa yang telah mengembang dengan sepenuhnya telah dikumpulkan dari 23 individu daripada tujuh spesies perumah mamalia kecil. Kesemua sampel telah dikaji untuk bakteria *Rickettsia* berdasarkan tindak balas berantai polimerase (PCR) menggunakan gen separa antigen 17-kDa. Hasil keputusan PCR yang diperolehi daripada kajian ini menunjukkan tiada jangkitan dan kehadiran *Rickettsia* sp. dalam semua sampel sengkenit. Penemuan kami mendedahkan bahawa tiada sampel sengkenit daripada kawasan hutan ini dijangkiti oleh patogen *Rickettsia*. Walau bagaimanapun, pengawasan yang lebih terperinci dan menyeluruh untuk *Rickettsia* daripada spesies sengkenit yang lain masih diperlukan di kawasan geografi yang lebih besar di seluruh Malaysia.

Kata kunci: Sengkenit, mamalia kecil, *Ixodes granulatus,* penyakit bawaan sengkenit

INTRODUCTION

Rickettsia is a gram-negative, non-motile, and obligate intracellular bacterium that have the ability to cause diseases in humans (Socolovschi et al. 2009). Transmission of *Rickettsia* bacteria to humans are mainly by infected arthropod vectors which includes ticks, fleas, mites and lice (Kuo et al. 2015). *Rickettsia* genus can be divided into two major groups which are the pathogenic group consist of flea-borne typhus group (TG) and tick-borne spotted fever group (SFG), and non-pathogenic group consist of *Rickettsia bellii* group and *R. canadensis* group (Parola et al. 2013; Merhej et al. 2014; Scarpulla et al. 2016). *Rickettsia typhi* and *R. felis* are the main causative agent of flea-borne TG and transmission commonly occur by oral route through blood meal intake, and fecal contamination (Bitam et al. 2010). Meanwhile, tick-borne SFG agents usually reported in Asia are *Rickettsia conorii, R. japonica, R. honei, R. tamurae, R. raoultii, R. heilongjiangensis,* and *R. sibirica* (Parola et al. 2013; Kho et al. 2017). *Rickettsia* bacteria associated with SFG are usually transmitted to vertebrates via the bites of hard ticks (Luce-Fedrow et al. 2015).

Several small rodents are important hosts for ixodid ticks (Che Lah et al. 2016; Ishak et al. 2018) and reservoirs for many tick-borne diseases including *Rickettsia* infection (Imhoff et al. 2015; Minichova et al. 2017). Besides, *Rickettsia* are commonly found as endosymbionts in ticks (Socolovschi et al. 2009). Investigation on *Rickettsia* bacteria in Malaysia have mainly focused on humans (Kho et al. 2016; Kho et al. 2017), monkeys (Tay et al. 2015), cats and wild rats (Tay et al. 2014). A few studies have reported the detection of *Rickettsia* bacteria from ticks and tissue samples of wild rodents (Tay et al. 2014; Minichova et al. 2017; Gajda et al. 2017). This study was therefore conducted to identify the presence of *Rickettsia* bacteria in ticks collected from small mammals in areas that are frequently visited by humans in Selangor, Malaysia. The information obtained from this study will be useful for surveillance, prevention and control for tick-borne diseases in Malaysia.

MATERIAL AND METHODS

Fieldwork and Ticks Sampling

A total of 200 collapsible cage traps were used to capture wild rodents in three different recreational forests (Ulu Yam, Sg. Congkak and Hulu Perdik) in Selangor, Malaysia. Different types of baits were used to attract the small mammals which include sweet potatoes with peanut butter, oil palm fruits, salted fish, and banana to attract small mammals (Shahrul Anuar et al. 2008; Ishak et al. 2018). Trapping were set up and checked once in the morning for five nights. All animals caught were collected and brought back to the Animal House (Universiti

Kebangsaan Malaysia) for host identification using morphometric measurement following description keys provided by Francis (2008). Small mammals were marked and identified to species level and other information such as gender, age and health status were also recorded. Fieldwork procedures and animal handling were approved by the Ethics Committee at Universiti Kebangsaan Malaysia (Reference: FST/2016/SHUKOR/18-MAY/750-MAY-2016-SEPT.-2018-AR-CAT2).

Each individual host was carefully checked for the presence of ticks and ticks were then removed from the host and stored in labelled cryo-vial containing 70% alcohol for preservation. Ticks were observed under stereo microscope and identified based on their morphological criteria up to genus level, sex, and life stages according to entomological keys (Walker et al. 2003; Barker & Walker 2014). Molecular approach was used to identify individual ticks until species level using 16S gene (Black & Piesman 1994) and COI gene (Folmer et al. 1994; Che Lah et al. 2016; Mohd-Taib et al. 2018). All tick species in this study have been identified and described in the previous study (Ishak et al. 2018).

DNA Extraction

Before DNA extraction, all tick samples were washed thrice in 70% ethanol and rinsed in sterile deionized water to remove any possible contaminants (Carpi et al. 2011). The samples were then dried and pulverized using sterile mortar and pestle. The resulting powder was resuspended in 500 µl of sterile phosphate-buffered saline (PBS) (Khoo et al. 2016). The samples were incubated at 56 ºC overnight in the presence of Proteinase K for complete lysis. DNA extractions were performed using MN-NucleoSpin® Tissue kit (MACHERY-NAGEL, Duren, Germany) according to manufacturer's protocol. The extracted DNA was then stored at -20°C until further use as template in PCR amplification.

PCR Amplification

Screening for *Rickettsia* species was accomplished by a conventional PCR assay, using a pair of primers for *Rickettsia*-specific 17-kDa gene producing a fragment of 600 bp (Oliveira et al. 2010). All PCR reactions were conducted in 20 µl volumes containing 10 µl of GoTaq® Green Master Mix (Promega, Madison, USA), 1 µmol/L of each forward and reverse primer, 6 µl nuclease free water and 2 µl of DNA template. The PCR was carried out using Thermal Cyclers (Applied Biosystems). The PCR conditions used were: initial denaturation at 95 ºC for 3 min, denaturation at 95 ºC for 15s, annealing at 42 ºC for 30s, extension at 72 ºC for 30s, and final extension at 72ºC for 4 min with a total of 40 cycles. For each PCR reaction, a negative control and positive control (DNA obtained from tick samples previously identified as PCR-positive for *Rickettsia* sp.) was used. The PCR products (2 µl) were electrophoresed on a 1.5% agarose gel stained with *Healthview*™ Nucleic Acid stain reagent at 100V for 60 min and their banding patterns were visualized using a UV trans-illuminator and photographed.

RESULTS AND DISCUSSION

A total of 106 individuals of ticks were collected from seven different host species (23 individual hosts) which were *Sundamys muelleri* (n=13), *Rattus tiomanicus* (n=3), *Rattus rattus* (n=2), *Maxomys whiteheadi* (n=4), *M. rajah* (n=1), *Sundasciurus tenuis* (n=2) and *Tupaia glis* (n=1) as in Table 1. All ticks were females, fully engorged and identified as *Ixodes granulatus*, *Dermacentor* sp. and *Amblyomma* sp. (Table 2) through morphological characters and molecular analysis based on the sequence analysis of the partial 16S rDNA and COI gene in the previous study (Ishak et al. 2018). Molecular screening of the ticks collected in this study showed that there was no *Rickettsia* sp. present in the DNA of all ticks. Samples were

interpreted as positive for *Rickettsia* sp. if band of 600 bp are obtained. Samples are negative if no band is detected. The results from this study are considered valid since positive control produced positive band of 600 bp, tick samples and negative control showed no band as in 1. Our findings were similar with previous study conducted by Madinah et al. (2014) where figure all tick samples were free from *Rickettsia* pathogens.

Table 1. List of host species and total number of ticks in each locality

Host species	Number of ticks			**Total**
	Ulu Yam	Sg. Congkak	Hulu Perdik	
Sundamys muelleri	34	40	6	**80**
Rattus tiomanicus	3	0	5	**8**
Rattus rattus	1	3	0	**4**
Maxomys whiteheadi	6	3	0	**9**
Maxomys rajah	0	2	0	**2**
Sundasciurus tenuis	1	1	0	**2**
Tupaia glis	0	1	0	**1**
Total	**45**	**50**	**11**	**106**

Table 2. List of tick species and total number of ticks in each locality

Ticks species	Number of ticks			**Total**
	Ulu Yam	Sg. Congkak	Hulu Perdik	
Ixodes granulatus	41	42	11	**94**
Dermacentor sp.	3	8	0	**11**
Amblyomma sp.	1	0	0	**1**
Total	**45**	**50**	**11**	**106**

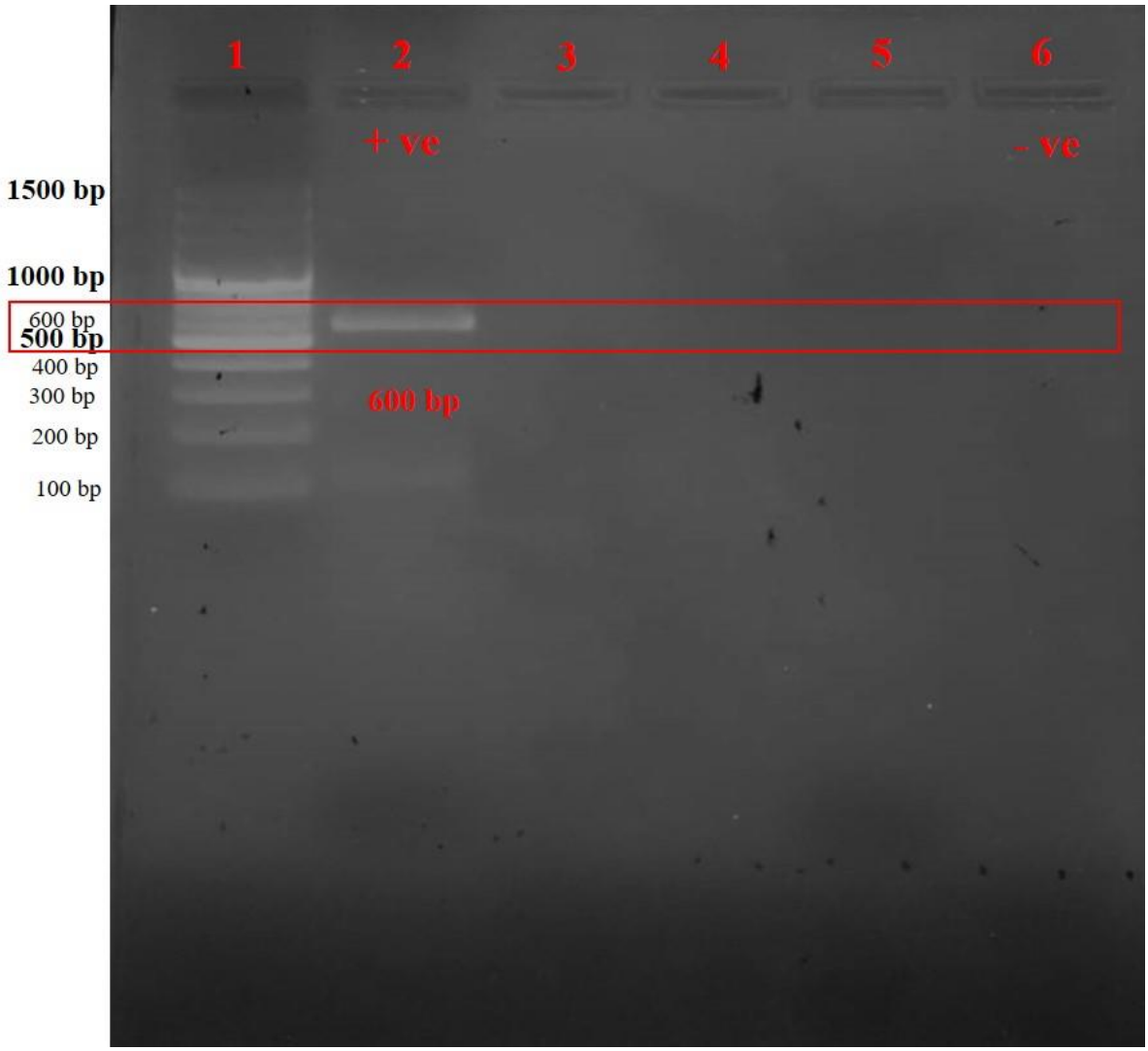

Figure 1. Lane 1-100 bp plus ladder, Lane 2- positive control of *Rickettsia* sp., Lane 3 to 5- DNA samples of ticks, Lane 6- negative control

However, there are several reasons and limitations to explain the absence of *Rickettsia* sp. in ticks collected from small mammals in this study. First, the sample size used in this study was relatively small compared to other research studies (Liyanaarachchi et al. 2015; Noh et al. 2017). Molecular screening of 900 tick's samples conducted by Benson et al. (2017) revealed only 1.7% (n=15) of tick samples harboured the pathogen in which only five ticks were recognized as pathogenic spotted fever group (SFG) *Rickettsia*. It thus showed that the percentage of infection was small. Therefore, continual sampling period is recommended in order to collect larger sample size and increase the chances of disease detection. Second, our study areas were limited to small areas and have no previous report, history and outbreak about *Rickettsia* disease except human and rodent leptospirosis cases (Zulfikli et al. 2016; Azhari et al. 2018) and detection of *Borrelia* sp. from *Ixodes granulatus* ticks (Khoo et al. 2018) in our sampling areas. Therefore, the transmission cycle of *Rickettsia* may not be present in the area of study. However, as rickettsial infections are likely to be under-diagnosed, the incidence of rickettsial infections here cannot be ruled out.

Our findings were also limited to only three tick species (*Ixodes granulatus, Dermacentor* sp. and *Amblyomma* sp.) and a small number of host species in which the prevalence of *Rickettsia* sp. may be absence or very low. A review of the literature shows that *Rickettsia* bacteria were reported from several other tick species in Malaysia. For examples, novel rickettsiae closely related to *Rickettsia tamurae* and *Rickettsia raoultii* were detected from *Amblyomma varanense* and *Amblyomma helvolum* respectively (Kho et al. 2015). *Rickettsia felis* was detected in a pool of *Haemaphysalis* sp. larvae from a microbiome study (Khoo et al. 2016). *Rickettsia asembonensis* was detected in the dog tick, *Rhipicephalus sanguineus* (Low et al. 2017). There is only one previous report of *Rickettsia* (*Rickettsia thailandii* sp. nov.) identified in *I. granulatus* ticks from the neighbouring Southeast Asian country, Thailand (Kollars et al. 2001). Other rickettsiae reported within the Southeast Asian region were primarily found from tick species other than *Ixodes* sp., including *Amblyomma, Haemaphysalis* and *Dermacentor* ticks, from both Laos and Thailand (Hirunkanokpun et al. 2003; Sumrandee et al. 2014; Taylor et al. 2016). On the other hand, *Rickettsia* bacteria have been detected from *I. granulatus* ticks in East Asian countries further away from Malaysia, including Taiwan and Japan (Fujita et al. 2008; Tsai et al. 2008).

The findings from our study and the other previously published studies suggest two possible scenarios. The first possible scenario would be the distribution of *Rickettsia* in three tick's species may be limited here, and therefore the role of these ticks in the transmission of potential rickettsial pathogens may be negligible in Malaysia or the Southeast Asian region. The second possible scenario would be the full extent of rickettsial distribution in *I. granulatus* (the most abundance tick species) is still under-investigated in this region as the previous published studies may have excluded *I. granulatus* ticks in their sampling populations. The sampling strategies from the previous publications generally focused on the sampling of other tick species. Improved efforts in surveillance, including extending the surveillance to larger number of *I. granulatus* ticks from greater expanse of geographical areas will be necessary to rule out either or both of the possible scenarios. As *I. granulatus* ticks are human-biting ticks and they feed on rodent hosts that are easily found in human-dwellings near forests, there are concerns of disease transmission by this tick species (Paperna 2006).

Last but not least, more sensitive assays, such as quantitative PCRs, may be utilized to improve the sensitivity of detection (Jiang et al. 2006). Since various rickettsial species have been reported from other tick species, animals as well as humans in Malaysia, it is imperative to continue with the efforts in the surveillance for rickettsiae and rickettsial infections in order to fully assess the risks of rickettsial diseases in Malaysia (Tay et al. 2015; Kho et al. 2016; Kho et al. 2017; Salgado Lynn et al. 2018).

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REFERENCES

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

Barker, S.C. & Walker, A.R. 2014. Ticks of Australia. *Zootaxa* 3816(1): 1-144.

Benson, C.I., Aboi, I., Abiodun, A.A., Elia, J.M., Anthony, I.O. & Larry, C.O. 2017. Molecular screening of ticks for the presence of *Rickettsia* species: A public health concern. *Asian Pacific Journal of Tropical Disease* 7(4): 199-204.

Bitam, I., Dittmar, K., Parola, P., Whitting, M.F. & Roult, D. 2010. Fleas and flea-borne diseases. *International Journal of Infectious Diseases* 14(8): e667–e676.

Black, W.C. & Piesman, J. 1994. Phylogeny of hard and soft-tick taxa (Acari: Ixodida) based on mitochondrial 16S rDNA sequences. *Proceedings of the National Academy of Sciences of the United States of America* 91(21): 10034–10038.

Carpi, G., Cagnacci, F., Wittekindt, N.E., Zhao, F., Qi, J., Tomsho, L.P., Drautz, D.I., Rizzoli, A. & Schuster, S.C. 2011. Metagenomic profile of the bacterial communities associated with *Ixodes ricinus* ticks. *Public Library of Science One* 6(10): e25604.

Che Lah, E.F., Yaakop, S., Ahamad, M., George, E. & Md. Nor, S. 2016. Precise identification of different stages of a tick, *Ixodes granulatus* Supino, 1897 (Acari: Ixodidae). *Asian Pacific Journal of Tropical Biomedicine* 6: 597–604.

Francis, C.M. 2008. *A Guide to the Mammals of South-East Asia*. United Kingdom: Princeton University Press.

Folmer, O.M., Black, M., Hoeh, R., Lutz, R. & Vrijenhoek, R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3: 294–299.

Fujita, H., Kadosaka, T., Nitta, Y., Ando, S., Takano, A., Watanabe, H. & Kawabata, H. 2008. *Rickettsia* sp. in *Ixodes granulatus* Ticks, Japan. *Emerging Infectious Diseases* 14(12): 1963-1965.

Gajda, E., Hildebrand, J., Sprong, H., Buńkowska-Gawlik, K., Perec-Matysiak, A. & Coipan, E.C. 2017. Spotted fever rickettsiae in wild-living rodents from south-western Poland. *Parasites & Vectors* 10: 413.

Hirunkanokpun, S., Kittayapong, P., Cornet, J.-P. & Gonzalez, J.-P. 2003. Molecular evidence for novel tick-associated spotted fever group rickettsiae from Thailand. *Journal of Medical Entomology* 40(2): 230-237.

Ishak, S.N., Yusof, M.A., Nor, S.M., Sah, S.A.M., Lim, F.S., Khoo, J.J. & Mohd-Taib, F.S. 2018. Prevalence of on-host ticks (Acari: Ixodidae) in small mammals collected from forest near to human vicinity in Selangor, Malaysia. *Systematic & Applied Acarology* 23(8): 1531–1544.

Imhoff, M., Hagedorn, P., Schulze, Y., Hellenbrand, W., Pfeffer, M. & Niedrig, M. 2015. Review: Sentinels of tick-borne encephalitis risk. *Tick and Tick-borne Diseases* 6(5): 592-600.

Jiang, J., Soeatmadji, D.W., Henry, K.M., Ratiwayanto, S., Bangs, M.J. & Richards, A.L. 2006. *Rickettsia felis* in *Xenopsylla cheopis*, Java, Indonesia. *Emerging infectious diseases* 12(8): 1281-1283.

Kho, K.L., Koh, F.X. & Tay, S.T. 2015. Molecular evidence of potential novel spotted fever group rickettsiae, *Anaplasma* and *Ehrlichia* species in *Amblyomma* ticks parasitizing wild snakes. *Parasites & Vectors* 8: 112.

Kho, K.L., Koh, F.X., Singh, H.K.L., Zan, H.A.M., Kukreja, A., Ponnampalavanar, S. & Tay, S.T. 2016. Spotted fever group rickettsioses and murine typhus in a Malaysian teaching hospital. *The American Journal of Tropical Medicine and Hygiene* 95:765-768.

Kho, K.L., Koh, F.X., Hasan, L.I.M., Wong, L.P., Kisomi, M.G., Bulgiba, A., Nizam, Q.N.H. & Tay, S.T. 2017. Rickettsial seropositivity in the indigenous community and animal farm workers, and vector surveillance in Peninsular Malaysia. *Emerging microbes & infections* 6(4): e18.

Khoo, J.J., Chen, F., Kho, K.L., Ahmad Shanizza, A. I., Lim, F.S., Tan, K.K., Chang, L.Y. & AbuBakar, S. 2016. Bacterial community in *Haemaphysalis* ticks of domesticated animals from the Orang Asli communities in Malaysia. *Ticks and Tick-borne Diseases* 79(5): 929-937.

Khoo, J.J., Ishak, S.N., Lim, F.S., Mohd-Taib, F.S., Khor, C.S., Loong, S.K. & AbuBakar, S. 2018. Detection of a *Borrelia* sp. from *Ixodes granulatus* ticks collected from rodents in Malaysia. *Journal of Medical Entomology* 55(6): 1642-1647.

Kollars, J.T., Tippayachai, B. & Bodhidatta, D. 2001. Thai tick typhus, *Rickettsia honei*, and a unique rickettsia detected in *Ixodes granulatus* (Ixodidae: Acari) from Thailand. *The American Journal of Tropical Medicine and Hygiene* 65(5): 535-537.

Kuo, C.C., Shu, P.Y., Mu, J.J. & Wang, H.C. 2015. High Prevalence of *Rickettsia* sp. infections in small mammals in Taiwan. *Vector-Borne and Zoonotic Diseases* 5(1): 13-20.

Liyanaarachchi, D.R., Rajakaruna, R.S. & Rajapakse, R.P.V.J. 2015. Spotted fever group *Rickettsia* in ticks infesting humans, wild and domesticated animals of Sri Lanka: one health approach. *Ceyclon Journal of Science* 44(2): 67-74.

Low, V.L., Prakash, B.K., Tan, T.K., Sofian-Azirun, M., Anwar, F.H.K., Vinnie-Siow, W.Y. & AbuBakar, S. 2017. Pathogens in ectoparasites from free-ranging animals: Infection with *Rickettsia asembonensis* in ticks, and a potentially new species of Dipylidium in fleas and lice. *Veterinary Parasitology* 245: 102-105.

Luce-Fedrow, A., Mullins, K., Kostik, A.P., St-John, H.K., Jiang, J. & Richards, A.L. 2015. Strategies for detecting rickettsiae and diagnosing rickettsial diseases. *Future microbiology* 10(4):537-64.

Madinah, A., Mariana, A. & Abdullah, M.T. 2014. Detection of rickettsiae in engorged ticks from small mammals in Malaysia. *Borneo Journal of Resource Science and Technology* 4(1): 34-41.

Merhej, V., Angelakis, E., Socolovschi, C. & Raoult, D. 2014. Genotyping: evolution and epidemiological findings of *Rickettsia* species. *Infection Genetic Evolution* 25: 122-137.

Minichova, L., Hamsikova, Z., Mahrikova, L., Slovak, M., Kocianova, E., Kazimirova, M., Skultety, L., Stefanidesova, K. & Spitalska, E. 2017. Molecular evidence of *Rickettsia* spp. in ixodid ticks and rodents in suburban, natural and rural habitats in Slovakia. *Parasites & Vectors* 10:158.

Mohd-Taib, F.S., Md. Lasim, A., Asyikha, R., Ali, R. & Ishak, S.N. 2018. A preliminary survey of ectoparasites of small mammals in Pangkor Island, Perak, Malaysia. *Serangga* 23(2): 73-82.

Noh, Y., Lee, Y.S., Kim, H.C., Chong, S.T., Kelin, T.A., Jiang, J., Richards, A.L., Lee, H.K. & Kim, S.Y. 2017. Molecular detection of *Rickettsia* species in ticks collected from the southwestern provinces of the Republic of Korea. *Parasites & Vectors* 10: 20.

Oliveira, K.A., Pinter, A., Medina-Sanchez, A., Boppana, D.V., Wikel, S.K., Saito, T.B., Shelite, T., Blanton, L., Popov, V., Teel, P.D., Walker, D.H., Marcio, A.M., Mafra, G.C. & Bouyer, D.H. 2010. *Amblyomma imitator* ticks as vectors of *Rickettsia rickettsia*, Mexico. *Emerging Infectious Diseases* 16(8): 1282-1284.

Paperna, I. 2006. The tick *Ixodes granulatus* infests *Rattus rattus* populating a small island offshore of Singapore. *Parasites* 13(1): 83-84.

Parola, P., Paddock, C.D., Socolovschi, C., Labruna, M.B., Mediannikov, O., Kernif, T., Abdad, M.Y., Stenos, J., Bitam, I., Fournier, P.E. & Raoult, D. 2013. Update on tick-borne rickettsioses around the world: a geographic approach. *Clinical Microbiology Review* 26(4): 657-702.

Salgado Lynn, M., William, T., Tanganuchitcharnchai, A., Jintaworn, S., Thaipadungpanit, J., Lee, H.M., Jalius, C., Daszak, P., Goossens, B., Hughes, T. & Blacksell, D.S. 2018. Spotted Fever Rickettsiosis in a Wildlife Researcher in Sabah, Malaysia: A case study. *Tropical Medicine and Infectious Disease* 3(29): 1-8.

Scarpulla, M., Barlozzari, G., Marcario, A., Salvato, L., Blanda, V., De Liberato, C., D'Agostini, C., Torina, A. & Macri, G. 2016. Molecular detection and characterization of spotted fever group rickettsiae in ticks from Central Italy. *Ticks and Tick-borne Diseases* 7(5): 1052-1056.

Shahrul Anuar, M.S., Nor Zalipah, M., Yusuf, A., Razlina, R., Abd. Muin, M., Nik Fadzly, N., Rashid, Y., Khairul, N., Muhd Fadhil, A.R., Mohd Shahril, A.M. & Nordin, A. 2008. A survey of understorey bats and non-volant small mammals at Taman Rimba Bukit Bauk. In: Razani, U., Koh, H.L., Rahim, M.R., Na'aman, J., Faridah-Hanum, I. & Latiff, A. (eds.). *Rimba Bandar Bukit Bauk. Terengganu: Pengurusan Hutan, Persekitaran Fizikal dan Kepelbagaian Biologi*, pp. 302–309. Kuala Lumpur: Forestry Department of Peninsular Malaysia.

Socolovschi, C., Mediannikov, O., Raoult, D. & Parola, P. 2009. The relationship between spotted fever group Rickettsiae and Ixodid ticks. *Veterinary Research* 40(2): 34.

Sumrandee, C., Hirunkanokpun, S., Doornbos, K., Kitthawee, S., Baimai, V., Grubhoffer, L., Trinachartvanit, W. & Ahantarig, A. 2014. Molecular detection of *Rickettsia* species in *Amblyomma* ticks collected from snakes in Thailand. *Ticks and Tick-borne Diseases* 5(6): 632-640.

Tay, S.T., Mokhtar, A.S., Low, K.C., Mohdzain, S.N., Jeffery, J., Abdulaziz, N. & Kho, K.L. 2014. Identification of rickettsiae from wild rats and cat fleas in Malaysia. *Medical and Veterinary Entomology* 28(1): 104–108.

Tay, S.T., Koh, F.X., Kho, K.L. & Sitam, F.T. 2015. Rickettsial infections in monkeys, Malaysia. *Emerging Infectious Diseases* 21(3): 545-547.

Taylor, A.J., Vongphayloth, K., Vongsouvath, M., Grandadam, M., Brey, P.T., Newton, P.N., Sutherland, I.W. & Dittrich, S. 2016. Large-scale survey for tick-borne bacteria, Khammouan Province, Laos. *Emerging infectious diseases* 22(9): 1635-1639.

Tsai, K.H., Wang, H.C., Chen, C.H., Huang, J.H., Lu, H.Y., Su, C.L. & Shu, P.Y. 2008. Isolation and identification of a novel spotted fever group rickettsia, strain IG-1, from *Ixodes granulatus* ticks collected on Orchid Island (Lanyu), Taiwan. *The American Journal of Tropical Medicine and Hygiene* 79(2): 256-261.

Walker, A.A., Bouatour, A., Camicas, J.L., Estadapena, A.A., Harok, I.G., Hatif, A.A., Pegram, R.G. & Preton, P.M. 2003. *Ticks of domestic animals in Africa: A guide to identification species*. United Kingdom: The University of Edinburgh.

Zulfikli, Z., Shariff, A.R.M. & Tarmidi, Z.M. 2016. Human leptospirosis distribution pattern analysis in Hulu Langat, Selangor. *IOP Conference Series: Earth and Environmental Science* 37: 012021.