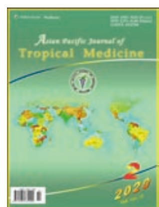

Letter to Editor
Asian Pacific Journal of Tropical Medicine
journal homepage: www.apjtm.org

doi: 10.4103/1995-7645.275418
Impact Factor: 1.77

First evidence of *Bartonella phoceensis* and *Candidatus* Mycoplasma haemomuris subsp. ratti in synanthropic rodents in Malaysia

Van Lun Low[1✉], Tiong Kai Tan[2✉], Jamaiah Ibrahim[3], Sazaly AbuBakar[1,4], Yvonne Ai Lian Lim[2,5]

[1]*Tropical Infectious Diseases Research and Education Centre (TIDREC), University of Malaya, Malaysia*
[2]*Department of Parasitology, Faculty of Medicine, University of Malaya, Malaysia*
[3]*Faculty of Medicine and Defence Health, National Defence University of Malaysia, Malaysia*
[4]*Department of Medical Microbiology, Faculty of Medicine, University of Malaya, Malaysia*
[5]*Centre of Excellence for Research in AIDS (CERIA), University of Malaya, Malaysia*

Rodent-borne leptospirosis is by far the most common bacterial zoonosis and it is an important emerging global public health concern in Southeast Asia. Bacterial pathogens associated with rodents, especially those that live in close association with humans have been underreported. To fill this knowledge gap, the present study was undertaken to explore other neglected disease agents that can naturally infect synanthropic rodents. Both *Bartonella* and *Mycoplasma* pose major health threats to various animal hosts and they are also emerging zoonoses and pathogens of public health concern. With these in mind, we aimed to detect the presence of *Bartonella* and *Mycoplasma* bacteria in synanthropic rodents from a densely populated capital city of Malaysia, Kuala Lumpur.

Rodent collection has been described elsewhere in detail[1]. Briefly, a total of 134 synanthropic rodents comprising *Rattus* (*R.*) *rattus diardii*, *R. norvegicus*, *R. argentiventer*, *R. tiomanicus*, and *R. exulans* were trapped from two human populated areas (Sentul and Chow Kit) in Kuala Lumpur using steel wire traps. Blood was collected from the heart using a needle and syringe and placed into EDTA tube. Genomic DNA was extracted from whole blood using the QIAamp DNA Blood Mini Kit (QIAGEN Inc.,Valencia, CA).

Bartonella DNA detection was performed using polymerase chain reaction (PCR) with primers (325-5'CTT CAG ATG ATG ATC CCA AGC CTT TTG GCG-3'), and (1100-5' GAA CCG ACG ACC CCC TGC TTG CAA AGC-3') which amplify a portion of the 16S-23S rRNA intergenic spacer region[2]. PCR amplification was performed in a final volume of 25 µL containing 25-50 ng genomic DNA, 12.5 µL of MyTaq Red Mix (Bioline Reagents Ltd., London, UK) and 10 pmol of each forward and reverse primer. PCR was performed using the Applied Biosystems Veriti 96-Well Thermal Cycler (Applied Biosystems, Inc., Foster City, CA) with the following thermal protocol: initial denaturation at 95 ℃ for 2 min followed by 55 cycles of denaturing at 94 ℃ for 15 s, annealing at 66 ℃ for 15 s, and extension at 72 ℃ for 15 s, and final extension at 72 ℃ for 1 min.

For *Mycoplasma* DNA detection, samples were subjected to *16S* rRNA gene amplification using a universal Mycoplasma primer pair (HBT-F-5' ATA CGG CCC ATA TTC CTA CG-3' and HBT-R-5' TGC TCC ACC ACT TGT TCA-3')[3], 25-50 ng genomic DNA in a total volume of 25 µL, with the following thermal protocol: 95 ℃ for 15 min and 50 cycles of 95 ℃ for 10 s, 55 ℃ for 15 s and 72 ℃ for 30 s, and 72 ℃ for 1 min.

Purified PCR amplicons were sequenced using an ABI PRISM 377 Genetic Analyzer (Applied Biosystems, Inc.). Representative sequences of *Bartonella* (MK953939-MK953940) and *Mycoplasma* (MK959182) generated from this study were deposited in the National Center for Biotechnology Information GenBank. A neighbour-joining (NJ) phylogenetic tree was plotted using MEGA6 (https://megasoftware.net/). The NJ bootstrap values were estimated using 1 000 replicates with Kimura's two-parameter model of substitution (K2P distance). *Brucella abortus* (X95889) and *Brucella*

✉To whom correspondence may be addressed. E-mail: vanlun_low@um.edu.my; stanley.monas@gmail.com

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-Non Commercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

©2020 *Asian Pacific Journal of Tropical Medicine* Produced by Wolters Kluwer-Medknow. All rights reserved.

How to cite this article: Low VL, Tan TK, Ibrahim J, AbuBakar S, Lim YAL. First evidence of *Bartonella phoceensis* and *Candidatus* Mycoplasma haemomuris subsp. ratti in synanthropic rodents in Malaysia. Asian Pac J Trop Med 2020; 13(2): 94-96.

Article history: Received 12 August 2019 Revision 14 December 2019
Accepted 16 December 2019 Available online 14 January 2020

melitensis (AY513568) were used as outgroups for the construction of *Bartonella* and *Mycoplasma* phylogenetic trees, respectively. Based on the 16S-23S fragment of *Bartonella*, successful PCR amplification was found in 5 out of 134 samples (3.73%). NJ phylogenetic analysis revealed that all five sequences clustered with *Bartonella* (*B.*) *phoceensis* sequence (AY515123) retrieved from the National Center for Biotechnology Information GenBank. *B. phoceensis* showed a sister relationship with *B. rattimassiliensis*, and distantly separated from the other *Bartonella* species (Figure 1). For *Mycoplasma*, three samples (2.24%) were positive to *16S* rRNA fragment and successfully sequenced. The three sequences formed a monophyletic clade with the sequences of *Candidatus* Mycoplasma (M.) haemomuris subsp. ratti (AB758434, AB758435 and AB758439) and showed a close relationship with *Candidatus* M. haemomuris subsp. musculi (Figure 2).

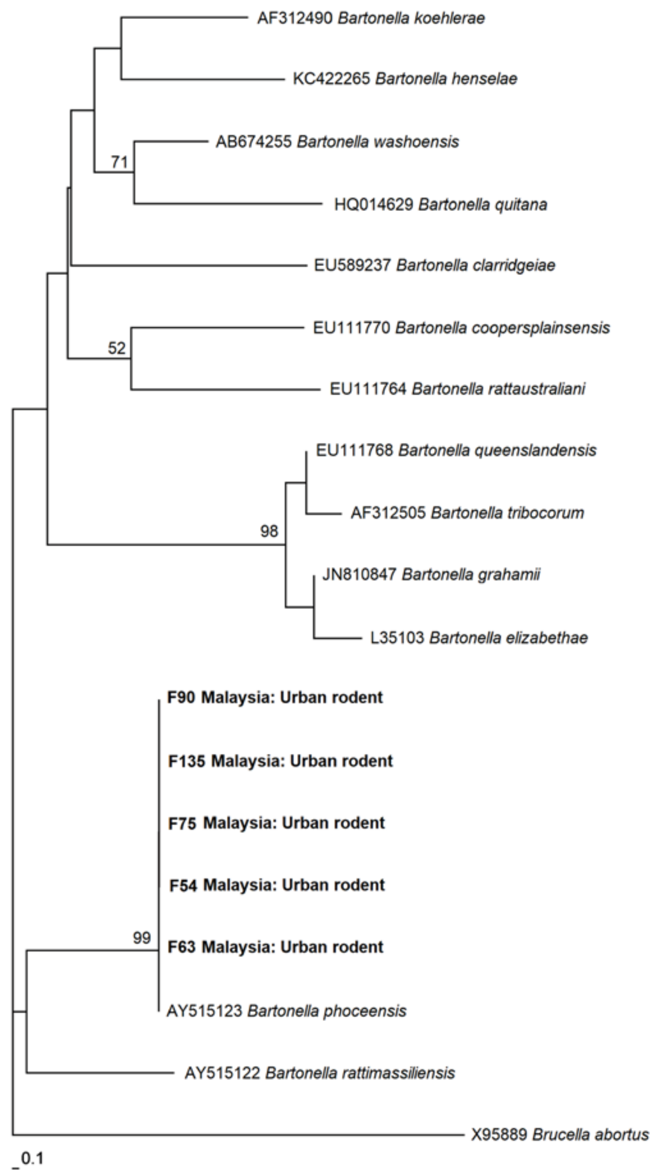

Figure 1. Neighbour-joining phylogenetic tree of *Bartonella* spp. based on the 16S-23S intergenic spacer region. Bootstrap values (NJ) are shown on the branches. Newly generated sequences are in bold.

This study provides the first evidence for the presence of *B. phoceensis* and *Candidatus* M. haemomuris subsp. ratti in synanthropic rodents in Malaysia. Their prevalence rates (3.73% for *Bartonella* and 2.24% for *Mycoplasma*), however, were considered low in comparison to that previously reported in Malaysia. In the earlier study, five different *Bartonella* species (*i.e.*, *B. tribocorum*, *B. rattimassiliensis*, *B. coopersplainsensis*, *B. elizabethae*, and *B. queenslandensis*) (13.7%) were isolated from kidney and spleen homogenates of rats[4]. Nevertheless, the detection protocols adopted (16S-23S *versus gltA* and *rpoB*; and blood *versus* kidney and spleen) may have contributed to the discrepancies in the recovered species in both studies. Among the detected species, *B. rattimassiliensis*, *B. tribocorum*, and *B. elizabethae* were implicated as causing human infections in Thailand[5]. The pathogenicity and zoonotic potential of *B. phoceensis* are unknown.

Mycoplasma spp. from various animals in Malaysia were reported at varying frequencies such as *M. haemofelis* (11.7%) in cats[6], *M. wenyonii* and *Candidatus* M. haemobos (69.0%) in cattle[7], and several species in various animal samples received in a veterinary diagnostic laboratory[8].

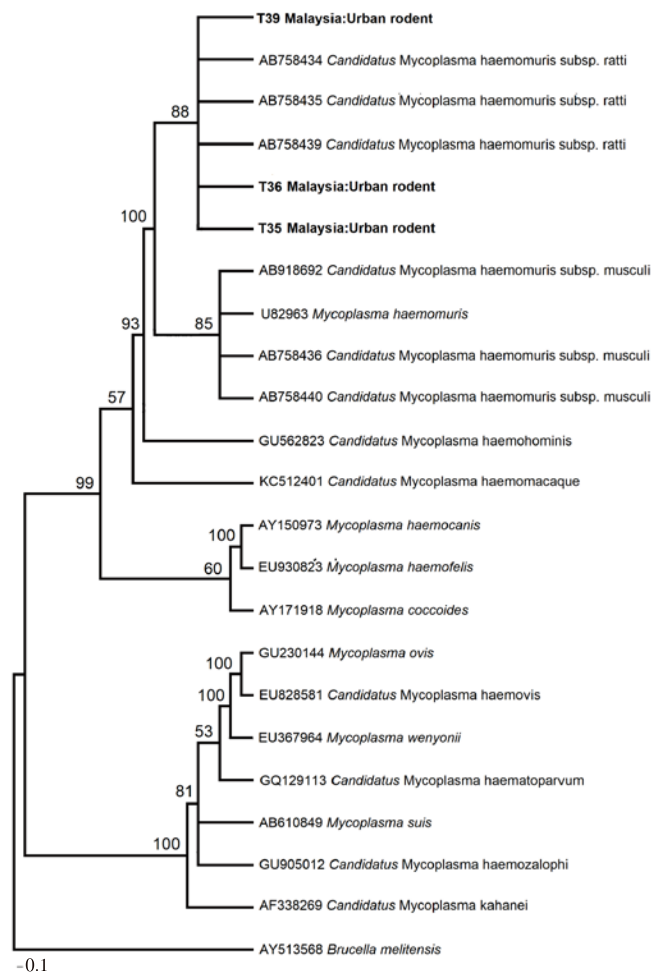

Figure 2. Neighbour-joining phylogenetic tree of *Mycoplasma* spp. based on the *16S* rRNA sequences. Bootstrap values (NJ) are shown on the branches. Newly generated sequences are in bold.

Investigation of *Mycoplasma* in rodents in Malaysia is underappreciated, though *M. arthritidis* was reported in three rats (out of 10 rats) in a previous study[9]. In contrast, in the present study, we detected *Candidatus* M. haemomuris subsp. ratti, a subspecies of *M. haemomuris* which was recently incriminated as the anaemic pathogen of rats in Japan, and differentiated from *Candidatus* M. haemomuris subsp. musculi which mainly infects mice[10]. Animal *Mycoplasma* spp. rarely infect humans, but their zoonotic potential could not be disregarded since cases of human haemoplasma infection have been documented.

In conclusion, we report here the occurrence of *B. phoceensis* and *Candidatus* M. haemomuris subsp. ratti in synanthropic rodents for the first time in Malaysia. Our results suggest that synanthropic rodents can serve as the reservoir for these vector-borne pathogens. Nevertheless, their routes of transmission and zoonotic potential require further investigation.

Ethics statement

The study protocol [PAR/20/09/2011/J (R)] was reviewed and approved by the Institutional Animal Care and Use Committee, University of Malaya, Malaysia.

Conflict of interest statement

The authors declare that there are no competing interests.

Acknowledgement

This study was financially supported by the University of Malaya research grant (RP021C-16SUS) and Higher Institution Centre of Excellence (HICoE) program (MO002-2019).

Authors' contributions

V.L.L. and T.K.T. wrote the manuscript and performed the experiments, J.I., S.A. and Y.A.L.L. contributed to the final version of the manuscript.

References

[1] Tan TK, Low VL, Ng WH, Ibrahim J, Wang D, Tan CH, et al. Occurrence of zoonotic *Cryptosporidium* and *Giardia duodenalis* species/genotypes in urban rodents. *Parasitol Int* 2019; **69**: 110-113.

[2] Diniz PP, Maggi RG, Schwartz DS, Cadenas MB, Bradley JM, Hegarty B, et al. *Canine bartonellosis*: Serological and molecular prevalence in Brazil and evidence of co-infection with *Bartonella henselae* and *Bartonella vinsonii* subsp. berkhoffi. *Vet Res* 2007; **38**: 697-710.

[3] Criado-Fornelio A, Martinez-Marcos A, Buling-Saraña A, Barba-Carretero JC. Presence of *Mycoplasma haemofelis*, *Mycoplasma haemominutum* and piroplasmids in cats from southern Europe: A molecular study. *Vet Microbiol* 2003; **93**(4): 307-317.

[4] Tay ST, Mokhtar AS, Zain SN, Low KC. Isolation and molecular identification of *Bartonellae* from wild rats (*Rattus* species) in Malaysia. *Am J Trop Med Hyg* 2014; **90**(6): 1039-1042.

[5] Kosoy M, Bai Y. *Bartonella* bacteria in urban rats: A movement from the jungles of Southeast Asia to metropoles around the globe. *Front Ecol Evol* 2019; **7**: 88.

[6] Aklilu E, Shaharulnizim N, Francis JJ, Anurrdin SH. Molecular investigation of *Mycoplasma haemofelis* in stray cats in Kota Bharu, Kelantan. *Trop Biomed* 2016; **33**(4): 608-612.

[7] Mohd Hasan LI, Kho KL, Koh FX, Hassan Nizam QN, Tay ST. Molecular evidence of hemoplasmas in Malaysian cattle and ticks. *Trop Biomed* 2017; **34**(3): 668-674.

[8] Dahlia H, Tan LJ, Zarrahimah Z, Chandrawathani P, Ramlan M. Isolation of *Mycoplasma* species from various animal hosts in Malaysia. *Malaysian J Vet Res* 2011; **2**(1): 53-57.

[9] Premaalatha B, Nurulaini R, Zawida Z, Norakmar I, Imelda LV, Adnan M, et al. A survey of bacterial and parasitic infections of rats caught in the Veterinary Research Institute (VRI), Ipoh. *Malaysian J Vet Res* 2010; **1**(1): 45-50.

[10] Harasawa R, Fujita H, Kadosaka T, Ando S, Rikihisa Y. Proposal for '*Candidatus* Mycoplasma haemomuris subsp. musculi' in mice, and '*Candidatus* Mycoplasma haemomuris subsp. ratti' in rats. *Int J Syst Evol Microbiol* 2015; **65**: 734-737.