Volume 1 (2026)
https://doi.org/10.22452/jtoh.vol1.3
JOURNAL OF TROPICAL One Health
SHORT COMMUNICATION

Absence of *Borrelia* spp. in Urban Pest Animals from Peninsular Malaysia

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ARTICLE HISTORY

Received: 14 Oct 2025
Revised: 7 Mar 2026
Accepted: 14 Apr 2026
Published 30 Jun 2026

ABSTRACT

Urbanization is rapidly influencing zoonotic disease dynamics, yet the presence of *Borrelia* spp., which are the causative agents of Lyme disease and relapsing fever, within Malaysia's high-density urban environments remains poorly characterized. This study provides the first molecular investigation of *Borrelia* prevalence among small mammal hosts in two major urban locations, namely Kuala Lumpur and Penang. Archived spleen DNA samples from 120 selected rodents and shrews were screened using an optimized nested PCR targeting the *flaB* gene, a highly sensitive and specific genetic marker for *Borrelia* identification. Despite the validated efficacy of the assay, all 120 screened samples yielded negative results, confirming an overall prevalence of 0% in the sampled populations. This non-detection contrasts sharply with reports from rural and forested regions of Malaysia, suggesting that urbanization may disrupt the vector-host transmission cycle through altered habitat and microclimate circumstances. These findings provide critical urban baseline data for *Borrelia* surveillance in Peninsular Malaysia and emphasize the need for integrated, One Health approaches, incorporating serological screening and tick vector assessments, to monitor potential shifts in *Borrelia* ecology within Malaysia's expanding urban landscapes.

Keywords: *Borrelia* spp., urban pest animals, urban ecology, zoonotic surveillance

Introduction

Borrelia spp. are tick-borne zoonotic spirochetes that cause infections in humans, specifically Lyme disease (LD) and relapsing fever (RF) (Steere et al., 2016; Madison-Anternucci et al., 2020). These bacteria are adapted to have flagella, and the core filament of the flagella is encoded by flagellin B (*flaB*) gene, which serves as a reliable molecular marker for their identification and characterization (Motaleb et al., 2000; Wodecka, 2011). Southeast Asia has been recognized as a hotspot for emerging zoonotic infections, with recent studies in Malaysia having detected *Borrelia* strains, including those related to LD and RF, in small mammals and ticks collected from rural areas, such as indigenous communities, forests, and oil palm plantations (Khoo et al., 2018; Lau et al., 2020; Mohd-Azami et al., 2023). These findings highlight the continuous prevalence of *Borrelia* spp. in small mammals, including rodents and shrews, as well as the increasing diversity of emerging *Borrelia* species across non-urban Malaysia.

Despite evidence of circulation in rural Malaysia, a significant knowledge gap remains regarding the prevalence and diversity of *Borrelia* in urban environments. Urbanization, which is associated with increased human-animal interactions, creates favorable conditions for urban pests to transmit zoonotic diseases, as these animals frequently live in close proximity to humans (Blasdell et al., 2022). This lack of understanding regarding the presence and transmissibility of *Borrelia* spp. by these animals in urban environments, combined with the possibility of underreporting and underdiagnosis of human *Borrelia* infection, complicates effective disease surveillance, diagnosis, and prevention strategies. Consequently, this poses a serious, unquantified public health threat.

Materials and methods

To address this knowledge gap, we screened archived spleen DNA from rodents and shrews collected in Kuala Lumpur and Penang, the two major urban areas in Peninsular Malaysia, using nested PCR targeting the flaB gene of *Borrelia* spp. The samples, obtained from the Tropical Infectious Diseases Research & Education Centre (TIDREC), Universiti Malaya, were collected under ethical approval from the Institutional Animal Care & Use Committee (IACUC), Universiti Malaya (G8/23122019/11102019-01-R).

Sampling was conducted within Kuala Lumpur (3° 08' 03.2"N, 101° 42' 55.9"E) and Penang (5° 25' 50.8"N, 100° 18' 42.3"E). Between January and October 2023, small mammals (rodents and shrews) were captured from urban habitats, including wet markets, residential and recreational areas, and green patches. Host species identification was confirmed using standard morphometric measurements and conventional PCR targeting the mitochondrial cytochrome *c* oxidase subunit 1 (*CO*1) gene (Robins et al., 2007). Genomic DNA was extracted from spleen tissues using a commercial kit (Nucleospin® Tissue Extraction Kit, Machery-Nagel, Düren, Germany) and stored at -20°C. A total of 120 selected DNA samples (70 from Kuala Lumpur and 50 from Penang) were screened for *Borrelia* species by amplifying a fragment of the *flaB* gene, which was selected due to its high sensitivity and specificity in *Borrelia* detection. The assay employed a two-step nested PCR approach with well-established primer pairs, originally described by Takano et al. (2010). Amplified products were visualized by agarose gel electrophoresis, with 1% agarose in 1x Tris-acetate-EDTA (TAE) buffer stained with SYBR® Safe DNA Gel Stain (Invitrogen). The gels were run at 85 volts (V) for 35 minutes. Successful amplification of the target *flaB* gene was confirmed by the presence of a band at the expected size of 345 bp under ultraviolet (UV) light.

Results and discussion

The host species composition was diverse, with seven different species identified through *CO*1 gene analysis (Table 1). The most abundant species was *Rattus rattus diardii* (n=115; 48.73%), followed by *Rattus norvegicus* (n=57; 24.15%) and *Rattus tanezumi* (n=47; 19.92%). Table 1 provides the full breakdown of host species composition, sampling effort, and molecular detection results by location.

Due to funding limitations, the nested PCR assay targeting the *flaB* gene was performed on randomly selected 120 archived DNA samples from Kuala Lumpur (n=70) and Penang (n=50) using the optimized protocol (Mohd-Azami et al., 2023). The assay was validated in every run, in which the *Borrelia*-positive control consistently yielded the expected ~345 bp amplicon, and the negative control showed no specific amplification. Despite the demonstrated efficacy of the assay, all 120 spleen DNA samples yielded negative results for *Borrelia flaB* gene sequences. The overall prevalence of *Borrelia* species across all screened small mammal populations was 0% (0/120). Representative gel electrophoresis results confirming the non-detection are presented in **Figure 1 (a)** and **(b)**. The current study is one of the first molecular investigations on the prevalence of *Borrelia* spp. in small mammal populations from the major urban centers of Kuala Lumpur and Penang, in Peninsular Malaysia. Despite utilizing a highly sensitive and optimized nested PCR targeting the *flaB* gene, all 120 screened spleen DNA samples yielded negative results, although both positive and negative controls performed as expected. This 0% prevalence is a significant epidemiological finding, indicating that *Borrelia* species associated with small mammal reservoirs may be absent or circulating at a prevalence below the detection limit of our assay in these specific urban areas.

This result contrasts sharply with recent *Borrelia* surveillance in Malaysia. Previous research has consistently confirmed the pathogen's presence in rodents and ticks collected from rural, semi-urban, and forested habitats across Selangor, Sarawak, Johor, and Perak, which includes strains related to both Lyme disease (*B. burgdorferi* s.s.) and relapsing fever (*B. miyamotoi*, *B. theileri*, *B. lonestari*) (Khoo et al., 2018; Lau et al., 2020; Mohd-Azami et al., 2023). The discrepancy between the positive findings in rural areas, forested areas, and natural settings, compared to the non-detection in urban environments such as Kuala Lumpur and Penang, suggests that *Borrelia* has a distinct non-urban epidemiological cycle in Malaysia. The absence of detection is most likely influenced by several ecological factors driven by urbanization. Firstly, habitat fragmentation and the lack of landscape connectivity in dense urban environments may restrict the migration of infected reservoir hosts and tick vectors from forested source areas (Shaw et al., 2024). Secondly, the altered urban microclimate, which is characterized by higher temperatures and lower humidity, is often detrimental to the survival and establishment of *Ixodes* ticks, the primary vectors for *Borrelia* (Heylen et al., 2019). The restricted or complete absence of tick vectors in these highly modified urban environments may therefore inhibit the maintenance of the *Borrelia* transmission cycle. The non-detection must be interpreted in consideration of the study's methodological limitations. Specifically, the temporal snapshot of sampling and the exclusive reliance on spleen tissue may have overlooked the low-level infections that were preferentially sequestered in other organs such as the bladder or skin (Bockenstedt et al., 2020).

Table 1: Host species composition and molecular detection summary for *Borrelia flaB* gene in small mammals from urban Kuala Lumpur and Penang

Location	Host species (n)							Total collected (n)	Total processed (n)	*Borrelia* positive (n)
	Rattus rattus diardii	*Rattus norvegicus*	*Rattus tanezumi*	*Suncus murinus*	*Tupaia glis*	*Rattus tiomanicus*	*Rattus argentiventer*			
Kuala Lumpur	81	2	45	3	6	2	0	139	70	0
Penang	34	55	2	5	0	0	1	97	50	0

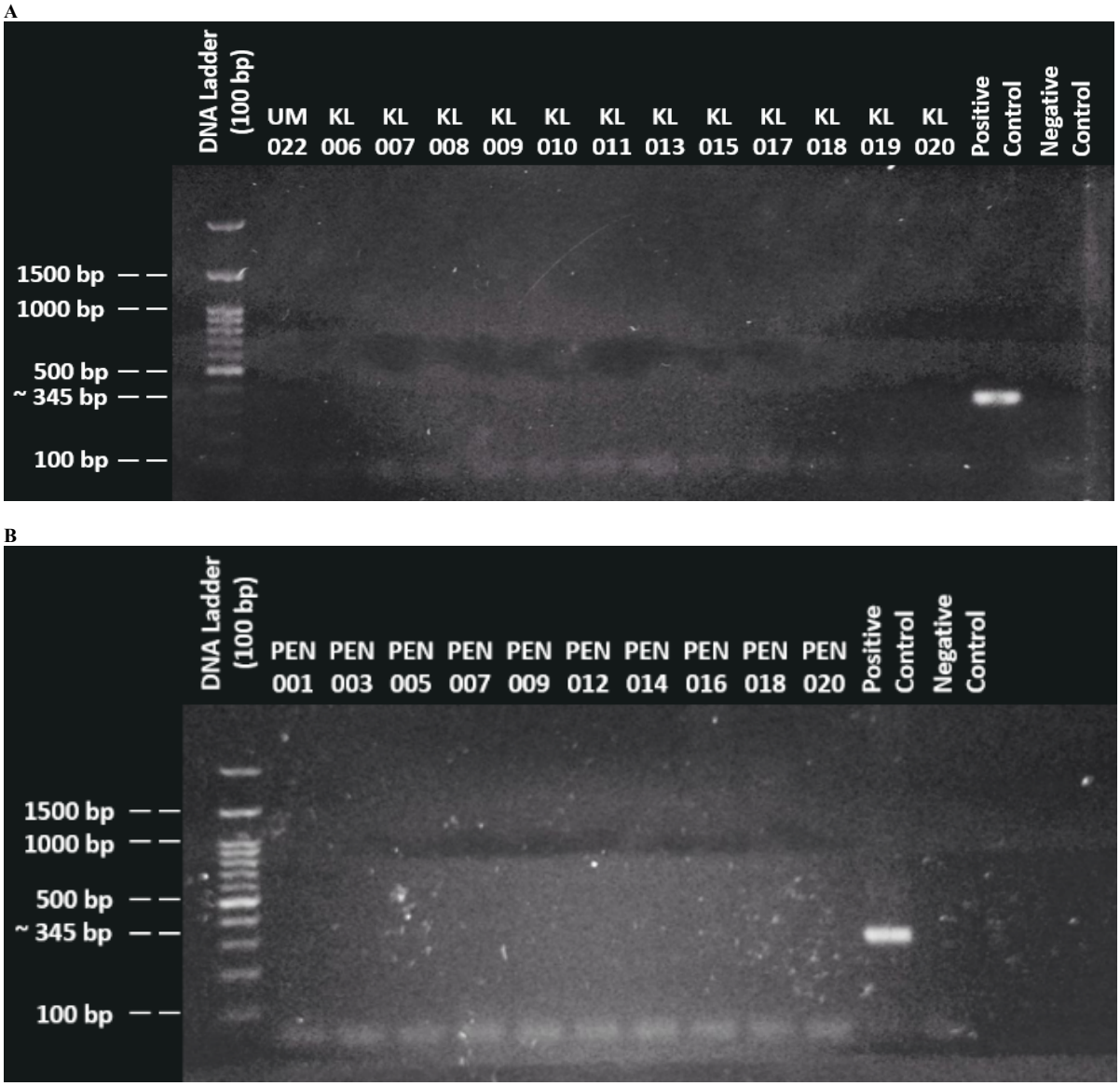

Figure 1 Amplification of the *Borrelia flaB* gene. (A): Representative gel electrophoresis result for nested PCR amplification of *Borrelia flaB* gene from spleen samples collected in Kuala Lumpur. (B): Representative gel electrophoresis result for nested PCR amplification of *Borrelia flaB* gene from spleen samples collected in Penang.

Conclusion

This study establishes baseline data on Borrelia prevalence in urban Malaysia, showing a 0% detection rate in small mammals from Kuala Lumpur and Penang, suggesting that Borrelia may not currently pose an immediate, high-priority public health concern in these urban environments compared to rural or semi-urban areas. This knowledge is essential for directing targeted public health efforts and optimizing resource allocation for zoonotic disease control, while emphasizing the importance of a One Health approach to monitor for the potential shifts in the urban *Borrelia* epidemiology (Mackenzie & Jeggo, 2019). Therefore, future research must adopt an enhanced strategy, including screening substantially larger sample sizes, expanding geographical scope to include peri-urban and rural areas for comparison, and analyzing multiple tissue types to increase sensitivity. Importantly, integrated surveillance must incorporate comprehensive tick collection and serological assays to provide a complete picture of *Borrelia* ecology and risk in Malaysia's rapidly evolving urban landscapes.

Acknowledgements

We sincerely thank Dr. Izzat Azmer Ahmad and the team from Seberang Perai City Council (MBSP), as well as the respective authorities from Kuala Lumpur City Hall (DBKL), for granting access to sampling sites and logistical support. We also extend our gratitude to Mohd Azlan Abd Majid, Nurul Naimah Kamal Bahrain, Nur Afifah Othman and Nurrin Jazlina Khalid from TIDREC, and Putri Wulan Dari, Aoha Yamamoto and Hsieh Ting Chuan from Universiti Sains Malaysia for their support in carrying out this work.

Funding

This research was funded by the Ministry of Higher Education, Malaysia for niche area research under the Higher Institution Centre of Excellence (HICoE) program (MO002-2019 and TIDREC-2023), Ministry of Higher Education under Dana Langganan SUKUK Pakej Rangsangan Ekonomi Prihatin Rakyat (SUKUK PRIHATIN) - Fasa 2 (MO002-2021) grant and Universiti Malaya (UM) Living Labs (FYP SDG@UM-051). No funders played a role in the study design, data collection or analysis, decision to publish, or manuscript preparation.

Conflict of interest statement

The authors declare no conflict of interest

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