*Borneo Journal of Resource Science and Technology (2014) 4(1): 34-41*

## SHORT COMMUNICATION

# Detection of Rickettsiae in Engorged Ticks from Small Mammals in Malaysia

MADINAH ADRUS[*1], MARIANA AHAMAD[2] & MOHD TAJUDDIN ABDULLAH[1,3]

*[1]Department of Zoology, Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia; [2]Unit of Acarology, Infectious Diseases Research Center, Institute for Medical Research, Jalan Pahang, 50588 Kuala Lumpur; [3]Kenyir Ecosystem Research Centre, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Malaysia*

**ABSTRACT**

Rickettsiae are intracellular bacteria found in ectoparasites such as ticks, lice, fleas, mites and chiggers that can cause emerging infectious diseases throughout the world. There is lacking of information on their distribution and the tick vectors involved. The aim of this study is therefore to determine whether engorged ticks extracted from small mammals (Insectivora, Rodentia and Scandentia) from 15 locations of study areas have any potential health risks to the public. Forty-eight engorged ticks were tested for the presence of rickettsiae DNA using Polymerase Chain Reaction (PCR) targeting a fragment of the rickettsial gene citrate synthase gene (*glt*A). There was no DNA of rickettsiae detected from the ticks. Further investigations are needed in order to generate more comprehensive information on the potential distribution of rickettsial disease in different locations and habitats that can establish an epidemiological data of rickettsiae in Malaysia.

Keywords: Engorged ticks, health risk, Polymerase Chain Reaction (PCR), Rickettsiae

Rickettsial diseases have been considered as emerging zoonoses worldwide and it is important to study as they can cause severe or fatal diseases in vertebrate animals as well as in human (Azad & Beard, 1998; Costa *et al*., 2002; Nadchatram, 2008; Sonenshine, 1993). Symptoms of the disease in human are rashes, fever for one to several weeks, headache, malaise, prostration and peripheral vasculitis (Sonenshine, 1993). The rickettsiae consist of a group of obligate intracellular, gram-negative, pleomorphic rod or coccoid-shaped bacteria that grow intracellularly as a symbiotic in ectoparasites such as mites, ticks and fleas (Raoult & Roux, 1997; Sonenshine, 1993). Transmission of rickettsiae to human or animals occurs via the infected ectoparasites, through exposure to an infected animal such as rodents and scandents, which are known as reservoir of diseases (Sonenshine, 1993; Brouqui *et al*., 2004; Guedes *et al*., 2005; Labruna *et al*., 2007).

*Corresponding author: *abdullahmt2@gmail.com; a.madinah@gmail.com*

Rickettsial diseases have been reported in Malaysia since 1927 (Institute for Medical Research, 1951). Cases such as Q-fever, tick typhus, murine typhus, and scrub typhus were reported (Nadchatram, 2008; Tay *et al*., 2002). Agents for Q-fever were found in three species of small mammals (*Sundamys muelleri*, *Maxomys rajah* and *Tupaia glis*) and three genera of ticks, namely, *Haemaphysalis*, *Dermacentor* and *Ixodes* (Institute for Medical Research, 1959; Nadchatram, 2008).

In 1958, six species of forest rodents and a species of tree shrew were sero-positive for tick typhus (Marchette, 1965). In that study, at least two genera and one species of ticks (*Haemaphysalis* spp. and *Ixodes granulatus*) were involved in the cycles of tick typhus (Marchette, 1965). Ticks of the genera *Ixodes*, *Dermacentor*, *Haemaphysalis* and a species, *I. granulatus* are capable of transmitting rickettsial agents in Malaysia (Nadchatram,

2008; Tay *et al*., 1996). In Sabah (Malaysian Borneo), Rickettsial DNA detected in 268 of 360 fleas (74.4%) extracted from 90 dogs were identified as *Rickettsia felis* (Kernif *et al*., 2012).

Little is known on the distribution of spotted fever group rickettsiae and their tick vectors in Malaysia. Hence, the objective of this study is therefore to detect the presence of these bacteria in engorged ticks collected from 15 study areas. It is also to determine whether the ticks found can pose any potential public health risk in those areas.

Forty-eight engorged ticks preserved in 100% alcohol were tested for rickettsial infection using polymerase chain reaction (PCR). All the engorged ticks were collected from 15 locations in Malaysia during ectoparasite surveys organized by the Department of Zoology, Universiti Malaysia Sarawak (UNIMAS) since 2008 until 2010. During these surveys, a total of 225 ticks from 44 hosts (Insectivora, Rodentia and Scandentia) were collected. However, many ticks did not provide sufficient concentration of DNA for PCR due to their small size (<0.1 mg) and were not engorged. Thus, only 48 tick samples were used in this study as listed in Table 1. The locations of study were selected based on our initial effort to investigate possible interactions of wildlife and their potential involvement in zoonotic disease transmission in protected forest and wildlife areas.

Preparation of ticks for extraction of DNA was based on modification of procedures by Institute for Medical Research of Kuala Lumpur, Labruna *et al*. (2004) and Ndip *et al*. (2004). Isolation of DNA from ticks was following these methods; each tick (<40mg) was washed with 1% hydrochloric acid (HCL) to remove any residual ethanol, subsequently rinsed in distilled water, dried on sterile filter paper, and cut into small pieces using sterile scissors in 1.5 ml micro centrifuge tube containing 80 µl PBS buffer. The DNA was then extracted using QIAamp DNA Mini kit following the manufacturer's protocol for DNA isolation from animal tissue. The yielded DNA was quantified using UV or visible spectrophotometer machine (Ultrospec 1100 Pro Amersham Pharmacia Biotech) at 260 nm wavelength prior to PCR. Purified DNA was stored at 4°C to avoid DNA degradation until used as template for PCR amplification.

DNA of each tick individual was used for PCR assays (Mycyler Biorad) with pimers CS-78 (forward - 5'-GCAAGTATCGGTGAGGA TGTAAT-3') and CS-323 (reverse - 5'-GCTT CCTTAAAATTCAATAAATCAGGAT-3'), which amplify a 401-bp fragment of the citrate synthase (*glt*A) rickettsial gene of the genera *Rickettsia*. The primer designed was selected based on previous study by Labruna *et al*. (2004). GoTaq® Flexi DNA polymerase PCR kit (Promega) was used to amplify the DNA products.

PCR amplication was performed using the following conditions; amplification volume was 25 µl containing 2.5 µl 5X colorless GoTaq® Flexi buffer, 1.75 µl of $MgCl_2$ solution (25 mM), 0.5 µl of dNTP mix (10 mM), 1.0 µl of each forward and reverse primers (10 mM), 12.95 µl of deionised distilled water ($ddH_2O$), 5.0 µl of DNA template and 0.3 µl GoTaq® DNA polymerase (5 u/µl). The amplification conditions included initial denaturation (3 min at 95ºC for 1 cycle), denaturation (15 sec at 95ºC), annealing (30 sec at 48ºC), and extension (30 sec 72ºC) for 40 cycles, final extension (7 min at 72ºC for 1 cycle) and soak (4ºC).

For each reaction, a negative control ($ddH_2O$) and a positive control (DNA of *Rickettsia parkeri*, supplied by Acarology Unit, Institute for Medical Research were included. Three microliters of each PCR product were taken and visualized by electrophoresis in 2% agarose gel (1.0 g of agarose powder added with 50 ml of 1X TAE buffer), stained with 1.0 µl EtBr (10 mg/ml) and examined using UV transillumination. A 100 bp DNA ladder (Promega) was used as the standard marker for comparison. The presence of rickettsiae was detected by comparing the band with the standard marker and the positive control.

Ticks collected were identified following Kohls (1957). Of the 48 individuals examined, only 16 ticks were able to be identified to species level i.e. *Ixodes granulatus*. The others were immature stages that have not yet developed certain characters for identification and were members of the following five genera; *Amblylomma* (n=3), *Dermacentor* (n=12), *Haemaphysalis* (n=12), *Ixodes* (n=4),

**Table 1.** List of ticks, animal hosts, and localities for the collection of ticks used for detection of rickettsiae.

| No | Genera/Species of tick | Host species | Locality of specimen |
|---|---|---|---|
| 1. | *Amblylomma* sp. | *Callosciurus prevostii* | Balambangan Island, Sabah |
| 2. | *Amblylomma* sp. | *Tupaia glis* | Endau Kluang Wildlife Reserves, Johore |
| 3. | *Amblylomma* sp. | *Ptilocercus lowii* | Sg. Menyarin Lanjak Entimau Wildlife Sanctuary, Sarawak |
| 4. | *Dermacentor* sp. | *C. prevostii* | Balambangan Island, |
| 5. | *Dermacentor* sp. | *Sundamys muelleri* | Balambangan Island, Sabah |
| 6. | *Dermacentor* sp. | *S. muelleri* | Balambangan Island, Sabah |
| 7. | *Dermacentor* sp. | *S. muelleri* | Balambangan Island, Sabah |
| 8. | *Dermacentor* sp. | *Rattus tiomanicus* | Balambangan Island, Sabah |
| 9. | *Dermacentor* sp. | *S. muelleri* | Balambangan Island, Sabah |
| 10. | *Dermacentor* sp. | *S. muelleri* | Kpg Giam Padawan, Sarawak |
| 11. | *Dermacentor* sp. | *S. muelleri* | Kpg Giam Padawan, Sarawak |
| 12. | *Dermacentor* sp. | *T. minor* | Kpg Giam Padawan, Sarawak |
| 13. | *Dermacentor* sp. | *T. glis* | Kpg Giam Padawan, Sarawak |
| 14. | *Dermacentor* sp. | *T. glis* | Kpg Giam Padawan, Sarawak |
| 15. | *Dermacentor* sp. | *S. muelleri* | Rumah Temuai Nanga Merit, Kapit, Sarawak |
| 16. | *Haemaphysalis* sp. | *S. muelleri* | Balambangan Island, Sabah |
| 17. | *Haemaphysalis* sp. | *S. muelleri* | Balambangan Island, Sabah |
| 18. | *Haemaphysalis* sp. | *S. muelleri* | Balambangan Island, Sabah |
| 19. | *Haemaphysalis* sp. | *S. muelleri* | Balambangan Island, Sabah |
| 20. | *Haemaphysalis* sp. | *S. muelleri* | Balambangan Island, Sabah |
| 21. | *Haemaphysalis* sp. | *S. muelleri* | Balambangan Island, Sabah |
| 22. | *Haemaphysalis* sp. | *S. muelleri* | Balambangan Island, Sabah |
| 23. | *Haemaphysalis* sp. | *T. glis* | Kubah National Park, Kuching, Sarawak |
| 24. | *Haemaphysalis* sp. | *S. muelleri* | Human Settlement, Kanowit, Sarawak |
| 25. | *Haemaphysalis* sp. | *Maxomys rajah* | Sg. Dusun Wildlife Reserve, Selangor |
| 26. | *Haemaphysalis* sp. | *M. rajah* | Sg. Dusun Wildlife Reserve, Selangor |
| 27. | *Haemaphysalis* sp. | *M. rajah* | Tasek Bera Ramsar Site, Pahang |
| 28. | *Ixodes granulatus* | *S. muelleri* | Bukit Aup Jubilee Park, Sibu, Sarawak |
| 29. | *I. granulatus* | *Lariscus insignis* | Endau Kluang Wildlife Reserves, Johore |
| 30. | *I. granulatus* | *Echinosorex gymnurus* | Human Settlement, Kanowit, Sarawak |
| 31. | *I. granulatus* | *R. rattus* | Human Settlement, Kanowit, Sarawak |
| 32. | *I. granulatus* | *M. rajah* | Lata Bujang Krau Wildlife Reserve, Pahang |
| 33. | *I. granulatus* | *R. rattus* | Kpg Giam Padawan, Sarawak |
| 34. | *I. granulatus* | *S. muelleri* | Kpg Giam Padawan, Sarawak |
| 35. | *I. granulatus* | *T. tana* | Kpg Giam Padawan, Sarawak |
| 36. | *I. granulatus* | *Niviventer cremoriventer* | Gunung Regu Padawan, Sarawak |
| 37. | *I. granulatus* | *N. cremoriventer* | Gunung Regu Padawan, Sarawak |
| 38. | *I. granulatus* | *R. exulans* | Gunung Regu Padawan, Sarawak |
| 39. | *I. granulatus* | *Leopoldamys sabanus* | Tasek Bera Ramsar Site, Pahang |
| 40. | *I. granulatus* | *M. rajah* | Tasek Bera Ramsar Site, Pahang |
| 41. | *I. granulatus* | *T. minor* | Mount Penrisen Padawan, Sarawak |
| 42. | *I. granulatus* | *S. muelleri* | Mount Penrisen Padawan, Sarawak |
| 43. | *I. granulatus* | *E. gymnurus* | Rumah Temuai Nanga Merit, Kapit, Sarawak |
| 44. | *Ixodes* sp. | *S. muelleri* | Bukit Aup Jubilee Park, Sibu, Sarawak |
| 45. | *Ixodes* sp. | *S. muelleri* | Human Settlement, Kanowit, Sarawak |
| 46. | *Ixodes* sp. | *S. muelleri* | Kpg Giam Padawan, Sarawak |
| 47. | *Ixodes* sp. | *T. glis* | Gunung Regu Padawan, Sarawak |
| 48. | *Rhipicephalus* sp. | *S. muelleri* | Niah National Park, Miri, Sarawak |

and *Rhipicephalus* (n=1). The ticks were extracted from 14 species of animal hosts, namely, *Echinosorex gymnurus* (Insectivora), *Callosciurus prevostii*, *Lariscus insignis*, *Leopoldamys sabanus*, *Maxomys rajah*, *Niviventer cremoriventer*, *Rattus exulans*, *R. rattus*, *R. tiomanicus*, and *Sundamys muelleri* (Rodentia), *Ptilocercus lowii*, *Tupaia glis*, *T. minor* and *T. tana* (Scandentia) (Table 1). All ticks were extracted from animals caught in four locations in Peninsular Malaysia and 11 locations in Malaysian Borneo as shown in Figure 1.

The PCR results obtained in this study showed that rickettsiae were not found in all the 48 engorged ticks (Figure 2). Findings from this study indicate that the ticks were free from rickettsiae. We cannot conclude that there is no potential risk of rickettsial disease to the local people living or near the study areas. This is due to the small sample size of this study and therefore warrants extensive investigation on more numbers of ectoparasites and hosts to determine better results on the status of rickettsial disease distribution in these areas. Rickettsiae can only be transmitted to humans or other animals by the infected ticks and infected host (Guedes *et al*., 2005). However, precautions should be taken whenever there is close and/or direct contact with ectoparasites and their hosts as rickettsiae have been detected in ticks collected in Sarawak and Peninsular Malaysia (Nadchatram, 2008; Tay *et al*., 1996).

There are several possible explanations as to why there was no DNA of rickettsiae detected from the engorged ticks. First, the study sites did not have previous or recent records of cases with rickettsial disease. Secondly, it could also be due to the absence or low number of rickettsiae in these samples. The difficulties in isolation of rickettsiae have been reported by Tay *et al*. (2002). Moreover, based on similar study on detection of rickettsiae in Peninsular Malaysia, *Orientia tsutsugamushi* (formerly known as *Rickettsia tsutsugamushi*) also was not detected in chiggers and animals tissue except for one blood sample of a rodent, *Leopoldamys sabanus* (Azima *et al*., 2013). The low prevalence of rickettsiae in ticks may probably made the isolation process very challenging. This is because interspecific competition for blood feeding between ticks on a host may result in uneven distribution of rickettsiae through transovarial transmission and thus may cause the low content of rickettsiae in the tick populations (Azad & Beard, 1998).

If similar study needs to be conducted in the future, it is suggested that the surveys should be conducted in areas with positive cases of rickettsial infection (Sagin *et al*., 2000), high incidence of sero-positive tick typhus infection and/or areas with the presence of TT118 rickettsiae natural infection in rodents (Ho *et al*., 1997). The rural areas of Sarawak such as upper Rejang River, Selangau, Lesong Laku and Long Murum should be further explored for data validation.

This study should be extended for at least two years to determine whether the presence of rickettsiae occur throughout the year. There is also a need to gain information for better understanding of possible factors such as meteorological parameters (temperature, humidity and rainfall) that causes the transmission of rickettsial. The diseases were showed to be affected by geographical distribution, seasonal activities of vectors and reservoirs, and human behaviors that place them at risk for tick attachment and succeeding infection (Chapman *et al*., 2006). In United States, these diseases tend to occur seasonally with the majority of cases occurred during the warmer spring and summer (Chapman *et al*., 2006). Therefore, the rickettsial diseases may probably occur during the warm and/or humid season in Malaysia.

Future studies should focus on various methods of rickettsiae detection including real time PCR assays with other possible primers and target genes, haemolymph test, direct immunoflourescence assay (DFAT) and fluorescence in-situ hybridisation (FISH) with peptide nucleic acid (PNA) probes. These methods have been proven for detection of rickettsiae in tick samples (Guedes *et al*., 2005; Kelly *et al*., 1994; Labruna *et al*., 2004; 2007; Moraes-Filho *et al*., 2009; Ndip *et al*., 2004). It is recommended to detect the presence of rickettsiae not only from tick samples but also from the blood of animals and human in the areas. Rickettsiae have been successfully detected by cell culture assay of DNA isolated from blood samples, PCR using isolation of

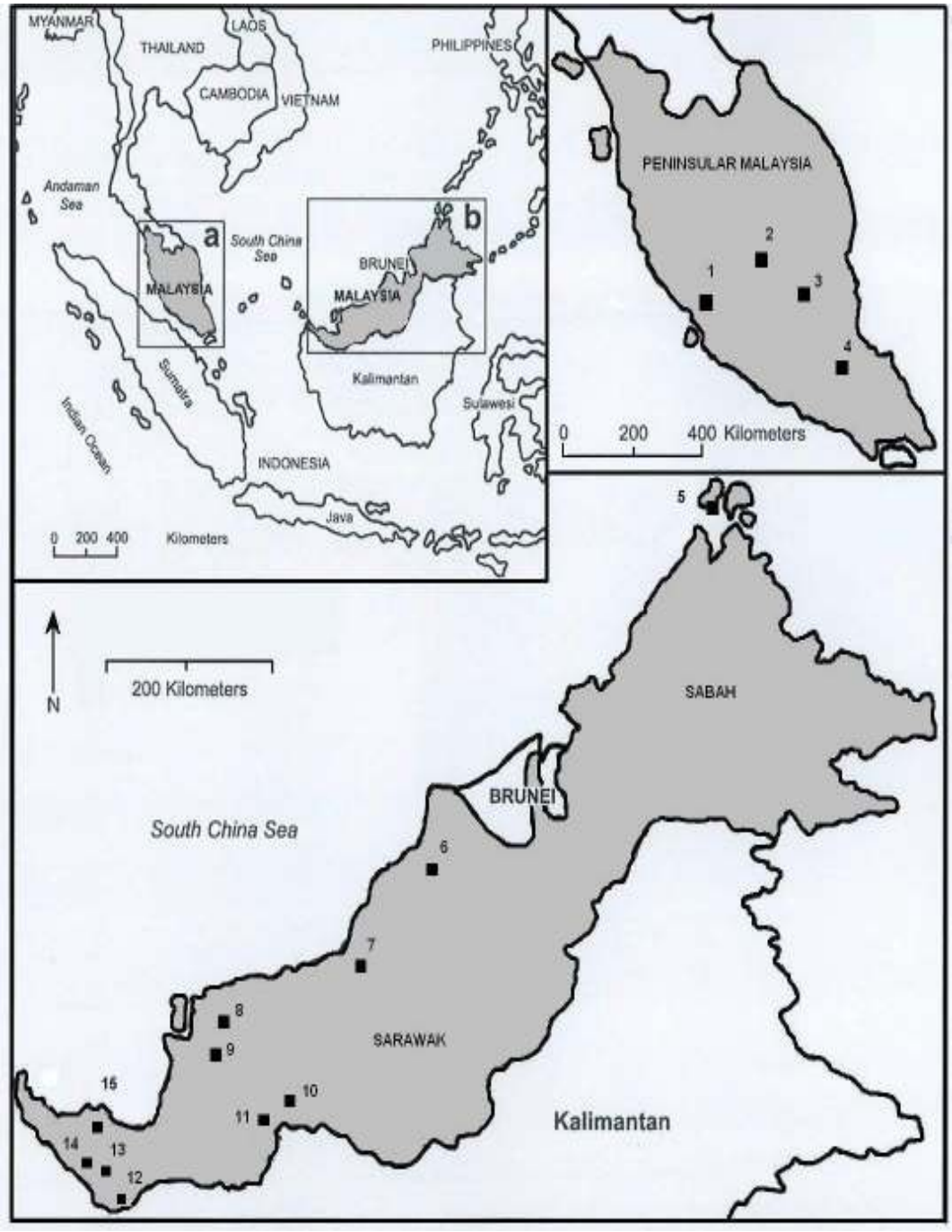


**Figure 1.** Maps showing the 15 localities of selected ticks used for the detection of rickettsial disease in Malaysia. 1- Sungai Dusun Wildlife Reserve, Selangor (SDWR); 2- Lata Bujang Krau Wildlife Reserve, Pahang (LBKWR); 3- Tasek Bera Ramsar Site, Pahang (TBRS); and 4- Endau Kluang Wildlife Reserve, Johore (EKWR); 5- Balambangan Island, Sabah (BIS); 6- Niah National Park, Miri, Sarawak (NNP); 7- Rumah Temuai Nanga Merit, Kapit, Sarawak (RTNM); 8- Bukit Aup Jubilee Park, Sibu, Sarawak (BAJP); 9- Human Settlement, Kanowit, Sarawak (HSK); 10- Sungai Bloh Lanjak Entimau Wildlife Sanctuary, Sarawak (SBLEWS); 11- Sungai Menyarin Lanjak Entimau Wildlife Sanctuary, Sarawak (SMLEWS); 12-Mount Penrisen, Sarawak (MPS); 13- Gunung Regu Padawan, Sarawak (GRP); 14- Kampung Giam Padawan, Sarawak (KGP); and 15- Kubah National Park, Kuching, Sarawak (KNP).

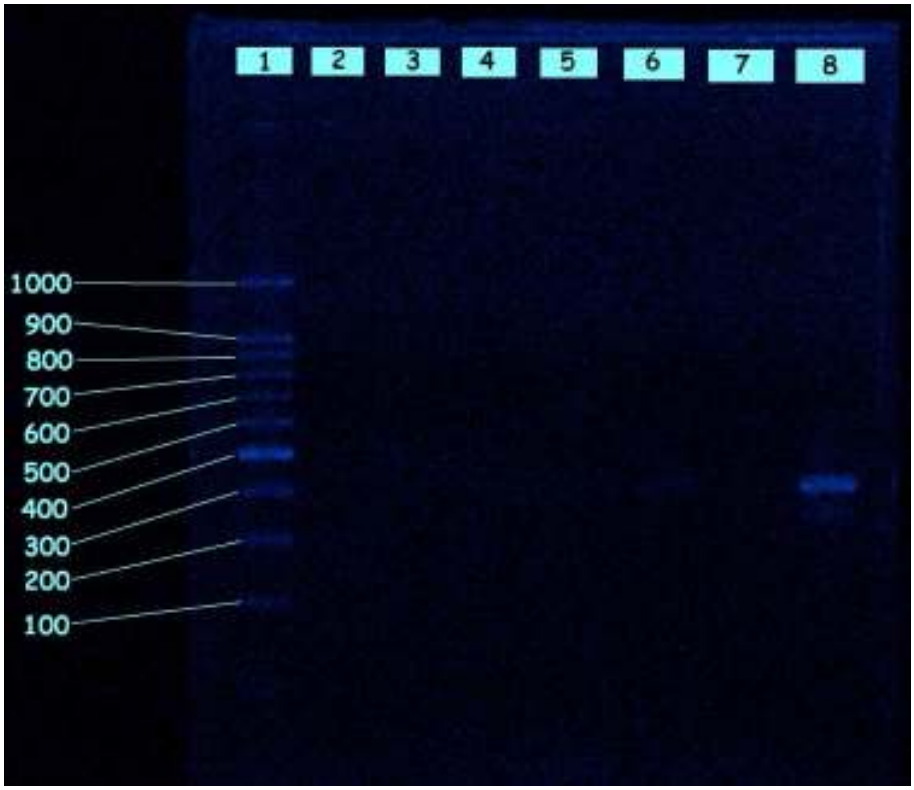


**Figure 2.** Polymerase chain reaction products show negative results with only positive control (*Rickettsia parkeri*) present. Products amplified with the CS-78 (forward) and CS-323 (reverse) primers and electrophoresis on 2% argarose gel. Lane 1-100 bp ladder; Lane 2-negative control, Lane 3 to 7- DNA extracted from five individuals of ticks; Lane 8-positive control (*R. parkeri*).

DNA from blood and organ samples, and indirect immunofluorescence assay (IFA), enzyme-linked immunosorbent assay (ELISA), Weil-Felix reaction and indirect immunoperoxidase reaction using blood serum (Moraes-Filho *et al*., 2009; Ndip *et al*., 2004; Richards *et al*., 2003; Sagin *et al*., 2000; Tay *et al*., 2002; Tzianabos *et al*., 1989). Other arthropod ectoparasites such as lice, fleas, chiggers and mites should also be examined as they have the potentials to transmit diseases like louse-borne typhus (lice), plague (fleas), scrub typhus (chiggers) and scabies (itch mites) (Azad & Beard, 1998; Azima *et al*., 2013; Kernif *et al*., 2012; Nadchatram, 2008; Roberts & Janovy, 2009).

Future study should generate more information on potential distribution areas of rickettsial disease, highlight the importance of ectoparasites as vectors of rickettsial disease and establishment of epidemiological data of rickettsiae in Malaysia for reference. Apart from that, future studies should include more coverage of survey areas and more research funding to enable larger size samples for detection of rickettsial disease.

## ACKNOWLEDGEMENTS

The authors wish to thank the Sarawak Forestry Corporation and Sarawak Forestry Department for permits NCCD 9074(IV).29, NCCD.907.4.4 (V)-70/Park Permit: 28/2010 and NCCD.907.4.4(V)-189/Park Permit No:104/2010, Department of Wildlife and National Park (DWNP) Kuala Lumpur, Sabah Wildlife Department and Sabah Forest Department, Kota Kinabalu, Sabah for their kind hospitality during study in Peninsular Malaysia and Sabah. We also wish to thank the Director-General of Health, Malaysia, for permission to publish this paper. We are grateful to the field team from the Department of Zoology, Faculty of Resource Science and Technology, Universiti Malaysia Sarawak (UNIMAS); and Mrs. Halimaton Ibrahim and Mr. Mohd Kulaimi Bujang from the Institute

for Medical Research (IMR) for guidance and confirmation of some Acarines. The authors wish to also thank Dr. Marcelo B. Labruna from University of São Paulo and Dr Claudio Mafra from University of Vicosa, Brazil for supplying us with the positive control and technical support. This study was supported by the UNIMAS Zamalah scholarships 2009/11, UNIMAS top-down grant on the zoonosis and EID [E14006 /F07 /06 /ZRC /03 /2007(03)] and FRGS/01(23)/766/2010(47) awarded to MTA and colleagues.

## REFERENCES

Azad, A.F. & Beard, C.B. (1998). Rickettsial pathogens and their arthropod vector. *Emerging Infectious Disease*, 4(2):179-186.

Azima, L.H., Mariana, A., Vishalani, V.N., & Ho, T.M. (2013). Detection of *Orientia tsutsugamushi* in chiggers and tissues of small mammals using polymerase chain reactions. *The Experiment,* 11(2):683-689.

Brouqui, P., Bacellar, F., Baranton, G., Birtles, R.J., Bjoërsdorff, A., Blanco, J.R., Caruso, G., Cinco, M., Fournier, P.E., Francavilla, E., Jensenius, M., Kazar, J., Laferl, H., Lakos, A., Lotric, S.F., Maurin, M., Oteo, J.A., Parola, P., Perez-Eid, C., Peter, O., Postic, D., Raoult, D., Tellez, A., Tselentis, Y., & Wilske, B. (2004). Guidelines for diagnosis of tick-borne bacterial disease in Europe. *Clinical Microbiology and Infection*, 10(12):1108-1132.

Chapman, A.S., Bakken, J.S., Bloch, K.C., Buckingham, S.C., Dasch, G.A., Dumler, J.S., Eremeeva, M.E., Folk, S.M., Krusell, A., Marshall, G.S., McQuiston, J.H., Nicholson, W.L., Ohl, C.A., Paddock, C.D., Sexton, D.J., Storch, G.A., Swerdlow, D.L., & Walker, D.H. (2006). *Diagnosis and management of tickborne rickettsial diseases: Rocky Mountain spotted fever, ehrlichioses, and anaplasmosis-United States (A practical guide for physicians and other health-care and public health professionals).* Volume 55. United State: Center for Health Information and Service, Center for Disease Control and Prevention and Department of Health and Human Services, Atlanta. pp 1-36.

Costa, P.S.G., Brigatte, M.E., Almeida, E.P. & Carvalho Valle, L.M. (2002). A typical fulminant *Rickettsia rickettsii* infection (Brazilian Spotted Fever) presenting as septic shock and adult respiratory distress syndrome. *Brazilian Journal of Infectious Diseases*, 6 (2):91-96.

Guedes, E., Leite, R.C., Prata, M.C.A., Pacheco, R.C., Walker, D.H., & Labruna, M.B. (2005). Detection of *Rickettsia rickettsii* in the tick *Amblyomma cajennense* in a new Brazilian Spotted Fever-endemic area in the state of Minas Gerais. *Memorias do Institute Oswaldo Cruz*, Rio de Janeiro, 100(8):841-845.

Ho, T.M., Tay, S.T., & Rohani, M.Y. (1997). A preliminary finding of arthropods-borne rickettsioses in Malaysia. *International Medical Research Journal*, 1:339-344.

Institute for Medical Research 1900-1950 (1951). *Studies from the Institute for Medical Research Federation of Malaya, Jubliee Issue (Volume 25)*. Kuala Lumpur: Institute for Medical Research Publication. pp 389.

Institute for Medical Research (1959). *Annual Report for 1958*. Kuala Lumpur: Institute for Medical Research Publication. pp104-106.

Kelly, D., Dasch, G.A., Chan, T.C., & Ho, T.M. (1994). Detection and characterization of *Rickettsia tsutsugamushi* (Rickettsiales: Rickettsiaceae) in infected *Leptotrombidium* (*Leptotrombidium fletcheri*) chiggers (Acari: Trombiculidae) with the polymerase chain reaction. *Journal of Medical Entomology*, 31:691-699.

Kernif, T., Socolovschi, C., Wells, K., Lakim, M.B., Inthalad, S., Slesak, G., Boudebouch, N., Beaucournu, J.C., Newton, P.N., Raoult, D., & Parola, P. (2012). *Bartonella* and *Rickettsia* in arthropods from the Lao PDR and from Borneo, Malaysia. *Comparative Immunology, Microbiology and Infectious Disease*, 35:51-57.

Kohls, G.M. (1957). Tick (Ixodoidae) of Borneo and Malaya. *Malaysian Parasites*, XVIII (28):65-94.

Labruna, M.B., Whitworth, T., Horta, M.C., Bouyer, D.H., McBride, J.W., Pinter, A., Popov, V., Gennari, S.M., & Walker, D.H. (2004). *Rickettsia* species infecting *Amblyomma cooperi* ticks from an area in the State of Sao Paulo, Brazil, where Brazilian spotted fever is endemic. *Journal of Clinical Microbiology*, 42 (1):90-98.

Labruna, M.B., Pacheco, R.C., Richtzenhain, L.J., & Szabo, M.P.J. (2007). Isolation of *Rickettsia rhipicephali* and *Rickettsia bellii* from *Haemaohysalis juxtakochi* ticks in the State of Sao Paulo, Brazil. *Applied and Environmental Microbiology*, 73(3):869-873.

Marchette, N.J. (1965). Rickettsiosis (tick typhus, Q-fever, urban-typhus) in Malaya. *Journal of Medical Entomology*, 2:339-371.

Moraes-Filho, J., Pinter, A., Pacheco, R.C., Gutmann, T.B., Barbosa, S.O., Gonzales, M.A.R.M., Muraro, M.A., Cecilio, S.R.M., & Labruna, M.B. (2009). New epidemiological data on Brazilian spotted fever in an endemic area of the State of Sao Paulo, Brazil. *Vector-Borne and Zoonotic Disease*, 9(1):73-78.

Nadchatram, M. (2008). The beneficial rain forest ecosystem with environmental effects on zoonoses involving ticks and mites (Acari), a Malaysian perspective and review. *Tropical Biomedicine*, 25(2):1-92.

Ndip, L.M., Fokam, E.B., Bouyer, D.H., Ndip, R.N., Titanji, V.P.K., Walker, D.H., & McBride, J.W. (2004). Detection of *Rickettsia africae* in patients and ticks along the coastal region of Cameroon. *American Journal of Tropical Medicine and Hygiene*, 71(3):363-366.

Raoult, D. & Roux, V. (1997). Rickettsioses as paradigms of new or emerging infectious disease. *Clinical Microbiology Reviews*, 10(4):694-719.

Richards, A.L., Ratiwayanto, S., Rahardjo, E., Kelly, D.J., Dasch, G.A., Fryauff, D.J., & Bangs, M.J. (2003). Serologic evidence of infection with Ehrlichiae and Spotted Fever Group Rickettsiae among residents of Gag Island, Indonesia. *American Journal of Tropical Medicine and Hygiene*, 68(4):480-484.

Roberts, L.S. & Janovy, Jr.J. (2009). *Gerald D. Schmidt and Larry S.Roberts's Foundations of Parasitology* (8th Edition). New York: The McGraw-Hill Companies Inc. pp.1-701.

Sagin, D.D., Ismail, G., Nasian, L.M., Jok, J.J. & Pang, E.K.H. (2000). Rickettsial infection in five remote orang ulu villages in upper Rejang River, Sarawak, Malaysia. *Southeast Asian Journal of Tropical Medicine and Public Health*, 31(4):733-735.

Sonenshine, D.E. (1993). *Biology of ticks* (Volume 2). New York: University Press, Inc. pp.194-255.

Tay, S.T., Ho, T.M., & Rohani, M.Y. (1996). Use of hemolymph test for detection of rickettsiae in Malaysian ticks. *Southeast Asian Journal of Tropical Medicine and Public Health*, 27:642-644.

Tay, S.T., Rohani, M.Y., Ho, T.M., & Devi, S. (2002). Isolation and PCR detection of rickettsiae from clinical and rodent samples in Malaysia. *Southeast Asian Journal of Tropical Medicine and Public Health*, 33(4):772-779.

Tzianabos, T., Anderson, B.E., & McDade, J.E. (1989). Detection of *Rickettsia rickettsii* DNA in clinical specimens by using polymerase chain reaction technology. *Journal of Clinical Microbiology*, 27(12):2866-2868.